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Comissão Julgadora:

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Prof. Dr. Ronald D. P. K. C. Ranvaud

Prof. Dr. Edson Amaro Júnior

_____

Prof. Dr. Gilberto Fernando Xavier

(orientador)

Ao meu pai, José Eurípedes,

que um dia me disse:

"Se não posso voar com você,

eu te ajudo a construir as tuas asas".

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SUMÁRIO

***** Esta dissertação foi estruturada segundo o formato de capítulos/artigos da USP, que estão redigidos em inglês. Ela contém uma introdução e uma discussão geral que abrange todos os assuntos presentes, além de introduções e discussões específicas para cada capítulo/artigo. Alguma repetição de conteúdo será encontrada nesses tópicos.

INTRODUÇÃO GERAL

Como seres sociais, nós humanos passamos boa parte do nosso tempo observando as outras pessoas, tentando entender o que elas estão fazendo e muitas vezes aprendendo com elas. Esta "comunicação primitiva" é essencial para estratégias de sobrevivência e sociabilidade do indivíduo. Contudo, como reconhecemos e entendemos as ações das outras pessoas? Quais as bases neurofisiológicas desta habilidade? A recente descoberta de neurônios-espelho tem inspirado uma série de estudos em busca destas respostas.

O reconhecimento de uma ação foi inicialmente concebido como baseado apenas no sistema visual; isto é, numa análise dos componentes visuais da ação específica, do agente envolvido, do objeto ao qual a ação é direcionada e do contexto no qual ela está inserida (Carey *et al*., 1997; Buccino *et al*., 2004a). Assim, a interação de todos estes elementos identificados visualmente permitiria ao observador reconhecer e entender uma ação feita por outra pessoa. Uma hipótese alternativa admite que a observação de uma ação estimularia uma "representação motora interna" que envolveria as mesmas estruturas neurais envolvidas na execução da ação observada; de acordo com esta concepção, embora nenhum movimento efetivo seja executado, a representação motora evocada pela observação permitiria o reconhecimento do significado do que é visto (Gallese *et al*., 1996; Umiltà *et al*., 2001; Rizzolatti e Craighero, 2004). Com a descoberta de que há ativação de neurônios na região do córtex pré-motor durante a observação de ações, os assim denominados "neurônios-espelho" (Gallese *et al*., 1996; Rizzolatti *et al*. 1996a) e, considerando que esta hipótese não exclui a possibilidade de que outros processos cognitivos, baseados na descrição do objeto e do movimento, possam participar desta função, esta hipótese motora vem ganhando cada vez mais

adeptos. Todavia, tem sido proposto que os neurônios-espelho formam um sistema que combina observação e execução.

Sistema de neurônios-espelho

Neurônios-espelho incluem grupos de neurônios cuja atividade aumenta durante a execução de uma ação motora particular ou da observação da mesma ação desempenhada por outro individuo (Gallese *et al*. 1996; Rizzolatti *et al.*, 2000, 2001; Rizzolatti e Craighero, 2004). Sua descoberta ocorreu durante experimentos com macacos envolvendo o controle motor de ações desempenhadas com as mãos, como por exemplo, pegar/manipular um objeto ou alimento (di Pellegrino *et al*., 1992). Rizzolatti e seus colaboradores implantaram eletrodos no córtex frontal inferior de macacos (região pré-motora, área F5) e registraram a atividade dos neurônios individualmente enquanto os animais alcançavam pedaços de alimentos (di Pellegrino *et al*., 1992, Rizzolatti *et al*. 1996). Eles observaram que alguns destes neurônios (situados no setor superior da área F5), disparavam não somente quando o macaco pegava o alimento, como também quando ele observava outro indivíduo (macaco ou humano) desempenhando esta ação, como se a mesma tivesse sido "refletida" no seu córtex motor. Estudos posteriores mostraram que em macacos, pelo menos 10% dos neurônios envolvidos no controle motor de ações desempenhadas com as mãos são "neurônios-espelho" (Gallese *et al*. 1996; Rizzolatti e Craighero, 2004).

A descoberta dos neurônios-espelho teve grande impacto no estudo do comportamento humano. Como parecem integrar observação e ação, eles têm sido foco de estudos sobre como o ser humano entende o próximo e em que extensão é capaz de compartilhar experiências. Rizzolatti e Sinigaglia (2010) sugerem que esse mecanismo básico de integração permite entender as ações dos outros a partir de uma representação interna. Tal "entendimento motor" seria o modo mais primitivo através do qual os

indivíduos se relacionam. Atualmente, tem sido sugerido que o sistema de neurônios-espelho esteja por trás de vários comportamentos sociais e processos cognitivos, tais como imitação, empatia, teoria da mente, e que alterações no seu funcionamento possam se relacionar com os distúrbios do espectro do autismo (Gallese et al., 2004; Rizzolatti et al., 2009).

Área F5 e o sistema de neurônios-espelho em macacos

No córtex pré-motor ventral de macacos, a área F5 é relacionada com o controle dos movimentos das mãos e da boca e possui características histoquímicas e citoarquitetônicas específicas (Rizzolatti *et al*., 1998; Umiltà *et al*., 2001; Buccino *et al*., 2004a). Os movimentos das mãos são representados em sua porção mais dorsal e os movimentos da boca tendem a ser representados mais ventralmente (Gallese *et al*., 1996). Pouco é conhecido sobre os neurônios relacionados aos movimentos da boca; diferentemente, as propriedades motoras e sensoriais dos neurônios relacionados ao controle dos movimentos das mãos têm sido extensivamente estudadas. Cerca de 81% destes neurônios apresentam seletividade e especificidade na forma particular como a resposta com a qual a sua atividade se relaciona se manifesta (preensão usando apenas alguns dedos, com os dedos em formato de pinça ou com toda a mão) (Rizzolatti *et al*., 1998; 1996). Além do tipo de preensão, a especificidade da resposta neuronal parece depender do tipo de interação com o objeto, incluindo "*grasping neurons"* (disparam com o movimento de uma mão se aproximando de um objeto e agarrando-o – trata-se do mais abundante), "*grasping-with-the-hand-and-the-mouth neurons"* (ativados com movimentos das mãos ou da boca), "*placing neurons"* (disparam quando o macaco move um objeto em direção a um plano ou suporte), "*manipulating neurons"* (disparam quando o macaco toca e move um objeto), "*holding neurons"* (disparam quando o macaco mantém um objeto na mão, segurando-o), entre outros (Rizzolatti et al., 1998,

Gallese *et al*., 1996). Essa especificidade foi demonstrada não somente durante a execução, mas também durante a observação destas ações executadas por outros indivíduos. Interessantemente, a observação da pantomima destas ações, isto é, a simulação da ação sem o objeto, não ativa tais neurônios (Rizzolatti et al., 1996b).

As propriedades espelho foram inicialmente demostradas em neurônios da área F5 por mera eventualidade. Devido às características do método empregado pelo grupo de Rizzolatti, a eletrofisiologia, o fenômeno foi observado nesta região justamente por ser este o local em que se encontravam implantados os eletrodos. A partir dos resultados de Perrett e colaboradores (1989; 1990), que mostram a ativação de uma região do córtex do sulco temporal superior (STS) quando o macaco observa ações desempenhadas por semelhantes, tais como movimentos da mão direcionados a objetos, e as semelhanças com as propriedades de F5, algumas questões a respeito dos mecanismos subjacentes à observação de ações e seu entendimento emergiram, possibilitando a exploração das propriedades espelho em outras regiões.

Baseado em estudos anatômicos que demostram a conectividade indireta entre as regiões de F5 e STS, através de áreas parietais (Petrides e Pandya, 1984; Rizzolatti et al., 1998), as propriedades da área PF (parietal inferior anterior em macacos) foram investigadas com o propósito específico de descobrir se essa região contém neurônios que respondem a movimentos desempenhados por semelhantes. Os resultados mostraram que cerca de 40% dos neurônios que foram registrados em PF se ativaram durante a observação de ações (Fogassi et al., 1998). As ações que mais ativaram esta região foram pegar um obejto, interação bimanual, segurar e alcançar um objeto. Cerca de metade dos neurônios registrados apresentaram especificidade para apenas um tipo de ação, ou seja, responderam apenas durante a observação de pegar ou de alcançar um objeto. A descoberta de que a maioria dos neurônios nesta região responde tanto durante

a observação quanto a execução da ação foi bastante importante. Eles foram, portanto, definidos como os neurônios-espelho, como no caso dos neurônios localizados em F5.

Assim, baseado em estudos anatômicos e funcionais, os neurônios-espelho, agora não mais restritos à área F5, passaram a ser vistos como parte de um sistema, o sistema de neurônios-espelho (MNS), ou circuito parieto-frontal (Rizzolatti et al., 2001). É importante notar que os neurônios registrados em STS não são ativados durante a execução de ações (Fabbri-Destro e Rizzolatti, 2008) e, portanto, não são denominados neurônios-espelho. Esta região, assim como várias outras que têm sido descritas recentemente (córtex frontal médio, cingulo anterior, córtex parietal superior), embora não façam parte do MNS, atuam como coadjuvantes do mecanismo espelho (Rizzolatti e Sinigaglia, 2010).

Sistema de neurônios-espelho em humanos

Atualmente, não existem evidências diretas da existência de neurônios-espelho em humanos. Porém, evidências indiretas, com estudos de populações de neurônios, sugerem a existência de neurônios-espelho em humanos. Dentre elas vale mencionar estudos eletrofisiológicos não invasivos envolvendo a ativação específica do sistema motor (Fadiga *et al*., 2005; Oberman *et al*., 2005; Pineda, 2005; Kessler *et al*., 2006; Bernier *et al.,* 2007) e estudos envolvendo técnicas de imageamento funcional por ressonância magnética (do inglês, functional Magnetic Ressonance Imaging, fMRI) (Grèzes *et al*., 2003; Buccino *et al*., 2004b; Constantiti *et al*., 2005; Gazzola *et al*., 2007).

O primeiro estudo a sugerir a existência de uma relação entre determinados ritmos eletrofisiológicos em humanos e a atividade de neurônios-espelho foi o de Altschuler *et al*. (1997), usando o ritmo *mu,* que foi descrito por Gaustaut e colaboradores já nos anos de 1950 (Gastaut e Bert, 1954 *apud* Rizzolatti e Craighero,

2004). O ritmo *mu* é uma oscilação sincronizada com freqüências dominantes entre as bandas 8 e 13 Hz, e 15 e 25 Hz, limitada a breves períodos de 0,5 a 0,2 segundos de duração e pode ser registrada no córtex sensoriomotor de humanos na ausência de movimentos. Interessantemente, este ritmo dessincroniza ou desaparece totalmente durante execução ou observação de movimentos ativos ou estimulação somatossensorial direta (Muthukumaraswamy *et al*., 2004, Oberman *et al*., 2005, Bernier *et al.,* 2007). Devido às propriedades motoras dos neurônios-espelho serem eletrofisiologicamente indistinguíveis daquelas referentes aos neurônios motores de áreas vizinhas, isto é, área pré-motora, motora e sensoriomotora, a supressão das ondas *mu* durante a execução de ações é provavelmente o resultado da ativação de vários sistemas neuronais dos córtices pré-motor e sensoriomotor. Entretanto, durante a observação de movimentos das mãos, o sistema de neurônios-espelho é a única rede ativa identificada nesta área do córtex. Isto sugere que a supressão do ritmo *mu* durante ações observadas poderia ser usada como uma medida seletiva da ativação do sistema de neurônios-espelho (Pineda, 2005).

Estudos envolvendo imageamento encefálico em humanos têm permitido a identificação de áreas corticais que podem conter neurônios-espelho. A observação de ações desempenhadas por outros ativa, além das áreas visuais, duas regiões corticais cujas funções são classicamente consideradas motoras: região anterior do lóbulo parietal inferior (IPL) e a parte inferior do giro pré-central (córtex pré-motor, PMC), além da região posterior do giro frontal inferior (IFG), a área de Broca (Rizzolatti *et al*., 1996b, Grafton *et al*., 1996, Buccino *et al*., 2001, Grèzes *et al*., 2003, Iacoboni *et al*., 2005).

Considerando que o PMC junto com IFG é considerado área homóloga a área F5, assim como IPL é considerada homóloga a área PF/PFG em macacos, parece haver uma grande congruência entre o MNS em macacos e humanos. Ainda, graças as características do método de fMRI, é possível analisar o encéfalo humano como um

todo, o que tem permitido estudar outras áreas ativas durante uma tarefa que investiga o funcionamento do MNS.

Imitação e o sistema de neurônios-espelho

Imitação é baseada em dois mecanismos distintos, porém relacionados: a capacidade de entender ações feitas por outros e a capacidade de replicar estas ações (Rizzolatti, 2005). Dados da literatura sugerem que o MNS possui uma função fundamental nestes dois mecanismos, tanto no entendimento, quanto na replicação de ações executadas por outros (Iacoboni *et al*., 1999, Grèzes *et al*., 2003, Vogt *et al*., 2003).

Entretanto, é interessante ressaltar as diferentes formas em que o termo "imitação" pode ser usado. Em estudos psicológicos, "imitação" se refere ao comportamento de indivíduos instruídos a replicar uma ação já presente em seu repertório motor. Já nos estudos etológicos, o foco é no aprendizado, isto é, "imitação" é a capacidade de adquirir uma habilidade motora previamente ausente no repertório motor do observador (Thorndike, 1898). Uma abordagem bastante interessante, proposta por Csibra (2007) defende que toda imitação se baseia, de alguma maneira, no repertório motor que o observador possui. Ele argumenta que quando alguém imita uma ação, ela escolhe certo nível de descrição da ação observada e o reproduz através da reconstrução em seu sistema motor. Deste modo, ele sugere que a imitação contém um forte componente de "emulação", isto é, a reprodução do objetivo final de uma ação pelos meios próprios do observador, utilizando o seu próprio repertório motor.

Considerando o modo hierárquico com que as ações são organizadas, a diferenciação entre imitação e emulação pode se tornar ainda mais difícil. Por exemplo, a execução de uma determinada ação, comer uma maçã, pode ser identificada como uma seqüência organizada de passos. Um deles é trazer a maçã à boca, o que pode ser

considerado como um sub-objetivo em direção ao objetivo de nível mais alto, que é comer a maçã. Este sub-objetivo exigirá também uma seqüência de atos como, por exemplo, pegar a maçã. Para executar este ato, é preciso realizar uma série de movimentos que, por sua vez, também podem ser analisados em unidades menores, no nível das ativações de músculos individuais. Suponha, por exemplo, que o modelo executou a ação de comer a maçã e escolheu realizá-la através dos atos descritos no lado esquerdo da Figura 1. Em resposta, um imitador pegou uma maçã, levou-a à sua boca e comeu. No entanto, ele pegou a maçã utilizando o seu polegar e o dedo indicador (em formato de pinça) em vez de utilizar todos os dedos. O imitador, de fato, imitou a ação do modelo? Se a reprodução da ação é avaliada em um nível básico, onde o tipo de preensão é definido, ele não imitou, mas emulou a ação observada. Se, no entanto, o tipo de ação é definido em um nível superior na hierarquia da organização da ação, a resposta é afirmativa, afinal, ele levou a maçã até a boca e não o contrário, e ele pegou-a usando sua mão. Isso não significa que a imitação, na verdade, não existe, mas que é satisfeita dependendo do nível da hierarquia da organização da ação que foi estabelecido.

Desta forma, é razoável definirmos imitação levando em consideração que nível da hierarquia da organização da ação de imitação irá ocorrer e identificando o grau de envolvimento do repertório motor do observador na reconstrução da ação observada, em vez de se pautar apenas pela presença (ou ausência) de aprendizado motor.

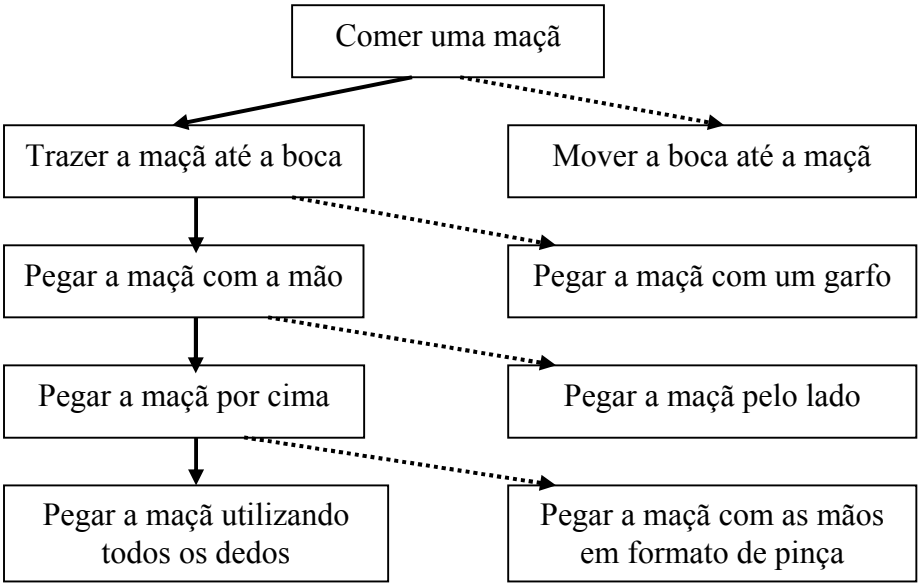

Figura 1. Exemplo de etapas da organização hierárquica da ação "comer uma maça" (modificado de Csibra, 2007).

O envolvimento dos neurônios-espelho na imitação foi sugerido pela primeira vez por Iacoboni e colaboradores (1999). Neste estudo, voluntários executaram movimentos com os dedos durante três condições: 1) observação do movimento do dedo a fim de imitar, 2) observação de uma mão estática com uma cruz em cima de um dos dedos, indicando que este deveria ser levantado (pista simbólica da ação), e 3) observação de um retângulo cinza com uma cruz em uma posição espacial (esquerda ou direita) que indicava o movimento do dedo alvo (pista espacial). Estas três condições também foram apresentadas em uma tarefa de simples observação, isto é, quando o voluntário apenas observa a ação, sem o requerimento de execução da mesma. Os resultados deste estudo mostraram que durante a tarefa de imitação as ativações do IFG e IPL foram mais pronunciadas quando comparadas com a tarefa de simples observação. Ainda, a atividade destas regiões está aumentada na condição em que o voluntário *observa* o movimento real dos dedos em comparação as condições com

pistas simbólicas e espaciais, sugerindo, segundo os autores, que estas áreas estão envolvidas com os mecanismos de imitação.

Buccino *et al*. (2004b) mostrou que a observação para fins de imitação de ações ausentes no repertório motor (acordes musicais) ativa o MNS mais intensamente quando comparada com a simples observação ou a geração de uma ação voluntária. Vogt *et al*., (2007), utilizando o mesmo protocolo, demonstrou que a observação para fins de imitação de acordes musicais não praticados, portanto, ausentes no repertório motor do voluntário, ativa mais intensamente o MNS (IFG e PMC) quando comparado com acordes conhecidos (praticados), mesmo num grupo de especialistas (músicos). Estes autores postulam que os neurônios-espelho atuam no mecanismo de ressonância dos atos motores primários que constituem a ação observada (levantamento de dedos, posicionamento relativo, configuração final do movimento), configurando e codificando-os motoramente. Quando a ação a ser imitada já está presente no repertório motor do observador, todos os atos motores que a compõem são codificados unitária e conjuntamente, sendo enviados imediatamente em direção a outras estruturas e assim replicado. Por outro lado, quando a imitação requer o aprendizado de um novo padrão motor ou uma nova seqüência motora, um mecanismo adicional é necessário. Os atos motores que compõem a ação precisam ser codificados separadamente e recombinados em um novo padrão motor ou nova sequência. Eventualmente, esse mecanismo adicional depende de outras estruturas que atuam conjuntamente com o MNS (Rizzolatti, 2005; Buccino *et al*., 2004b).

Todavia, se existem diferenças na atuação do MNS durante a imitação de ações presentes e ações ausentes no repertório motor de um indivíduo, é possível que este sistema seja modulado durante o processo de aprendizado de uma habilidade motora. Além disso, é possível que, mesmo depois de aprendida, uma mesma ação possa ativar

diferentemente o MNS dependendo de algumas contingências, tais como: característica do estímulo, contexto do experimento, fins para os quais a ação é executada, nível de aprendizado.

OBJETIVOS

O objetivo geral do presente estudo é identificar, por meio de fMRI, estruturas nervosas envolvidas especificamente na observação de uma ação motora desconhecida (em seu nível hierárquico superior) com vistas à sua imitação, antes e depois da aprendizagem dessa ação, com foco especial nas regiões do sistema de neurônios-espelho (incluindo córtex pré-motor, giro frontal inferior, córtex intraparietal e parietal inferior). Almejamos ainda identificar estruturas neurais do sistema de neurônios-espelho envolvidas na representação de uma ação motora durante a observação da ação, preparação e execução propriamente dita.

Capítulo I

Encephalic hemodynamic changes during action observation for imitation of either known or unknown actions: a fMRI study

ABSTRACT

There have been proposals that the mirror neuron system (MNS), including premotor cortex, inferior frontal gyrus and inferior parietal lobule, is involved in action recognition, motor imagery and imitation of simple movements already represented in the motor repertoire of primates. However, it has also been suggested that the MNS plays a key role during acquisition of new motor abilities by way of imitation. These figures raise the question on how the MNS participates in the imitation of unknown actions. The purpose of this experiment was to investigate hemodynamic changes in the MNS by way of functional Magnetic Ressonance Imaging (fMRI), during observation of either untrained or trained actions. Naïve non-musician volunteers were instructed to observe video clips of either a hand or kinematically comparable moving dots performing guitar chords "as if they were to imitate the movement later", but without performing any movement, both before and after extensive practice training with these chords. Concurrently with these observation sessions the subjects were exposed to fMRI procedures. The results showed that observation of both types of video clips activated the MNS and that the degree of activation was stronger for untrained as compared to trained actions. These results lend support to the notion that the MNS is critical for observing aiming at imitation of both untrained and trained actions. In addition, these data support the hypothesis that increments of neural efficiency throughout learning processes lead to improvements of the information processing, with simultaneous minimization of the costs both in terms of computation and attentional resources thus rendering the general functioning of the system efficient and economical.

1.1 INTRODUCTION

Acquisition of a new motor skill may require substantial amounts of attention at early stages. However, as procedural knowledge about the perceptuo-motor skill is acquired its performance gradually becomes automatic, thus reducing the attentional load for task performance. This transition depends upon the previous perceptuo-motor repertoire in order to build the new schema. This internal perceptuo-motor representation is refined by repeated execution of the task; possibly, this automatization process is associated with an increase of specificity in neural circuit connections underlying performance of the task which then may be controlled by a much smaller amount of neural tissue, with consequent energetic economy.

There have been proposals that the mirror neuron system (MNS), including the premotor cortex (PMC), inferior frontal gyrus (IFG), inferior parietal cortex (IPL) and intraparietal sulcus (IPS), is involved in action recognition, motor imagery and imitation of simple movements already represented in the motor repertoire of the individual (Rizzolatti and Sinigaglia, 2010). By using functional Magnetic Ressonance Imaging (fMRI), Buccino et al. (2004) showed that the MNS is involved in acquisition, by way of imitation, of complex hand actions. In their experiment musically naïve participants were asked to imitate guitar chords after observing models presented by an expert guitar player. The MNS was found to be active in every phase of the motor learning process, including observation of the model up to its execution. The authors suggested that the MNS plays a key role during the acquisition of a new motor pattern, which involves re-arranging elementary motor acts present in the participants' motor repertoire in order to fit their action to the provided model.

Several studies have proposed that this operation involving translation of action representation into own's action with the participation of the MNS occurs

particularly in the early stages of the learning and also engage subcortical and associative areas (Byrne, 2002; Rizzolatti, 2003; Buccino and Riggio, 2006). This involvement of the MNS in motor learning is supported by evidence demonstrating that action observation by itself leads to the formation of motor memories similar to those underlying practice-induced changes in motor representations (Mattar and Gribble, 2005; Stefan et al., 2005). In addition, Mattar and Gribble (2005) showed that this effect is also achieved when the possible use of conscious strategies is minimized. These authors exposed subjects to videos showing another person learning a complex motor task requiring dynamic adaptations concurrently with performance of an attention-distracting arithmetic task. Despite the attention deviation, the participants learned the action, leading the authors to conclude both that learning relied on implicit engagement of neural systems for movement planning and control and that during the observation phase there was no use of a cognitive strategy for learning.

There have been proposals that increment of neural efficiency throughout the learning process leads to stabilization and improvement of the information processing, with simultaneous minimization of the costs in terms of computation and attentional resources (e.g., Squire and Kandel, 1999; Reithler et al., 2010). As a matter of fact, neuroimaging studies show a reduction of the activation pattern along learning (Poldrack et al., 1998, Hikosaka et al., 2002, Muhlau et al., 2007).

However, the context in which an action is observed for later performance also interferes with the hemodynamic changes in the MNS. For instance, Calvo-Merino et al. (2005) showed that activation of the MNS is stronger in ballet dancers and capoeira players when they observe an action already present in their respective motor repertoire. That is, while ballet dancers exhibit stronger activation of the MNS when observing ballet dancers, as compared to capoeira players exhibiting kinematically comparable

actions not belonging to their repertoire, capoeira players exhibit stronger activation when observing capoeira players as compared to ballet dancers exhibiting kinematically comparable actions. These results suggest that action observation is more likely to recruit MNS areas when the observed action is represented in the subject's personal motor repertoire. Congruently, subjects not exposed to either ballet or capoeira training did not exhibit differences in hemodynamic changes when observing ballet dancers or capoeira players. Together, these results suggest that action observation may induce an internal motor simulation of the observed movement. However, it seems important to note because of the instructions given to the subjects these studies did not involve observing for imitating (see below).

Buccino et al. (2004), using musicaly naïve participants exposed to non-practiced chors, and Vogt et al. (2007) using a similar experimental design involving musicians and non-musicians exposed to either practiced or non-practiced chords, demonstrated that the MNS is strongly involved during action observation for imitation of unknown actions; this activation was stronger as compared to that seen for action observation for imitation of actions already known by the volunteer. This latter result is intriguing concerning the group of musicians: despite of their prior training with a variety of chords, there was stronger MNS activation when they were exposed to non-practiced chords. Thus, the effects of practice in action observation for imitation of specific movements on activation of the MNS is an open question.

The purpose of this study was to investigate hemodynamic changes in the MNS during observation of either known or unknown actions. Specifically, we asked whether the activation of the MNS follow the above mentioned optimization of neural processes after learning, with consequent reduction of the MNS activation. This question was addressed in naïve non-musician volunteers exposed to videos clips of

guitar chords before and after a practice session. Further, we used a video involving symbolic cues (moving dots) of the action, in addition to a video clip showing a hand performing the required action, in order to investigate whether activation of the MNS is restricted to movements performed by a biological structure.

1.2. MATERIAL AND METHODS

1.2.1 Subjects

Twenty healthy female subjects (ages varying between 19 and 32 years, mean, 25.3 years), right-handed according to the Edinburgh Handedness Inventory, were recruited for the study. Four participants were later excluded due to image acquisition failures. They were students of the University of São Paulo recruited through e-mail advertisements. Exclusion criteria involved (1) ability to play any musical instrument even modestly and informally, (2) current or previous history of neurological diseases, (3) current or previous history of psychiatric disorders, (4) consumption of addictive drugs, and (5) any signs of claustrophobia. The quality of their imitation during the experiments was evaluated and also considered as an exclusion criterion. All subjects had normal or corrected-to-normal visual acuity. All subjects gave their written informed consent to participate in the experimental procedure, which had been approved by the Ethical Committee of the Faculty of Medicine at the University of São Paulo.

1.2.2 Experimental Devices and Stimuli

Stimuli were presented via Eloquence Invivo screen (1024 x 768) located above the participant's head. E-prime 2.0 Software (Psychology Software Tools, Inc.) was used for displaying the colored cues (squares with 2 degrees of visual angle) and video clips (448 x 336 pixels, 25 frames per second, 25 degrees in the horizontal and 19 degrees in the vertical visual angle). All video clips were recorded using a classical

guitar played by a skilled guitarist. He used his left hand so that the chords appeared as a counter mirror image of the participant's left hand. This perspective was chosen in order to match the anatomy and preserve the details seen when one observes another person performing an action right in front of ones.

In the video clip showing a hand performing the chord, the model's index, middle and ring finger moved from the resting position below the guitar neck to the specific chord position and returned to the resting position; this was repeated three times. This resulted in a rhythmical 3-s-duration press release strings pattern that continued throughout the video clip exhibition. Every of the four chosen chords (A, C, D and G) involved three fingers, each of them on a different string and involving only the three upper frets. No chord involved the small finger. For the moving dots video clip, a frame of the "hand" video clip with the hand in a resting position was taken in order to project the circles above the fingers of the guitar player. An animation was made such that the moving dots simulated the movement of the fingers thus exhibiting the chord for the same amount of time as the "hand" video clip; the Sony Vegas Pro 9.0 (trial version) software was used to prepare this animation.

A wooden guitar neck (height 42 cm) without strings and frets was used for practice training. It was placed horizontally along the volunteer's body, next to the volunteer's hips so that it could be comfortably held during the practice session and during the experiment involving imitation (see Chapter 2). However, during this especific experiment the volunteers did not hold the guitar neck. The participants were instructed to perform the chords in the same rhythm as shown in the video clips.

A black screen with a cross in its centre was used as the baseline for the contrasts. The participants were instructed to avoid any movement during the scanning

sessions. During the practice session, the participants had no visual feedback of their movements.

1.2.3 Design and Experimental Conditions

The experiment involved two observation sessions, one executed before and the other after extensive imitative motor training (see Figure 1.2.1 for a session illustration).

The first of these sessions, named initial observation session (iOBS), was run before any training of the subjects, therefore, they had no familiarity with the actions observed. The participants were exposed to a video clip showing either a hand performing a guitar chord on a guitar neck (Figure 1.2.2A) or a similar image in which the hand was maintained still and independent moving dots exhibited a comparable kinematic movement relative to that of the hand (Figure 1.2.2B). Each video clip presented the same chord four times in a 12-second long event. The subjects were instructed to observe the video clip as if they were to perform the same action later but that no action should be performed. Each video presentation was followed by a rest period of 16-18 seconds, used as the baseline. These two events (video clip presentation and resting period) formed a trial. Each session included 24 trials. While in the first 12 trials the video clip showed a hand performing the chords, in the 12 remaining trials the chords were showed by the video clip with moving dots. The subjects were instructed to avoid any movement at any time during this observation session.

The imitative motor training involved exposure of the subjects to either a video clip showing a hand performing a chord or a moving dots video clip showing a chord (12 seconds), followed by the action execution (6 seconds). This procedure was repeated along 20 minutes (30 trials). This training, executed outside the MRI

equipment but as if the subject was inside the equipment, allowed both acquisition on how to hold the guitar neck and acquisition on how to move the fingers on the guitar neck in order to imitate the shown chord. The participants were instructed to attentively observe the model chord and to reproduce it with maximal accuracy, using their left hand. An experimenter was present in the scan room all time to help the participant to position their fingers on the guitar neck correctly. During this training participants had no visual feedback of their movements. Then, the subjects participated in an imitation experiment lasting about 25 minutes, run inside the MRI equipment (see Chapter 2 for details). In this experiment the subjects had to observe a video clip and then to execute one of the required tasks. Hence, the entirely practice session lasted about 45 minutes.

The second session, named final observation session (fOBS), was run following the same procedures described for the iOBS. Therefore, as in iOBS section, they were instructed to avoid any movement at any time.

Because the subjects had no prior experience with the chords in the iOBS but were exposed to a reasonable amount of imitation training with the chords before the fOBS, their ability to identify and to execute the corresponding movements were incorporated in their repertoire. Thus, comparison of the iOBS and the fOBS should reflect this experience.

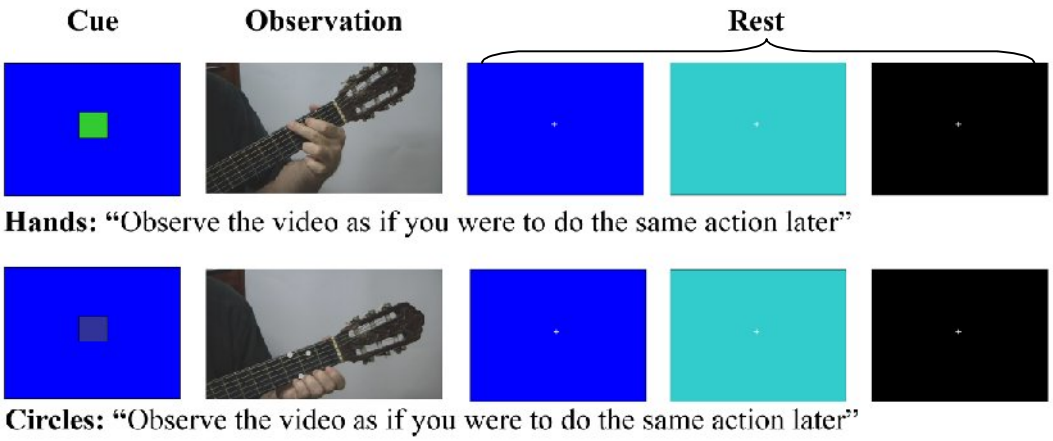

Figure 1.2.1 Experimental design with the conditions "Hands" (superior images raw) and "Moving Dots" (inferior images raw) illustrated. Each trial began with the presentation of a cue (a colored square) in order to prompt the participants. Subsequently, the participants observed the video followed by the rest (blue, ciano and black screen). Duration of the events: Cue 2s; Observation 12s; Rest 16-18s.

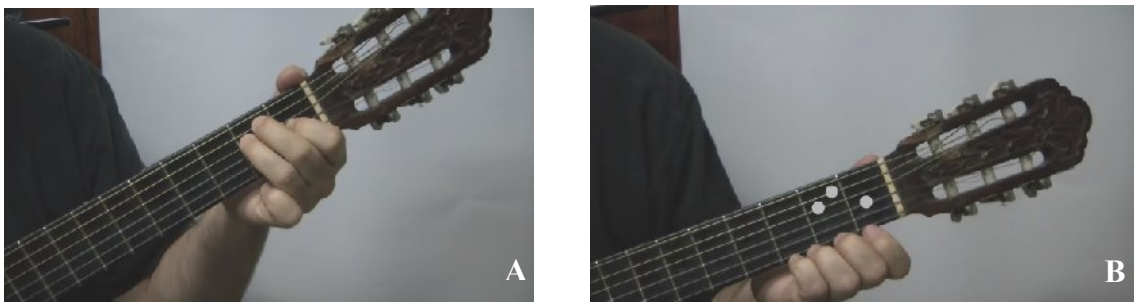

Figure 1.2.2 A – A frame of the video clip showing a hand performing the chord. The fingers move from the resting position below the guitar neck to the chord position and returns to the resting position. **B** – A frame of the video clip showing moving dots with the hand in the resting position and the chord as indicated only by the moving dots. Thus, the moving dots corresponded to a symbolic cue for the fingers movement. The moving dots started their movement from the fingertips towards the chord position and then return to the resting position.

1.2.4 Image Acquisition and Settings for the fMRI

The experiment was carried out using a 3T scanner with an eight-channel head coil (3T Philips Achieva, Institute of Radiology, HC – FMUSP). The operation of this equipment was assessed throughout the study by daily quality control. The MRI exam

employed procedures known to minimize artifacts caused by head movements, for instance, the use of a tape on the forehead and of two foam pads on the head sides, thus providing good immobilization of the head, without causing discomfort. All participants were instructed to avoid movements during image acquisition, particularly those of the head.

Forty transversal AC-PC slices T1 weighted echo-planar images were obtained from a gradient-echo sequence used to measure task-related changes in blood oxygen level dependent (BOLD) signal as an index of regional neuronal activity. The following parameters were used: echo time TE 28 ms, repetition time TR 2 s, flip angle 8, field of view FOV 240 mm, slice thickness 1.875 x 1.875 x 3 mm, interslice gap 0.4mm, in-plane resolution 3.125 x 3.125mm. The forty slices covered the whole brain from the top of cerebellum through to the vertex.

12.5 Data Processing and Statistical Analysis

1.2.5.1 Pre-Processing

The initial five volumes of each subject's scan were not collected in order to wait until full T1 saturation. Data analysis was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, which is part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). A Fourier-space time-series phase-shifting was applied to correct for the temporal offset between the slices acquired in one scan. Non-brain voxels were removed using BET (Smith, 2002). A temporal high-pass filter (Gaussian-weighted least-squares straight line fitting) with a cut-off frequency of 1/54 Hz was used for baseline correction of the signal, in order to correct for large drifts. Spatial smoothing using a Gaussian kernel of FWHM 5.3 mm as well as mean-based intensity normalization of all volumes by the same factor was applied to the functional data. To

align the individual functional data slices onto the corresponding 3D stereotactic coordinate reference system (MNI), a rigid linear registration with six degrees of freedom (three rotational, three translational) was carried out using FLIRT (Jenkinson and Smith, 2001).

2.5.2 Whole Brain Analysis

The whole brain analysis involved two different approaches. The first, involving data from each subject, the onsets and durations of each of the stimuli were modeled using the General Linear Model (GLM) according to the experimental conditions and events. Data were motion-corrected using MCFLIRT (Jenkinson et al., 2002) and affine spatial normalization 12DOF. The design matrix was composed of 4 regressors according to the type of video clip (either observation of a hand or observation of moving dots showing the chords) for each session (iOBS and fOBS). The regressors were convolved with the gamma basis functions (12 additional regressors per session were included in the GLM to account for voxel intensity variations due to absolute and differential head movement. In the second approach the respective contrast images from the first stage for each subject were entered into one-sample t tests (random effects analysis, Friston et al., 1999). Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$ (Worsley, 2001). The standard coordinates of the local maxima within areas of significant activity change were determined for all conditions. The tables showing anatomical localization of the local maxima and clusters were assessed by reference to Talaraich Coordinates after appropriate coordinate transformation and checked visually by a radiologist. The activation maps were made using MRIcron Software

(www.mricron.com, 12/2009 Version) and illustrates the activations with 20% of air/skin threshold and 8mm of search depth.

1.3. RESULTS

1.3.1 Initial Observation (iOBS)

The results of the present study showed bilateral and significant hemodynamic changes in the entire MNS (Figure 1.3.1 and Table 1.3.1), including the PMC (dorsal and ventral portions), IFG, IPL and IPS, during observation without any performance of both the video clip showing a hand performing a chord and the video clip showing moving dots representing a chord, when the subjects were exposed to the video clips without any previous experience with the chords.

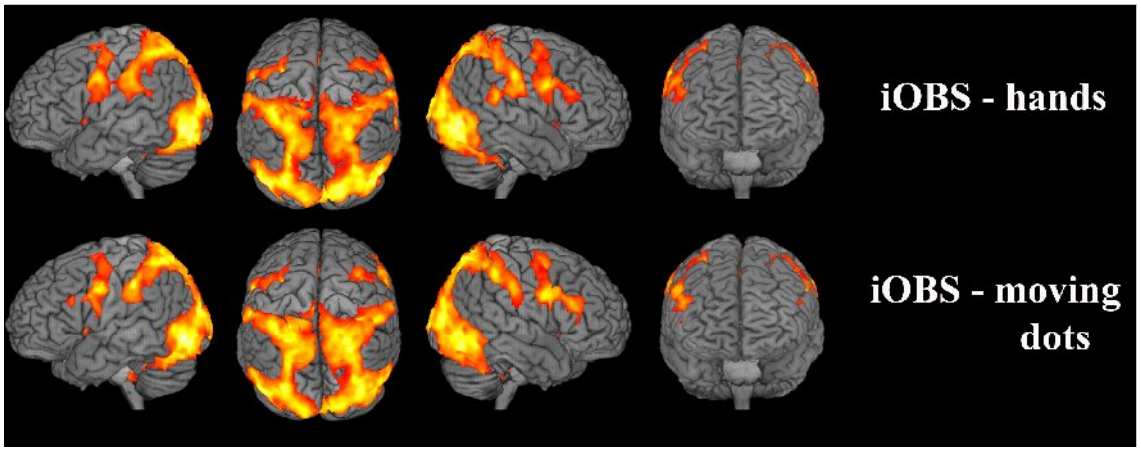

Figure 1.3.1 Cortical areas activated during the initial observation in which participants observed either a guitarist hand executing the guitar chords (iOBS – hands) or moving dots exhibiting a movement kinematically comparable (iOBS – circles). All conditions were contrasted with observation of a black screen (baseline).

In addition to the MNS activation, there was, as expected, activation in the occipital lobe. Furthermore, there was bilateral activation in the mesial cortices, parahippocampal gyrus, insula, basal nuclei and the medial frontal gyrus (pre-

supplementary motor area – preSMA) during observation of both video clips (see Supplementary Material).

Table 1.3.1. Local Maxima of Activated Foci, expressed in Talairach Coordinates, during the iOBS.

				Coordinates		
Brain Region	Brodmann Area	Side	Z score	x	y	z
Hands						
Fusiform Gyrus	BA 19	Left	6.37	-36.33	-72	-8
Cuneus	BA 17	Right	6.34	18.99	-94	-4
Inferior Temporal Gyrus	BA 37	Right	6.23	47.54	-68	-2
Lingual Gyrus	BA 18	Left	6.16	-2.44	-92	-14
Cerebellum		Left	6.09	-32.79	-82	-14
Cerebellum		Right	3.93	8.04	-28	-8
Inferior Occipital Gyrus	BA 19	Right	5.97	42.16	-78	-6
Inferior Frontal Gyrus	BA 9	Right	4.91	54.55	10	30
Middle Frontal Gyrus	BA 6	Right	4.43	33.09	-4	54
Precentral Gyrus	BA 6	Right	4.34	38.43	-6	50
Inferior Frontal Gyrus	BA 44	Right	4.1	45.55	12	18
Parahippocampal Gyrus	BA 27	Left	5.3	-11.47	-32	-2
Parahippocampal Gyrus	BA 27	Right	4.91	25.97	-30	-2
Parahippocampal Gyrus	BA 35	Right	4.21	20.56	-30	-8
Thalamus	Pulvinar	Left	3.68	-8.05	-24	6
Lentiform Nucleus	Putamen	Left	3.41	-29.5	-2	-2
Insula	BA 13	Left	3.3	-36.65	14	-2
Lentiform Nucleus	Lateral Globus Pallidus	Left	3.27	-18.81	0	-6
Moving dots						
Fusiform Gyrus	BA 19	Left	6.43	-36.33	-72	-8
Lingual Gyrus	BA 17	Right	6.25	18.98	-94	-6
Lingual Gyrus	BA 18	Left	6.03	-9.59	-88	-14
Fusiform Gyrus	BA 19	Left	5.92	-36.37	-80	-12
Inferior Temporal Gyrus	BA 37	Right	5.89	45.72	-66	-2
Precentral Gyrus	BA 6	Left	5.17	-43.59	4	34
Sub-Gyral	BA 6	Left	5.15	-18.61	-6	52
Precentral Gyrus	BA 6	Left	4.45	-34.73	4	32
Middle Frontal Gyrus	BA 9	Left	4.33	-41.88	10	30
Inferior Frontal Gyrus	BA 9	Left	4.23	-38.32	6	24
Medial Frontal Gyrus	BA 6	Left	3.94	-6.22	4	58
Inferior Frontal Gyrus	BA 9	Right	5.21	54.56	8	30
Middle Frontal Gyrus	BA 6	Right	4.77	29.53	-4	52
Precentral Gyrus	BA 6	Right	4.77	49.16	2	28
Parahippocampal Gyrus	BA 35	Right	5.23	22.42	-28	-10
Parahippocampal Gyrus	BA 27	Left	4.85	-11.53	-32	-2
Parahippocampal Gyrus	BA 27	Right	4.43	25.92	-30	-4
Parahippocampal Gyrus	BA 28	Left	4.42	-22.27	-26	-6
Thalamus		Left	4.93	-15.09	-30	2
Thalamus	Pulvinar	Right	4.82	22.38	-30	4

1.3.2 Final Observation (fOBS)

The results showed significant hemodynamic changes in the main areas of the MNS during observation of both types of video clips (Figure 1.3.2 and Table 1.3.2).

When observing the video clip showing a hand performing the chords the subjects exhibited bilateral activation in the dorsal portion of the PMC; however, this activation was smaller in the right hemisphere. Differently, the ventral portion of the PMC and the IFG were activated only in the left hemisphere. The IPL and IPS showed bilateral activation.

In constrast, when observing the video clip showing moving dots there was activation in the PMC (dorsal and ventral portions), IFG and IPL, which was restricted to left hemisphere. The IPS exhibited bilateral activation.

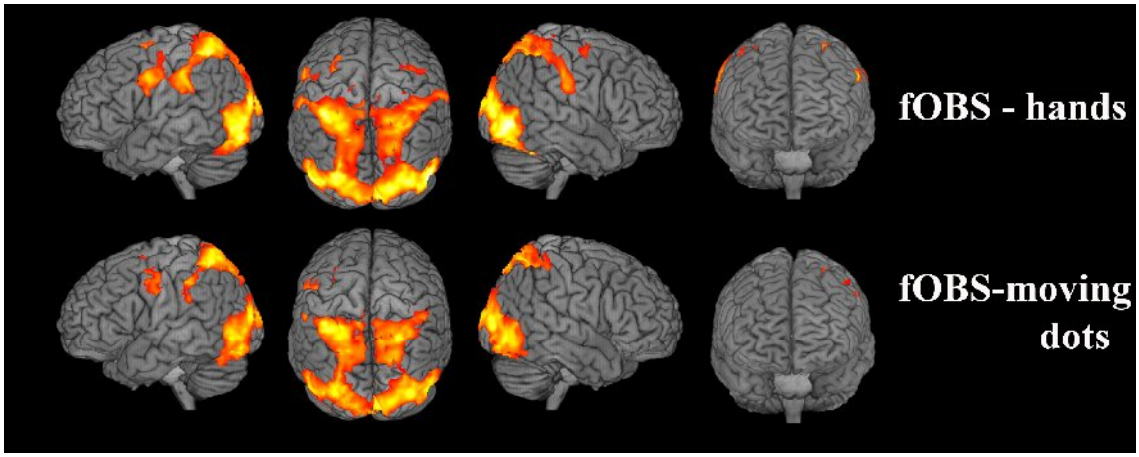

Figure 1.3.2 Cortical areas activated during the final observation in which participants observed a guitarist hand executing the guitar chords (fOBS – hands) and the moving dots exhibiting the chord (fOBS – moving dots). All conditions were contrasted with observation of a black screen (baseline).

The occipital activation during the fOBS was similar to that seen during the iOBS (Figure 1.3.2 and Table 1.3.2). Differently, the mesial cortices activation was smaller in the fOBS as compared to iOBS (Figure 1.3.2 and Table 1.3.2). Interestingly,

while the parahippocampal gyrus was bilaterally activated during observation of the video clip showing a hand performing the chords, its activation was restricted to the left hemisphere during observation of moving dots exhibiting the chords (Figure 1.3.2 and Table 1.3.2).

Table 1.3.2. Local Maxima of Activated Foci, expressed in Talairach Coordinates, during the final observation (fOBS).

				Coordinates		
Brain Region	Brodmann Area	Side	Z score	x	y	z
Hands						
Cerebellum		Left	3.07	0.82	-36	-6
Cerebellum		Right	2.61	6.14	-36	-6
Fusiform Gyrus	BA 19	Right	6.39	40.41	-78	-12
Inferior Occipital Gyrus	BA 17	Right	6.21	11.84	-92	-10
Lingual Gyrus	BA 18	Left	6.19	-7.79	-90	-12
Middle Frontal Gyrus	BA 6	Right	4.09	31.27	-4	54
Middle Occipital Gyrus	BA 19	Right	6.37	49.34	-74	-8
Parahippocampal Gyrus	BA 27	Right	4.66	25.94	-30	-8
Parahippocampal Gyrus	BA 30	Left	4.9	-16.86	-32	-4
Parahippocampal Gyrus	BA 30	Right	4.17	16.97	-32	-4
Parahippocampal Gyrus	BA 35	Left	4.91	-16.86	-28	-8
Precentral Gyrus	BA 6	Right	2.95	45.45	-4	58
Thalamus	Pulvinar	Left	3.91	-8.03	-26	8
Thalamus	Pulvinar	Right	3.41	20.47	-26	10
Thalamus		Left	3.39	-4.5	-20	2
Circles						
Brainstem	Red Nucleus	Left	2.59	-1.02	-24	-2
Cerebellum		Left	3.13	-0.97	-36	-4
Cuneus	BA 17	Left	5.52	-18.59	-84	10
Lingual Gyrus	BA 18	Left	5.81	-7.86	-90	-12
Lingual Gyrus	BA 18	Right	5.49	13.53	-88	-12
Parahippocampal Gyrus	BA 30	Left	4.98	-15.09	-32	-4
Superior Parietal Lobule	BA 7	Left	5.6	-31.08	-54	60
Thalamus	Pulvinar	Left	3.06	-17.04	-30	14

1.3.3 Contrasts Between iOBS and fOBS

Comparisons between iOBS and fOBS data showed that the four major areas of the MNS exhibited stronger activation in the initial observation session as compared

to the final observation session, independently on the type of video clip presented (Figure 1.3.3 and Table 1.3.3).

During the iOBS of the video clip showing hands performing a chord, the ventral PMC and the IFG exhibited stronger activation in the right hemisphere. In contrast, the dorsal PMC, the IPL and the IPS exhibited bilateral activation.

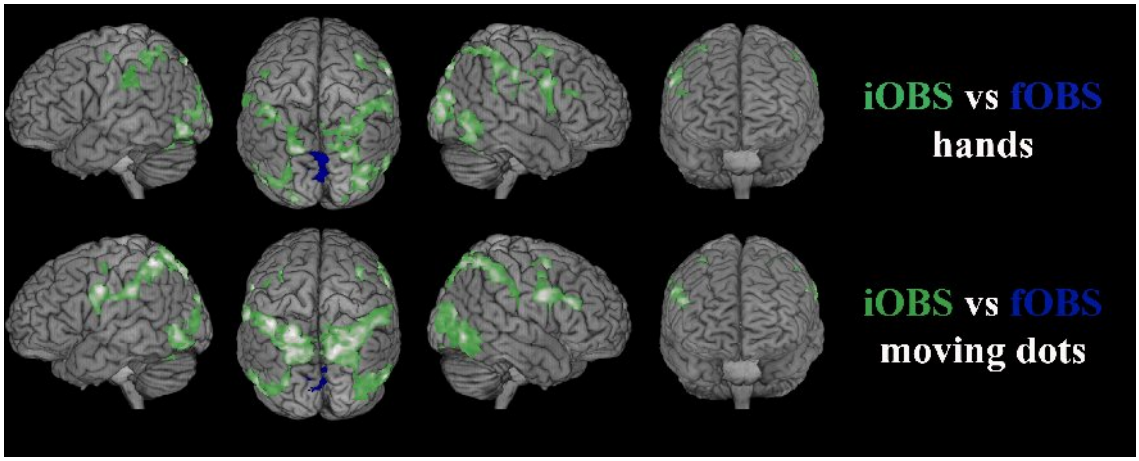

Figure 1.3.3 Comparison between iOBS and fOBS when the observation sessions involved either hands or moving dots performing a chord. The top row of images shows the areas exhibiting stronger activation in iOBS (green) relative to fOBS (blue) during obsevation of the hands performing a chord. The bottom row of images shows the areas exhibiting stronger activation in iOBS (green) in relation to fOBS (blue) during observation of the moving dots representing a chord. P<0,05. Abbreviations as in Figure 1.3.1 and 1.3.2.

Table 1.3.3 Local Maxima of Activated Foci, expressed in Talairach Coordinates, for the contrasts between iOBS and fOBS for condition Hands and Circles.

				Coordinates		
Brain Region	Brodmann Area	Side	Z score	x	y	z
iOBS > fOBS - Hands						
Cingulate Gyrus	BA 24	Left	3.75	-18.79	-6	44
Fusiform Gyrus	BA 19	Left	6.28	-36.51	-72	-12
Inferior Frontal Gyrus	BA 9	Right	-2.41	43.71	12	28
Inferior Parietal Lobule	BA 40	Left	6.46	-36.5	-40	48
Middle Frontal Gyrus	BA 6	Left	3.93	-22.37	-8	48
Middle Frontal Gyrus	BA 6	Right	-0.92	27.62	8	56
Middle Frontal Gyrus	BA 10	Right	-1.66	34.77	36	16
Middle Occipital Gyrus	BA 19	Right	-0.79	34.89	-84	8
Middle Temporal Gyrus	BA 37	Right	-1.72	49.21	-58	2

Middle Temporal Gyrus	BA 39	Right	-0.92	38.48	-76	16
Precentral Gyrus	BA 4	Right	-3.22	58.07	-18	42
Precentral Gyrus	BA 6	Left	5.09	-34.87	-10	54
Precentral Gyrus	BA 6	Right	-1.15	31.2	-6	50
Precuneus	BA 19	Right	0.17	25.97	-82	46
Precuneus	BA 7	Left	3.83	-11.53	-78	46
Sub-Gyral	BA 6	Left	4.26	-18.74	-8	56
fOBS >iOBS - Hands						
Cerebellum - Anterior Lobe		Left	2.16	-0.92	-54	-2
Cuneus	BA 17	Right	0.58	18.74	-78	8
Cuneus	BA 18	Left	2.84	-4.46	-88	18
Cuneus	BA 19	Right	1.10	9.78	-78	32
iOBS > fOBS - Circles						
Inferior Frontal Gyrus	BA 9	Right	-2.16	47.3	10	22
Inferior Parietal Lobule	BA 40	Left	6.16	-31.15	-40	44
Middle Frontal Gyrus	BA 46	Right	-1.85	47.33	30	24
Middle Frontal Gyrus	BA 6	Right	0.08	24.1	-2	44
Precuneus	BA 7	Right	1.93	17.08	-72	50
Superior Parietal Lobule	BA 7	Left	6.87	-32.89	-48	60
Superior Parietal Lobule	BA 7	Right	0.46	29.55	-60	52
fOBS >iOBS - Circles						
Cerebellum -Posterior Lobe		Right	0.46	16.93	-70	-10
Cingulate Gyrus	BA 31	Left	2.99	-6.25	-56	26
Cuneus	BA 18	Left	3.40	-9.81	-78	20

1.4. DISCUSSION

The results of the present study revealed that observing video clips of unknown actions as if they were to be imitated later, but with no actual movement, promotes a strong activation of the structures integrating the MNS. In addition, extensive practice involving imitation of the seen movements resulted in a significant reduction of activity in these structures. These results lend support to hypotheses proposing that increments of neural efficiency throughout learning processes lead to improvements of the information processing, with simultaneous minimization of the costs both in terms of computation and attentional resources (e.g., Squire and Kandel, 1999; Reithler et al., 2010).

An alternative interpretation for this pattern of results is that it occurred habituation to repeated exposure of the video clips (see Grill-Spector *et al*. 2001, 2006, Krekelberg et al. 2006) associated with a substantial decline in the attention level (see Büchel and Friston, 1997), and not to the incorporation of a novel motor representation in the subject's repertoire. However, in a similar study involving musicians and non-musicians exposed to practiced and non-practiced chords, Vogt *et al.* (2007) showed a marked difference between the pattern of the MNS activation for practiced chords as compared to non-practiced chords; in addition, this difference was also present for musicians. Together, these results indicate that it is not the repeated exposure to video clips showing chords that leads to differences in the hemodynamic responses but the actual knowledge about the related actions the critical feature.

1.4.1 Effects of Practice on the Mirror Neuron System

Different proposals have been advanced for explaining why the structures of the so called MNS seem to constitute a functional unit. For instance, while Ramachandran (2006) proposed that it is the result of filogenetic evolution standing as an innate system, Catmur et al. (2007) and Heyes (2010) proposed that is built step by step and throughout life, associatively. The idea of an innate system seems to require quite ellaborated explanations, for instance, about causal relations difficult to establish. It seems plausible to admit the occurrence of Hebbian changes in both perceptual and motor representational systems associated with the acquired information, as a result of correlated sensorimotor experience. Thus, neurons that fire together wire together. This concept seems to allow explanation on how the MNS could be built through experience. An individual exposed to a sensorimotor experience, in which the observation and execution of a particular action are correlated or contingent, is likely to associate them

(Catmur et al., 2009). Even more interesting is the possibility to reverse this effect through periods of 'counter-mirror' sensorimotor training. Heyes and co-workers (2005) showed that following periods of training during which the execution of one first action (e.g., hand open) is paired with the observation of a second action (e.g., hand close) the small reaction time for imitative responses (see hand opening and do the same action) may be abolished due the new association.

Along these lines, the stronger activation in the MNS in our initial observation session, when the chords stimuli have no motor counterpart in the observes's motor repertoire, as compared to the final observation session when the subjects had already practiced the chords, is consistent with this idea.

Prior studies have demonstrated that besides the pre-supplementary motor area (preSMA), the anterior cingulate cortex, ventral to the preSMA, may also contribute for acquisition of new sequences, especially when performance monitoring is most needed. In addition, the mesial and subcortical areas play a crucial role at the beginning motor learning, when multiple neural mechanisms are interacting (Hikosaka et al, 2002). Signals originating from the cerebral cortex are optimized in terms of their sensorimotor accuracy, by projecting through the basal ganglia and cerebellar loop circuits, respectively. This feedback is likely to be a critical during the process of motor action learning. After the action has been learned, the critical importance of this process is reduced, thus decreasing the activity of these regions. Our results corroborate this idea. It is possible to note the significant decrease of activation of mesial regions when comparing the initial and final sessions. Also, cortical areas involved in attention and awareness may be needed early in the learning as the prefrontal and parietal cortices, known to be important for visual attention. After the acquisition of the skill, these areas may become less important as learning proceeds (Squire and Kandel, 1999).

Vogt et al. (2007) emphasized the critical importance of the instructions provided for the subjects before task performance in the hemodynamic changes. In the present study the participants where instructed to carefully observe the video as if they were to execute the same action later. In other studies, which found the MNS more active during observation of known actions, the instructions included to judge 'how tiring' each movement was (Calvo-Merino et al., 2005 and 2006), how well they could dance each movement (Cross et al., 2006), or simply watch different actions (Buccino et al., 2004b). Buccino et al. (2004) demonstrated that in a pure chord observation condition the activation of the MNS relative to an imitation condition is reduced. It is plausible that the different outcomes between these studies and ours reflect the different aims of the observation (Grèzes et al., 1998). We suggest that during observation in order to imitate, novel actions tend to induce stronger activations in the MNS than familiar actions due the substantial amounts of motor attention requiered at early stages. Once the skill has been acquired, the system becomes more efficient and now requires the activation of specific regions, increasing the specificity of neural circuit connections underlying performance of the task which may be controlled by a much smaller amount of neural tissue, with consequent energetic economy.

1.5 CONCLUSIONS

Our results lend support to the notion that the MNS is critical for observing aiming at imitation of both untrained and trained actions. In addition, these data support the hypothesis that increments of neural efficiency throughout learning processes lead to improvements of the information processing, with simultaneous minimization of the costs both in terms of computation and attentional resources thus rendering the general functioning of the system efficient and economical.

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1.7 SUPPLEMENTARY MATERIAL

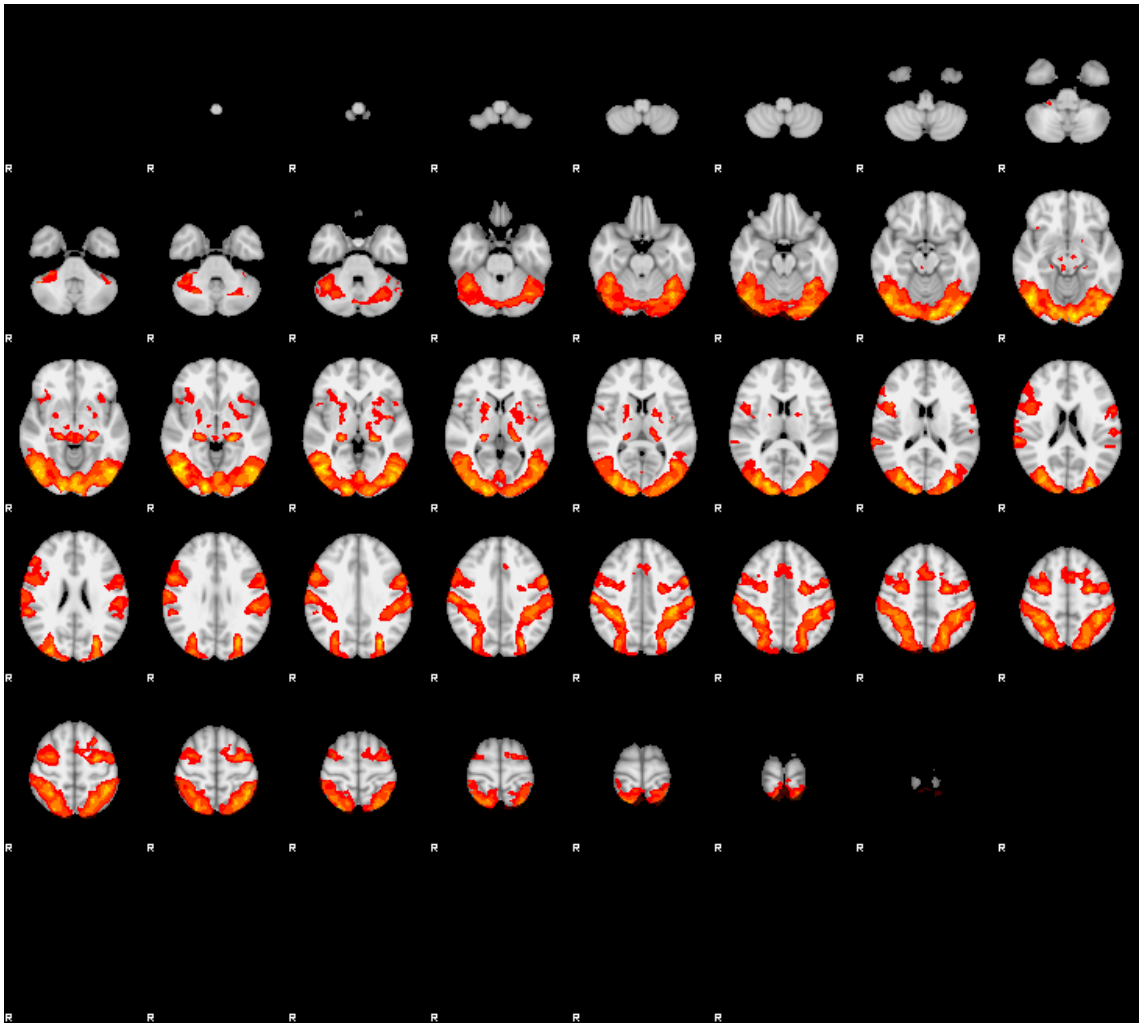

Figure 1.7.1 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the **inicial observation** using the video with a **guitar player**. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

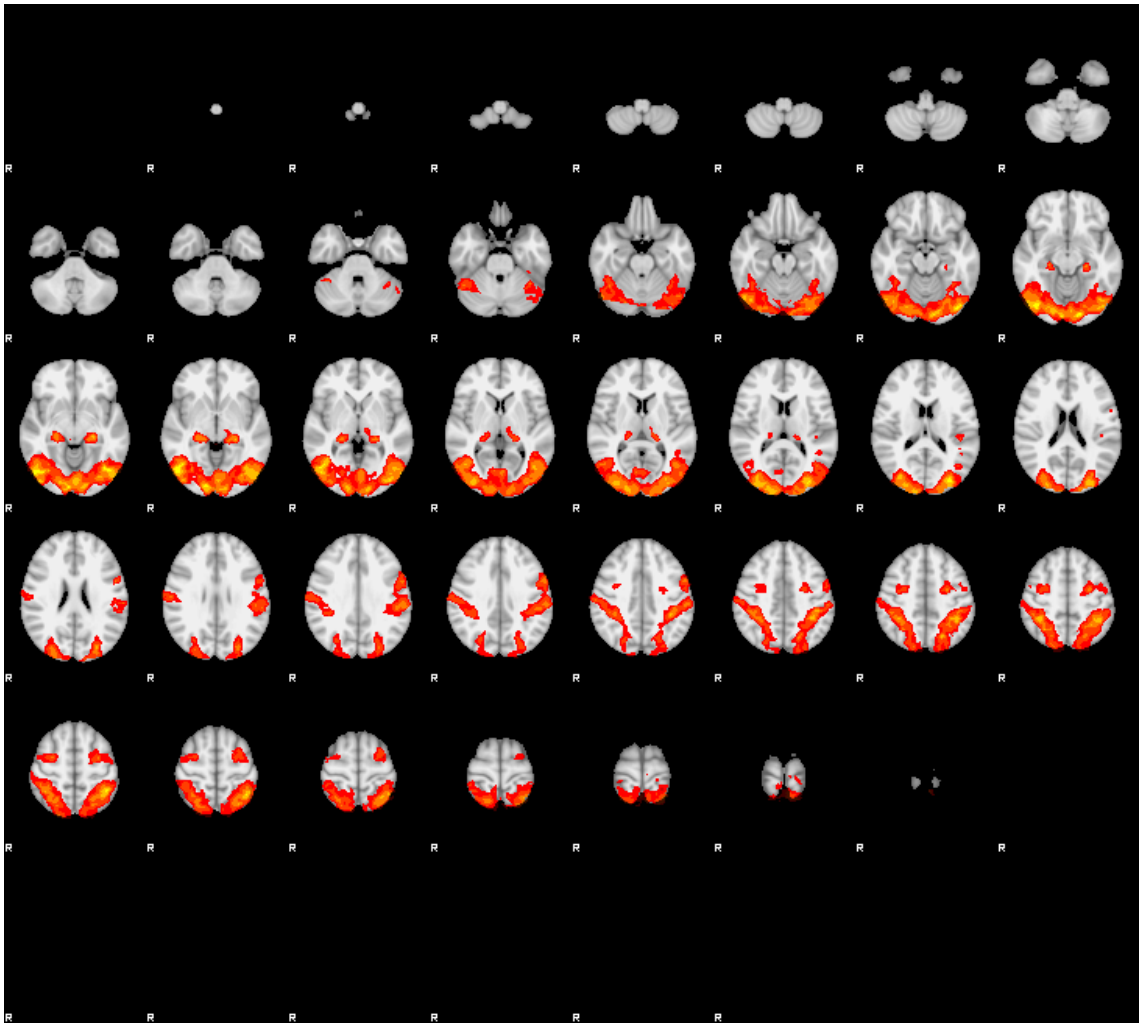

Figure 1.7.2 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the **final observation** using the video with a **guitar player**. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

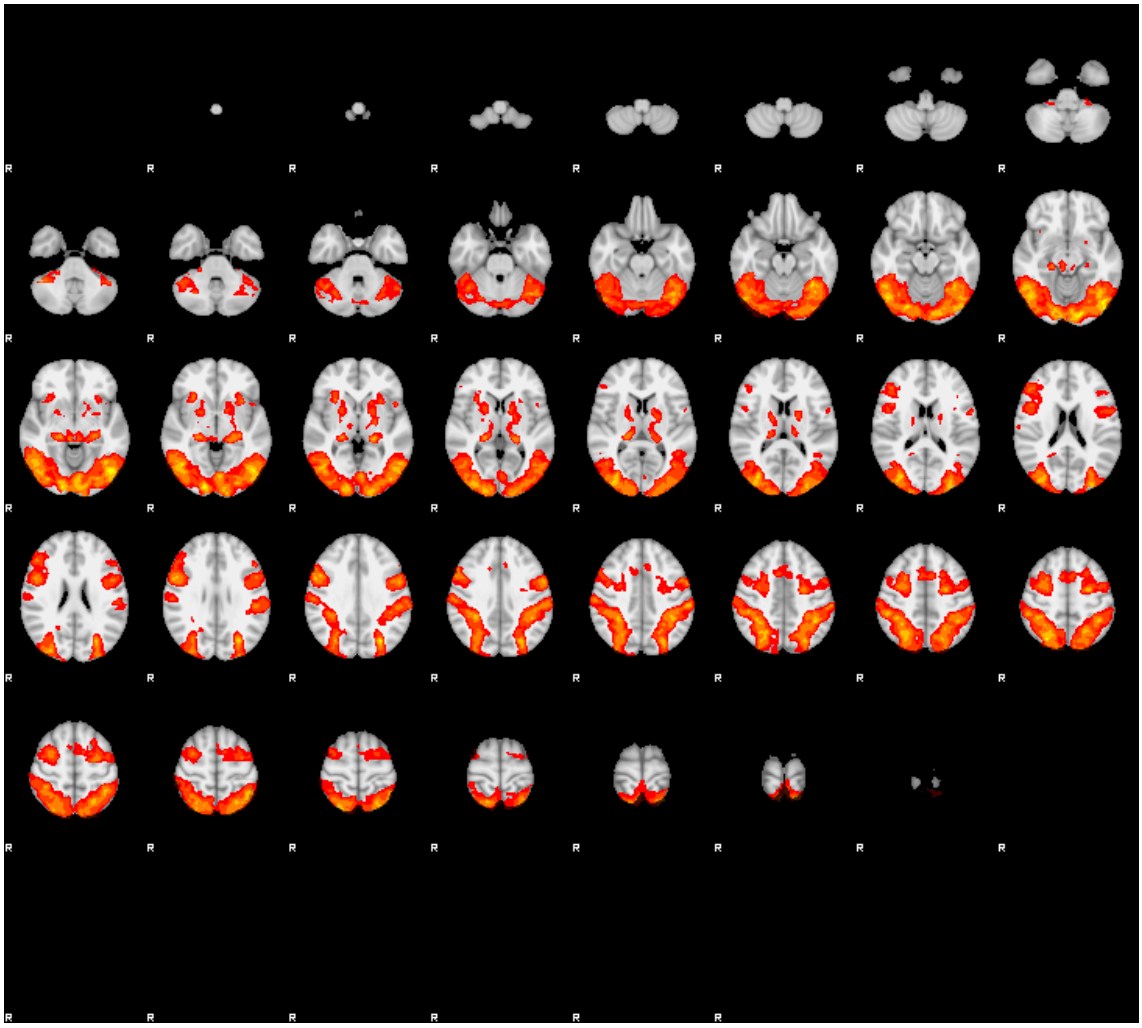

Figure 1.7.3 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the **inicial observation** using the video with a **moving dots**. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

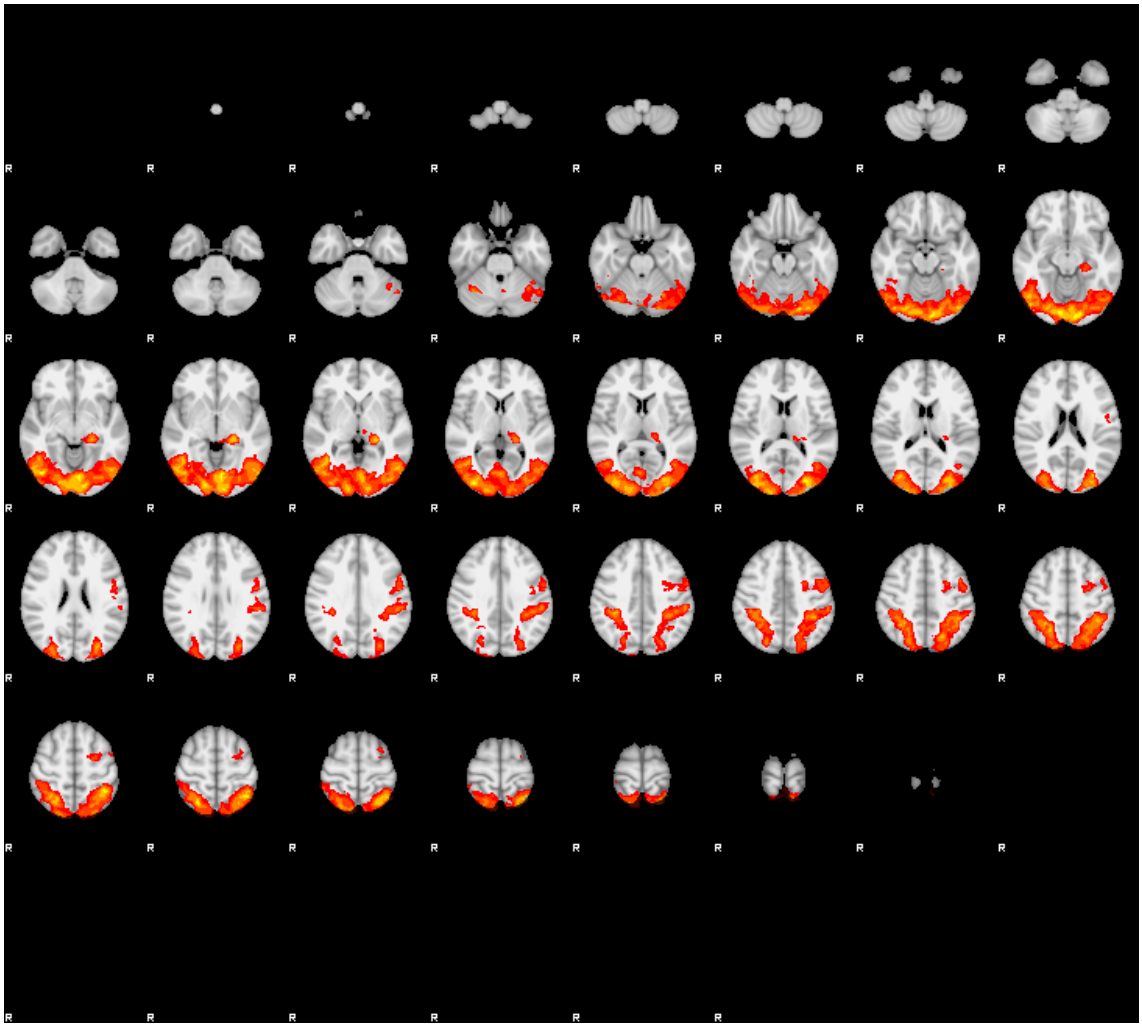

Figure 1.7.4 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the **final observation** using the video with a moving dots. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

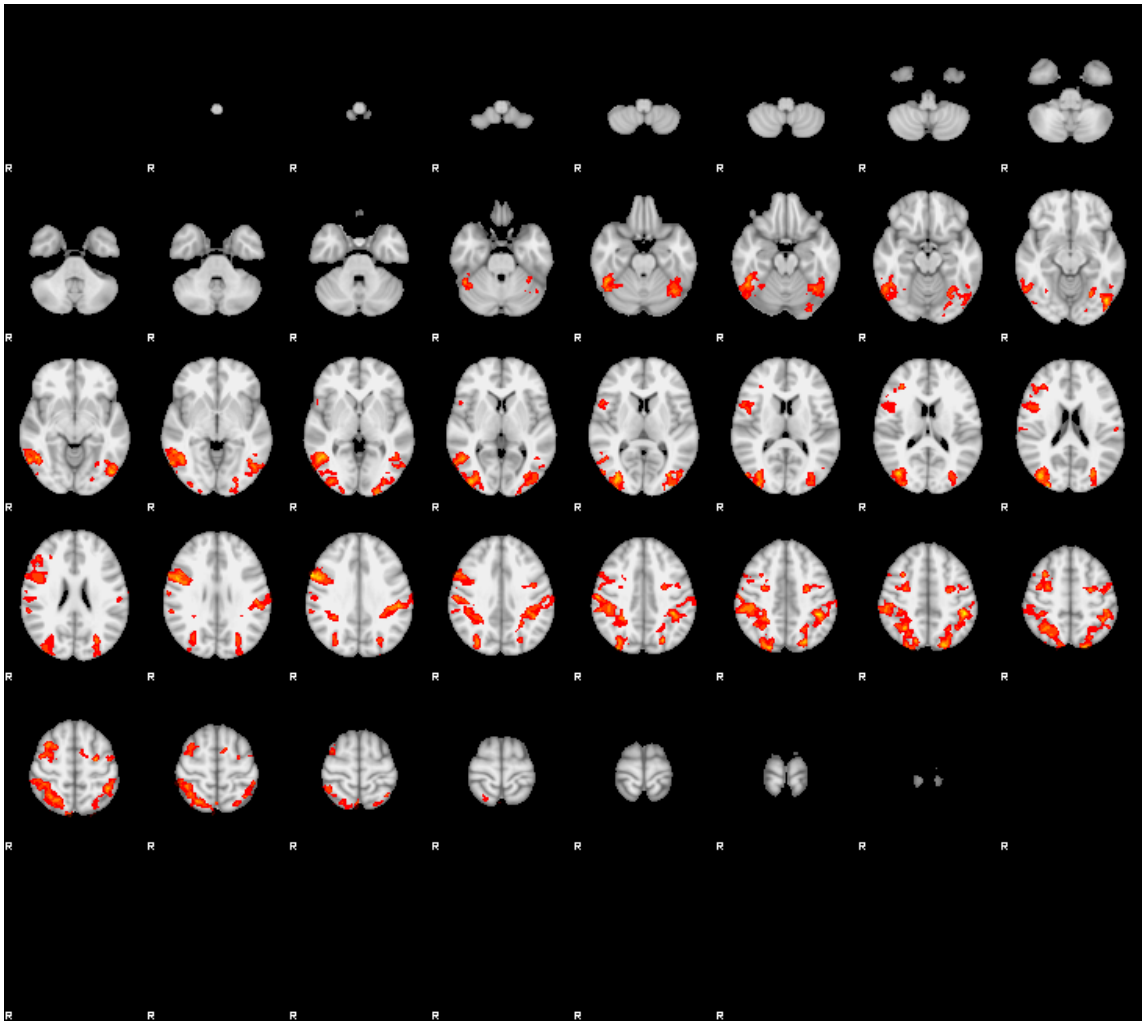

Figure 1.7.5 Comparison between iOBS and fOBS when the observation sessions involved **hands** to performing a chord. The figure shows the regions with increased activity during iOBS in relation to fOBS. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

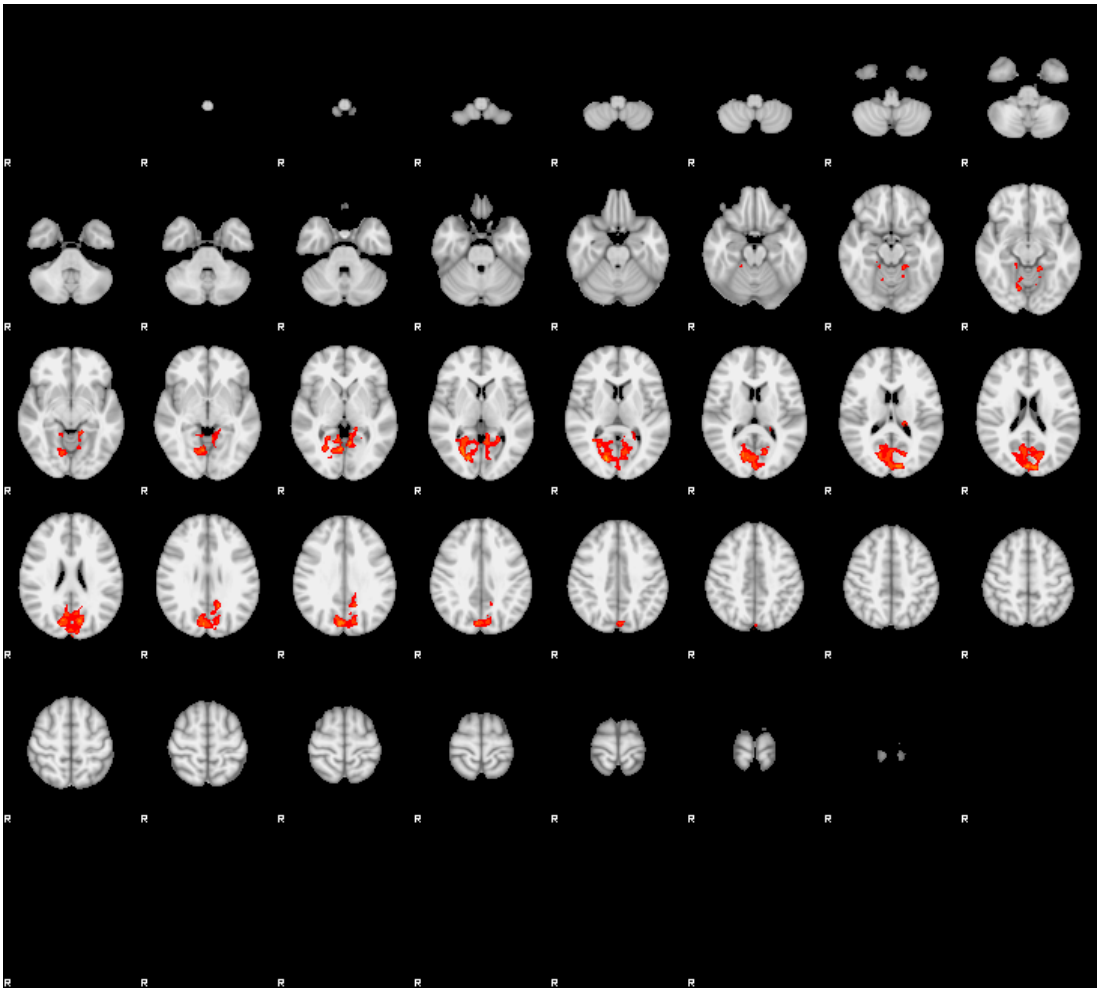

Figure 1.7.6 Comparison between iOBS and fOBS when the observation sessions involved **hands** to performing a chord. The figure shows the regions with increased activity during fOBS in relation to iOBS. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

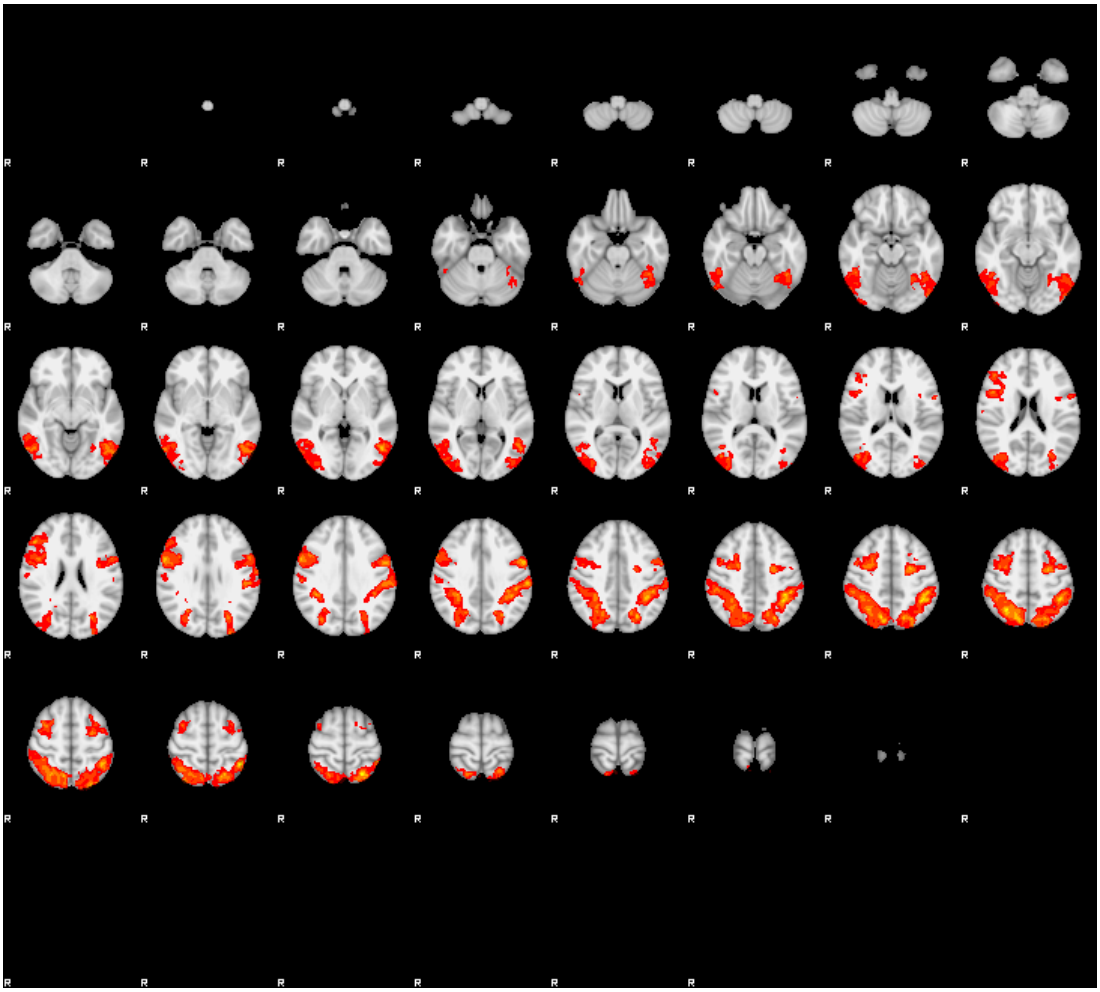

Figure 1.7.7 Comparison between iOBS and fOBS when the observation sessions involved **moving dots** performing a chord. The figure shows the regions with increased activity during iOBS in relation to fOBS. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

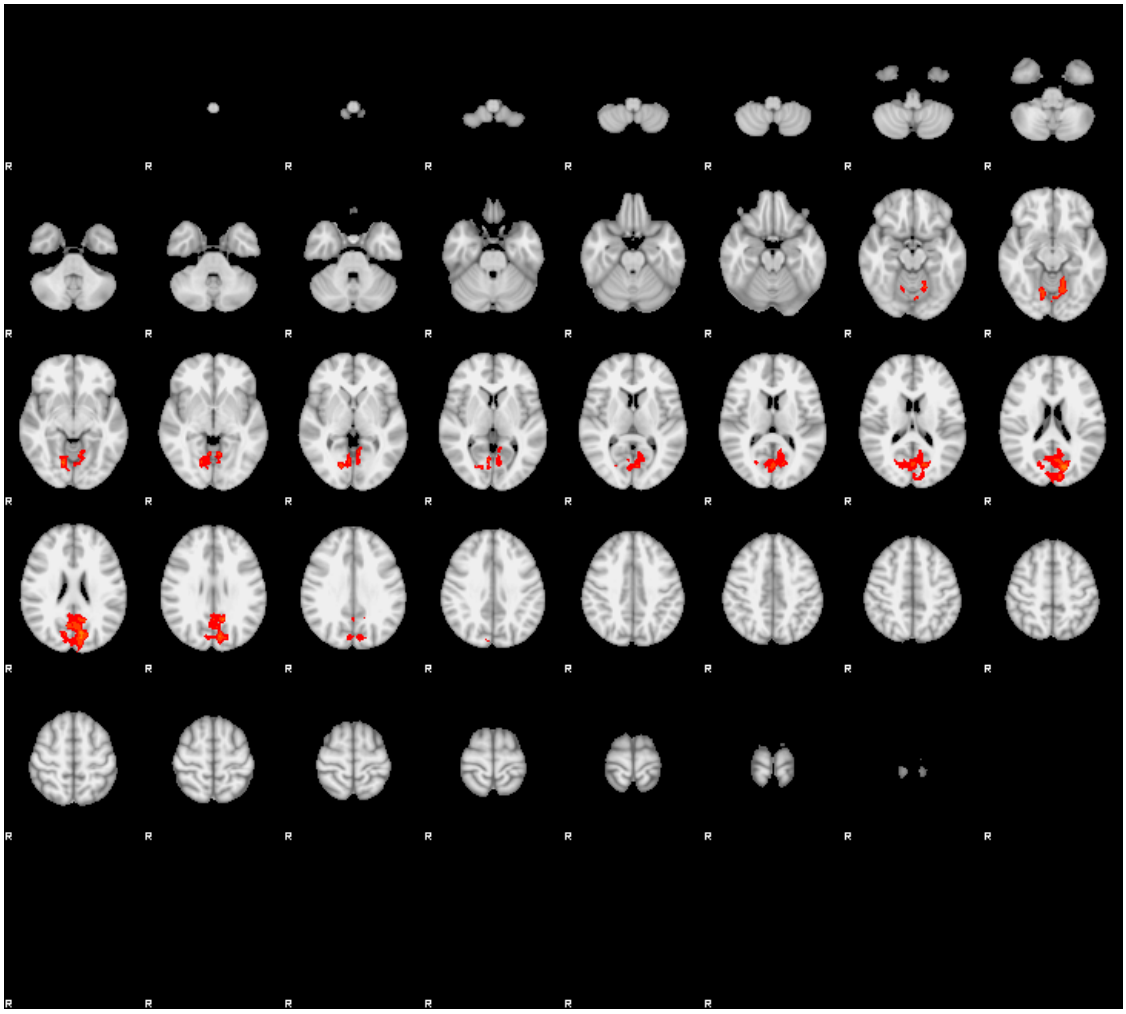

Figure 1.7.8 Comparison between iOBS and fOBS when the observation sessions involved **moving dots** performing a chord. The figure shows the regions with increased activity during fOBS in relation to iOBS. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

Capítulo II

Encephalic hemodynamic changes during action observation for imitation, preparation and execution: a fMRI study

Este artigo encontra-se ainda em fase de preparação.

ABSTRACT

Imitation requires translation of visual perception into a motor action similar to that observed. It has been suggested that the mirror neuron system (MNS), including premotor cortex, inferior frontal gyrus and inferior parietal lobule, underlies imitative behaviors and also contributes for understanding others' actions. This study examined human brain hemodynamic changes, by way of functional Magnetic Ressonance Imaging (fMRI), while previously trained volunteers (1) watched to a video clip showing either a hand performing a chord (tasks IMI and IDE) or independent moving dots representing execution of a chord (tasks IMIc and ANO), both on a guitar neck (event 1), (2) maintenance of the information about the observed action and preparation for task execution (event 2), (3) actual execution of a task involving either to perform, as accurately as possible, the same (tasks IMI and IMIc) or another (task ANO) chord, or identification of the exhibited chord by way of a distinct hand movement (task IDE) (event 3). This experimental design aimed at evaluating if these three stages of imitative behavior (events 1, 2 and 3) engage similar brain regions. In addition, the involvement of the MNS during performance in different cognitive tasks was evaluated using a within-subject schedule of testing. Before presentation of the video clip showing the critical action for that specific trial, the subjects were exposed to a cue indicating which type of task should be performed later. Therefore, when observing the video clip (event 1) they already knew the task that should be performed later (event 3). Except for small variations, the results showed activation of all structures of the MNS during preparation and execution phases of all tasks (IMI, IMIc, IDE and ANO). Similarly, all structures of the MNS were activated during the observation phase of the IMIc, IDE and ANO tasks. Interestingly, however, during observation phase of the IMI task there was activation of the parietal areas of the MNS but not of the frontal areas of the MNS, suggesting that the context in which the subject observes a specific action, including the aim of observing that action, interferes with the activity of the different components of the MNS.

2.1 INTRODUCTION

The description of mirror neurons in primates stimulated an intense debate in neuroscience research in the last years. These neurons, located initially in the the ventral premotor cortex (F5) and the rostral part of the inferior parietal lobule (7b, PF) of monkeys, fire both when the subject acts and also when he only observes the same action performed by another subject (Di Pellegrino et al. 1992, Gallese et al. 1996, Rizzolatti et al. 1996). A central issue this debate relates to the role of these neurons in imitation and in understanding actions performed by others (Rizzolatti et al. 2001), processes that are critical for primates learning and social behaviour.

Imitation involves translating visual perception into a motor action similar to that observed. This requires both visual mapping of the action to be performed and selection and activation of motor control neural networks available in the subject's repertoire as an attempt to match, as closely as possible, the observed action. That is, specific visual features of actions previously associated with motor programs elicit a search for internal representations of the most similar actions already represented in the subjects repertoire. This "direct matching" between the action seen and the observer's motor repertoire has been considered as the core of the perception-to-action translation (Rizzolatti, 2001). It is not clear to which extent there is a single system underlying direct matching, or if several systems interact to allow its occurrence. Supporters for the first interpretation claim that the system should be necessary and sufficient to accomplish all tasks at the core of the concept of "direct matching" (Rizzolatti et al., 2001). Investigations of brain circuits underlying these mechanisms and how they are supported by other cognitive processes should contribute for this discussion.

Translating perception about others' action into own's action requires perception of the action itself and also identification/recognition of the better

alternatives available for performing that action using observer's repertoire. This information would be encoded and maintained in working memory. During action preparation this information is compared to the available motor repertoire, thus allowing to select the actions closely related to that seen, which are then used for orienting the actual execution of the movements (Menz *et al*., 2009). It is likely that both the mechanisms and their underlying neural substrates overlap along this process.

Also, the proposal that fronto-parietal "mirror neuron" system (MNS) play a critical role in imitation is suggested due to the role of this system in recognize and understand actions done by others (Iacoboni, 1999; Heyes et. al, 2005; Vogt *et al*., 2007). In humans these system include (1) the premotor cortex (PMC) and the inferior frontal gyrus (IFG - mainly the BA44), which together is considered homologous to the PMC of macaques (F5 area) (Rizzolatti and Arbib, 1998; Makuuchi, 2005; Rizzolatti and Sinigaglia, 2010), and (2) the inferior parietal lobe (IPL - BA 40), considered homologous to the IPL of macaques (PF/PFG area) (Rizzolatti et al. 2001; Rizzolatti, 2005). In addition, both brain areas related to working memory, including the dorso lateral prefrontal cortex (D'Esposito *et al*., 1999, Vogt *et al*., 2007) and the supplementary motor area (SMA) (Petit *et al*., 1998), and brain areas related to visual perception and action processing, including the dorsal and ventral visual pathways (Goodale and Milner, 1992), have also been shown to be engaged during performance of tasks involving imitation. Furthermore, when action is directed to an object, the intra-parietal sulcus (IPS), the IPL and the middle frontal gyrus become active, particularly when the subject grasps or manipulates the object (Binkofski *et al*., 1999; Johnson and Grafton, 2003). Also, the borders of the intraparietal sulcus, the superior parietal lobe and the posterior cingulate gyrus have been reported to become active during the

integration of object properties (Binkofski *et al*., 1999; Grezes *et al*., 2003; Mecklinger *et al*., 2002).

Buccino et al., (2004) and Vogt et al. (2007) a made a series of experiments using an experimental design that allowed separate and operate at least three stages of imitative behavior: action observation, preparation to execution and actual execution (imitation) of the observed action. These studies allowed understanding how the purposes of imitation influences the way of an action is observed and how much it is dependent on the observer's motor repertoire. However, while these studies have succeeded to investigate the course of time when the imitative behavior occurs, they failed to separate some basic cognitive processes that are embedded to an observation of action regardless if the purpose is to imitate. It would be interesting to explore the stages of imitative behavior not only temporarily but also cognitively. The processes by which an observed action is recognized and the preparation to execution of an action can be very similar to those in which imitation is not required and the role of the MNS could be different.

In the present study, we investigated human brain hemodynamic responses, the so-called BOLD effect, in healthy non-guitar players during their attempt to directly match performance of practiced hand actions (guitar chords), and thus contribute for the identification of brain structures thought to underlie imitative behaviors. Non-guitar players were asked to observe videos showing performance of guitar chords (played by an expert guitarist) and to replicate them “as accurately as possible” after a period of preparation to action execution. Brain hemodynamic responses during performance of this imitation task were compared with those seen in three other conditions, including execution of a chord after seeing a video showing moving symbolic cues (dots) representing the chord, single identification of the shown chord, without its

performance, and execution of another guitar chord different from that seen in the video. Comparisons of hemodynamic responses in these conditions allowed (1) assessing to which extent observation of symbolic cues representing an action and its later execution activates similar brain circuits relative to those activated during observation of a conspecific executing that action associated with later imitation, (2) identifying brain areas engaged in representation of an action without its actual performance and without imitative behavior, and (3) evaluating to what extent brain circuits underlying motor imitative actions overlap with those involved in internally generated actions. In addition, the time interval between observation and execution (preparation to action execution) was such that it allowed comparing the effects of motor preparation during action observation.

We hypothesized that the MNS would be more active during the observation of guitar chords played by an guitarist than during a video showing moving dots based on the ideia that imitation of biological movements facilitates action execution, which is evidenced by behavioral studies showing that humans are faster at imitating finger movements than at performing the same movement in response to non-biological spatial cues (Brass et al., 2000, 2001; Jonas et al., 2007; Kessler et al., 2006). Also we expect to clarify other mechanisms involved in the identification of a chord and the generation of a voluntary action that are beyond the MNS but act together.

2.2 MATERIAL AND METHODS

2.2.1 Subjects

Twenty healthy female volunteers (19 to 32 years old, mean, 25.3 years), undergraduate and graduate students at the University of Sao Paulo, right-handed according to the Edinburgh Handedness Inventory, participated in the study. Exclusion

criteria included the ability to play any musical instrument even informally, age less than 18 or more than 35 years, history of neurological or psychiatric disorders, regular consumption of psychiatric medication or substances of abuse, and claustrophobia. An additional exclusion criterion involved a poor quality of the imitation, checked by way of a video tape. All volunteers exhibited normal or corrected-to-normal visual acuity and signed a written informed consent to participate in the experiments. The experimental protocol complying with national and international rules and guidelines for the involvement of human subjects in experimental research was approved by the Ethical Committee of the Biosciences Institute at the University of São Paulo.

2.2.2 Experimental Devices and Stimuli

A wooden guitar neck heighting 42 cm was placed close and longitudinal to the participants' body such that they could comfortably move their hands on it throughout the scanning periods. In order to reduce tactile exploration, strings and frets were removed. During non-active events (Events 1, 2, and 4), participants placed their index, middle, and annular finger in a rest position on the down side of the fretboard. Stimuli were presented via Eloquence Invivo screen (1024 x 768 pixels) located above the participant's head. E-prime 2.0 Software (Psychology Software Tools, Inc.) was used for displaying colored stimuli (2 degrees of visual angle) and video clips (448 x 336) pixels, 25 frames per second, 25 horizontal and 19 vertical visual angle). All video clips were recorded using a classical guitar played by a skilled guitarist. He used his left hand so that the chords appeared as a counter mirror image of the participant's left hand. This perspective was chosen in order to match the anatomy and preserve the details that are seen when the participants observe another person performing an action right in front of them.

In the “actual hand” video clip, the index, middle and ring finger moved from the rest position to the chord position and returned to the rest position for four times. This resulted in a rhythmical pattern of 3s duration, ensuring that the fingers could pause on the gutar neck before returning to the rest position. Participants were instructed to perform the chord observed at least three times. The four chords chosen for the present study, (A,C,E and G), involved three fingers, each of them positioned on a different string and using only the upper three frets; no chord involved the small finger.

In the “moving dots” video clip, a frame of the “hand” video clip in the rest position was taken in order to project the dots on the fingers of the guitar player. An animation was made with the dots, not the hand, moving towars the chord locations, thus simulating a guitar chord, by using the software Sony Vegas Pro 9.0 (trial version).

The participants’ hand performance was videotaped during scanning in order to score the quality of the imitation, based on the position of fingers related to string and frets lines.

2.2.3 Design and Experimental Conditions

The present experiment (named imitation experiment) is part of a set of learning and imitation study involving the same volunteers exposed to training with the chords. This experiment was carried out between the two sessions of the experiment described in Chapter 1. Volunteers started this experiment after a practice session (see Instructions and Procedures), in which they learned to execute and to identify all four chords. In addition, the subjects received detailed explanations about the experimental design in order to familiarize them with the trials and sequence of events of the experiment.

Figure 1 illustrates the general procedure and the tasks the subjects were required to perform including (1) imitating (***IMI),*** "as precisely as possible", the actual hand action observed in the video clip, (2) imitating (***IMIc),*** "as precisely as possible", moving dots ("symbolic cues") observed in the video clip indicating the location of each finger, (3) identification (***IDE)*** of the chord presented in the video clip by naming it, and (4) performance of another (***ANO)*** chord relative to that shown by way of moving dots indicating the fingers location on the gutar neck, at their own choice.

Each trial started with exposure of the volunteer to a cue, either a green, blue, yellow or red square, along 1.2-2.0s, indicating the nature of the task to be performed, respectively, IMI, IMIc, IDE or ANO (see Figure 2.2.1), followed by Event 1 (observation), i.e., presentation of a 12-second video clip showing either a hand performing a chord on a gutar neck or moving dots showing the locations where the fingers should be positioned to perform the chord. Each chord was showed four times during each video presentation. At the end of the video clip presentation, a blue screen with a gray fixation cross was presented during 4-8s indicating the Event 2 (preparation). In order to alert the volunteer that the next event was about to start, the fixation cross changed its color (to the same color of the cue) 1s before the event 3. The event 3 (execution) was a green screen in which the volunteer should keep his eyes on the fixation point and executed the chord on the neck of the guitar. The event 4 (rest) the volunteer should repositioned his fingers down the guitar neck and stand still. Since event 4 was taken as the baseline for most contrasts, particular attention was paid to the motor behavior of participants during this event. Specifically, images of the first 2s of this event were included in the analysis as part of the Event because over this time participants were still repositioning their hand on the guitar neck.

Each volunteer was exposed to 48 trials, 12 in each of the four tasks, in a quasi-random sequence. For instance, in a trial involving the ***IMI task (Green Cue),*** during Event 1 the participants were required to watch a 12-s video clip showing the hand of a guitar player performing the same guitar chord four times, in order to subsequently imitate it. During Event 2, corresponding to presentation of a blue screen, the participants were required to keep the video information in mind and to prepare to answer in the next event. Subjects were instructed to avoid any kind of movement during this event. Along the last second of Event 2, a cross having the same color as that of the square was shown in the center of the screen to remind the participants the kind of task they should perform and to indicate that Event 3 was about to start. During Event 3 a cyan screen was presented indicating for the participants that they should imitated the chord seen, using their left hand. Event 4 corresponded to presentation of a black screen, indicating to the participants that they should place their left hand in a rest position on the guitar neck located inside the scanner, maintaining it still. In a trial involving the ***IMIc task (Blue Cue),*** events were the same as in the IMI task, except for Event 1, which video clip showed moving dots, instead of a hand, indicating the location of the fingers to perform the required guitar chord. In a trial involving the ***IDE task– (Yellow cue)*** the participants were required to watch a 12-s video clip showing the hand of a guitar player performing the same guitar chord four times during Event 1 and to identify it during Event 3. The idenfication was expressed by positioning the fingers on the guitar neck illustrating the numbers: 1 (one finger), 2 (two fingers), 3 (three fingers) and 4 (four fingers). This code was assigned by the volunteer itself during the practice session. In a trial involving the ***ANO task (Red Cue)*** the participants watched to a video clip showing moving dots indicating the location of the fingers, and, during Event 3, had to perform a chord different from that shown, at their own choice.

Note that Events 2 and 4 were had different ("jittered") durations across trials, and that tasks randomly varied from trial to trial to ensure that all brain regions were sampled in different stimulus time points and to avoid antecipation by the volunteer.

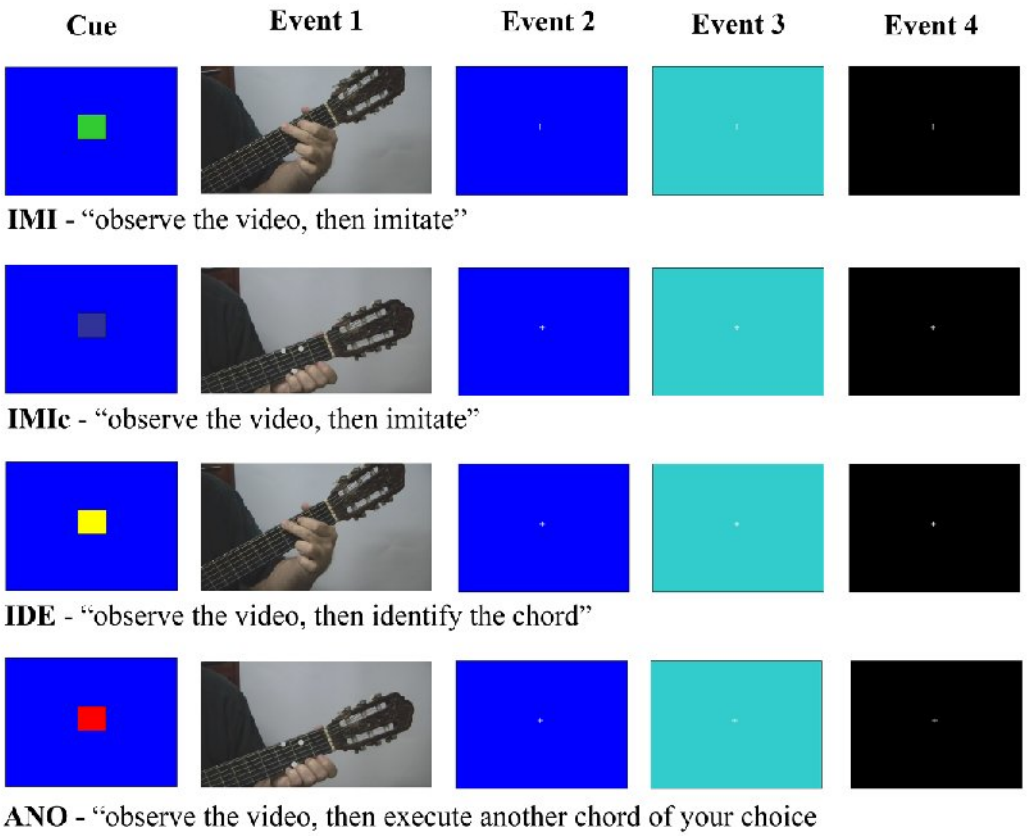

Figure 2.2.1. The events constituting each of the four types of trials, including IMI, IMIc, IDE, and ANO, are illustrated. Each trial began with the presentation of a cue (a colored square) which color indicated the experimental condition. The purpose was to prompt participants to the task to be performed. Subsequently, the events illustrated occurred one after the other. In each task, Event 3 was preceded by presentation of a cross with the same color as that of the square. The cross was presented 1s before the end of Event 2. Its disappearance from the screen was the signal for participants to start the required task. Participants were instructed to reposition their hands on the guitar neck immediately after the task completion. Duration of the events, Cue: 1.2-2s; Event 1: 12s; Event 2: 4-8s; Event 3: 6s; Event 4: 4–10s.

Abbreviations: IMI, imitation of actual hand movement; IMIc, imitation of moving dots; IDE, identification of the chord; ANO, choice of another chord.

2.2.4 Instructions and Procedures

During practice sessions participants learned to execute and name the four chords. Practice was performed with the participants lying on the MR table and stimuli presented in the same way as during the experiment. Subjects were introduced to the guitar neck, to the hand's rest position, and to the rhythmical press-release pattern in which chords were to be performed. Participants were instructed to observe the video clip chords carefully and to reproduce the chords with maximal accuracy. The participants used their left hand to perform the required actions and had no visual feedback of their movements. An experimenter was present in the scan room to help the participants to position their fingers correctly. In a second part of the practice session, the tasks and events were presented to the participants in order to familiarize them with the experimental design. For this aim a full block of 16 trials was run, 4 of each type of task, with exactly the same structure as that of the main experiment. For the ANO task, participants were asked not to use the same chord across trials, but also not to excessively engage in the chord's choice. The practice session lasted about 20 minutes and was directly followed by the main experiment.

2.2.5 Image Acquisition and Settings for the fMRI

The experiment was carried out using a 3 Tesla scanner with an eight-channel head coil (3T Philips Achieva, Institute of Radiology, Clinics Hospital, Medical School, University of São Paulo). The system performance was assessed throughout the study by a daily quality control program. Artifacts related to head movements during the RMI

exam were minimized by positioning a tape on the forehead and by inserting foam pads in each side of the head; this provided good immobilization of the head, without causing discomfort. All participants were instructed to maintain their heads immobile during image acquisition.

Forty transversal AC-PC slices T1 weighted echo-planar images were obtained from a gradient-echo sequence used to measure task-related changes in blood oxygen level dependent (BOLD) signal as an index of regional neuronal activity. The following parameters were used: echo time (TE) 28 ms, time repetition (TR) 2s, flip angle (FA) 8 degrees, field of view (FOV) 240 mm, slice thickness 3 mm, interslice gap 0.4mm, in-plane resolution 3.125 x 3.125 mm. The forty slices covered the whole brain from the top of the cerebellum throughout to the vertex.

2.2.6 Data Processing and Statistical Analysis

2. 2.6.1 Pre-Processing

The first five volumes of each subject's scan were not collected in order to wait until full T1 saturation. Data analysis was carried out using FMRI Expert Analysis Tool (FEAT) version 5.98, part of the FMRIB's Software Library (FSL, ww.fmrib.ox.ac.uk/fsl). In order to correct for the temporal offset between the slices acquired in one scan, a Fourier-space time-series phase-shifting was applied. Non-brain voxels were removed using BET tool (Smith, 2002). Large drifts were corrected by way of a temporal high-pass filter (Gaussian-weighted least-squares straight line fitting) with a cut-off frequency of 1/54 Hz; this allowed signal baseline correction. Spatial smoothing using a Gaussian kernel of FWHM 5.3 mm as well as mean-based intensity normalization of all volumes by the same factor was applied to the functional data. In order to align the individual functional data slices onto the corresponding 3D

stereotactic coordinate reference system (MNI), a rigid linear registration with six degrees of freedom (three rotational and three translational) was carried out using FLIRT (Jenkinson and Smith, 2001).

2.2.6.2 Whole Brain Analysis

Two whole brain analyses were performed. In the first analysis, the onsets and durations of each of the stimuli were modeled using the General Linear Model (GLM) according to the experimental conditions and events, for each subject. Data were motion-corrected using MCFLIRT (Jenkinson et al., 2002) and affine spatial normalization 12DOF. The design matrix was composed of regressors according to the experimental conditions (Events 1, 2, and 3 for each of the tasks including IMI, IMIc, IDE and ANO). The regressors were convolved with the gamma canonical HRF (12 additional regressors per session were included in the GLM to account for voxel intensity variations due to absolute and differential head movement). In the second analysis, the respective contrast images from the first stage for each subject were entered into one-sample t tests (random effects analysis, Friston et al., 1999). Z-statistic images were thresholded using clusters determined by z-voxel>2.3 and a (corrected) cluster significance threshold of $p<0.05$ (Worsley, 2001). The standard coordinates of the local maxima within areas of significant activity change were determined for all conditions. The tables showing anatomical localization of the local maxima and clusters was assessed by reference to Talaraich Coordinates after appropriate coordinate transformation and checked visually by a radiologist. The activation maps were made using MRIcron Software (www.mricron.com, 12/2009 Version) and illustrates the activations with 20% of air/skin threshold and 8mm of search depth.

2.2.6.3 Data presentation

Comparative hemodynamic responses in each event of the perceptually similar but cognitively distinct tasks focused not only on areas related to the MNS but also in other brain areas found active in the whole analysis. Data are referred to considering the type of task performed associated with a number that represents the corresponding event. For instance, IMI-1 corresponds to the first event of the imitation task and IDE-3 corresponds to the third event of identification task). Every event was contrasted with its corresponding baseline, i.e., Event 4 (black screen).

2.3. RESULTS

2.3.1 Brain Activity Related to Observation (Event 1)

Observation of either a hand performing (IMI-1 and ANO-1) or of moving dots representing (IMIc-1 and IDE-1) a guitar chord produced hemodynamic responses represented in Figure 2.3.1 and local maxima of the activated clusters shown in Table 2.1. As expected, visual brain areas were activated during all tasks.

In addition, as can be seen in Figure 2.3.1 and Table 2.1, during IMI-1 there was bilateral activation of the IPL and IPS. Interestingly, during IMIc-1 involving a similar cognitive task compared to IMI-1, in addition to bilateral activation of IPL and IPS, there was also activation of frontal areas. The IFG was bilaterally activated with a larger cluster in the left hemisphere. The PMC showed active clusters bilaterally, one in the left ventral portion of the precentral gyrus and another more dorsally in the precentral gyrus. When comparing directly IMI-1 and IMIc-1, i.e., tasks with similar cognitive requirements except for the video clip using moving dots in this latter, higher hemodynamic changes were detected in the ventral PMC and IPL (left) during IMI-1

(Figure 2.3.2 and Table 2.2); in contrast, higher hemodynamic changes were detected in the dorsal PMC during IMIc-1 (Figure 2.3.2 and Table 2.2).

During IDE-1 the ventral portion of the PMC and the IFG were bilaterally activated, although with larger clusters in the right hemisphere (Figure 2.3.1 and Table 2.1). In addition, the IPL and the IPS were bilaterally activated. Direct comparisons of IMI-1 and IDE-1, i.e., tasks involving the same video clip but different cognitive demands, revealed greater hemodynamic changes in the IFG and IPS (right hemisphere) during IDE-1 (see Figure 2.3.2 and Table 2.2).

During ANO-1 there was bilateral activation of the PMC (ventral portion) and the IFG, although this latter exhibite a smaller cluster (Figure 2.3.1 and Table 121). The IPL and IPS were activated bilaterally. Direct comparisons of IMIc-1 and ANO-1, i.e., tasks involving exposure to the same stimulus, revealed greater hemodynamic changes in the IPL (left hemisphere) during IMIc-1 as compared to ANO-1, and greater hemodynamic changes in the medial portion of the PMC during ANO-1 as compared to IMIc-1 (Figure 2.3.2 and Table 2.2).

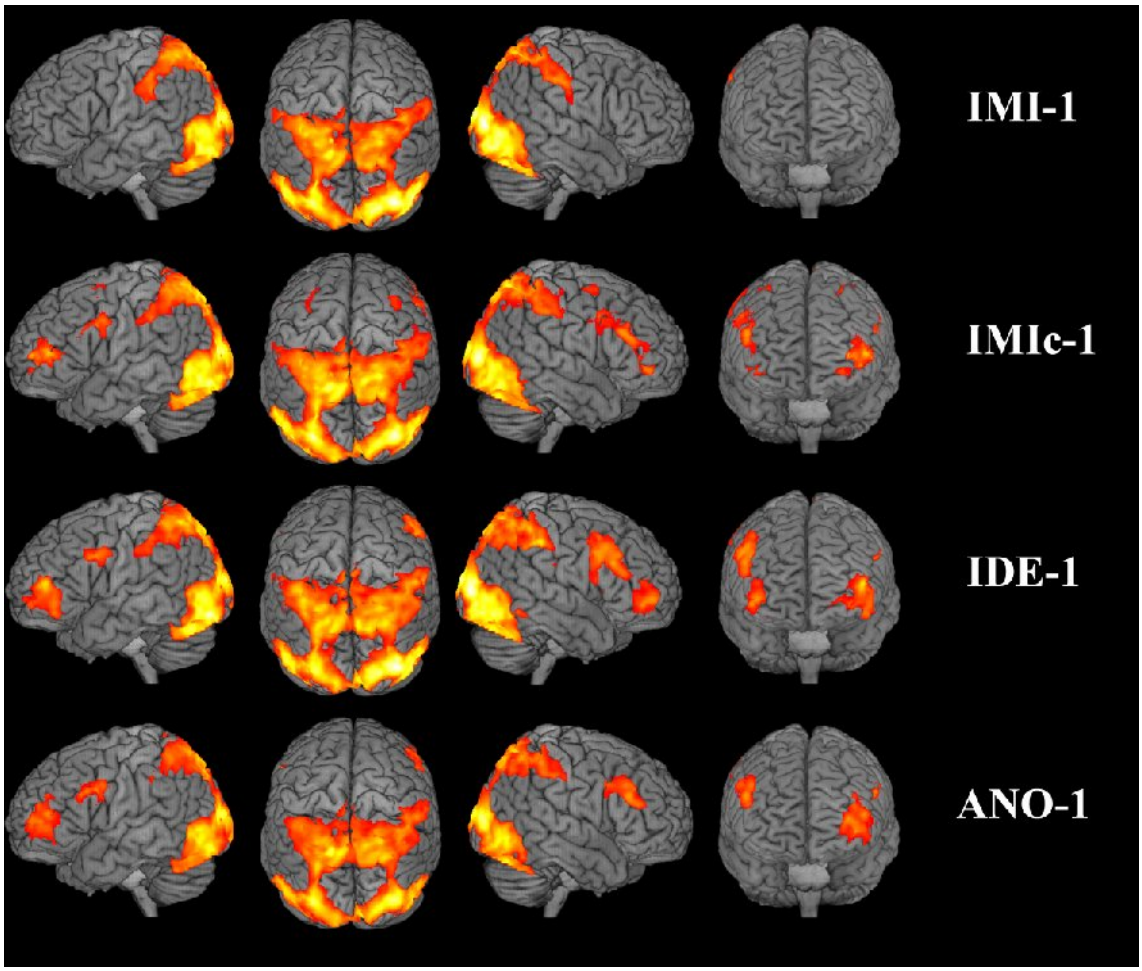

Figure 2.3.1 Cortical areas activated during observation of either a hand performing (IMI-1 and ANO-1) or of moving dots representing (IMIc-1 and IDE-1) a guitar chord associated with distinct instructions about the task to be performed later (see Figure 1 for details). Event 1 of every task was contrasted with its corresponding event 4, taken as a baseline. The number associated with identification of each task indicates the event number. Abbreviations as in Figure 2.2.1.

Table 2.1. Local Maxima of Activated Foci, expressed in Talairach Coordinates, during Event 1 of the tasks IMI, IMIc, IDE and ANO.

Brain Region	Brodmann Area	Side	Z score	Coordinates		
				X	Y	Z
IMI-1						
Fusiform Gyrus	BA 19	Left	7.92	-34.6	-72	-12
Inferior Occipital Gyrus	BA 18	Right	0.57	40.36	-80	-12
Middle Occipital Gyrus	BA 18	Right	-0.1	45.7	-78	-12
Middle Occipital Gyrus	BA 19	Right	1.88	26.07	-98	14
Middle Temporal Gyrus	BA 37	Left	8.49	-41.75	-60	0
IMIc-1						
Caudate		Right	-1.63	34.79	-30	-4
Cerebellum		Left	3.02	-6.2	-34	-6
Cerebellum		Right	1.14	8.03	-46	-6
Cingulate Gyrus	BA 24	Left	3.9	-16.95	-6	46
Fusiform Gyrus	BA 19	Left	7.82	-32.73	-72	-12
Inferior Occipital Gyrus	BA 17	Right	2.23	20.78	-92	-8
Middle Frontal Gyrus	BA 10	Left	6.54	-36.5	50	10
Middle Frontal Gyrus	BA 6	Left	3.94	-25.96	-4	58
Middle Frontal Gyrus	BA 6	Right	-0.48	25.85	-6	42
Middle Frontal Gyrus	BA 10	Right	-1.51	36.62	46	-4
Middle Frontal Gyrus	BA 8	Right	-3.09	50.88	12	40
Middle Frontal Gyrus	BA 9	Right	-2.16	47.36	30	26
Middle Occipital Gyrus	BA 18	Left	6.85	-22.02	-90	10
Middle Occipital Gyrus	BA 19	Right	1.02	36.87	-88	6
Parahippocampal Gyrus	BA 27	Left	4.47	-13.27	-32	-2
Parahippocampal Gyrus	BA 30	Right	0.36	16.97	-32	-6
Sub-Gyral	BA 6	Left	3.88	-18.76	4	54
Superior Frontal Gyrus	BA 10	Left	5.11	-24.03	60	8
Superior Frontal Gyrus	BA 6	Left	3.48	-20.6	8	60
Superior Frontal Gyrus	BA 11	Right	-1.56	33.01	44	-14
Thalamus		Right	0.92	24.22	-32	2
IDE-1						
Cerebellum		Left	3.71	-11.57	-34	-8
Fusiform Gyrus	BA 19	Left	8.09	-34.52	-80	-14
Inferior Frontal Gyrus	BA 10	Left	5.71	-38.38	46	0
Inferior Frontal Gyrus	BA 9	Left	5.47	-47.44	16	22
Inferior Occipital Gyrus	BA 19	Left	7.68	-36.36	-70	0
Middle Frontal Gyrus	BA 10	Left	6.47	-32.91	52	10
Middle Frontal Gyrus	BA 10	Right	-1.74	41.99	58	-2
Middle Frontal Gyrus	BA 11	Left	5.83	-40.17	46	-14
Middle Frontal Gyrus	BA 11	Right	-1.14	34.84	44	-8
Middle Frontal Gyrus	BA 9	Left	6.31	-40.16	14	30
Middle Frontal Gyrus	BA 9	Right	-2.6	50.91	18	36
Middle Frontal Gyrus	BA 46	Right	-1.69	45.59	28	24
Middle Occipital Gyrus	BA 18	Right	1.1	38.67	-88	4
Middle Occipital Gyrus	BA 19	Right	1.85	26.13	-98	14
Parahippocampal Gyrus	BA 27	Right	0.71	18.77	-34	-2

Parahippocampal Gyrus	BA 30	Right	0.65	15.16	-34	-6
Precentral Gyrus	BA 6	Left	6.01	-43.79	2	34
Precentral Gyrus	BA 9	Right	-1.88	41.97	18	36
Superior Frontal Gyrus	BA 10	Left	4.64	-25.88	64	-4
Thalamus		Right	-0.12	25.9	-32	6
ANO-1						
Cerebellum		Right	1.99	22.52	-82	-18
Inferior Frontal Gyrus	BA 44	Left	5.59	-45.63	12	20
Inferior Frontal Gyrus	BA 9	Right	-4.14	54.34	14	28
Inferior Occipital Gyrus	BA 17	Right	2.11	20.73	-92	-6
Middle Frontal Gyrus	BA 10	Left	5.19	-27.62	48	-2
Middle Frontal Gyrus	BA 46	Left	5.54	-40.23	22	20
Middle Frontal Gyrus	BA 9	Left	6.56	-43.74	20	30
Middle Frontal Gyrus	BA 9	Right	-2.2	43.71	28	36
Middle Frontal Gyrus	BA 8	Right	-2.47	36.48	16	42
Middle Occipital Gyrus	BA 18	Left	6.28	-22.1	-92	10
Middle Occipital Gyrus	BA 18	Right	0.9	33.23	-88	4
Middle Occipital Gyrus	BA 19	Right	0.81	35.02	-90	10
Superior Frontal Gyrus	BA 10	Left	4.61	-31.28	62	2
Superior Frontal Gyrus	BA 9	Left	4.48	-27.69	48	28

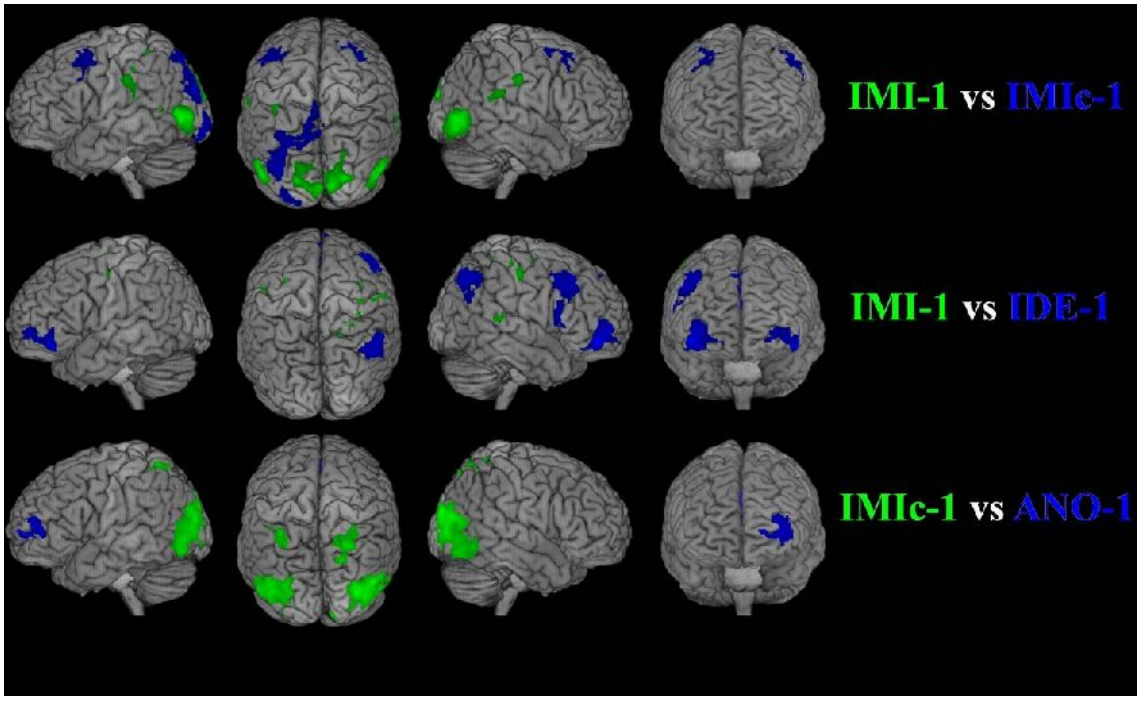

Figure 2.3.2 Contrasts of hemodynamic changes reflecting activations in different cortical regions when comparing pairs of tasks. The top row shows areas exhibiting stronger activation during IMI-1 (green) and during IMIc-1 (blue) when these two tasks are compared. Note that these tasks involved different stimuli, either moving hands or moving dots, but similar cognitive demands. The middle row shows areas exhibiting stronger activation during IMI-1 (green) and during IDE-1 (blue) when these tasks are

compared. Note that these tasks involved the same stimulus and different cognitive demands. The bottom row shows areas exhibiting stronger activation during IMIc-1 (green) and during ANO-1 (blue), tasks involving the same stimulus but distinct cognitive demands. P<0,05. Abbreviations as in Figure 2.2.1.

Table 2.2 Local Maxima of Activated Foci, expressed in Talairach Coordinates, when contrasting pairs of tasks during Event 1.

Brain Region	Brodmann Area	Side	Z score	Coordinates		
				X	Y	Z
IMI-1 > IMIc-1						
Cerebellum - Posterior		Right	2.69	49.9	-63.02	-20
Cuneus	BA 17	Right	4.82	15.89	-80.62	12
Cuneus	BA 18	Left	4.15	-12.41	-79.71	18
Cuneus	BA 18	Right	3.92	15.02	-71.71	16
Fusiform Gyrus	BA 19	Right	3.37	42.62	-65.5	-10
Inferior Parietal Lobule	BA 40	Left	2.98	-33.35	-38.47	56
Inferior Temporal Gyrus	BA 37	Right	4.71	49.2	-55.84	0
Insula	BA 13	Right	3.23	53.17	-19.46	24
Middle Occipital Gyrus	BA 19	Right	4.64	49.2	-55.84	-6
Middle Temporal Gyrus	BA 37	Left	5.05	-40.41	-62.53	2
Middle Temporal Gyrus	BA 21	Left	2.23	-49.42	-47.21	6
Middle Temporal Gyrus	BA 22	Left	2.23	-49.94	-41.86	8
Postcentral Gyrus	BA 2	Left	2.94	-47.79	-25.42	40
Postcentral Gyrus	BA 3	Left	2.38	-57.8	-19.18	36
Postcentral Gyrus	BA 3	Right	2.57	62.14	-15.02	22
Postcentral Gyrus	BA 40	Right	2.68	57.6	-26.25	20
Precentral Gyrus	BA 6	Right	3.03	50.61	-12.5	34
Precuneus	BA 31	Right	4.78	26.72	-75.99	22
Sub-Gyral	BA 37	Right	4.80	48.5	-48.7	-2
IMIc-1 > IMI-1						
Fusiform Gyrus	BA 19	Left	3.58	-20.03	-78.64	-12
Lingual Gyrus	BA 18	Left	3.90	-12.06	-83.28	-8
Middle Frontal Gyrus	BA 6	Left	2.16	-38.06	9.72	56
Middle Frontal Gyrus	BA 6	Right	2.02	29.02	16.07	62
Middle Frontal Gyrus	BA 8	Right	1.24	35.08	31.04	44
Precuneus	BA 7	Left	3.67	-19.56	-64.18	50
Superior Frontal Gyrus	BA 6	Right	2.54	19	22.34	54
Superior Frontal Gyrus	BA 8	Left	1.40	-42.82	20.06	50
Superior Frontal Gyrus	BA 8	Right	1.73	27.98	26.78	50
Superior Occipital Gyrus	BA 19	Left	3.55	-27.83	-75.77	26
IMI-1 > IDE-1						
Insula	BA 13	Right	2.79	41.3	-13.38	22
Middle Frontal Gyrus	BA 6	Left	2.26	-14.3	-2.44	56
Middle Frontal Gyrus	BA 6	Right	2.81	40.08	-0.89	46
Paracentral Lobule	BA 3	Right	2.97	19.17	-37.1	56
Paracentral Lobule	BA 5	Right	2.85	21.55	-42.28	52
Postcentral Gyrus	BA 3	Right	2.33	15.79	-41.03	64
Postcentral Gyrus	BA 5	Right	2.85	21.38	-40.49	68

Precentral Gyrus	BA 4	Right	2.28	35.24	-28.38	64
Precentral Gyrus	BA 6	Right	2.9	50.26	-8.93	30
Precuneus	BA 7	Right	2.88	16.31	-46.38	54
Sub-Gyral	BA 6	Left	2.32	-21.41	-6.72	56
Sub-Gyral	BA 6	Right	2.61	21.62	-4.44	52
Superior Frontal Gyrus	BA 6	Left	2.19	-25.66	-1.72	62
IDE-1 > IMI-1						
Inferior Frontal Gyrus	BA 13	Right	1.86	42.71	29.98	6
Inferior Frontal Gyrus	BA 47	Left	2.27	-50.34	39.18	-14
Inferior Parietal Lobule	BA 40	Right	3.39	50.2	-46.76	46
Medial Frontal Gyrus	BA 8	Left	1.40	-1.89	43.76	38
Medial Frontal Gyrus	BA 8	Right	1.31	4.57	35.36	40
Medial Frontal Gyrus	BA 9	Left	1.97	1.84	44.12	26
Middle Frontal Gyrus	BA 10	Left	1.30	-45.61	48.62	-8
Middle Frontal Gyrus	BA 10	Right	3.02	34.67	54.46	-10
Middle Frontal Gyrus	BA 11	Left	0.88	-29.02	51.99	-12
Middle Frontal Gyrus	BA 8	Right	2.03	34.9	32.84	44
Middle Frontal Gyrus	BA 9	Right	1.72	36.25	38.37	36
Precentral Gyrus	BA 9	Right	1.83	34.26	20.17	36
Superior Frontal Gyrus	BA 10	Left	1.07	-34.96	55.03	-2
Superior Frontal Gyrus	BA 10	Right	1.92	30.08	63.01	-4
Superior Frontal Gyrus	BA 8	Left	1.62	-4.92	36.27	46
Superior Frontal Gyrus	BA 8	Right	1.95	37.81	22.31	48
IMIc-1 > ANO-1						
Lingual Gyrus	BA 18	Right	3.32	17.93	-82.25	-4
Middle Occipital Gyrus	BA 19	Left	3.13	-33.42	-76.31	14
Middle Occipital Gyrus	BA 19	Right	3.50	42.8	-67.28	6
Middle Occipital Gyrus	BA 37	Left	3.05	-36.85	-60.42	0
Middle Temporal Gyrus	BA 19	Left	2.97	-37.03	-58.64	10
Middle Temporal Gyrus	BA 19	Right	3.57	49.55	-59.43	16
Middle Temporal Gyrus	BA 37	Left	2.98	-37.03	-58.64	4
Middle Temporal Gyrus	BA 39	Right	3.49	37.21	-67.81	22
Postcentral Gyrus	BA 40	Left	1.86	-37.6	-33.49	50
Postcentral Gyrus	BA 5	Left	1.92	-36.73	-42.41	60
Precuneus	BA 31	Left	3.35	-22.59	-71.68	24
Sub-Gyral	BA 7	Left	2.63	-25.03	-46.7	52
Superior Occipital Gyrus	BA 19	Right	3.89	35.69	-71.55	22
Superior Parietal Lobule	BA 7	Left	2.47	-28.75	-47.05	64
Superior Parietal Lobule	BA 7	Right	3.02	26.15	-50.85	58
ANO-1 > IMIc-1						
Anterior Cingulate	BA 32	Left	1.26	-10.68	37.52	16
Anterior Cingulate	BA 32	Right	1.29	8.47	33.93	20
Cingulate Gyrus	BA 32	Right	0.86	5.27	28.21	32
Inferior Frontal Gyrus	BA 10	Left	0.96	-42.23	52.54	4
Medial Frontal Gyrus	BA 6	Left	2.38	-4.22	29.14	34
Middle Frontal Gyrus	BA 10	Left	1.30	-35.13	56.82	2
Sub-Gyral	BA 6	Left	1.13	-37.99	47.54	2
Superior Frontal Gyrus	BA 10	Left	1.22	-36.82	54.86	12

2.3.2 Maintenance of the Information About the Observed Action and Preparation for Task Execution (Event 2)

The event 2 of the experiment corresponded to the maintenance of the information about the observed action and later preparation for task action execution. Note that during this event subjects had only to "get prepared" to perform the required task; the actual execution of the task occurred during Event 3 (see below). That is, participants had to prepare themselves either to imitate the observed action (IMI-2 and IMIc-2), to identify the chord (IDE-2) or to execute a different chord compared to that observed (ANO-2).

The results associated with event 2 are presented in Figure 2.3.3 and Table 2.3. As can be seen, the four tasks employed in the present study induced similar patterns of hemodynamic changes during event 2. The brain regions showing major hemodynamic changes included frontal areas. For instance, the dorsal portion of the PMC was activated in each of the four tasks bilaterally and the ventral portion showed activation restricted to the right hemisphere. The IPS and IPL were bilaterally activated in each of the tasks, and the anterior IPS showed a slight lateralization, with a smaller cluster in the left hemisphere. Interestingly, the IFG exhibited activation restricted to the anterior portion of the left hemisphere during performance of the IMIc-2 task, thus contrasting with a bilateral and greater activation of this brain region during performance of the remaining tasks.

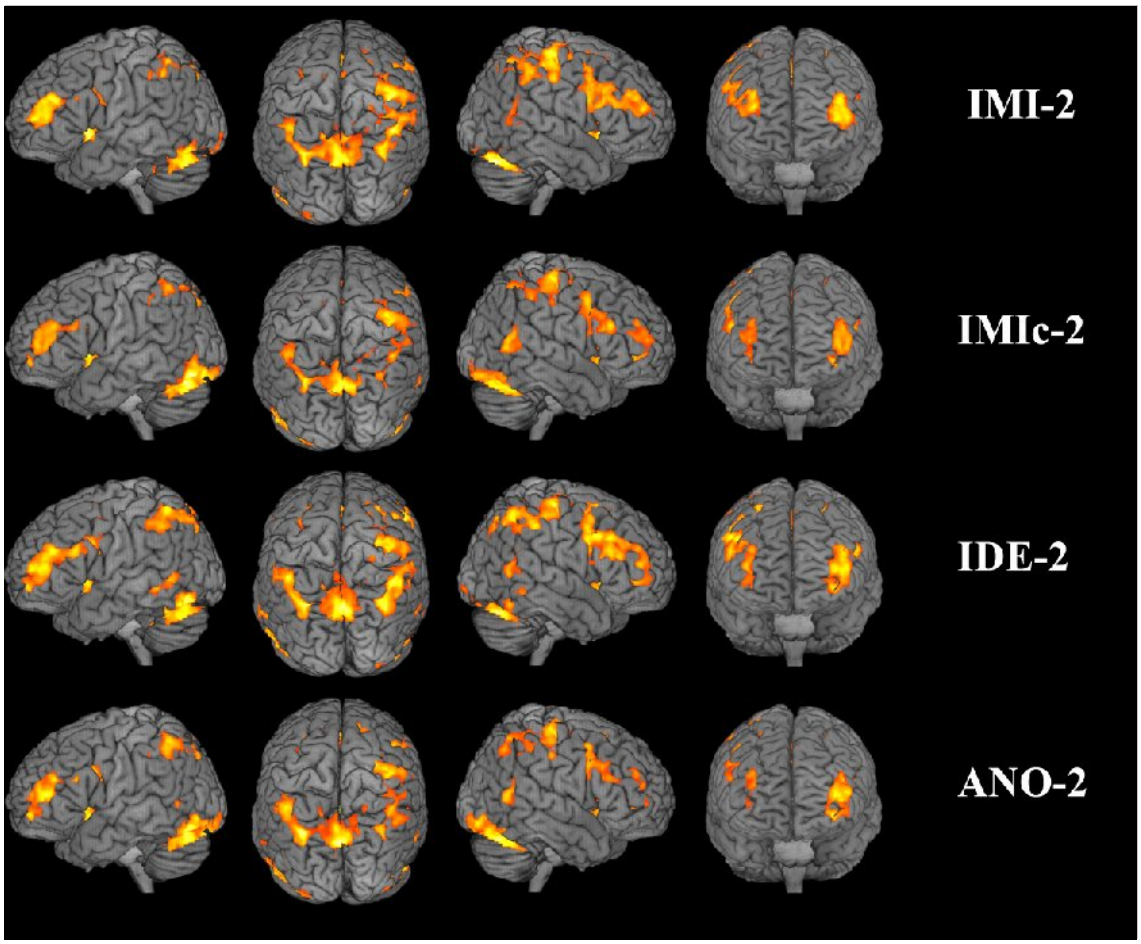

Figure2.3.3 Cortical areas activated during event 2 involving maintenance of the information about the observed action and preparation for task execution. Event 2 of every task was contrasted with its corresponding event 4, taken as a baseline. In IMI-2 and IMIc-2 tasks, after seeing a video showing a guitar chord demonstrated by way of either a hand or moving dots, respectively, participants had to rehearse it and get prepared for its later execution (note that there was no execution during this event, i.e., execution occurred in the next event). In IDE-2 task, after seeing a video showing moving dots representing a chord, the subjects had to maintain this information in order to identify it later, during the next event. In ANO-2, after seeing a video showing a hand executing a chord, participants had to maintain this information in order to freely choose a different chord during the next event. Abbreviations and conventions as in Figure 2.2.1.

Table 2.3. Local Maxima of Activated Foci, expressed in Talairach Coordinates, during Event 2 of the tasks IMI, IMIc, IDE and ANO.

Brain Region	Brodmann Area	Side	Z score	Coordinates		
				X	Y	Z
IMI-2						
Cerebellum		Left	2.83	-6.21	-38	0
Cerebellum		Right	4.00	45.69	-66	-26
Cingulate Gyrus	BA 31	Right	2.55	15.13	-36	32
Claustrum		Left	2.21	-29.37	16	-2
Extra-Nuclear	BA 13	Left	2.17	-25.83	20	-6
Hippocampus		Left	3.07	-24.12	-36	0
Inferior Frontal Gyrus	BA 47	Right	4.12	36.73	24	-6
Inferior Parietal Lobule	BA 40	Right	4.07	40.3	-52	48
Insula	BA 13	Left	2.26	-32.94	14	-6
Lentiform Nucleus		Left	2.12	-22.27	22	0
Lingual Gyrus	BA 30	Left	3.02	-20.54	-40	0
Medial Frontal Gyrus	BA 6	Left	1.12	-16.98	2	58
Middle Frontal Gyrus	BA 10	Left	4.14	-34.74	50	10
Middle Frontal Gyrus	BA 46	Left	4.20	-40.17	22	20
Middle Frontal Gyrus	BA 6	Left	1.24	-25.96	-8	58
Paracentral Lobule	BA 5	Right	2.64	8.01	-36	48
Posterior Cingulate	BA 23	Right	2.67	6.29	-30	24
Precentral Gyrus	BA 4	Right	4.17	33.17	-18	58
Precentral Gyrus	BA 6	Left	0.39	-31.22	8	26
Superior Frontal Gyrus	BA 10	Left	4.09	-31.18	50	14
Superior Frontal Gyrus	BA 6	Left	1.00	-8.11	6	62
Superior Parietal Lobule	BA 7	Left	4.92	-23.95	-68	42
IMIc-2						
Cerebellum		Left	7.01	-41.74	-68	-16
Cerebellum		Right	5.83	47.49	-56	-26
Cingulate Gyrus	BA 23	Left	1.84	-0.87	-30	26
Cingulate Gyrus	BA 23	Right	1.79	2.67	-22	26
Cingulate Gyrus	BA 31	Right	1.65	13.34	-36	36
Cingulate Gyrus	BA 32	Right	-0.06	2.58	16	38
Claustrum		Left	1.29	-29.39	16	2
Extra-Nuclear	BA 13	Left	1.22	-24.09	18	-10
Extra-Nuclear	BA 47	Left	1.33	-33.02	16	-8
Inferior Frontal Gyrus	BA 45	Right	4.92	45.57	22	4
Inferior Frontal Gyrus	BA 47	Left	1.26	-27.67	22	-4
Inferior Frontal Gyrus	BA 47	Right	5.04	36.67	26	-4
Inferior Parietal Lobule	BA 40	Left	7.79	-31.06	-46	40
Insula	BA 13	Right	5.14	29.57	26	2
Middle Frontal Gyrus	BA 6	Left	3.09	-25.96	-6	56
Medial Frontal Gyrus	BA 6	Right	-0.07	2.57	12	44
Middle Frontal Gyrus	BA 10	Left	5.06	-34.77	50	6
Middle Frontal Gyrus	BA 46	Right	4.93	45.65	24	18
Middle Frontal Gyrus	BA 8	Right	4.90	47.38	10	44
Middle Temporal Gyrus	BA 21	Right	2.86	61.56	-50	8
Middle Temporal Gyrus	BA 39	Right	2.98	52.71	-56	6
Postcentral Gyrus	BA 3	Right	6.85	40.31	-22	56
Posterior Cingulate	BA 23	Right	1.74	6.3	-30	24
Precentral Gyrus	BA 4	Right	6.87	38.53	-22	62

Precuneus	BA 7	Left	7.65	-20.36	-70	42
Precuneus	BA 7	Right	7.30	6.41	-76	48
Sub-Gyral	BA 37	Right	3.05	47.29	-46	-2
Sub-Gyral	BA 6	Left	3.00	-18.74	2	58
Superior Frontal Gyrus	BA 10	Left	4.99	-29.42	46	18
Superior Frontal Gyrus	BA 6	Right	-0.11	6.14	8	52
Superior Temporal Gyrus	BA 22	Right	3.00	50.86	-48	8
Superior Temporal Gyrus	BA 39	Right	2.95	54.47	-52	12
Thalamus		Right	1.65	13.39	-30	14
IDE-2						
Caudate		Left	1.17	-20.58	26	2
Cerebellum		Left	3.28	-40.02	-64	-16
Cingulate Gyrus	BA 23	Left	0.01	-2.73	-26	28
Cingulate Gyrus	BA 31	Right	-0.18	11.51	-36	34
Claustrum		Left	1.26	-27.64	16	2
Extra-Nuclear	BA 13	Left	1.24	-25.81	18	-8
Inferior Frontal Gyrus	BA 13	Left	1.36	-34.81	12	-12
Inferior Frontal Gyrus	BA 47	Right	2.29	34.93	22	-8
Insula	BA 13	Left	1.34	-31	14	-2
Middle Frontal Gyrus	BA 10	Left	2.28	-34.76	50	10
Middle Frontal Gyrus	BA 9	Left	2.33	-38.28	12	28
Posterior Cingulate	BA 23	Left	-0.04	0.84	-36	22
Posterior Cingulate	BA 23	Right	-0.11	6.27	-28	24
Precentral Gyrus	BA 6	Left	2.26	-32.93	6	26
Precuneus	BA 7	Left	3.07	-23.97	-68	40
Precuneus	BA 7	Right	2.62	9.96	-70	44
Superior Parietal Lobule	BA 7	Right	2.24	38.51	-54	48
ANO-2						
Caudate		Left	2.07	-18.77	22	6
Caudate		Left	2.02	-15.23	26	12
Caudate		Left	1.97	-11.72	18	12
Cerebellum		Left	6.98	-39.99	-70	-16
Cerebellum		Right	6.99	29.63	-60	-20
Cingulate Gyrus	BA 32	Right	3.54	9.82	32	24
Claustrum		Left	2.19	-27.59	16	2
Extra-Nuclear	BA 13	Left	2.17	-25.82	18	-8
Extra-Nuclear	BA 47	Right	0.41	36.58	22	-4
Inferior Frontal Gyrus	BA 47	Left	2.26	-32.95	16	-8
Inferior Frontal Gyrus	BA 47	Right	0.51	29.37	32	0
Inferior Frontal Gyrus	BA 9	Left	0.43	-34.79	6	26
Inferior Frontal Gyrus	BA 9	Right	4.95	43.78	14	22
Insula	BA 13	Right	0.29	45.39	14	2
Medial Frontal Gyrus	BA 32	Right	3.50	13.44	14	44
Medial Frontal Gyrus	BA 8	Left	3.66	0.95	20	46
Middle Frontal Gyrus	BA 10	Left	5.04	-32.94	42	22
Middle Frontal Gyrus	BA 10	Right	5.06	34.85	42	18
Middle Frontal Gyrus	BA 46	Left	5.13	-40.11	26	24
Middle Frontal Gyrus	BA 46	Right	4.90	47.35	26	22
Middle Frontal Gyrus	BA 6	Right	4.95	43.79	10	48
Middle Frontal Gyrus	BA 9	Left	0.48	-38.33	12	28
Middle Frontal Gyrus	BA 9	Right	4.92	45.56	22	30
Postcentral Gyrus	BA 3	Right	6.85	40.34	-22	56

Posterior Cingulate	BA 23	Right	2.69	4.61	-30	24
Precentral Gyrus	BA 6	Left	0.43	-34.94	0	36
Precuneus	BA 7	Left	7.67	-22.11	-70	42
Precuneus	BA 7	Right	7.34	2.89	-78	42
Superior Frontal Gyrus	BA 10	Left	4.92	-24.01	62	-6
Superior Frontal Gyrus	BA 6	Left	3.95	-20.51	8	60
Superior Frontal Gyrus	BA 6	Right	3.43	18.74	16	50
Superior Parietal Lobule	BA 7	Left	7.70	-23.9	-70	46

2.3.3 Execution of the Required Task (Event 3)

During event 3 the participants either executed the same (IMI-3 and IMIc-3 tasks) or a different (ANO-3 task), or just identified the chord previously shown (IDE-3 task), using the guitar neck to perform each of these tasks.

The results are shown in Figure 2.3.4 and Table 2.4. The activations were remarkably similar during performance of each of the four tasks. In all of them there was a strong bilateral activation of the PMC, IFG, IPS and IPL.

2.3.4 Brain Areas Activated in Common During Action Observation of the Video Clip Showing a Chord, Maintenance of this Information, and Preparation for Task Execution (Imitation, Identification or Execution of Another Chord)

The Figure 2.3.5 shows brain areas activated in common during observation of the video clip (event 1), maintenance of this information, and preparation for task execution (event 2). In addition to stimulation of visual cortical areas, the overlay maps show IFG and the PMC co-localized at the right hemisphere. The IPL exhibited a slight overlap, mainly concentrated in the border of the IPS.

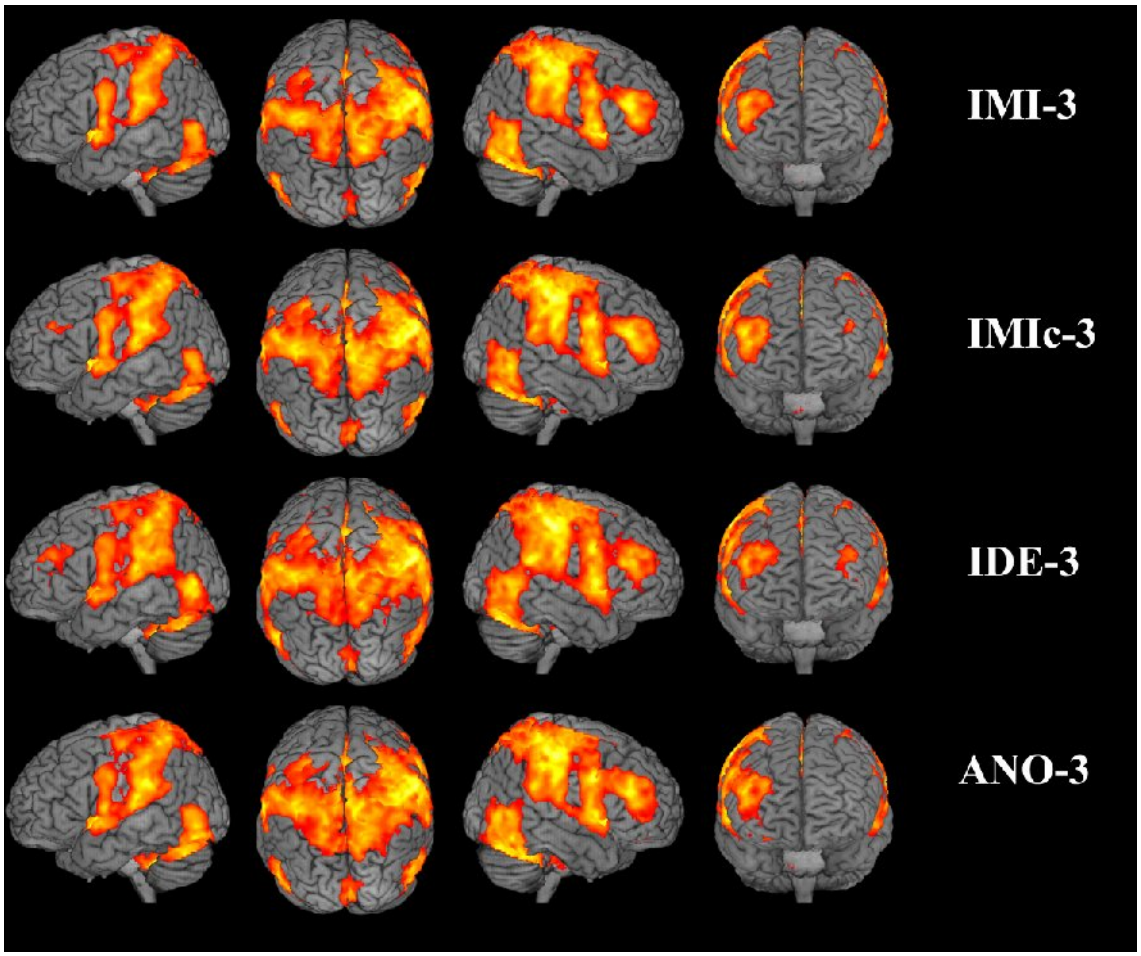

Figure 2.3.4 Cortical areas activated during event 3 involving the execution of the required action. Event 3 of every task was contrasted with its corresponding event 4, taken as a baseline. In the IMI-3, IMIc-3 and ANO-3 tasks the subjects executed chords on the provided guitar neck; in the IDE-3 the subjects positioned their fingers on the guitar neck in order to express their identification of the chord. Abbreviations and conventions as in Figure 2.2.1.

Table 2.4. Local Maxima of Activated Foci, expressed in Talairach Coordinates, during Event 3 of the Conditions IMI, IMIc, IDE and ANO.

Brain Region	Brodmann Area	Side	Z score	Coordinates		
				X	Y	Z
IMI-3						
Cerebellum - Posterior Lobe		Left	6.12	-11.36	-62	-22
Cerebellum - Posterior Lobe		Right	1.45	35.04	-54	-34
Cerebellum - Anterior Lobe		Left	6.34	-14.94	-58	-24
Cerebellum - Anterior Lobe		Right	4.76	1.12	-58	-10
IMIc-3						
Anterior Cingulate	BA 32	Left	4.18	-24.15	34	16
Cerebellum - Anterior Lobe		Left	7.21	-20.24	-54	-30

Cerebellum - Anterior Lobe		Right	5.14	1.18	-58	-10
Cerebellum - Posterior Lobe		Left	6.31	-11.32	-62	-22
Cerebellum - Posterior Lobe		Right	4.23	6.49	-78	-24
Middle Frontal Gyrus	BA 9	Left	5.23	-27.65	40	28
Superior Frontal Gyrus	BA 10	Left	3.52	-22.41	52	24
IDE-3						
Cerebellum - Anterior Lobe		Left	7.01	-20.26	-54	-30
Cerebellum - Posterior Lobe		Right	1.93	35.1	-60	-30
Middle Frontal Gyrus	BA 10	Left	5.55	-34.83	46	10
Middle Frontal Gyrus	BA 9	Left	5.65	-29.41	36	30
Postcentral Gyrus	BA 2	Right	0.14	49.34	-28	52
Superior Frontal Gyrus	BA 10	Left	4.64	-25.91	52	22
Superior Frontal Gyrus	BA 9	Left	5.54	-31.23	44	26
Superior Temporal Gyrus	BA 13	Right	-1.14	58.22	-40	22
ANO-3						
Cerebellum - Anterior Lobe		Left	7.29	-20.25	-56	-30
Cerebellum - Posterior Lobe		Left	5.98	-9.57	-64	-22
Cerebellum - Posterior Lobe		Right	4.6	1.11	-58	-12

2.3.5 Brain Areas Activated In Common During Action Observation And Task Execution

The Figure 2.3.6 shows brain areas activated in common during observation of the video clip (event 1) and task execution (event 3). As can be seen, the PMC, IFG, IPL and IPS at the right hemisphere exhibited overlapping patterns of activation during action observation and task execution. In contrast, in the left hemisphere similar patterns of activation were restricted to the parietal areas. In addition, the PMC exhibited a small cluster in the left hemisphere and hemodynamic changes were absent in all tasks.

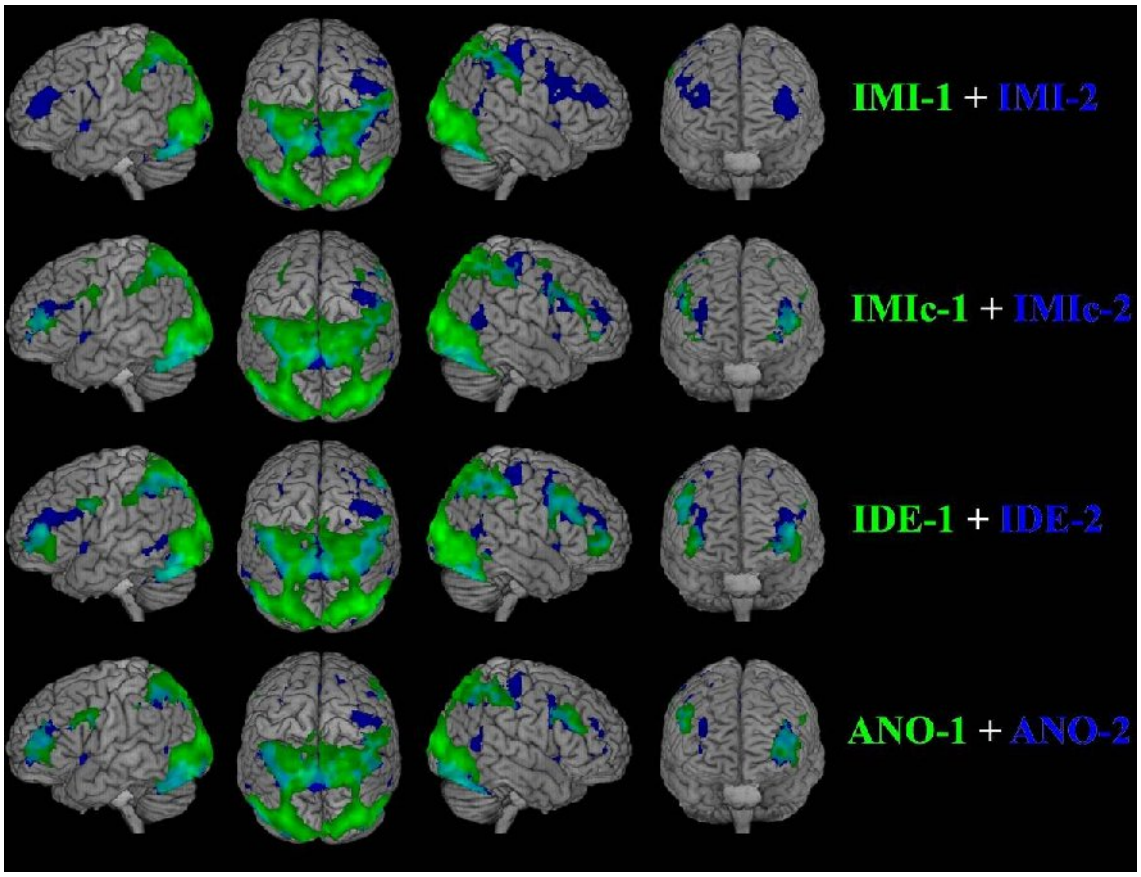

Figure 2.3.5. Brain areas activated during observation of video clips showing chords (event 1, green), maintenance of this information, and preparation for task execution (event 2, dark blue). Note that even though each task involved presentation of a video clip showing a chord, the subjects were required to prepare for later execution of either the same (IMI and IMIc tasks) or another (ANO task) chord; in IDE task the subjects were asked to get prepared to identify the chord by exhibiting a distinct hand display. Brain areas activated in common were represented in light blue.

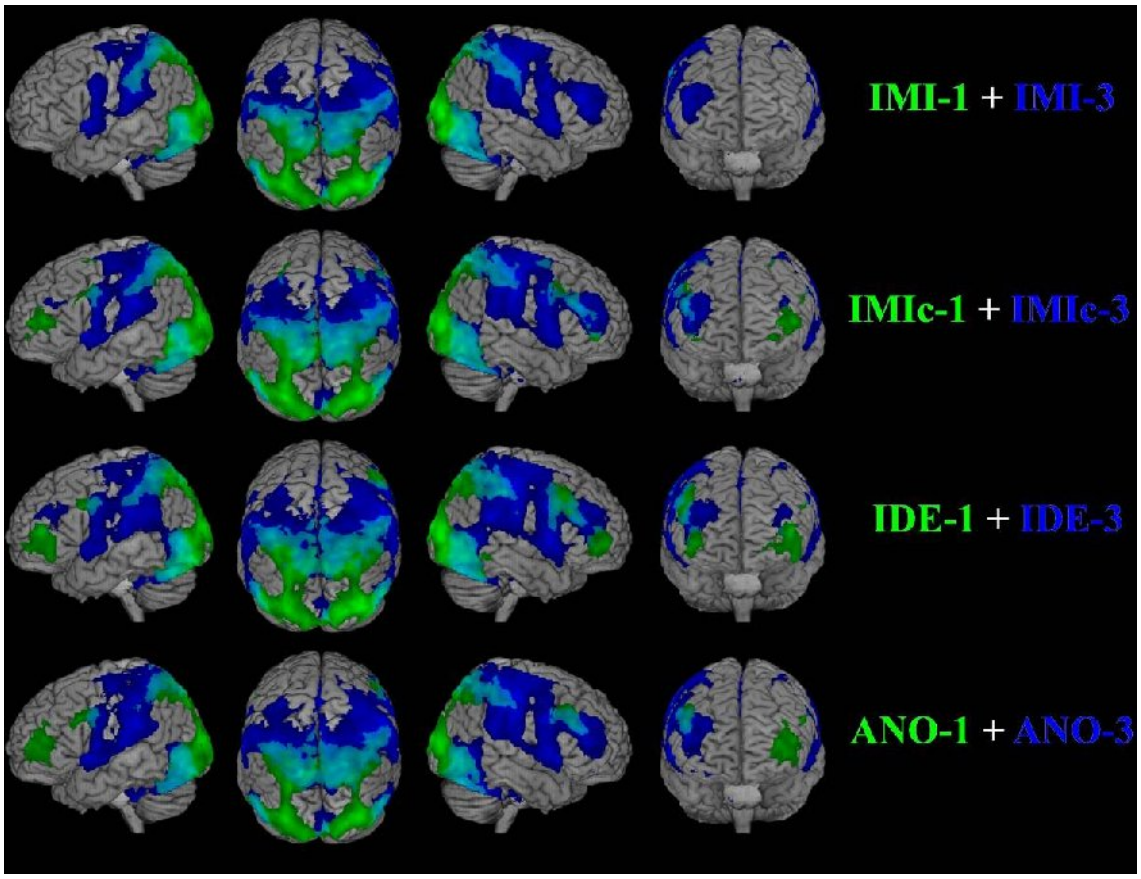

Figure 2.3.6. Brain areas activated during observation of video clips showing chords (event 1, green) and during task execution (event 3, dark blue). Note that even though each task involved presentation of a video clip showing a chord, the subjects were required to execute the same chord in the IMI and IMIc tasks, to execute a different chord in the task ANO and to identify the shown chord by exhibiting a distinct hand display (IDE). Brain areas activated in common were represented in light blue.

2.3.6 Other Brain Areas Exhibiting Hemodynamic Changes During Imitation

In addition to the expected activation of the PMC, IFG, IPL and IPS, we found other brain areas active.

2.3.6.1 Observation of video clips showing guitar chords (Event 1)

During the observation of the video clips, several areas of the occipital lobe were strongly activated independently on the task to be performed, including the

cuneos, lingual gyrus, fusiform gyrus and the border of the calcarine sulcus. Areas located in the superior parietal lobule were also activated. Hemodynamic changes were also observed in mesial cortices, hippocampus, superior colliculus, lateral geniculate bodies, and pulvinar nuclei. In the prefrontal cortex the border of superior frontal sulcus and the *pars triangularis* of the IFG (BA45) were also activated. All regions are showed on Figure 2.3.1 and Table 2.1, for mesial regions see Supplementary material.

2.3.6.2 Information maintenance and preparation for task execution (Event 2)

During information maintenance and preparation for task execution there were hemodynamic changes in mesial cortices, insula and hippocampus, bilaterally. The thalamus, anterior cingulate gyrus, supplementary motor area, lateral geniculate bodies, and superior colliculus were also activated. Areas located in the occipital lobe were activated during maintenance of the information and preparation for task execution, including the pre-cuneus, fusiform gyrus (bilaterally) and lingual gyrus; this activity, however, was less prominent than that seen during observation. The superior parietal lobule, and the middle and superior frontal gyrus were also activated bilaterally, and the right pre- and post-central gyrus were activated in the left hemisphere. All regions are showed on Figure 2.3.3 and Table 2.3, for mesial regions see Supplementary Material.

2.3.6.3 Task execution (Event 3)

During task execution of either the same (tasks IMI and IMIc) or different (task ANO) chords, and during identification of the chord by way of a distinct hand movement (IDE), there were also activations in the right primary motor cortex, primary and secondary sensory cortex bilaterally (superior, inferior and intra-parietal cortex),

with a slight predominance to the right hemisphere, the supplementary motor area, anterior cingulate gyrus, basal nuclei, putamen, thalamus, insula, temporo-occipital transition and cerebellum. Left prefrontal cortex activations were virtually absent. The hemodynamic changes in the right hemisphere did not differ substantially in the four tasks. All regions are showed on Figure 2.3.4 and Table 2.4, for mesial regions see Supplementary Material.

2.4. DISCUSSION

This study examined brain hemodynamic changes while volunteers (1) watched to a video clip showing either a hand performing a chord (tasks IMI and IDE) or independent moving dots representing the execution of a chord (tasks IMIc and ANO), both on a guitar neck (event 1), (2) maintained the information about the observed action and prepared themselves for task execution (event 2), or (3) executed a task involving either to perform, as accurately as possible, the same (IMI and IMIc tasks) or another (ANO task) chord, or identified the exhibited chord by way of a distinct hand movement (IDE task) (event 3). This within-subjects experimental design aimed at evaluating to which extent these three stages of imitative behavior (events 1, 2 and 3) engage brain regions usually included in the MNS. The two main tasks (IMI and IMIc) were designed relying on Iacoboni's et al. (1999) study showing increased activation of MNS during imitation of biological movements (finger lift) as compared to symbolic and spatial cues. The ANO and IDE tasks included in this study as control tasks allowed evaluation of brain hemodynamic changes associated with both recognition and memory maintenance, either for chord identification (IDE) or for self-generation of a distinct action (ANO). Before presentation of the video clip showing the chord to be used for that specific trial, the subjects were exposed to a cue indicating

which type of task, i.e., either IMI, IMIc, IDE or ANO, should be performed later. Therefore, when observing the video clip during event 1 the subjects already knew the task to be performed during event 3. The tasks, event sequences and chords were trained previously to the scanning session.

Previous studies have proposed that a set of brain areas, including the PMC, IFG, IPL and IPS, collectively named MNS, becomes active when monkeys either observe a conspecific performing an action or perform themselves the same action, i.e., imitates it (Iacoboni *et al.*, 1999; Koski *et al*., 2003; Buccino *et al*., 2004; Chaminade *et al*., 2005; Cunnington *et al*., 2006; Makuuchi *et al*., 2005b, Vogt *et al*., 2007). However, other studies have failed to show activation in frontal and/or in parietal areas (see Morin and Grèzes, 2008; Molenberghs *et al*., 2009; Turella *et al*., 2009; Caspers *et al*., 2010, for reviews). In addition, a recent meta-analysis pointed out that almost half of studies (nine out of twenty) reporting positive activation of structures included in the MNS during action imitation may be questioned after correction for multiple comparisons in the whole brain analysis (see Molenberghs *et al*., 2009). In addition, as pointed out by Turella et al. (2009) control tasks for imitative tests not always are appropriate, i.e., frequently the studies does not distinguish between activity time-locked to observation, execution, and imitation and the overlapping os activation among these events (Iacoboni et al., 1999, Tanaka et al. 2001, Molnar-Szakacs et al., 2005, for a complete list, see Turella et al., 2009). Therefore, the crucial involvement of the structures usually included in the MNS specifically for imitation is still matter of debate.

2.4.1 Brain Activity Related To Observing An Action (Event 1)

Observation of video clips showing either hands or independent moving dots displaying a chord (event 1) promoted activation in the parietal lobule (IPL and IPS)

independently on the type of task to be performed later (IMI, IDE, IMIc and ANO) (Figure 1). This observation confirms data of several prior studies reporting the participation of the IPL and IPS in imitative behavior (Mühlau et al., 2005, Gazzolla et al, 2007, Jonas et al., 2007, for a review, see Molenberghs et al., 2009). In contrast, frontal lobe (PMC and IFG) activation during the video clip observation did dependent upon the task that should be performed later. That is, while single observation for later performance of the IDE, IMIc and ANO tasks (event 1) did increase frontal lobe activity (Figure 1), observation for later performance of the IMI task (event 1) did not (Figure 1). Note that event 1 for IMI and IDE tasks involved observation of the same video clip exhibiting a hand performing a chord; therefore, the unique distinction between these tasks at this stage (event 1) was the knowledge by the subjects about which task, IMI or IDE, should be performed later (during event 3). On the other hand, event 1 for IMI and IMIc tasks involved, respectively, observation of a video clip showing a hand performing a chord (IMI) and observation of a videlo clip showing independent moving dots exhibiting a chord (IMIc); at this stage (event 1) the subjects knew that the task to be performed later (during event 3) was the same, i.e., to imitate the observed chord. Hemodynamic changes revealed no frontal activation when the subjects observed the video clip showing hands performing a chord knowing that it should be imitated later (IMI) (Figure 2.3.1); in contrast, there was frontal activation both when the subjects observed the same video clip knowing that the exhibited chord should be identified later (IDE) and when they observed the moving dots video clip knowing that it should be either imitated (IMIc) or avoided (ANO) later (Figure 2.3.1). This result is surprising face to data of prior studies showing that observing an actual hand movement for later imitation activates the entire MNS, including the frontal areas IFG and PMC (see Molenberghs et al., 2009 and Caspers et al., 2010 for reviews).

In the present study, the subjects were exposed practice sessions both to learn how to execute and name the chords and to get familiar with the experimental design involving different types of trials. Vogt et al. (2007) showed that activation of the MNS is stronger for non-practised actions as compared to practiced actions. These results were interpreted as evidence that participation of the MNS in early stages of imitation learning is stronger. In this context, the lack of frontal activation during observation of the video clip knowing that an IMI task should be performed later could be interpreted as consequence of the prior practice given to the subjects. Note, however, that the same amount of practice was also provided for the IMIc, IDE and ANO tasks, and that there was frontal activation when these subjects observed either the same (IDE) or another (IMIc and ANO) video clip knowing that they should perform these tasks later. It is possible that practice acquisition associated with hands executing a guitar chord is quicker due to a direct matching of an internal representation acquired along our daily live experience of imitating hand movements; this would provide quicker acquisition of practice which could reflect on smaller activation of the frontal components of the MNS, as shown by Vogt et al. (2007). In addition, even though both IMI and IMIc tasks require imitation of the observed action, this latter task requires, in addition, a translation of symbolic cues (independent moving dots) into finger movements, which could render its acquisition slower. An alternative interpretation is that the IMIc task engage frontal activity exactly for translating the movement of independent dots into finger movements. Along with this rationale, IDE-1 would require maintenance of information about the number of the chord and ANO-1 would require both inhibition of performance of the observed chord and the choice for another chord for later performance; these additional task requirements could explain the increase in frontal activation when observing for later performance of these tasks.

Hence, it is possible that the activity of the PMC and IFG is directly linked to the activation of other frontal areas (dorso lateral prefrontal, medialfrontal gyrus, anterior cingulate, orbitofrontal cortex) and, if IMI-1 requires no other engagement than the motor, the lack of frontal activity could be explain by the lack of the necessity of modulatory input from prefrontal areas in this task.

Jonas et al. (2007) investigated the occurrence of hemodynamic changes in the posterior inferior frontral gyrus (pIFG) and the anterior inferior parietal lobule (aIPL) during observation and imitation of a simple finger movement task. Their study involved observation of four types of stimuli, including a dot color change, a moving finger, a moving dot, and a simultaneous finger-dot movement (dot movements matched the finger movements spatially and kinematically). Faster performances were seen when the volunteers imitated a finger movement as compared to either dot movements or dot color changes. Hemodynamic changes (fMRI) revealed no significant increase of the pIFG activity during both observation of finger movements for later imitation and observation of a dot color change. In contrast, there was a significant increase of activity in the pIFG during observation of the finger-dot movement and an almost significant increase of activity during observation of the moving dot alone. In parallel, there was aIPL activation during observation of the finger-dot and of the dot alone movement, a trend activation during observation of the finger movement and a lack of activation to a dot color change. In addition, there was activation of the left anterior intraparietal sulcus during observation of all types of moving stimuli. Our study shows, in addition to similarities in experimental design, some similar results as Jonas' study. We also used dots as a symbolic cue of the action to be executed (imitated), simulating the movement. An important difference is that the baseline used in the Jonas's study was the static image of a hand while we use a black screen without any visual stimulus. However, the

lack of activation of IFG during the observation of fingers doing the action is consistent with our finding, as well as the activation of this area during observation of moving dots. The authors argue this difference could be due the context of a reaction task in which participants have to produce a response as fast as possible may have invoked stimulus processing based on common spatial coding rather than action observation – execution matching. In the present study the subjects received the same instructions before observing either the video clip showing moving fingers (IMI-1) or the video clip showing independent moving dots (IMIc-1). The task to be performed after this observation phase was the same, that is, to plan and execute the presented chord. Since we do not used a reaction task, we argue that hemodynamic differences related to these tasks are due to the nature of the stimuli and differential coding required. It is possible that the moving fingers observed during IMI-1 were interpreted as an "integrated moving object", whereas the moving dots observed during IMIc-1 were interpreted as "independent moving objects" dispersed in the space such that performance required integrating the movement of the three independent dots as if they were a "single object"; this would involve not only maintenance of information about their movement but also its manipulation in working memory. As a mater of fact, Smith and Jonides (1999) demonstrated that ventral portions of the frontal lobe (BA 45 and 47) are involved in the information maintenance in the working memory. They showed, in addition, that the information about objects involves BA46 and BA9. Specifically, the dorsal portion of the BA9 is related to special working memory and the dorsolateral prefrontal cortex (BA46) get engaged in operations of selection and combination of individual elements. Relying on these evidence, we propose that frontal activations during IMIc-1 are related to the necessary rearrangements to translate independent moving dots into finger movements. In favor of this interpretation, data of the present study revealed bilaterally

dorsal PMC activation during the video observation in the IMIc-1 task, but not during observation of the same video in the ANO-1 task in order to choose a distinct chord for later execution.

The ventral PMC was activated bilaterally during observation of the video clip for later performance of the IMIc-1, IDE-1 and ANO-1 tasks. Previous studies have shown that this brain region becomes active during hand action observation, finger movements and/or objects grasping (Binkofski et al., 1999; Iacoboni et al., 1999; Gerardin et al., 2000), and hand motor imagery (Grafton et al., 1996; Gerardin et al., 2000), indicating its engagement in hand motor representation (Rizzolatti et al., 2001, for a review, Molenberghs *et al*., 2009; Caspers *et al*., 2010). The IFG (BA44 portion) is thought to transform visual information into knowledge coded at an abstract level and might be involved in understanding the action meaning as have been proposed by some authors (Nishitani et al., 2005; Rizzolatti & Craighero, 2004). The PMC together with the IFG, it is considered the human homologue of the F5 area of the macaque brain (Rizzolatti and Arbib, 1998; Makuuchi, 2005b; Rizzolatti and Sinigaglia, 2010). Concurrent activation of these regions during observation in IMIc-1, IDE-1 and ANO-1 tasks, even though they involved different instructions (either imitate, identify and choose another chord for later performance, respectively), whilst required formulation of a motor representation in order to perform the required tasks, favor this interpretation. In contrast, these regions were not activated during observation in IMI-1 task that also require formulation of a motor representation. The reasons for this apparently conflicting results may be related to the amount of previous training, as discussed above.

The posterior part of the parietal lobe has been traditionally considered as a typical association cortex. The activation of the IPL and IPS during all conditions may

not merely be related to the observation of an action per se but also due the involvement in the (implicit) processing of object features and their integration within the observed motor act (Caspers et al., 2010). In the parietal cortex, there is a sensory association of afferents from two or more sensory modalities which is the basis for some types of percepts, such as space (Fogassi et al., 2005). In addition, it is known that the PMC and IPL are anatomically connected (Frey et al., 2008; Zubicaray et al., 2010), suggesting a possible functional circuit, involving both IPL and PMC for action organization and intention understanding (Bonini et al., 2010) based on the object affordances.

Although many studies have demonstrated the involvement of the IPL and IPS during the observation and execution of hands actions and reaching-to-grasp protocols, suggesting the idea of its mirror properties and involvement in other's action linking (Rizzolatti, 2005; Molnar-Szakacs et al., 2006), it is possible that the parietal cortex activation could reflect the monitoring of one's future course of own action besides predicting of outcome of others' actions. This view comes from the observation that several parietal structures are concerned with various types of predictive mechanisms (Colby *et al.* 1996; Desmurget and Grafton, 2000) which is present in our experiment, once the participants had to first observe an action and later execute an action equal or very similar to that observed. In addition, this proposal is based on the idea of forward model, which argues that the internal simulation of observed actions allows the observer to predict the kinematics and outcome of his or her own observed action and this may be also used for predicting others' actions. (Miall and Wolpert, 1996; Shmuelof and Zohary, 2007). Thus, we argue that the involvement of parietal cortex during observation of actions performed by others is due to direct matching of the action observed and the internal simulation/preparation to the action execution (imitation of the action).

2.4.1.1 Contrasts Between Conditions During Observation

The direct comparison between conditions shows any difference that there was among conditions, even if the absolute value of each condition was below the statistical threshold. Since the only difference between IMI-1 and IMIc-1 was the type of video that the participants observed, we assign the results take into account the specific characteristics of each video. In this sense, considering the finding of hands specificity coding of the IPL (Rizzolatti, 2001), the higher BOLD signal change in this region during IMI-1 in relation to IMIc-1 reflects the processing of moving hands and fingers in the first. The involvement of the higher dorsal PMC BOLD signal change in IMIc-1, as discuss later, is due the engaged in operations of selection and combination of individual elements required by the moing dots video and engaged in formulate the appropriate motor program.

Considering the identification component that is implicit in the observation of the chords addressed by the IMI-1 and IDE-1, the higher BOLD signal change in IFG and IPS during IDE-1 in contrast with IMI-1, which used the same kind of stimulus, could be due 1) the inner speech that may occur even in absence of the participant's awareness (Grèzes and Decety, 2001; Heyes, 2001), once they probably evoked the nomination of the chord in order to identify the chord as 1; 2; 3 or 4, which was not necessary during IMI-1 and 2) the fact that during IDE-1, through the parietal areas, the participant can build a bottom-up simulation of the chord more intensely than during IMI-1, once during the task of identification the participant extracts the necessary information (which is the chord?) implicitly in opposition to the IMI-1 in which information is extracted explicitly from the video.

In addition, to differentiate the engagement that exists for the observation of an action that latter will be entirely copied from that the action is self-generated, without collecting information from stimulus about the details of action to be performed, we

compared IMIc-1 with ANO-1 (same stimulus). The results suggest that during IMIc-1 there is a higher sensorimotor integration in the IPL due to the complexity of the information that is taken from the stimulus observed. During ANO-1 is probably that the participant engages the attention on the presented stimulus strongly in the first few seconds in order to identify which is the chord, and thus choose what will be the chord that it will perform. This is reflected in higher activation of the mesial PMC (supplementary motor area) during ANO-1 in relation to IMIc-1, since the participant has already begun rehearsing the chosen chord.

2.4.2 Preparation to Action Execution

The preparation to action execution had a very similar pattern of activation in the MNS compared to action observation. The actions that would be executed after this preparation event were very similar between conditions. Even for the IDE condition, where the participants should prepare to perform a different action of a chord, a kinematic similarity was maintained among tasks regard the action that should be executed. In this event, participants should identify the chord observed using their fingers, making rhythmic movements in the same way as they executed the chords in other conditions.

A large number of functional neuroimaging studies have demonstrated that motor imagery/preparation is associated with the specific activation of the neural circuits involved in the early stage of motor controla and shares the same structures of the action execution (Decety, 1996, Jeannerod, 2001, Cisek and Kalaska, 2004, Hanakawa et al., 2008, Munzert et a., 2009). This similar pattern of activation was hypothesized considering the idea that the MNS is a system that contains the representation of the actions and any stimulus that elicit this representation will active

this system, based on the ideia that “neurons that fire together wire together”. Thus, our results show that the. mental representations during observation of actions performed by others, and even more during simulation of one's own actions, share common neural mechanisms with other covert aspects of motor performance, such as planning and programming.

2.4.3 Others Neural Structures Involved in Action Observation and Action Preparation

The activation of the visual system (cuneos, lingual gyrus, fusiform gyrus, border of the calcarine sulcus) was strongly present during the observation of the video and very weak during the preparation to action execution, mainly because of the kind of stimulus, which was a static blue screen during preparation in the later. The activation of the superior parietal lobule and the involvement during action observation was reported in studies in which participants observed actions with the aim to imitate them later or imitated it online (Iacoboni et al., 1999; Tanaka et al., 2001; Tanaka and Inui, 2002) but not during the mere action observation (Buccino et al., 2004). In fact, when Rizzolatti and Matelli (2003) proposed that the dorsal pathway is actually composed by two distinct systems, they considered that the dorso-dorsal pathway connects the visual cortex to the superior parietal lobe and it is quite exclusively involved in the organization of the motor activities. In the present experiment, since all conditions were followed by action execution and, considering also the fundamental role of the superior parietal lobule in proprioception demonstrated in nonhuman primates (Lacquaniti et al., 1995), we interpret its activation as due to a proprioceptive representation of the action that the individual will later execute. Also, our study is in line with the lack of finding activation of the mesial areas during the mere observation of known hand action, even if the purpose of observation is the imitation (Rizzolatti et al., 1996; Grafton et al., 1996;

Iacoboni et al., 1999; Buccino et al., 2004; Vogt et al., 2007). Our previous findings (see Chapter 1) confirm the notion that mesial activation during imitation tasks is dependent of the knowledge of the action. Nevertheless, a strong activation of these areas was present during the preparation to action execution in all experimental condition. We argue that during the mere observation the impetus to initiate an action is lower than during the declared preparation. Indeed, the role of mesial areas as a control system of the MNS areas involved in action initiation activity was demonstrated by Hikosaka and co-wokers (2000). Thus, while the participants wait for permission to execute the chord, the activation of these areas in our task may reflect their role in the control of action execution, mainly when there is a necessity to inhibit the selected action until its execution is allowed.

2.4.4 Action Execution

In addition to the MNS, all areas involved with the execution of an action were activated as expected. As all actions executed by volunteers were very similar, with exception of IDE-3, in which the volunteers do not executed a chord but rather an indication of the number of the chord with the fingers (see material and methods). Our findings are consistent with several studies that use execution of actions with similar characteristics, i.e., hand movements, sequencing and positioning of the fingers. (Rao et al., 1997, Moritz et al., 2000, Vogt et al., 2007, Sisti et al., 2011). Once the action was executed with the left hand, the right lateralization of primary sensory and motor areas are consistent with findings from studies involving motor action execution (Gazzaniga, 2006).

2.4.5 Common Activation During Action Observation, Preparation and Action Execution

There is a greater overlapping of the neural structures that is activated not only during the action observation and the action execution, which is the classical definition of the mirror neurons (Rizzolatti et al., 1996), but also during the preparation to action execution (Decety, 1996, Jeannerod, 2001, Cisek and Kalaska, 2004, Hanakawa et al., 2008, Munzert et a., 2009). According to Fuster (2008), the neuronal substrate for the production of any action is identical to the substrate for its representation and, considering our results, we suggest that the MNS could be thought actually a system where all forms of motor representation are depicted.

2.5 CONCLUSIONS

In our study, we describe a set of fMRI data that suggest that observing an action may share similar cortical motor representations according to various types of cognitive demandand. We speculate that the automaticity which an action could achieve can significantly reduce the activation of the MNS during action observation, even when a later execution is required. Also, we suggest that the MNS is involved not only with translation of biological stimuli, as report by extensive literature, but also with a symbolic cue that represent the observed action.

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2.7 SUPPLEMENTARY MATERIAL

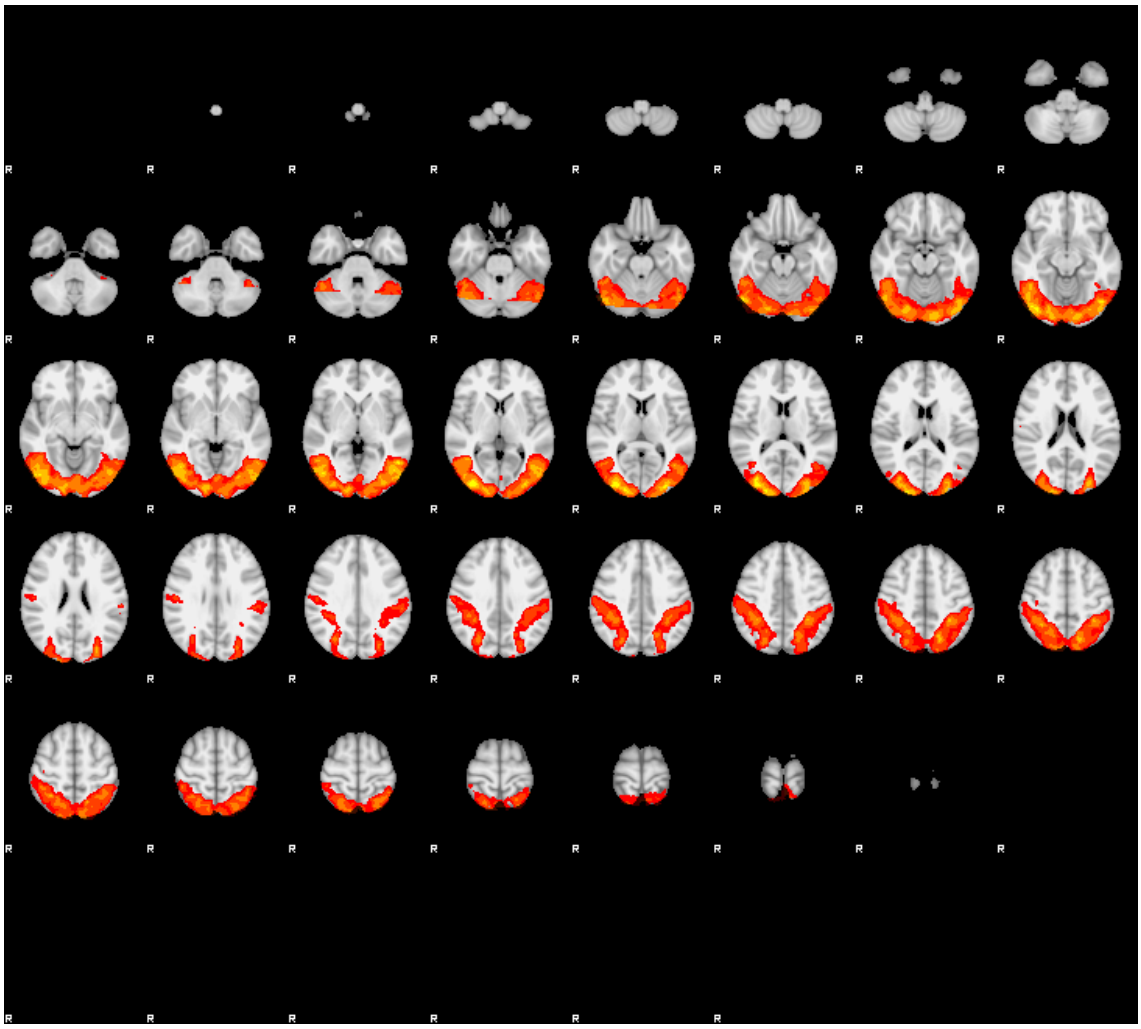

Figure 2.7.1 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the observation (event 1) of IMI task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

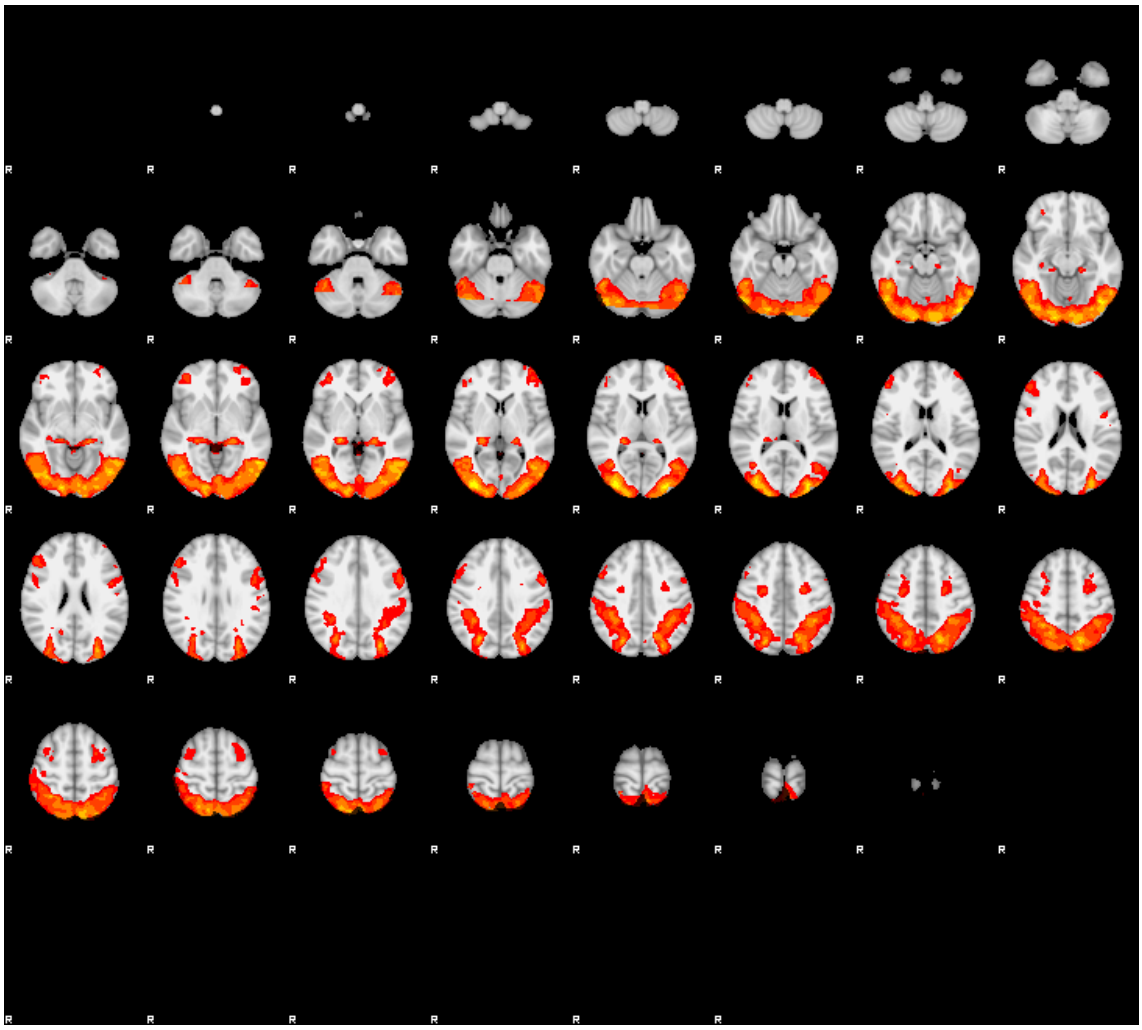

Figure 2.7.2 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the observation (event 1) of IMIc task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

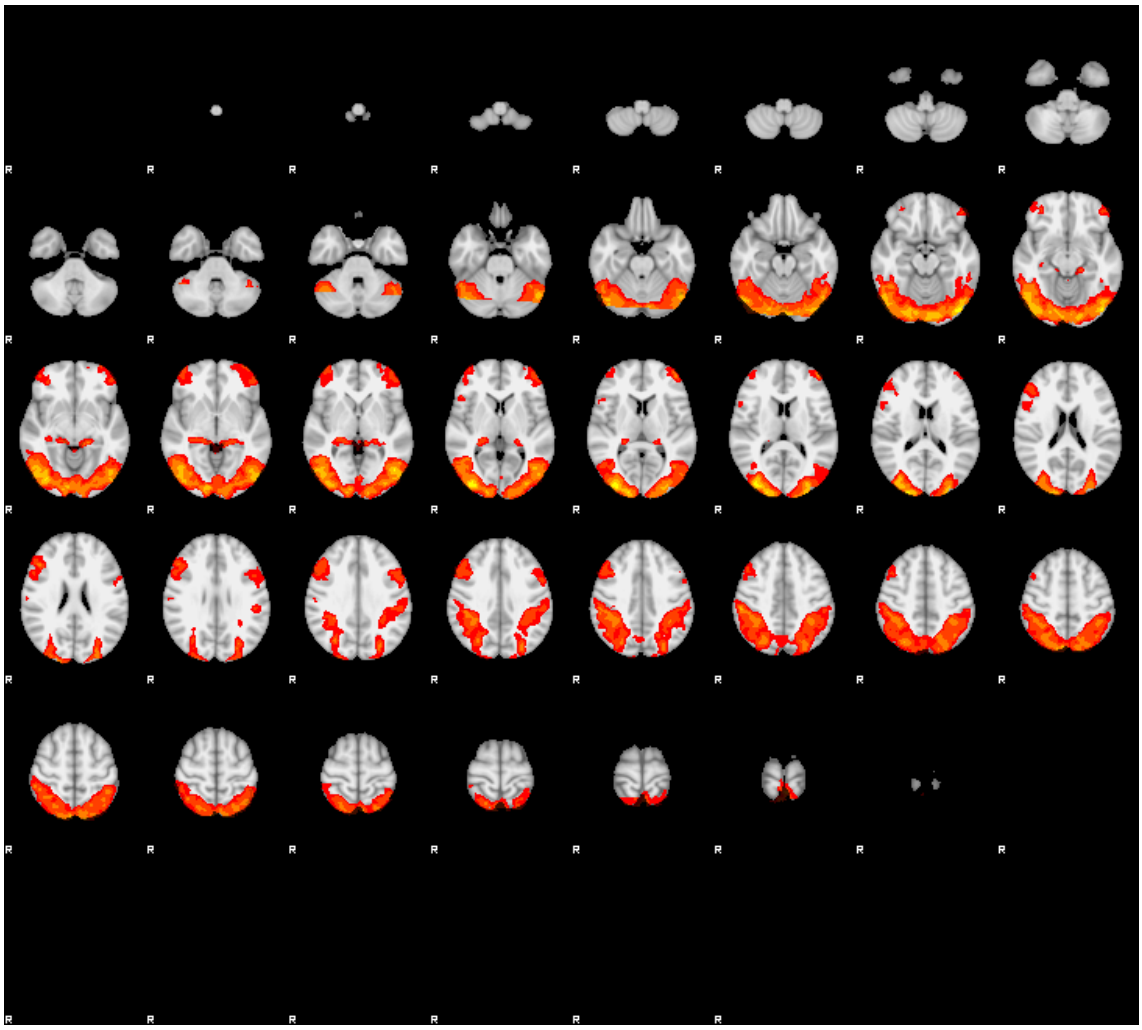

Figure 2.7.3 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the observation (event 1) of IDE task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

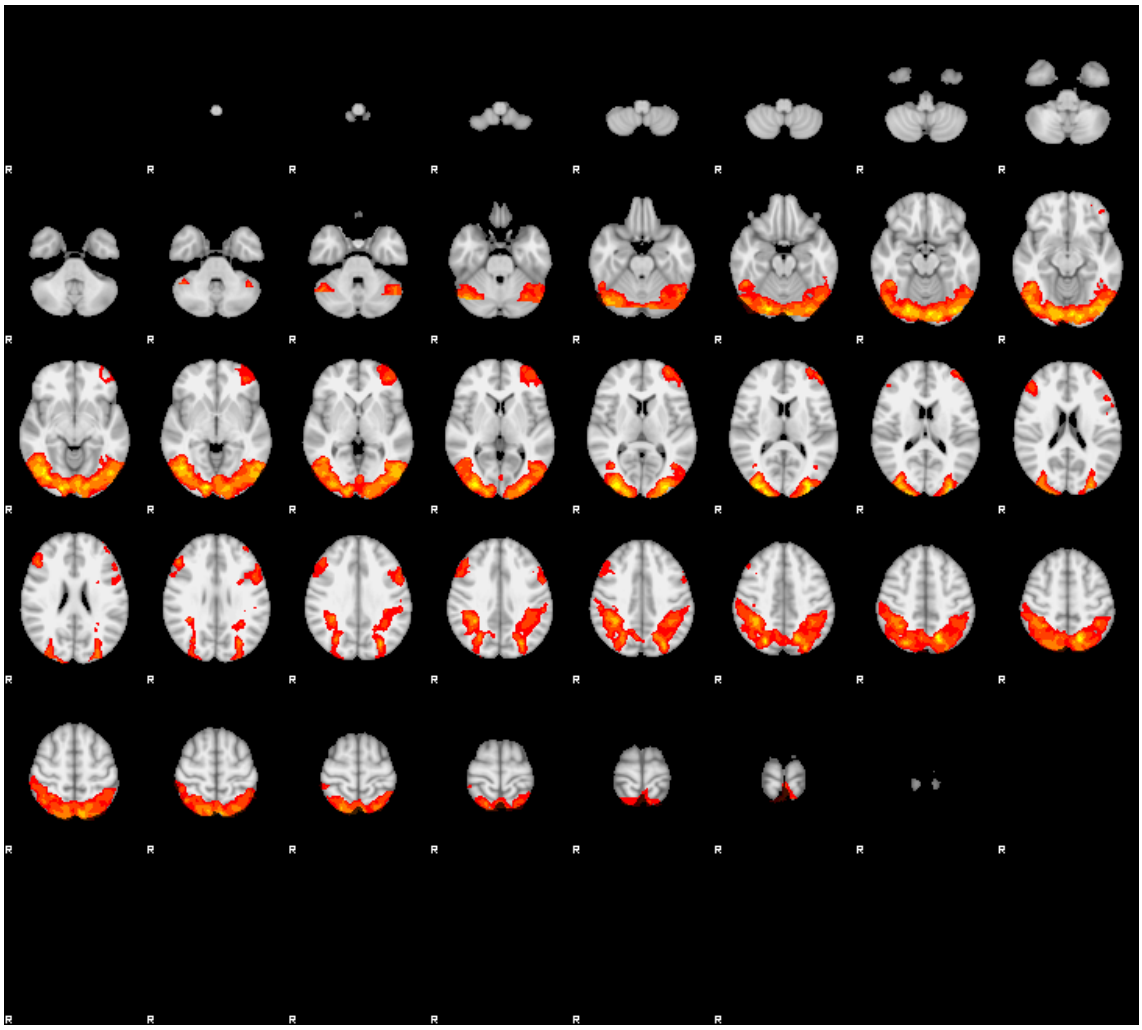

Figure 2.7.4 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the observation (event 1) of ANO task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

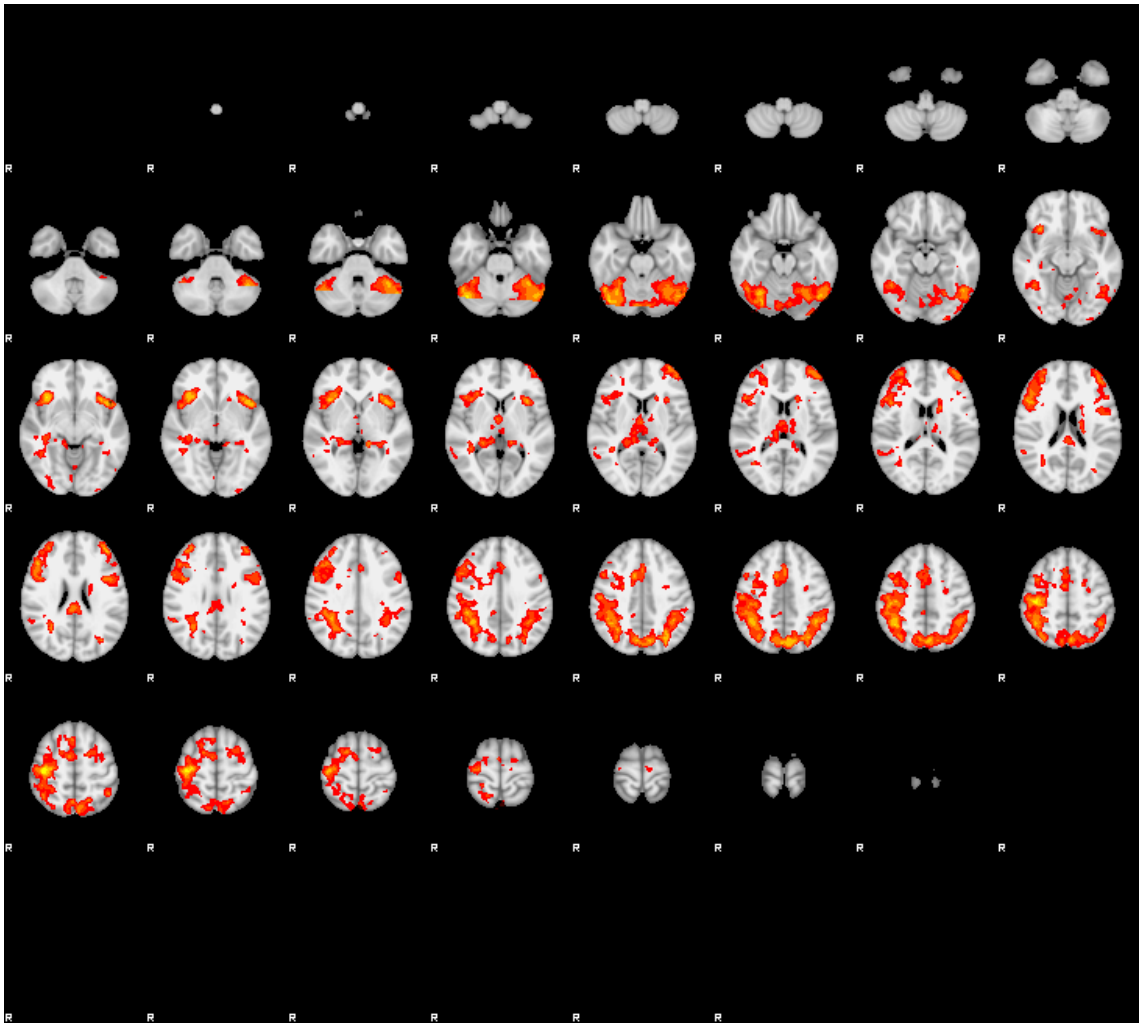

Figure 2.7.5 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the preparation to action execution (event 2) of IMI task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

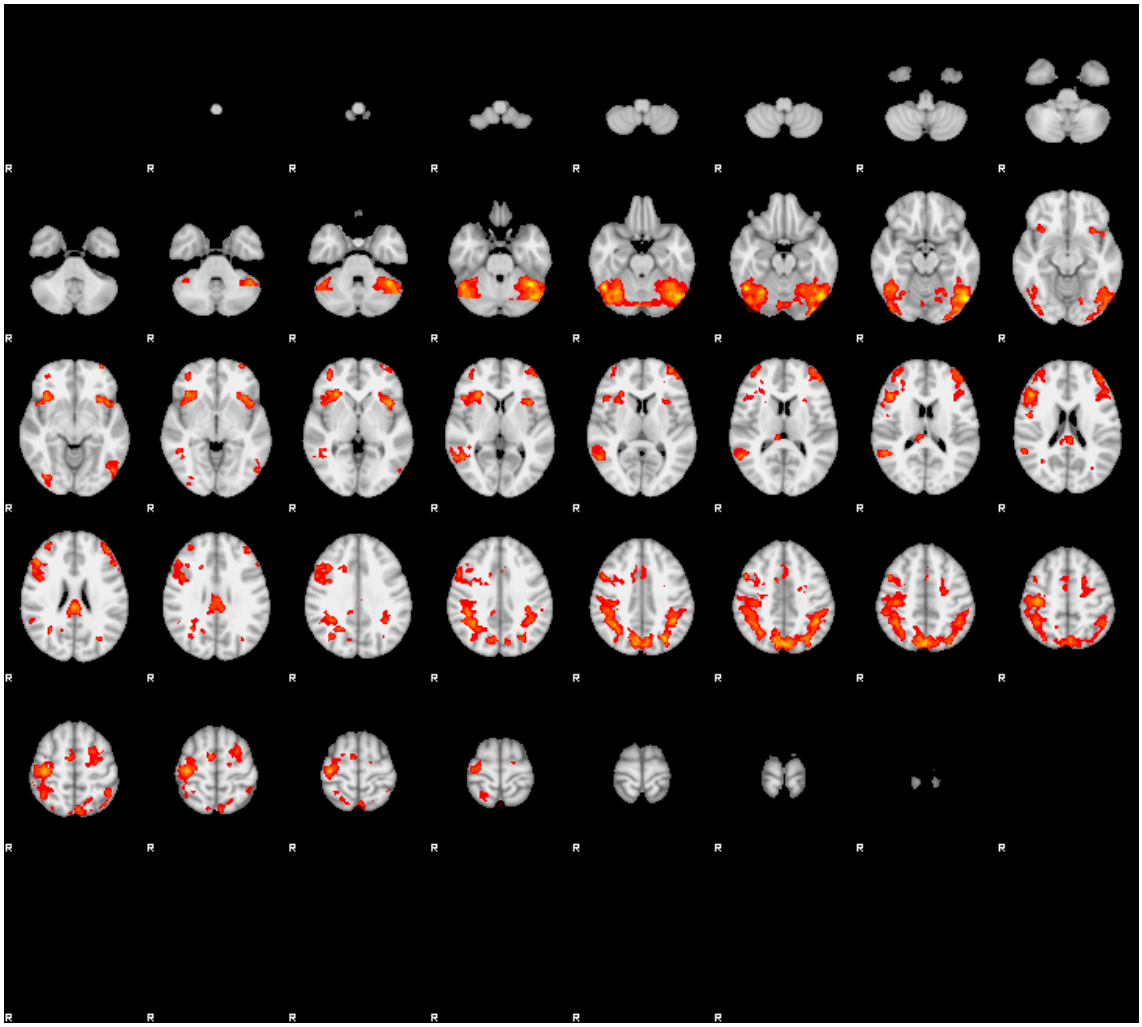

Figure 2.7.6 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the preparation to action execution (event 2) of IMIc task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

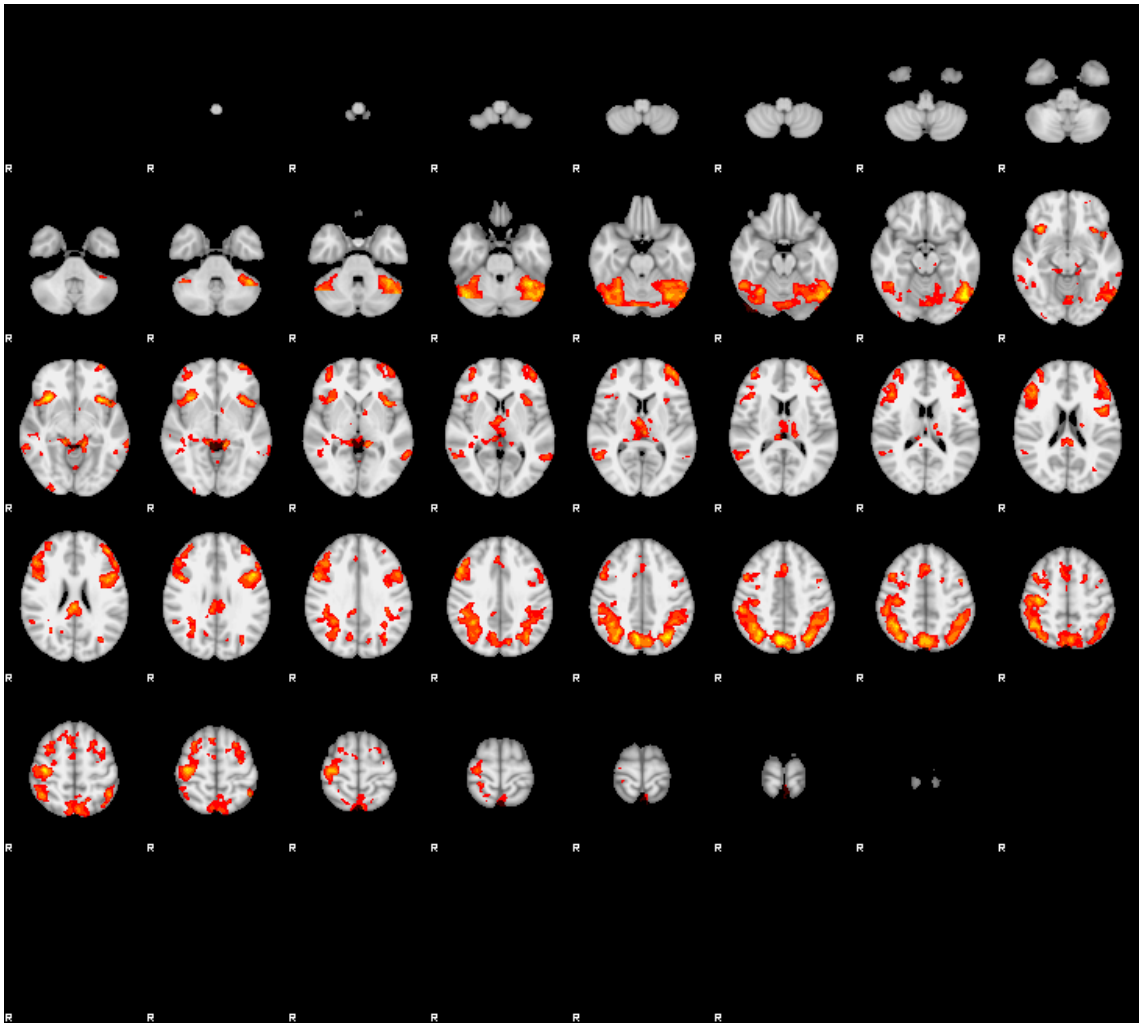

Figure 2.7.7 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the preparation to action execution (event 2) of IDE task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

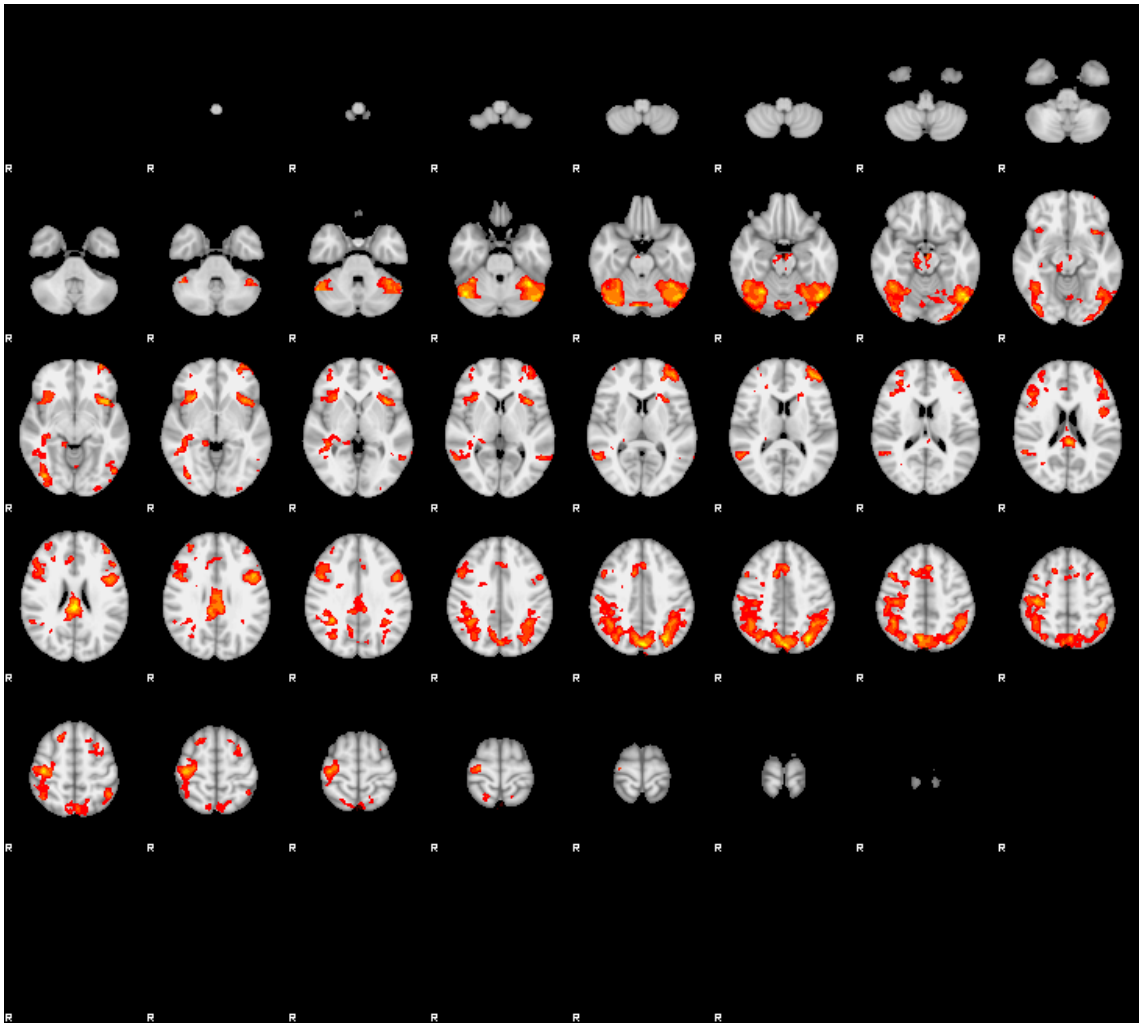

Figure 2.7.8 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the preparation to action execution (event 2) of ANO task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

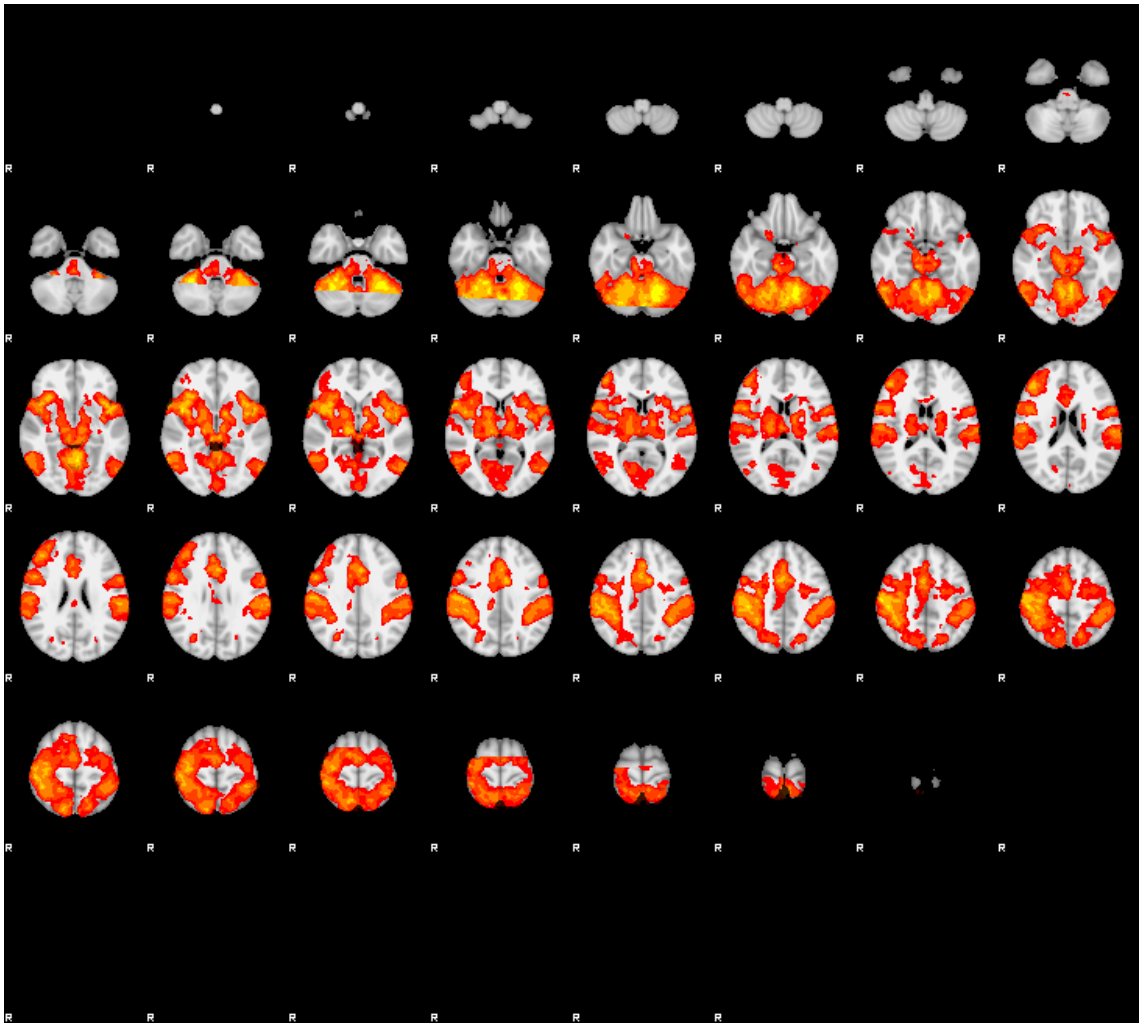

Figure 2.7.9 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the action execution (event 3) of IMI task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

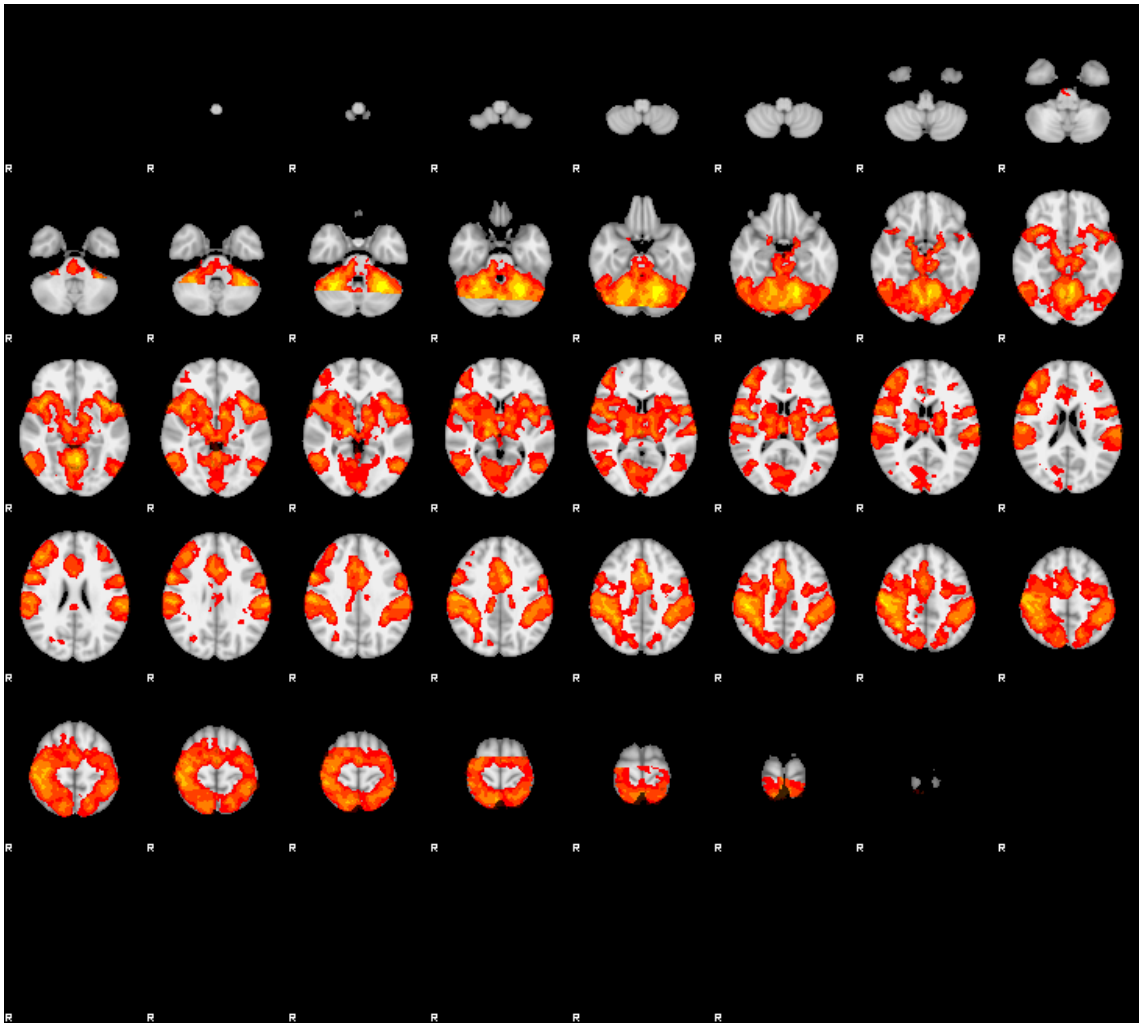

Figure 2.7.10 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the action execution (event 3) of IMIc task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

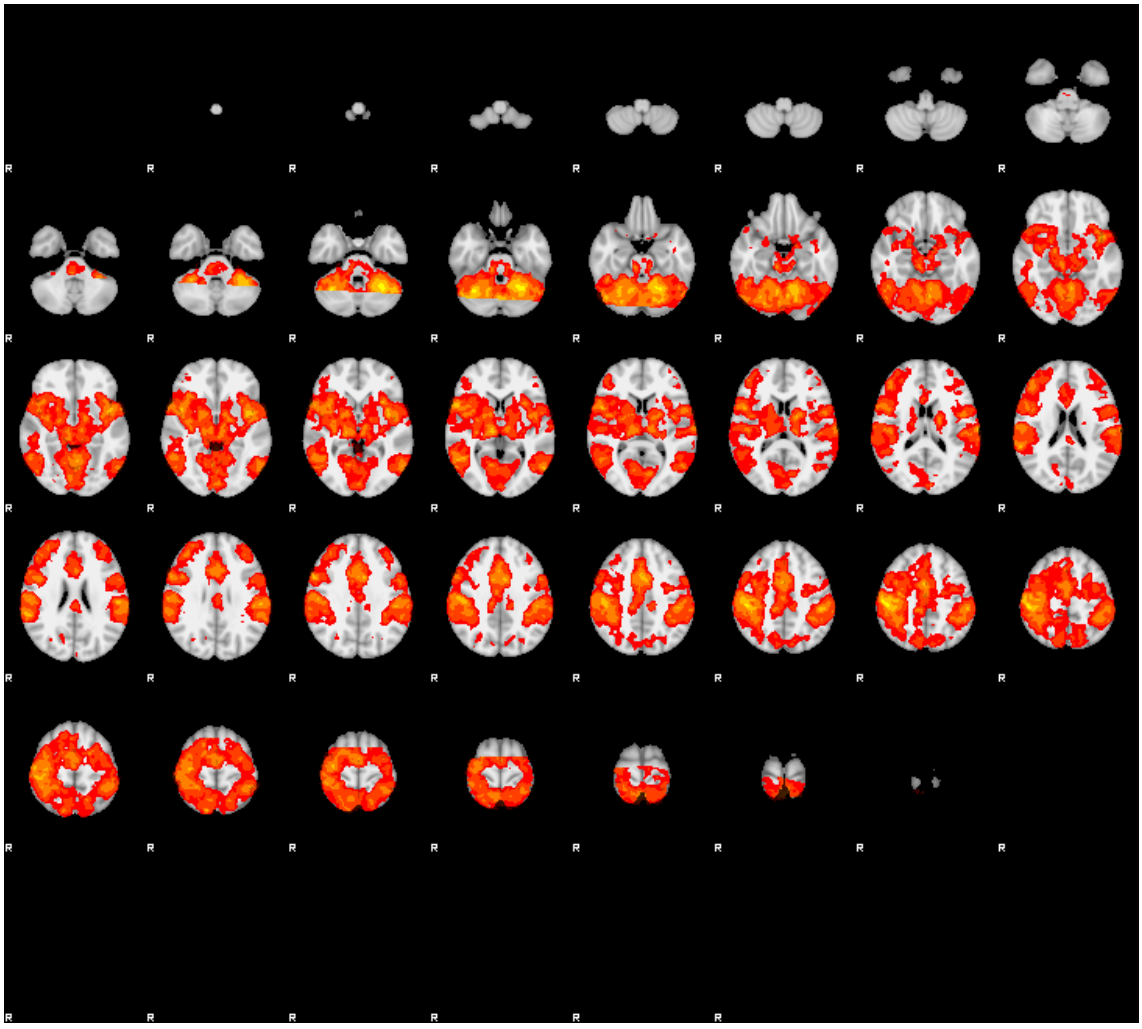

Figure 2.7.11 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the action execution (event 3) of IDE task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

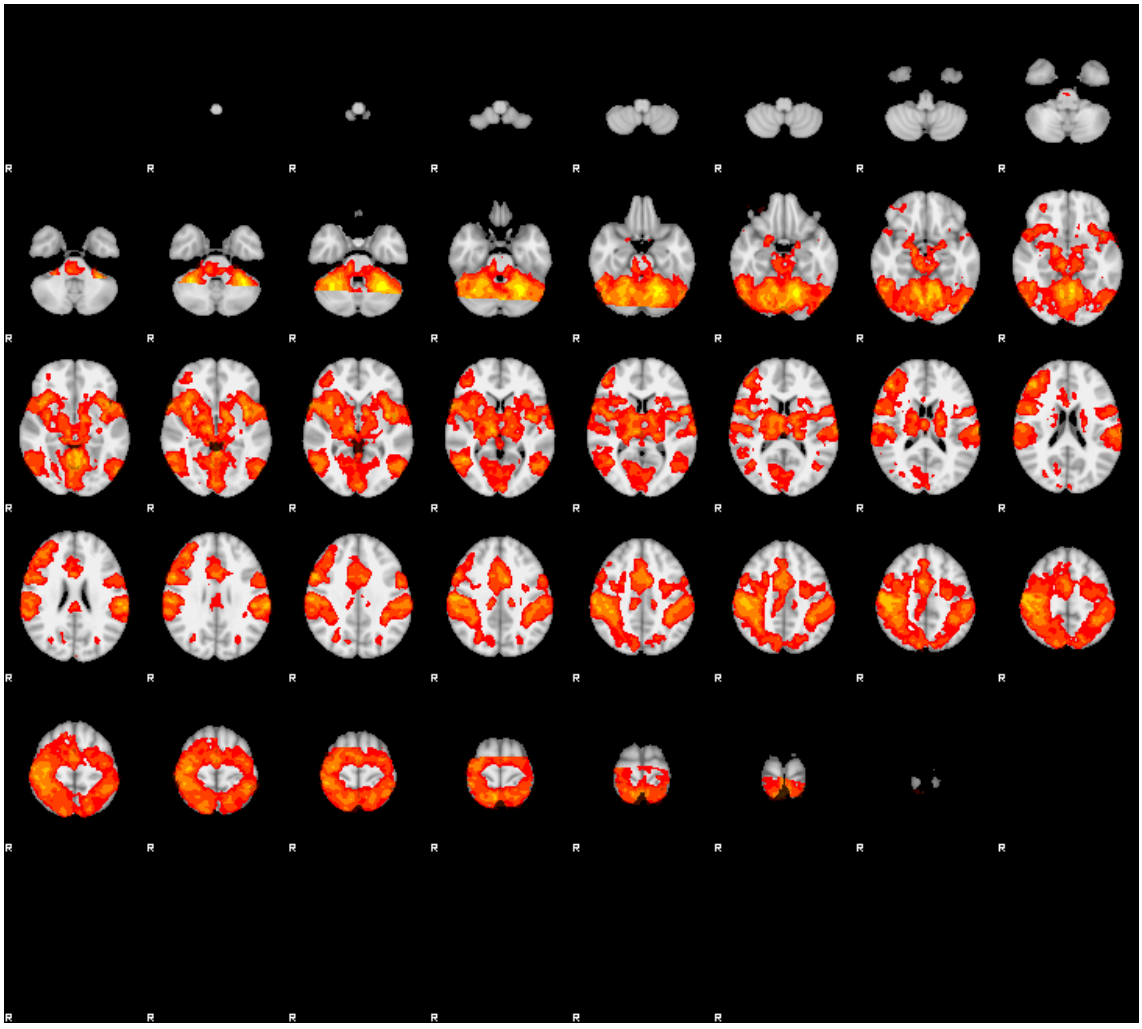

Figure 2.7.12 Statistical fMRI map activation patterns for the group in transversal projections through the whole brain during the action execution (event 3) of ANO task. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

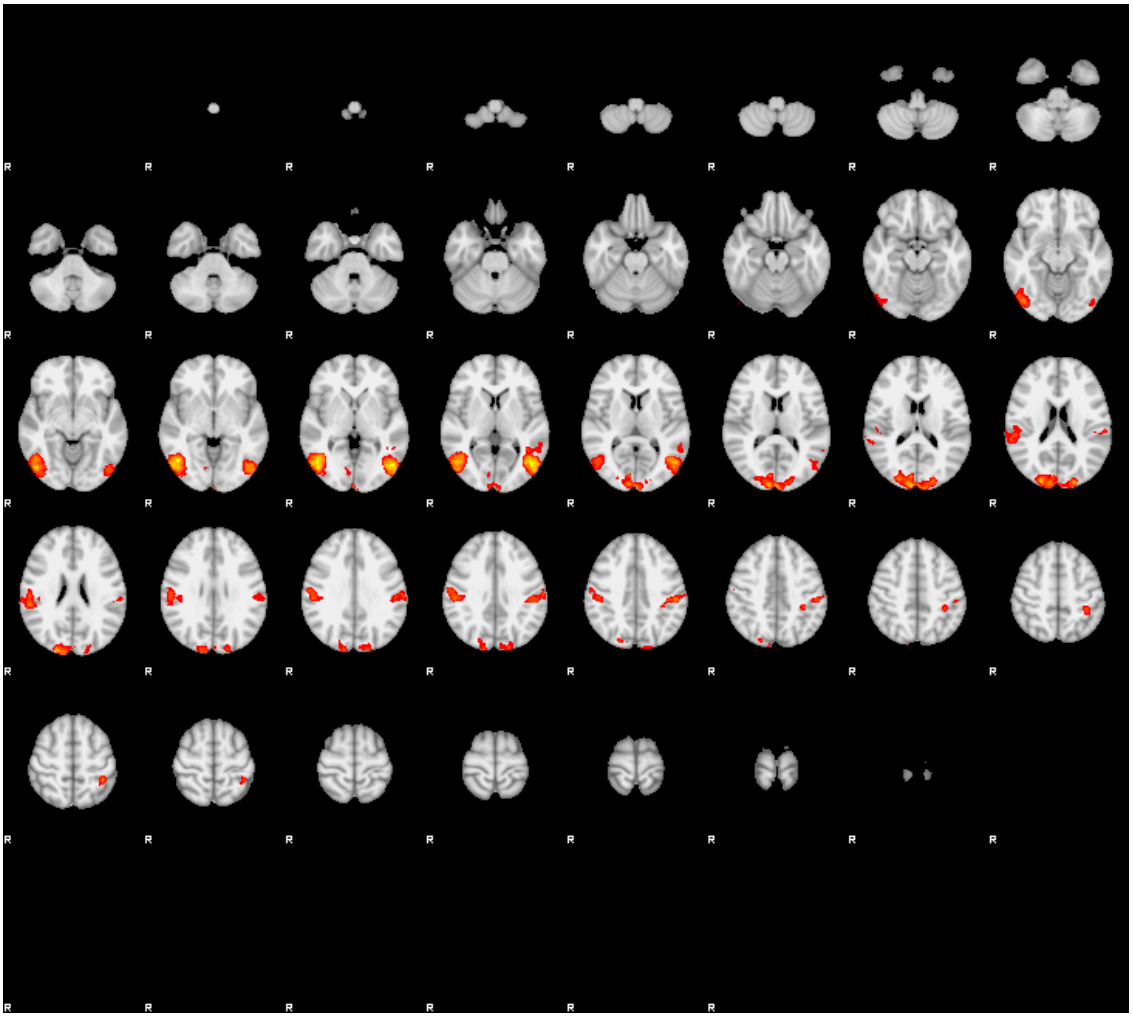

Figure 1.7.13 Comparison between IMI and IMIc during the observation of chords (event 1). The figure shows the regions with increased activity during IMI in relation to IMIc. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

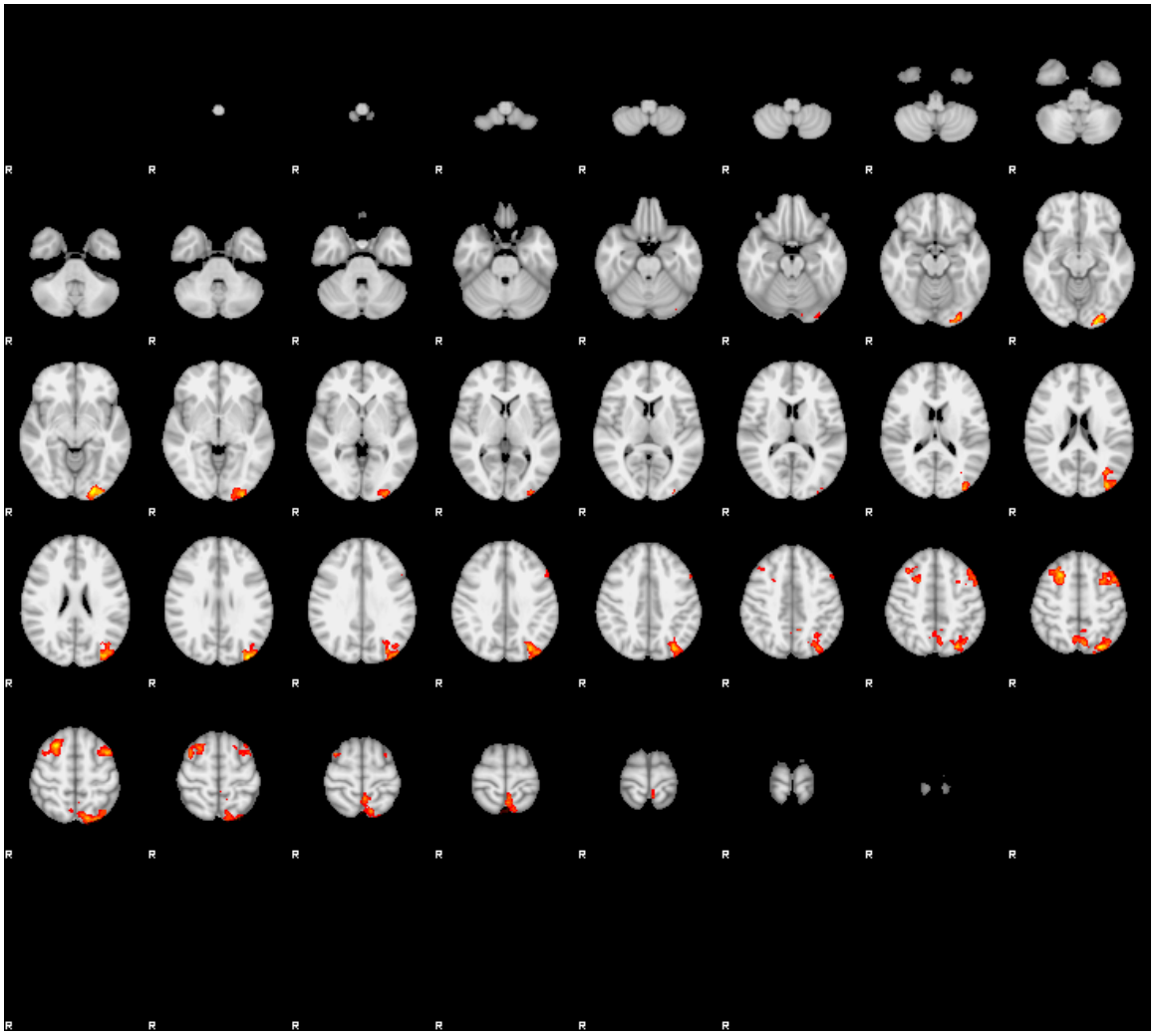

Figure 1.7.14 Comparison between IMI and IMIc during the observation of chords (event 1). The figure shows the regions with increased activity during IMIc in relation to IMI. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

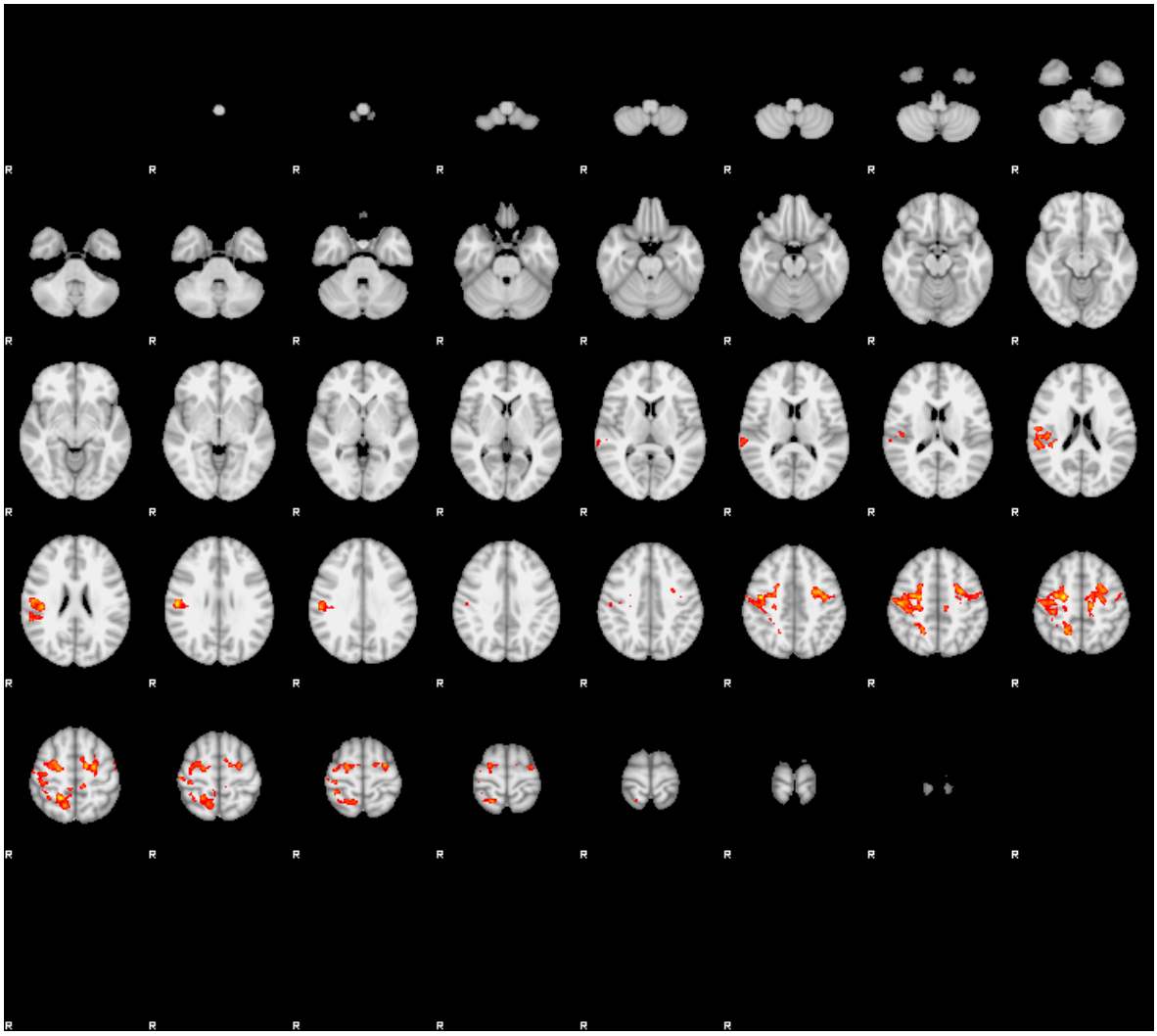

Figure 1.7.15 Comparison between IMI and IMIc during the observation of chords (event 1). The figure shows the regions with increased activity during IMI in relation to IDE. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

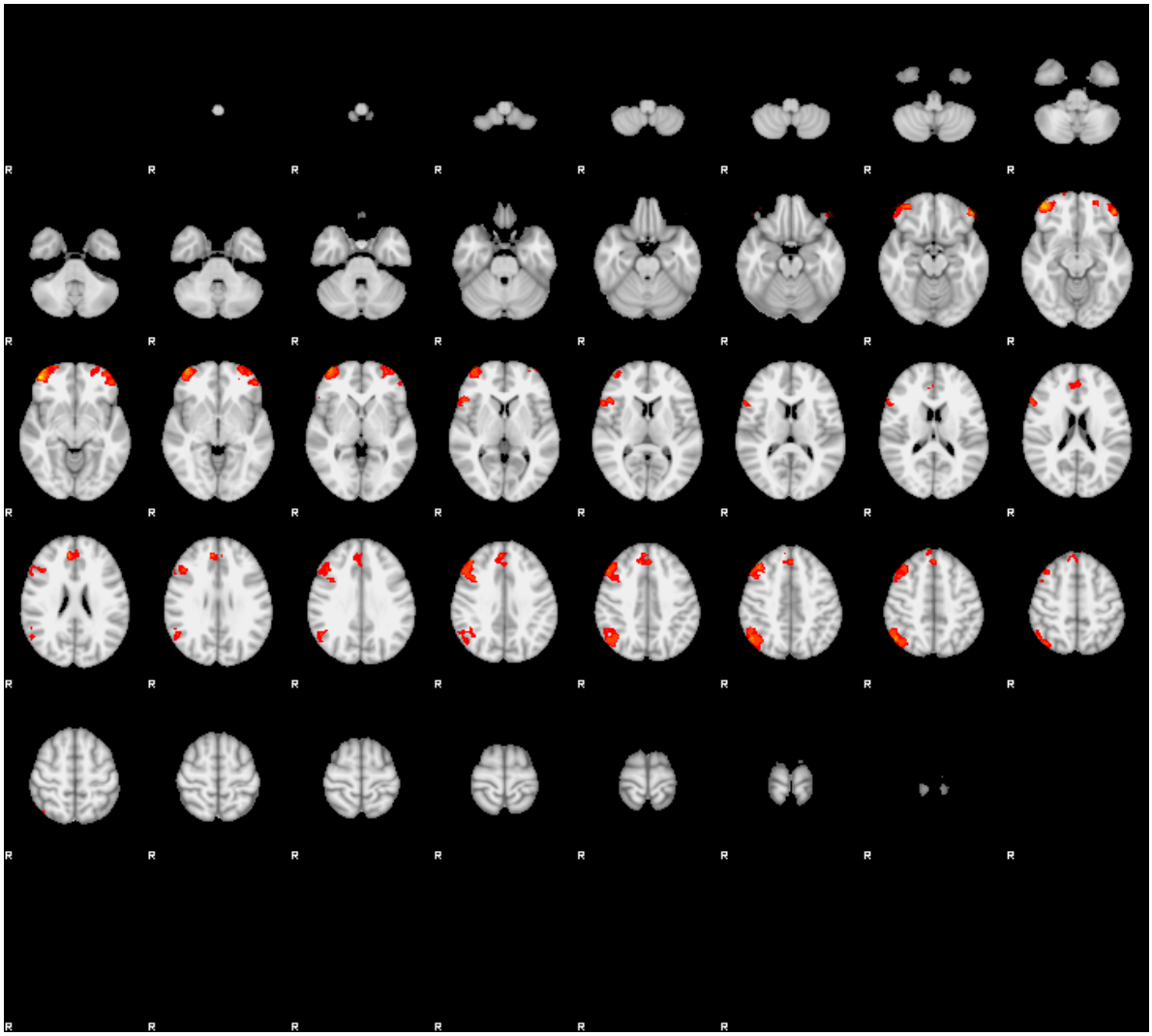

Figure 1.7.16 Comparison between IMI and IMIc during the observation of chords (event 1). The figure shows the regions with increased activity during IDE in relation to IMI. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

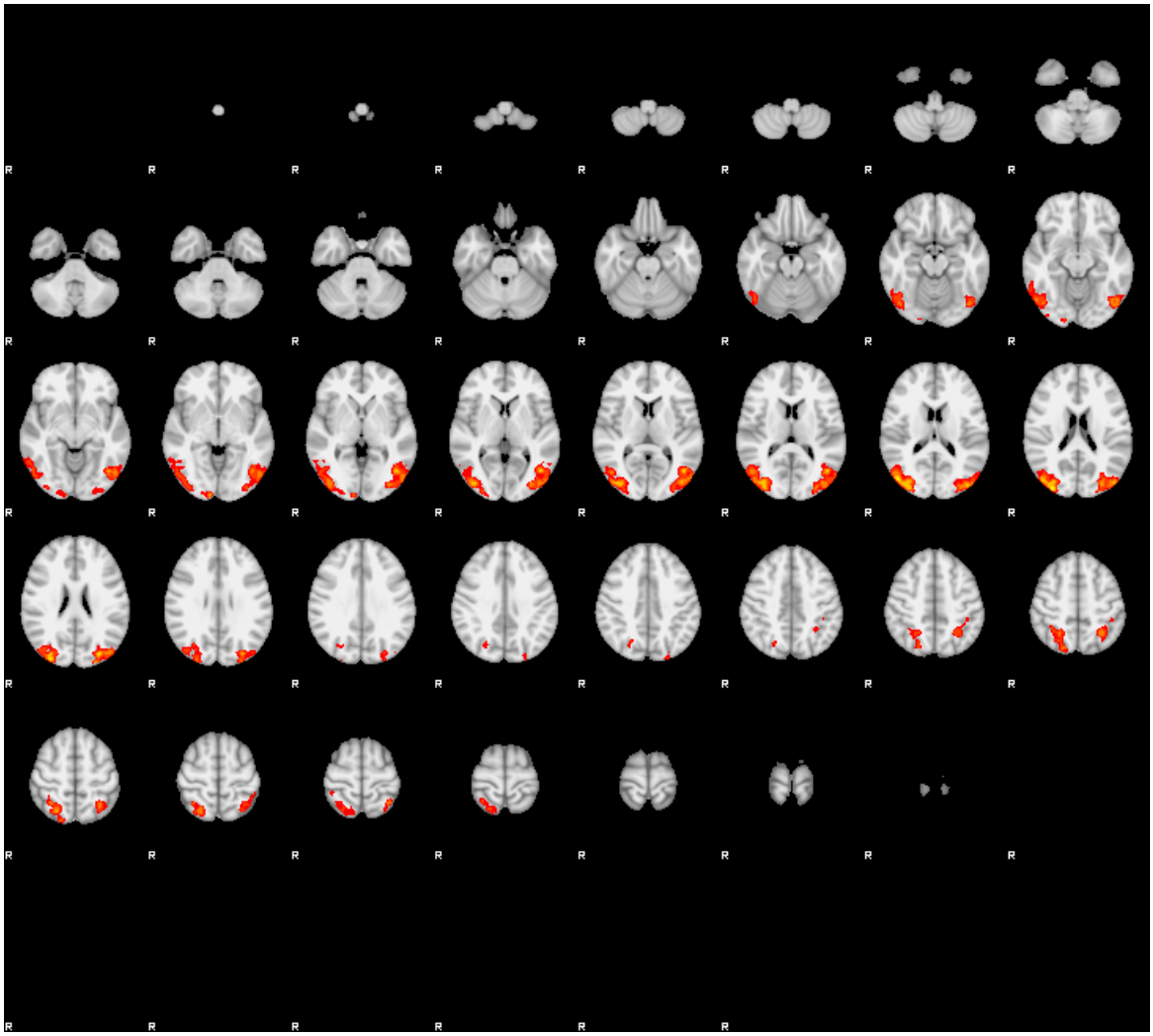

Figure 1.7.17 Comparison between IMI and IMIc during the observation of chords (event 1). The figure shows the regions with increased activity during IMIc in relation to ANO. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

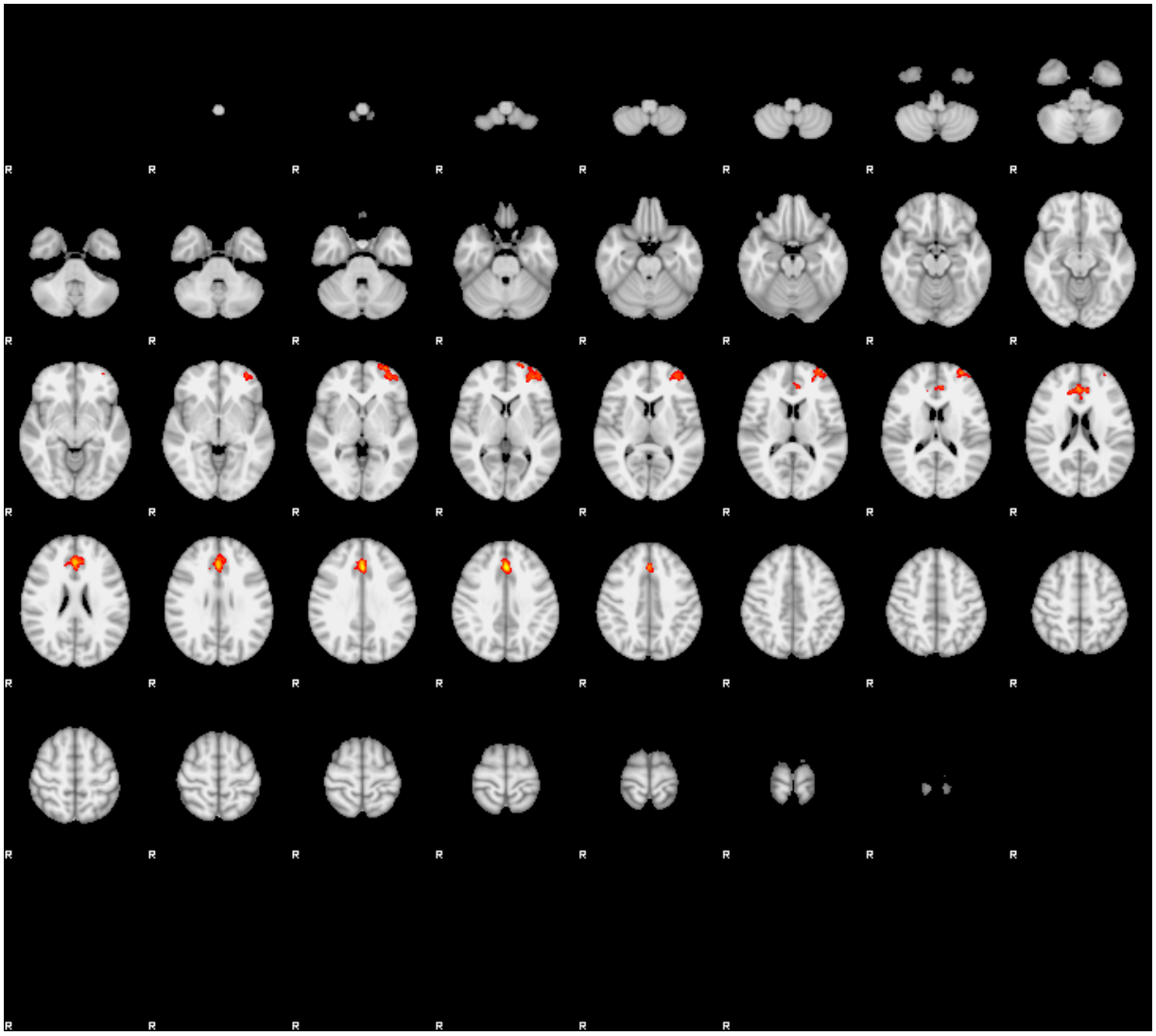

Figure 1.7.18 Comparison between IMI and IMIc during the observation of chords (event 1). The figure shows the regions with increased activity during ANO in relation to IMIc. Cluster threshold activation images (min. red, max yellow) Z-statistic images were thresholded using clusters determined by z-voxel > 2.3 and a (corrected) cluster significance threshold of $p < 0.05$.

DISCUSSÃO GERAL E CONCLUSÕES

Como podemos traduzir uma ação que observamos outra pessoa desempenhar em atos motores capazes de produzir a mesma ação observada, principalmente quando ainda não sabemos executá-la? Esta questão bastante simples tem sido foco de vários estudos sobre comportamento humano. A descoberta dos neurônios-espelho tem contribuído bastante para essa discussão. Estes neurônios, agora pensados como um sistema (MNS), incluem grupos de neurônios em várias regiões do encéfalo cuja atividade aumenta durante a execução de uma ação motora particular ou da observação da mesma ação desempenhada por outro indivíduo (Gallese *et al*. 1996; Rizzolatti *et al.*, 2000, 2001; Rizzolatti e Craighero, 2004). Estudos envolvendo os mais diversos protocolos sujerem que estes neurônios estão relacionados com a tradução de percepção em ação através de uma combinação direta entre o que é observado e a representação motora interna que o observador tem desta ação. Especialmente, estudos sobre comportamento imitativo abordam exatamente esta questão, uma vez que baseada a imitação se baseia em dois mecanismos básicos: a capacidade de entender ações feitas por outros e a capacidade de replicar estas ações (Rizzolatti, 2005).

No presente trabalho demonstramos que o MNS possui papel fundamental durante a observação de uma ação com o intuito de imitá-la. Nas etapas iniciais da imitação, isto é, quando a ação a ser imitada é desconhecida, nossos resultados sujerem que o MNS atua na decomposição da ação em pequenos atos motores, presentes no repertório motor do observador e possíveis de serem executados. Um mecanismo adicional, envolvendo outras áreas corticais, é necessário para que estes atos motores sejam recombinados e organizados em uma nova sequência motora capaz de reproduzir de maneira fiel a ação observada. Após o treino efetivo da execução desta ação através

do aprendizado por observação seguido de execução, o processo de replicação da ação se torna mais eficiente e econômico, uma vez que a combinação direta entre a ação observada e o repertório motor do observador acontece na ação como um todo, sem a necessidade de decomposição e recombinação dos atos motores, requerindo menos atividade do MNS. Além disso, a ativação do sistema de neurônios-espelho pode ser alterada dependendo do contexto em que a ação está inserida. Mesmo durante a imitação de ações conhecidas, o MNS pode atuar conjuntamente com outros sistemas se houver uma demanda adicional na interpretação da ação observada, como por exemplo, quando a ação é representada por pistas simbólicas não biológicas.

Assim, concluímos que o MNS atua fundamentalmente no processo de combinação direta entre a ação observada e a representação motora interna desta ação. Este sistema possui um papel crítico na interpretação das ações observadas e na busca pela representação mais próxima disponível no repertório motor do observador.

RESUMO

Neurônios espelho são ativados tanto durante a execução de uma ação como durante a observação desta mesma ação desempenhada por outra pessoa. Como parecem integrar observação e ação, os neurônios espelho têm sido foco de estudos sobre como o ser humano entende o próximo e em que extensão é capaz de compartilhar experiências. Esta integração inclui uma "representação interna" que envolve as mesmas estruturas nervosas envolvidas na execução da ação observada e tem sido sugerida como parte fundamental da facilitação do aprendizado por imitação. Este trabalho teve como objetivo, além de investigar o papel do sistema de neurônios-espelho no comportamento imitativo, investigar como ações motoras desconhecidas passam a ser reconhecidas e incorporadas ao repertório motor no contexto atual de neurônios espelho. Para isso, 20 voluntários foram treinados a executar acordes musicais em tarefas envolvendo imitação. Nossos resultados mostram que o sistema de neurônios-espelho possui um crítico papel durante a observação de uma ação com o intuito de imitá-la. Além disso, a ativação do sistema de neurônios-espelho pode ser alterada dependendo do contexto em que a ação está inserida.

Palavras-chave: neurônios espelho, imitação, fMRI.

ABSTRACT

Mirror neurons are activated both during action execution and during observation of this same action performed by another person. As they seem to integrate observation and action, mirror neurons have been the focus of studies on how humans understand the other and to what extent is able to share experiences. This integration includes an "internal representation" that involves the same neural structures involved in the execution of an observed action and has been suggested as a fundamental part of the facilitation of learning by imitation. This study aimed, besides investigating the role of the mirror neuron system in imitative behavior, investigating how unknown motor actions are recognized and incorporated into the repertoire after practice in the current context of motor mirror neurons. For this, 20 volunteers were trained to perform tasks involving musical chords in imitation context. Our results show that the mirror neuron system has a critical role during the observation of an action in order to imitate it. Moreover, activation of mirror neuron system may be altered depending on the context in which the action is inserted.

Keywords: mirror neurons, imitation, fMRI.

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Anexos

Universidade de São Paulo
Instituto de Biociências

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

(maiores de 18 anos)

ESTUDO: Aquisição de habilidades motoras pela observação: contribuição do Sistema de Neurônios-espelho

Você está sendo convidado(a) a participar do projeto de pesquisa acima citado. O documento abaixo contém todas as informações necessárias sobre a pesquisa que estamos fazendo. Sua colaboração neste estudo será de muita importância para nós.

Eu, (inserir o nome)....., residente e domiciliado na....., portador da Cédula de identidade, RG ....., e inscrito no CPF/MF..... nascido(a) em ___/___/_____, abaixo assinado(a), concordo de livre e espontânea vontade em participar do estudo "***Aquisição de habilidades motoras pela observação: contribuição do Sistema de neurônios-espelho***" e esclareço que obtive todas as informações. Estou ciente que:

I) Tenho a liberdade de desistir ou de interromper a colaboração neste estudo no momento em que desejar, sem necessidade de qualquer explicação;

II) A desistência não causará nenhum prejuízo à minha saúde ou bem estar físico;

III) Os resultados obtidos durante este ensaio serão mantidos em sigilo, mas concordo que sejam divulgados em publicações científicas, desde que meus dados pessoais não sejam mencionados;

IV) Caso eu desejar, poderei tomar conhecimento dos resultados, ao final desta pesquisa

( ) Desejo conhecer os resultados desta pesquisa.

( ) Não desejo conhecer os resultados desta pesquisa.

São Paulo, de de 2010

Pesquisador Responsável pelo Projeto: *Renata Pereira Lima*

Telefone para contato:_____

Email:_____

QUESTIONÁRIO DE EDINBURGH (adaptado)

Se você é destro, já teve alguma tendência a ser canhoto? _____
Existe algum canhoto na sua família?_____

Algumas atividades requerem as duas mãos, neste caso, a questão é em relação à parte da atividade **explicitada entre parênteses**.

Tente responder todas as questões e somente deixe em branco caso você não tenha absolutamente nenhuma experiência com o objeto da tarefa descrita.

Indicar a preferência manual nas atividades abaixo. Assinale:

- ✓ "XX" na coluna apropriada quando a preferência for tão forte que você **nunca use a outra mão**.
- ✓ "XX" e "X" nas colunas apropriadas quando preferir usar **uma das mãos em relação à outra**.
- ✓ "XX" nas duas colunas quando usar indistintamente **qualquer uma das mãos**.

Atividades	**Esquerda**	**Direita**
Escrever		
Desenhar		
Jogar uma pedra		
Usar uma tesoura		
Usar um pente		
Usar uma escova de dentes		
Usar uma faca (sem o uso do garfo)		
Usar uma colher		
Usar um martelo		
Usar uma chave de fendas		
Usar uma raquete de ping-pong		
Usar uma faca (com o garfo)		
Usar uma vassoura (mão superior)		
Usar um rodo (mão superior)		
Acender um fósforo		
Abrir um vidro com tampa (mão que segura a tampa)		
Distribuir cartas		
Enfiar a linha na agulha (mão que segura a linha)		
Qual o olho você usa, se forçado a usar somente um ?		
Total (deixar em branco)		

Dominância pedal (chutar uma bola) _____

Duração média do sono _____

Horário preferido para acordar _____

Medicamentos em uso _____

Hábito de Brincar com jogos eletrônicos:
Sim ( ) Não ( )

Mulheres:

Data da última menstruação _____ **Regularidade do ciclo** _____

Quociente de lateralidade [(D-E)/(D+E)] _____
(deixar em branco)

Sobre música:.

1. Toca/já tocou algum instrumento musical?

□ Sim. Qual? _____ Há/por quanto tempo? _____

□ Não.

2. Canta?

□ Sim. Há quanto tempo? _____

□ Não.

3. Outros familiares são músicos? Quantos? Que grau de parentesco? Tocam profissionalmente?

_____

4. Tem alguma experiência com vídeo-game?

V A M S – Pré

INSTRUÇÕES: Avalie como você se sente agora em relação aos itens abaixo e marque cada linha com um traço vertical no ponto que melhor descreve seus sentimentos. O centro de cada linha indica como você habitualmente se encontra e as extremidades indicam o máximo de cada condição.

ALERTA	_____	SONOLENTO
CALMO	_____	AGITADO
FORTE	_____	FRACO
CONFUSO	_____	COM IDÉIAS CLARAS
ÁGIL	_____	DESAJEITADO
APÁTICO	_____	DINÂMICO
SATISFEITO	_____	INSATISFEITO
PREOCUPADO	_____	TRANQUILO
RACIOCÍNIO DIFÍCIL	_____	PERSPICAZ
TENSO	_____	RELAXADO
ATENTO	_____	DISTRAÍDO
INCAPAZ	_____	CAPAZ
ALEGRE	_____	TRISTE
HOSTIL	_____	AMISTOSO
INTERESSADO	_____	DESINTERESSADO
RETRAÍDO	_____	SOCIÁVEL

CLASSIFICAÇÃO SÓCIO ECONÔMICA

a) Instrução do Participante (você)

( ) analfabeto/primário completo
( ) primário completo/ginasial incompleto
( ) ginasial completo/colegial incompleto
( ) colegial completo/superior incompleto
( ) superior completo

Instrução do chefe da família

( ) analfabeto/primário completo
( ) primário completo/ginasial incompleto
( ) ginasial completo/colegial incompleto
( ) colegial completo/superior incompleto
( ) superior completo

b) Ítens de conforto familiar - critério ABA

Ítens de posse	Não tem	Quantidade possuída					
	0	1	2	3	4	5	6 e +
televisor							
rádio (excluindo o do carro)							
banheiro							
automóvel							
empregada mensalista							
aspirador de pó							
máquina de lavar roupa							

c) Itens de conforto Familiar - critério ABIPENE

Ítens de posse	Não tem	Quantidade possuída					
	0	1	2	3	4	5	6 e +
automóvel							
televisor em cores							
rádio (excluindo o do carro)							
banheiro							
empregada mensalista							
aspirador de pó							
máquina de lavar roupa							
vídeo cassete / DVD							
geladeira comum ou c/ freezer							

O(A) SR(A). PODERIA, POR FAVOR, RESPONDER ÀS SEGUINTES PERGUNTAS A RESPEITO DE SUA SAÚDE:

1.	Tem dores de cabeça freqüentes?.....	SIM □	NÃO □
2.	Tem falta de apetite?.....	SIM □	NÃO □
3.	Dorme mal?.....	SIM □	NÃO □
4.	Assusta-se com facilidade?.....	SIM □	NÃO □
5.	Tem tremores na mão?.....	SIM □	NÃO □
6.	Sente-se nervoso(a), tenso(a) ou preocupado(a)?.....	SIM □	NÃO □
7.	Tem má digestão?.....	SIM □	NÃO □
8.	Tem dificuldade de pensar com clareza?.....	SIM □	NÃO □
9.	Tem se sentido triste ultimamente?.....	SIM □	NÃO □
10.	Tem chorado mais do que de costume?.....	SIM □	NÃO □
11.	Encontra dificuldades para realizar com satisfação as suas atividades diárias?.....	SIM □	NÃO □
12.	Tem dificuldades para tomar decisões?.....	SIM □	NÃO □
13.	Tem dificuldades no serviço (seu trabalho é penoso, lhe causa sofrimento)?.....	SIM □	NÃO □
14.	É incapaz de desempenhar um papel útil em sua vida?.....	SIM □	NÃO □
15.	Tem perdido o interesse pelas coisas?.....	SIM □	NÃO □
16.	Você se sente uma pessoa inútil, sem préstimo?.....	SIM □	NÃO □
17.	Tem tido a idéia de acabar coma vida?.....	SIM □	NÃO □
18.	Sente-se cansado(a) o tempo todo?.....	SIM □	NÃO □
19.	Tem sensações desagradáveis no estômago?.....	SIM □	NÃO □
20.	Você se cansa com facilidade?.....	SIM □	NÃO □

INVENTÁRIO PARA DEPRESSÃO DE BECK

Neste questionário existem grupos de afirmativas. Por favor leia cada uma delas e selecione a afirmativa que melhor descreva como você se sentiu NA SEMANA QUE PASSOU, INCLUINDO O DIA DE HOJE. Desenhe um círculo ao lado da afirmativa que tiver selecionado.
Se várias afirmativas no grupo parecem aplicar-se igualmente bem, circule cada uma delas. Certifique-se de ter lido todas as afirmativas antes de fazer sua escolha.

---

1)
0 Não me sinto triste.
l Sinto-me triste.
2 Sinto-me triste o tempo todo e não consigo sair disso.
3 Estou tão triste e infeliz que não posso agüentar.

---

2)
0 Não estou particularmente desencorajado quanto ao futuro.
l Sinto-me desencorajado quanto ao futuro.
2 Sinto que não tenho nada por que esperar.
3 Sinto que o futuro é sem esperança e que as coisas não podem melhorar.

---

3)
0 Não me sinto fracassado.
l Sinto que falhei mais do que o indivíduo médio.
2 Quando olho para trás em minha vida, tudo que vejo é uma porção de fracassos.
3 Sinto que sou um fracasso completo como pessoa.

---

4)
0 Obtenho tanta satisfação com as coisas como costumava fazer.
l Não gosto das coisas da maneira como costumava gostar.
2 Não consigo mais sentir satisfação real com coisa alguma.
3 Estou insatisfeito ou entediado o tempo todo.

---

5)
0 Não me sinto particularmente culpado.
l Sinto-me culpado boa parte do tempo.
2 Sinto-me muito culpado a maior parte do tempo.
3 Sinto-me culpado o tempo todo.

---

6)
0 Não sinto que esteja sendo punido.
1 Sinto que posso ser punido.
2 Sinto que estou sendo punido.

---

7)
0 Não me sinto desapontado comigo mesmo.
l Sinto-me desapontado comigo mesmo.
2 Sinto-me aborrecido comigo mesmo.
3 Eu me odeio.

---

8)
0 Não sinto que seja pior que qualquer outra pessoa.
l Critico minhas fraquezas ou erros.
2 Responsabilizo-me o tempo todo por minhas falhas.
3 Culpo-me por todas as coisas ruins que acontecem.

---

9)
0 Não tenho nenhum pensamento a respeito de matar.
l Tenho pensamentos sobre me matar mas não os levaria adiante.

2 Gostaria de matar.
3 Eu me mataria se tivesse uma oportunidade.

---

l0) 0 Não costumo chorar mais que o habitual.
l Choro mais agora do que costumava fazer.
2 Atualmente choro o tempo todo.
3 Eu costumava conseguir chorar, mas agora não consigo, mesmo que queira.

---

ll) 0 Não me irrito mais agora que em qualquer outra época.
l Fico molestado ou irritado mais facilmente do que costumava.
2 Atualmente sinto-me irritado todo tempo.
3 Absolutamente não me irrito com as coisas que costumavam irritar-me.

---

12) 0 Não perdi o interesse nas outras pessoas.
l Interesso-me menos do que costumava pelas outras pessoas.
2 Perdi a maior parte do meu interesse nas outras pessoas.
3 Perdi todo o meu interesse nas outras pessoas.

---

l3) 0 Tomo decisões mais ou menos tão bem como em qualquer outra época.
l Adio minhas decisões mais do que costumava.
2 Tenho maior dificuldade em tomar decisões mais do que antes.
3 Não consigo mais tomar decisões.

---

l4) 0 Não sinto que minha aparência seja pior do que costumava ser.
l Preocupo-me por estar parecendo velho ou sem atrativos.
2 Sinto mudanças permanentes em minha aparência que me fazem parecer sem atrativos.
3 Considero-me feio.

---

l5) 0 Posso trabalhar mais ou menos tão bem quanto antes.
l Preciso de um esforço extra para começar qualquer coisa.
2 Tenho que me forçar muito até fazer qualquer coisa.
3 Não consigo fazer nenhum trabalho.

---

l6) 0 Durmo tão bem quanto de hábito.
l Não durmo tão bem quanto costumava.
2 Acordo l ou 2 horas mais cedo do que de hábito e tenho dificuldade de voltar a dormir.
3 Acordo várias horas mais cedo do que costumava e tenho dificuldade de voltar a dormir.

---

l7) 0 Não fico mais cansado que o hábito.
l Fico cansado com mais facilidade do que costumava.
2 Sinto-me cansado ao fazer quase qualquer coisa.
3 Estou cansado demais para fazer qualquer coisa.

---

l8) 0 Meu apetite não está pior do que de hábito.
l Meu apetite não é tão bom como costumava ser.
2 Meu apetite está muito pior agora.
3 Não tenho mais nenhum apetite.

---

l9) 0 Não perdi muito peso se é que perdi algum ultimamente.
l Perdi mais de 2,5 Kg.

2 Perdi mais de 5,o Kg
3 Perdi mais de 7,5
Estou deliberadamente tentando perder peso, comendo menos:( ) Sim ( ) Não.

---

20) 0 Não me preocupo mais de hábito com minha saúde.
1 Preocupo-me com problemas físicos, como dores e aflições no estômago ou prisões de ventre.
2 Estou muito preocupado com problemas físicos e é difícil pensar em muito mais que isso.
3 Estou tão preocupado com meus problemas físicos que não consigo pensar em outra coisa.

---

21) 0 Não tenho observado qualquer mudança recente em meu interesse sexual.
1 Estou menos interessado por sexo do que costumava.
2 Estou bem menos interessado em sexo atualmente.
3 Perdi completamente o interesse por sexo.

TOTAL: _____

V A M S pós

Nome: ..... Horário: .... horas .... min.
Data: ..../..../.....

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HOSTIL	_____	AMISTOSO
INTERESSADO	_____	DESINTERESSADO
RETRAÍDO	_____	SOCIÁVEL

Questionário de Avaliação do Experimento

1) O (A) Sr.(a) sentíu alguma dor relacionada à posição do exame ?

2) O (A) Sr. (a), caso necessário, voltaria para fazer este exame ?

3) O (A) Sr. (a) gostaria de perguntar algo sobre o exame ?

4) Dê uma nota de 0 (ruim) a 10 (excelente) para a sua impressão deste exame: