"Role of Broca's area in motor cognition"

Clerget, Emeline

Abstract
Processing hierarchy is crucial in humans, allowing us to perform complex behaviours from expert activities to everyday-life actions, including linguistic skills. A common feature of many of these behaviours is that they rely on structured sequences, which must obey certain syntactic rules. Because of its crucial role in linguistic syntax, Broca's area, located in the left inferior frontal cortex of the human brain, has been considered as a possible candidate to support syntactic function across multiple domains; accordingly, this area has been named the "supramodal syntactic processor". Despite a constantly growing number of studies trying to gain further insight into this issue, experimental evidence supporting this view remains scarce. In the present thesis, a series of six experiments was conducted, taking advantage of the TMS technique, to determine the causal implication of Broca’s area in various "motor syntax" related tasks. We started our investigations by testin...

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Role of Broca’s area in motor cognition

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AF</td>
<td>Arcuate fasciculus</td>
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<tr>
<td>AGL</td>
<td>Artificial grammar learning</td>
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<tr>
<td>ASD</td>
<td>Autism spectrum disorders</td>
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<tr>
<td>BA</td>
<td>Brodmann area</td>
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<tr>
<td>BOLD</td>
<td>Blood-oxygen-level dependence</td>
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<tr>
<td>DLPFC</td>
<td>Dorso-lateral PFC</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>ECF</td>
<td>Extreme capsule fasciculus</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalography</td>
</tr>
<tr>
<td>ELAN</td>
<td>Early LAN</td>
</tr>
<tr>
<td>ERP</td>
<td>Event related potential</td>
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<tr>
<td>fMRI</td>
<td>functional MRI</td>
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<tr>
<td>GLI</td>
<td>Grey level index</td>
</tr>
<tr>
<td>IFG</td>
<td>Inferior frontal gyrus</td>
</tr>
<tr>
<td>IPL</td>
<td>Inferior parietal lobule</td>
</tr>
<tr>
<td>LAN</td>
<td>Left anterior negativity</td>
</tr>
<tr>
<td>LGG</td>
<td>Low-grade glioma</td>
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<tr>
<td>MEG</td>
<td>Magneto-encephalography</td>
</tr>
<tr>
<td>MEP</td>
<td>Motor evoked potential</td>
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<td>MNS</td>
<td>Mirror neuron system</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MTG</td>
<td>Middle temporal gyrus</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>PMC</td>
<td>Premotor cortex</td>
</tr>
<tr>
<td>PMd</td>
<td>Dorsal premotor cortex</td>
</tr>
<tr>
<td>PMv</td>
<td>Ventral premotor cortex</td>
</tr>
<tr>
<td>rMT</td>
<td>Resting motor threshold</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of interest</td>
</tr>
<tr>
<td>RT</td>
<td>Reaction time</td>
</tr>
<tr>
<td>SLF</td>
<td>Superior longitudinal fasciculus</td>
</tr>
<tr>
<td>SMA</td>
<td>Supplementary motor area</td>
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<tr>
<td>SRTT</td>
<td>Serial reaction time task</td>
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<tr>
<td>STG</td>
<td>Superior temporal gyrus</td>
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<tr>
<td>STS</td>
<td>Superior temporal sulcus</td>
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<tr>
<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
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<td>VLPFC</td>
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Chapter 1. Introduction

Broca’s area is primarily known as a brain region crucially involved in speech production. So, the title of the present manuscript, namely “Role of Broca’s area in motor cognition” highlights the unexpected association between Broca’s area and motor functions.

First, the concept of “motor cognition” should be clarified. It refers to the close relationship between the motor system and cognitive functions through high-level mental processes and internal representations. This could be made clearer by illustrating with an example taken among the human everyday life behaviours. “Say you have lost your car keys and you are anxious as time passes because you might miss your morning flight. An efficient way to find them is to stop wandering around, then retrace in your mind what you have done the previous evening, where in your house you have been, with whom you have interacted, and so forth. Such a mental simulation reactivates, amongst other things, your motor representations in working memory and hopefully will help you to spot your keys” (Jackson and Decety, 2004). Motor cognition encompasses the planning, preparation and production of successive movements accomplished to reach a specific goal, or to react to an event, which occurs in the environment. Such an embodiment of cognition into action also allows anticipating and predicting our own actions and, in addition, recognizing, interpreting, imitating and understanding the actions of others.

Second, one might ask the reason why a language area is concerned with motor cognition. A direct answer is that viewing Broca’s area only as a language centre is now outdated. In the contemporary literature, numerous studies report a contribution of Broca’s area to non-linguistic domains (Fadiga et al., 2009); it concerns especially the motor domain, from action execution to action observation, encompassing imitation and mental imagery of movement, learning ruled-based sequences, prediction of repeated sequential patterns and so on. Different hypotheses have been formulated to explain these astonishing findings and, amongst them, a particularly promising assumption considers the multiple influences of Broca’s area as an indicator of supramodal function. More exactly, this assumption considers Broca’s area as a centre responsible for dealing with structural rules, namely a syntax-like process that governs a wide range of behaviours. While data arguing in favour of a role of Broca’s area in this syntactic process accumulate, causal evidence is too sparse to reach a more detailed and complete view of such function.
Chapter 1. Introduction

First and foremost, the aim of the present manuscript is to gain further insights about the diverse implications of Broca’s area, with a particular focus on the action domain through a review of the literature presented in the introductory chapter. Then, this work will intend to shed some light on the aforementioned hypothesis through our investigations about syntactic/hierarchical processing of action-related sequences, a mechanism falling under motor cognition and being subserved by the Broca’s area.

Because Broca’s area is steeped in history, we put aside our central question for the moment to backtrack to its discovery, to the early knowledge about its anatomy and function and, whenever appropriate, to deal with important issues from different points of view. These range from the historical (e.g. localisationist theories, language evolution ...) to the modern (e.g. mirror neurons) and include clinical (e.g. aphasia, autism ...) perspectives. It is not intended to provide a complete overview for all these issues but to offer a picture of the complexity surrounding Broca’s area.

This Introduction is divided into five parts.

1. The first part aims at presenting the brain area at the core of our research, Broca’s area. It is probably the most famous cortical area of the human brain as it was the first area associated with a precise function, speech production. Thus we start with a synopsis of the discovery of Broca’s area and its impact on cognitive neuroscience history. Next, we look at special issues related to the confusion that has arisen in the literature from the discrepancy between the area originally identified by Pierre Paul Broca and the current definition of Broca’s area, as well as from the inconsistency in the terms used to refer to this cortical area, without delimiting it clearly. This brings us to the following step, to give a picture of the current definition of Broca’s area. A description of its anatomy is given and the associated terminology explored. Nowadays, the most commonly used term “Broca’s area” refers, from an anatomical point of view, to the portion of the inferior frontal gyrus1 comprising the pars opercularis and the pars triangularis. According to the Brodmann’s nomenclature, these two regions correspond, respectively, to Brodmann areas 44 and 45.

2. The second section is about the linguistic function of Broca’s area. It starts with an updated report of its role as a main language centre for speech production and continues with an integrated view of this function in the language organization of the brain. However, based

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1 The inferior frontal gyrus is composed of three parts, respectively, from the more posterior to the more anterior, the pars opercularis, the pars triangularis and the pars orbitalis.
on linguistic research, it appears clearly that its contribution may be not restricted to speech production as originally claimed. Broca’s area is also involved in speech comprehension and in syntactic processing, as demonstrated in several studies using syntactic anomaly detection or judgment, artificial grammar learning or studies playing with syntactic complexity, in which this cortical area is found activated.

3. The third part moves to the issue of the different roles played by Broca’s area beyond language. Indeed, with constant advances in brain investigation techniques, a new view about Broca’s area has progressively emerged, providing for the first time the idea that its contribution may be not restricted to linguistic functions. Recent studies, predominantly using brain imaging, have revealed activations corresponding to Broca’s area even though they were designed initially to study different cognitive functions such as calculation, music or motor-related functions. Therefore, in the first sub-part, we mention such implications of Broca’s area far away from the linguistic domain. In the second sub-part, we focus on the motor domain since it is the most relevant to the experiments in this thesis. In the third sub-part, we highlight another point, the role of Broca’s area in the mirror neuron system in particular, also related to our investigations. All these facts taken from the literature underline the manifold contributions of Broca’s area, but also raise the question of the involvement of a main language centre in non-linguistic domains. This will be covered in the last sub-part.

4. The fourth section is a more detailed discussion about one of the hypotheses mentioned in part 3, to explain the implications of Broca’s area in various domains. At first glance, the tasks owning to different domains seem completely distinct from each other. However, on closer inspection it appears that most of these tasks have the common property of being rule-based, combining discrete units - such as pitches for music, numbers for calculation, motor acts for motor-related function - into longer sequences that are in this way hierarchically structured. So, despite using distinct experimental tasks and designs, all these studies can be considered as “syntax studies”. According to its basic definition, syntax corresponds to the order of words (elements) in a sentence (sequence) and to the linguistic (motor) rules that determine the way of combining them. Any behaviour, ranging from the learned or instinctive motor sequences of animals to the complex skills of humans, can be considered as a sequence of movements with syntax-like properties. These properties or rules allow the hierarchical association of individual movements into meaningful sequences. All these facts lead one to suppose that Broca’s area may be specialized for syntax, or an extended form of syntax. According to this view, linguistic syntax becomes “simply one” of the countless hierarchically organized behaviour that humans can perform. This fourth part consists of first describing this assumption and second, reviewing all of the corroborating findings from the literature.
5. The fifth and last part is composed of some concluding remarks about the Introduction section and a concise outline of my thesis. The latter aims at introducing the purpose of the thesis, some important information about the technique of investigation used (i.e., Transcranial Magnetic Stimulation or TMS), and the experimental questions addressed through our different research projects. That research is detailed in the Chapter 2 of this manuscript.

To summarize, this introductory chapter proceeds from the classical view to the more recent findings that underlie the multiple functions attributed to Broca’s area apart from language. Importantly, it also addresses the issue about how to explain these non-linguistic implications of the Broca’s area, with particular emphasis on the assumption that Broca’s area is endowed with the ability to process hierarchically organized sequences across domains. It is worth noting that particular attention has been paid to provide a view about the evolution of knowledge, both for the anatomy and function, from early findings to the more recent ones.
Chapter 1. Introduction

1. Broca’s area

1.1. Historical considerations

1.1.1. A brief overview of its discovery

Early in the history of the neurosciences, a major concern was to determine the neural substrate of human mental activities (Feinberg and Farah, 2000). The first most remarkable attempt is attributed to Franz Joseph Gall (1758-1828) who developed phrenology, originally based on the idea that the different mental faculties are represented in specific portions of the brain, recognizable onto the scalp through its relief, with a particular focus on traits of character such as intelligence or courage (Simpson, 2005). Actually, his theory originated when he was young with the remark that, among his classmates, individuals having memory facilities could be identified according to their faces. Later, he collected evidence for his primarily observation by conducting many palpations of skulls of “peculiar” individuals from artists to criminals. Obviously, phrenology encountered many criticisms and is now considered a pseudo-science (Simpson, 2005). However, retrospectively, it has undeniably been influential in other ways (Simpson, 2005). A common expression still used in French, “avoir la bosse des maths”, meaning to be endowed with mathematic facilities, comes from this theory.

The first, scientific, demonstration of functional localisation is attributed to Pierre Paul Broca (1824-1880, Figure 1.1) (Feinberg and Farah, 2000; Monod-Broca, 2001; Crank and Fox, 2002; Jay, 2002), who evidenced that a circumscribed frontal region of the brain is associated with a precise function, namely the production of speech (Broca, 1861a, c, b, 1863, 1865, 2006). If Pierre Paul Broca remains famous for that discovery, at his time he was also a renowned surgeon. Interested by many other fields of medicine, he studied for instance cancers and their propagation through metastases, rachitism, blood transfusion and even medical statistics (Simpson, 2005).

Figure 1.1: Pierre Paul Broca (1824-1880).

The French scientist (surgeon, anatomist, neurologist, anthropologist ...) famous for having localised for the first time a precise function in the human brain: the third frontal convolution as the seat of speech production. From (Monod-Broca, 2001).
Chapter 1.  Introduction

1.1.2.  First evidence for Broca’s area involvement in speech production

The historically pioneering brain localisation of Pierre Paul Broca arises from the clinical evaluation of patients suffering from language trouble; two cases are particularly renowned. His first patient was Mr Leborgne, whose nickname "Tan" reflected his linguistic deficit as he consistently repeated the word "tan" instead of any other words or sentences. When the patient died due to gangrene of the leg, an autopsy revealed a lesion of the lateral part of the frontal lobe in the left hemisphere (Broca, 1861b, a) (For an English-translated version see Broca, 2006). Shortly thereafter, Pierre Paul Broca examined a second patient, Mr Lelong. This patient also exhibited a clear impairment in speech production and, at autopsy, a lesion was observed around the same region i.e. the second and third convolutions of the left frontal lobe. In comparison with Leborgne’s case, the impairment in speech production was slightly more pronounced and the lesion was more circumscribed. These two clinical cases led Pierre Paul Broca to posit that the centre for speech production is localised in the left third frontal convolution of the brain. Since it was the first time that a brain region was associated to a given function, Pierre Paul Broca is largely considered as the father of the functional localisation approach in humans (Cubelli and De Bastiani, 2011).

Actually, the conclusion of Pierre Paul Broca was twofold: the left hemisphere is dominant for language and part of it is crucial for articulated speech. However, it is worth noting that similar observations had been made earlier, especially by Marc Dax (1770-1837) and his son, Gustave Dax (1815-1893). This point is frequently neglected in the literature (Finger and Roe, 1999; Buckingham, 2006) mostly because no written traces earlier than the publication of Broca in 1861 could be found. Henceforth, the frontal region identified as responsible for speech production has become known as “Broca’s area”, and the deficit associated to its lesion known primarily as “aphemia” and then as “Broca’s aphasia” (renamed by Armand Trousseau (1801-1867)), a term still used nowadays.

Since its original discovery, the involvement of Broca’s area in speech production has been repeatedly confirmed by clinical studies. Patients having a lesion that invades the left inferior frontal gyrus (IFG), in the neighbourhood of the Broca’s area, show speech deficits. Such patients, so-called “Broca’s aphasics”, typically search for words, speak slowly and short so that their production is weak and effortful; they also suffered frequently from agrammatism resulting in telegraphic speech (e.g. Goodglass, 1997). “Expressive aphasia”, “non-fluent aphasia” and “agrammatic aphasia” are other terms also employed to refer to Broca’s aphasia and illustrated clearly the characteristics of the impairment. Importantly, in Broca’s aphasia, there is no motor deficit related to speech production i.e. patients are able to use their tongue
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and mouth muscles normally. In general, global comprehension is preserved in such patients, but could be degraded in the case of sentences having a complex structure and relying on elaborate syntax (see section 2.1.4 about speech comprehension). There are few statistics about the incidence of Broca’s aphasia but millions of people worldwide might suffer from this deficit. The incidence of aphasia, with all types taken into account, is estimated at 1/250 individuals in the US according to the “National Aphasia Association” (http://www.aphasia.org). Some famous people who had suffer from Broca’s aphasia are, for instance, Charles Baudelaire and Lenin (Teive et al., 2011).

Apart from clinical studies in Broca’s aphasic patients, the involvement of Broca’s region in speech production has been confirmed experimentally on several occasions. Historically, the first occasion was during direct electrical stimulation of this region (Penfield and Roberts, 1959). The technique of electrical stimulation of the cortex in awake patients during brain surgery was developed originally by Wilder Penfield in order to spare mental faculties or, at least, to reduce the side-effects of invasive surgical treatments. Patients who had to undergo this surgery were drug-resistant epileptic cases and therefore the only possible treatment consisted of removing brain tissue responsible for epilepsy. Special care was required to preserve language abilities: for each patient, the precise part of the cortex being critical for speech production was determined; if a region were critical to this language function, electrical stimulation of it should interrupt speech production, a phenomenon called “speech arrest”. Penfield and colleagues showed that the electrical stimulation of a frontal zone corresponding to Broca’s area resulted in speech arrest. They demonstrated also that in fact, the same effect occurred following the electrical stimulation of a number of regions located in the motor, premotor and temporal cortices (Penfield and Roberts, 1959) (Figure 1.2) (see section 2.1.1.).

![Diagram of speech arrest sites](image.png)

Figure 1.2: Sites of speech arrest.

On this drawing each red dot represent a site for which its electrical stimulation induced speech arrest according to Penfield and Roberts (1959). From (Purves et al., 2005).

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2 This technique also allowed Penfield to propose a map of the human motor cortex, known as Penfield’s homunculus (Penfield, 1950).
1.1.3. Broca’s area as a main centre of the brain’s language circuit

Broca’s area was not the only language area for long. Indeed, several years after the discovery of Pierre Paul Broca, Carl Wernicke evidenced similarly a causal relationship between brain lesions, located in the left superior temporal gyrus (STG), and impairments in understanding speech (Wernicke, 1874). The area responsible for language comprehension has been called “Wernicke’s area” and the associated deficit named “Wernicke’s aphasia” or “fluent aphasia”. Few years later, a pathway connecting both areas, the *arcuate fasciculus* (AF) was evidenced. It was believed that a lesion to this tract causes “conduction aphasia” by disconnecting receptive (Wernicke’s area) from expressive (Broca’s area) language areas (Dejerine, 1895). Following these successive discoveries arose the first model of the organization of language function in the brain: the “Wernicke-Geschwind model” (Figure 1.3, left) proposed by Norman Geschwind (1970). In addition to these frontal and temporal language centres, Norman Geschwind foresaw the importance of a third region located in the parietal lobe and, more precisely, in the inferior parietal lobule\(^3\) (comprising both the angular gyrus (BA39) and the supramarginal gyrus (BA40). Later, this region, so-called the “Geschwind’s territory” has been found to be connected to both Broca’s and Wernicke’s areas (Catani et al., 2005).

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\(^3\) Geschwind proposed that the inferior parietal lobule is responsible for apprehending the semantic aspects that allow us to identify and classify information and that this area is a seat for verbal comprehension explaining the impairment, in patients suffering from limb apraxia following left parietal lesions, in executing gestures on verbal command (see Goldenberg, 2009).
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According to this model, the comprehension and production are processed serially by distinct areas in the brain, anatomically and functionally interconnected. More precisely, it postulates that information is first processed by primary areas, either the primary auditory, somatosensory or visual cortices (as illustrated in Figure 1.3, right), depending on the input modality, and then sent to the inferior parietal lobule. From this multimodal associative area responsible for processing phonological/articulatory (supramarginal gyrus) and semantic (angular gyrus) aspects, information is transmitted to the Wernicke's area where a meaningful sentence is conceived and conveyed via the AF to the Broca's area. The latter is finally in charge of sending the instructions for word articulation to the primary motor cortex to produce speech.

This earlier model, in which Broca’s area was responsible for speech production, dominated the view about language organization in the brain for a long time. Later, an alternative model was proposed by Marsel Mesulam (Mesulam, 1990). This second model makes the assumption that language-related information is processed according to its level of complexity. However, this model still focuses on two language centres in the left hemisphere, i.e. Broca’s area, the seat of speech production, and Wernicke’s area, the seat of auditory comprehension.

Lastly, both Geschwind’s and Mesulam’s locationist models of language were criticized as they encountered an important limitation: by categorizing language simply into receptive and production fields, they failed to account for the variety of aphasic symptoms (Poeppel and Hickok, 2004); thus production and comprehension should not be split up as such (Papathanassiou et al., 2000). The use of modern techniques (functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI), transcranial magnetic stimulation (TMS), Intra-Cranial Electrophysiology (ICE) and so on) has allowed the proposal of new and more realistic models of language organization (For reviews see, Bookheimer, 2002; Martin, 2003; Poeppel and Hickok, 2004; Hagoort, 2005; Stowe et al., 2005; Vigneau et al., 2006; Friederici, 2009; Hickok, 2009a; Price, 2010). Broca’s area occupies a central position in all these models (see section 2.1.3.).

1.2. Confusion about the definition of Broca’s area

For a long time, Broca’s area was defined as a frontal portion of the left hemisphere but was not clearly delimited. As a consequence, with the growing number of studies published over the years, in an increasing number of them this region has been found, rightly or wrongly,
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to be implicated in diverse tasks that have been the source of misleading conclusions about its exact function(s). As we will see, the anatomical definition of Broca’s area has evolved to become more restrictive than the original description. With technological advances, progress in describing this brain region has been made, especially in terms of its structural organization (cyto- and recepto-architecture, connectivity), a key feature for elucidating its functions (Zilles et al., 2002; Zilles and Amunts, 2009; Amunts et al., 2010).

1.2.1. The starting point

As previously stated, since Pierre Paul Broca’s discovery, the approximate region of the left frontal convolution identified as responsible for deficits in speech production has become known as Broca’s area. In his famous 1861 paper, Pierre Paul Broca said that "Le lobe frontal de l’hémisphère gauche est ramolli dans la plus grande partie de son étendue (...)" but that "(...) le foyer principal et le siège primitif du ramollissement, est la partie moyenne du lobe frontal gauche; c’est là que l’on trouve les lésions les plus étendues, les plus avancées et les plus anciennes" (Broca, 1861a). Two years later, in the 1863 report, with accumulating evidence he refined more precisely the location of the lesion responsible for the deficit of articulate speech: "Ainsi voilà huit faits où la lésion a siégé dans le tiers postérieur de la troisième circonvolution frontale" (Broca, 1863).

Actually, Pierre Paul Broca attributed the speech deficits of his patients to an area more restricted than it should have been. Indeed, Pierre Paul Broca limited the description of the brain lesions to a visual inspection of the surface; he underestimated the extent of the lesions, ignoring for instance the possible contribution of subcortical structures. This has been confirmed by a recent study (Dronkers et al., 2007): as the brains of his two famous patients, Mr Leborgne (Figure 1.4A and 1.4B) and Mr Lelong (Figure 1.4C and 1.4D), were kept intact by Pierre Paul Broca and preserved4, both lesioned brains have been inspected again by means of high resolution MRI in order to describe more precisely the location and extent of the lesions. Dronkers and collaborators found that the lesions described by Broca extended significantly into other regions, and thus that the deficits observed by Broca are attributable to larger lesions that originally claimed (Dronkers et al., 2007).

4 These brains are specimens at the Musée de l’Homme in Paris as well as Pierre Paul Broca’s brain itself.
Figure 1.4: Photographs of the brains of Leborgne and Lelong, Pierre Paul Broca’s first two aphasic patients.

(A) Lateral view of the brain of the first patient, Leborgne. The external lesion is clearly visible in the inferior frontal lobe. The softening in the area superior and posterior to the lesion suggests further cortical and subcortical involvement. (B) Close-up of the visible lesion in Leborgne’s brain. (C) Lateral view of Broca’s second patient, Lelong. The frontal, temporal and parietal lobes have retracted due to severe atrophy, exposing the insula. (D) Close-up of the visible lesion in Lelong’s brain. Note that only the most posterior part of what is currently called Broca’s area is infarcted; the anterior portion is completely spared. From (Dronkers et al., 2007).

1.2.2. Current definition of Broca’s area

According to the more widespread view in the current literature, Broca’s area is now assigned to a precise portion of the left IFG. It is classically considered as being composed of two parts, a posterior and an anterior part, respectively, the *pars opercularis* and the *pars triangularis*. The major sulcal landmarks used to delineate Broca’s area and its two parts are the precentral sulcus, the inferior frontal sulcus, the ascending and horizontal branches of the Sylvian/lateral fissure and the Sylvian/lateral fissure itself (Figure 1.5, upper part). Thus, Broca’s area lies between the Sylvian/lateral fissure and the inferior frontal sulcus and is demarcated by the precentral gyrus and by the horizontal ramus of the Sylvian/lateral fissure. The anterior ascending ramus of the Sylvian/lateral fissure divides this region into its two parts. According to the Brodmann’s classification - the most frequently used map for the human cortex (Brodmann, 1909) - the *pars opercularis* corresponds to Brodmann area (BA) 44 whereas the *pars triangularis* corresponds to BA45. Other cytoarchitectonic maps, less used, refer to Broca’s area as areas 57–59 for Riegele, areas 56–59 for Vogt and areas 57–59 (and 65) for Knauer (Keller et al., 2009b). Originally the classification of Brodmann was established according to the visual inspection of stained histological sections and by using classical...
histological criteria such as the type of cells and their densities, the variation of cortical laminar thickness and so on. More recent cytoarchitectonic studies, using an observer-independent method (Schleicher et al., 1999) that is a statistical estimation of the changes in the laminar patterns through the grey level index (GLI, (Schleicher and Zilles, 1990)), confirmed the cytoarchitectonic distinction between BA44, dysgranular, and BA45, granular (Amunts et al., 1999; Amunts et al., 2003) (Figure 1.5, lower part). This distinction between dysgranular or granular refers to, respectively, the light or strong presence of layer IV.

Figure 1.5: Cytoarchitectonic map (adapted from Brodmann) and cytoarchitecture of BA 44 and 45.

Upper part: The region of interest contains areas 44 and 45 as well as parts of the neighboring areas 4, 6, and 47. Note that Brodmann’s map does not show the ventral border of area 44, 45, and 6 in the depth of the lateral fissure. ab, ascending branch of the lateral fissure; cs, central sulcus; hb, horizontal branch of the lateral fissure; ifs, inferior frontal sulcus; lf, lateral fissure; prcs, precentral sulcus. From (Amunts et al., 2010)

Lower part: Cytoarchitecture of BA 44 and 45 in coronal, cell-body stained sections of a postmortem brain. The cytoarchitecture of both areas is characterized by large pyramidal cells in deep layer III, which exceed those of layer V in size. Whereas granular BA 45 shows a clearly visible layer IV, the layer IV of the dysgranular BA 44 is thinner and not clearly delineable from neighboring layers, since pyramidal cells from layers III and V invade into layer IV. Cortical layers are numbered. Scale bars, 0.5 mm. From (Amunts et al., 2004).

The description of the “modern Broca’s area” is further detailed in section 1.3.1.
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1.2.3. A persistent confusion in terminology

The term “Broca’s aphasia” leads to the idea that this deficit in due to a lesion of Broca’s area. Actually, Broca's aphasia occurs typically following stroke or cerebral vascular accident involving the left middle cerebral artery that supplies a large part of the lateral cortex. Figure 1.6 contrasts the region causally involved in Broca’s aphasia with a smaller zone considered to correspond to Broca’s area.

![Figure 1.6: Schematic representations of the approximate region of infarction causing Broca’s aphasia.](image)

Incidentally, the proportion of Broca’s aphasics having a lesion in Broca’s area has been estimated as high as 85% and, conversely, 50-60% of patients having a lesion in Broca’s area suffer from Broca’s aphasia (Dronkers, 2000). Lesions to Broca’s area (Alexander et al., 1989; Hillis et al., 2004) or to the underlying frontal operculum of the insular cortex (Dronkers, 1996) also conduct to another type of language deficit, and especially a motor speech planning deficit called apraxia of speech (Ogar et al., 2005). Thus, it is clear that there is no clear one-to-one mapping between aphasia and lesion location (Mohr, 2006). The consequence of this discrepancy between the approximate region originally described by Broca and the region causally involved in Broca’s aphasia was a source of great controversy about the definition of Broca’s area and subsequently about its assigned roles (Kljajević, 2011).
Furthermore, Broca’s area has been a term often used to identify all, part of or even neighbouring cortical regions surrounding the IFG; sometimes other terms such as Broca’s region, Broca’s complex or Broca’s territory are also used to reinforce the idea that this cortical portion could not be considered as a single area because of its organisational and functional heterogeneity as we will see later in this manuscript. An interesting study conducted a search in the literature from 1994 to 2004 for published articles using the term “Broca’s area” (Lindenberg et al., 2007). The first surprising result was that 21% of all the articles included in the study (n=542) used the term “Broca’s area” without defining it. Second, for the remaining 79% that provided a definition (anatomical definition for 77% and functional description for 2%), a huge variation in the definition of the “Broca’s area” was observed. While for 27% of all articles, Broca’s area referred to BA44 and BA45 (respectively, pars opercularis and pars triangularis; Figure 1.7B), for some authors Broca’s area comprised BA44 and BA45 but also BA47, mainly because it corresponded to the pars orbitalis that constitutes the third part of the IFG (Figure 1.7C). Broca’s area has also been defined as only one area (only pars opercularis/BA44, Figure 1.7A or only pars triangularis/BA45, Figure 1.7E) or as a combination of a variable number of areas amongst BA44, BA45, BA47, BA46 and BA6.

**Figure 1.7:** Schematic illustrations of the various macroscopic definitions of “Broca’s area”.

In all drawings the region considered to correspond to Broca’s area appears shaded in yellow. (A) traditional pars opercularis, bound caudally by the inferior precentral sulcus (ipcs) and rostrally by the anterior ascending ramus (ar) of the Sylvian fissure (e.g. (Tomaiuolo et al., 1999); (B) traditional pars opercularis (ipcs-ar) and pars triangularis (ar-hr) measured in unison (e.g. (Falzi et al., 1982)) or separately (e.g. (Knaus et al., 2006; Keller et al., 2007; Knaus et al., 2007)); (C) Entire IFG, bound caudally by the ipcs and rostro-ventrally by the lateral orbital sulcus (los), which has been measured in total with subsequent separation into the pars opercularis (ipcs-ar), pars triangularis (ar-hr), pars orbitalis (hr-los), triangularis caudalis (ar-ts) and triangularis rostralis (ts-hr) (Albanese et al., 1989); (D) Pars opercularis extending caudally into the precentral gyrus by using the anterior subcentral sulcus as the posterior border (e.g. (Foundas et al., 2001b) and (Foundas et al., 1998)); (E) traditional pars triangularis (ar-hr) (e.g. (Foundas et al., 2001a), (Foundas et al., 1995) and (Foundas et al., 1996)) and (F) pars opercularis and posterior half of the pars triangularis (ipcs-ts) measured in unison (Wada et al., 1975). From (Keller et al., 2009b).
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In addition, some authors used more general terms to refer to Broca’s area such as the prefrontal cortex (PFC) and more precisely the ventrolateral part of the PFC (VLPFC) (Figure 1.8). For yet other researchers, Broca’s area is considered as part of the premotor cortex (PMC) and more precisely of its ventral part (PMv), extending the posterior part of Broca’s area to a portion of the precentral gyrus (part of BA6) (Figure 1.7D and Figure 1.8).

Figure 1.8: Schematic of major anatomical sub-divisions in the frontal lobes.

Boundaries and Brodmann areas (BA) are only approximate. Arrows indicate anatomical directions of anterior/rostral (front) versus posterior/caudal (back) and dorsal (up) versus ventral (down). From caudal to rostral, labeled areas include motor cortex, dorsal and ventral PMC, dorsal and ventral aspects of anterior PMC, ventro-(VLPFC) and dorsolateral PFC (DLPFC), and lateral frontal polar cortex. From (Badre, 2008).

Furthermore, the regions of interest are sometimes described according to different cytoarchitectonic classifications (e.g. Brodmann’s classification (1909), von Economo and Koskinas’s classification (1925), Riegele’s classification (1931), Sarkissov and collaborators classification (1949), etc...) and/or in terms of macro-structural anatomy. The problem is that both these micro- and macro-structural delimitations do not necessarily coincide exactly (Figure 1.9) (Amunts et al., 1999). In addition to this variability of the area’s delimitations according to sulci, there is a large inter-subject variability of the sulcal landmarks (Tomaiuolo et al., 1999). Consequently, both parts of Broca’s area greatly vary in shape, surface and volume across individuals (Keller et al., 2007; Keller et al., 2009a).

5 Within Broca’s area, the Brodmann parcellation into BA44/BA45 does not follow exactly the sulcal landmarks division into pars opercularis/pars triangularis (Amunts et al., 1999).
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Figure 1.9: Illustration of the intersubject variability for BA44/45.

Lateral views of 3D reconstructions (surface rendering) of 10 individual postmortem brains (nos. 1 – 10) with BA 44 (red) and 45 (yellow) of the left and right hemispheres after cutting into histological sections, staining for cell bodies, and observer-independent definition of cytoarchitectonic borders on serial histological sections (Amunts et al., 1999). arlf, denotes ascending branch of the lateral fissure; ds, diagonal sulcus; hrlf, horizontal branch of the lateral fissure; ifs, inferior frontal sulcus; prcs, precentral sulcus. Note the high intersubject variability with respect to (i) differences in shape and size of both areas, (ii) variability in the sulcal pattern, and (iii) in the relationship of areal borders to surrounding sulci. From (Amunts et al., 2004).

To summarize, the original delineation of Broca’s area by Broca himself led to a long controversy about the precise location of Broca’s area. As pointed out by Mohr “In retrospect, had Broca emphasized the extent of the lesion topography in his two cases, he might have prevented over a century of controversy” (Mohr, 2006, p. 391). As a consequence, it is easy to imagine that the functions attributed to this area have been probably overestimated, as it will be discussed later in this Introduction.
1.3. The “modern” Broca’s area

1.3.1. Anatomical description

The previously described anatomical parcellation within Broca’s area has been validated and completed by studies using modern methods such as DTI (Anwander et al., 2007; Klein et al., 2007) and quantitative receptor autoradiography (Amunts et al., 2010). The latter is a particularly interesting mapping tool since it enables the generation of a “functional” parcellation based on the distribution of receptors for different neurotransmitters; the rationale is that a similar receptor pattern could signal a similar function (Zilles and Amunts, 2009). Using this method, Amunts and collaborators have proposed a novel parcellation map of Broca’s region (Amunts et al., 2010). The authors have evidenced a subdivision of BA44 into a dorsal and a ventral parts (BA44d and BA44v), and a subdivision of BA45 following an antero-posterior gradient (BA45a and BA45p) (Amunts et al., 2010) (Figure 1.10). This complex segregation within the IFG could explain why so many functions have been attributed to Broca’s area as we will see later in this Introduction. This new parcellation of Broca’s area should be taken into account to interpret the results of functional studies even if the spatial resolution of these methods might still be insufficient to target a precise sub-region of BA44 or BA45 (But see Molnar-Szakacs et al., 2005).

![Figure 1.10: New parcellation of Broca’s area.](image)

Extent of delineated areas projected to the lateral surface of an individual postmortem brain. From (Amunts et al., 2010).
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Currently, the consensus is that Broca’s area is composed of BA44 and BA45, roughly corresponding to *pars opercularis* and *pars triangularis*, excluding BA47 (*pars orbitalis*) and BA6 (PMC). Recent support comes from the aforementioned quantitative receptor architectonic study in which a hierarchical cluster analysis revealed that the Euclidean distance between areas 44 and 45 is very small indicating a highly similar receptor-architectonic organization. Conversely, the Euclidean distance between this BA44/BA45 group and BA47 as well as BA6 is large, suggesting different functional properties (Figure 1.11) (Amunts et al., 2010). Thus, it seems unlikely that either BA47 or BA6 are part of Broca’s area.

![Figure 1.11: Hierarchical cluster analysis of the posterior inferior-frontal areas.](image)

Euclidean distances were calculated as a multivariate measure for inter-area differences. A small Euclidean distance between areas, e.g., between areas 44 and 45 or areas op8 and op9, indicates a high similarity in their receptor architectonic organization. The graph shows that areas 47 and 4 differ maximally from the group of areas. Areas 44 and 45 were not divided in 44d and 44v, or 45a and 45p, because all these areas were not present in all brains studied here. From (Amunts et al., 2010).

Aware of these considerations, we should point out that, for the sake of clarity, the term Broca’s area that includes BA44/*pars opercularis* and BA45/*pars triangularis* will be most often used across the present work, although sometimes the term IFG is used as a synonym. Since among the studies we refer to, some provided details regarding the part of Broca’s area more precisely involved in the task at hand, such details will be specified whenever possible.
1.3.2. Connectivity of Broca’s area

If, in non-human primates, tracer experiments are the gold standard for studying connectivity, it was difficult to study more precisely and non-invasively the connectivity in the human brain until the tractography procedure emerged, exploiting the development of novel diffusion imaging methods to map white matter tracts. Indeed, DTI (Le Bihan et al., 2001) is a particular MRI protocol based on the degree of difference of the diffusion of water molecules in neural tissue, providing images of the structural connectivity. With this technique, in addition to the well-known AF, another tract connecting Broca’s area was first evidenced (Catani et al., 2005). This tract is referred as to an indirect pathway because it connects Broca’s area to Wernicke’s area via a third region, Geschwind’s territory (in the inferior parietal lobe) and via two distinct segments: the anterior and posterior (Figure 1.12).

![Figure 1.12: Tractography reconstruction of the AF.](image)

Numbers indicate the cortical targets of the different segments: 1, superior temporal lobe; 2, middle temporal lobe; 3, inferior frontal and precentral gyrus; 4, middle frontal and precentral gyrus; 5, supramarginal gyrus; 6, angular gyrus. From (Catani and Mesulam, 2008).

It should be mentioned that Wernicke proposed that, in addition to the AF, a more ventral pathway for language may exist but his proposal was rejected and forgotten (see Weiller et al., 2011 for a historical account about this ventral pathway). Modern DTI studies (Croxson et al., 2005; Anwander et al., 2007; Frey et al., 2008; Glasser and Rilling, 2008; Saur et al., 2008; Friederici, 2009) exploring the Broca’s region have completed and detailed its connectivity pattern and, importantly, have confirmed the presence of a ventral pathway. Even if still controversial (see Bernal and Ardila, 2009; Bernal and Altman, 2010), the consensus view is

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6 Bernal and collaborators suggest that the AF does not connect the Broca’s area but rather the premotor cortex.
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that Broca's area is connected to Wernicke’s area in the inferior parietal lobe (IPL) and to several regions in the temporal lobe, the superior (STG) and middle (MTG) temporal gyrus as well as the superior temporal sulcus (STS), via two pathways\(^7\), a dorsal and a ventral one (Figure 1.13). The dorsal pathway connects Broca's area to the temporal lobe either directly or indirectly through the IPL via the superior longitudinal fasciculus (SLF) that includes the classical AF. The ventral pathway connects Broca’s area to the temporal lobe via the uncinate fasciculus (UF) and/or the extreme capsule fasciculus (ECF).

Figure 1.13: Fiber tracking of DTI data for the IFG (frontal cluster) and STG/STS/MTG (temporal cluster) regions.

The tracking reveals that these regions (in red) are connected via two separate white matter pathways, a dorsal connection via AF/SLF (in yellow) and a ventral connection via the UF/ECF (in blue). The pathways are shown onto the transparent smoothed white matter skeleton. The temporal cluster includes indications for the borders between STG, STS, and MTG. From (Brauer et al., 2011)

Moreover, it appears that BA44 and BA45 exhibit a different connectivity pattern (Rogalsky and Hickok, 2010; Friederici et al., 2011a): whereas BA44 is mainly connected via the dorsal-AF/SLF pathway, BA45 is mainly connected via the ventral-U/ECF pathway. Broca’s area is also connected with the medial frontal cortex (Ford et al., 2010) and again, differently for its two parts: whereas BA45 is connected to the prefrontal cortex (BA8 and BA9), BA44 is connected to the supplementary motor area (SMA) and pre-SMA (Ford et al., 2010). Alltogether, parcellation based on connectivity is appropriate to subdivide Broca’s area and generally, the parcellation data obtained from diffusion imaging studies in humans are in accordance with monkey tracer literature (e.g. Petrides and Pandya, 2002).

\(^7\) Details about the functionality of these two pathways are given in section 2.2.
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2. The current linguistic account of Broca’s area

2.1. Speech production: an update

While elocution deficits in Broca’s aphasic patients have provided non-negligible evidence for the involvement of Broca’s area in speech production, this view is corroborated by speech arrest induced either by electrical or magnetic stimulation of this area. However, as we will see, Broca’s area is neither totally irreplaceable nor unique for speech production. Indeed, data from clinical studies of patients with damage to Broca’s area have raised the need to re-evaluate the function of Broca’s area and more recent studies, especially functional imaging ones, have emphasized that speech production requires a complex network of additional areas.

2.1.1. Speech arrest following Broca’s area stimulation

Initiated by Penfield and collaborators (see section 1.1.2.), the technique of electro-cortical stimulation is still used nowadays as a mapping approach (Tharin and Golby, 2007) to preserve as much as possible the speech faculty after a surgery that requires tissue resection, for instance the removal of tumours or epileptic foci\(^8\). An important side effect of electrical stimulation protocols is that they can induce intraoperative seizures (Szelenyi et al., 2007). Recently, a case-report study dealing with a patient suffering from a brain tumour located near Broca’s area has demonstrated that such a seizure risk could be reduced by using weaker stimulation (14.5 ms, 1-2 Hz rather than 1-3 s, 50 Hz as in the classic protocol) (Axelson et al., 2009), improving the clinical use of this method. Importantly, results from recent electrical stimulation studies demonstrate that if speech arrest could be obtained following the stimulation of several cortical sites, it is most reliably induced by stimulating the posterior part of Broca’s area (left BA44, pars opercularis) (Figure 1.14) (Ojemann et al., 1989; Duffau et al., 2003; Ojemann et al., 2008).

\(^8\) Sometimes patients suffer from drug resistant epilepsy and a removal of the epileptic foci is the last available treatment.
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![Cortical sites for which the electrical stimulation induced speech arrest.]

Figure 1.14: Cortical sites for which the electrical stimulation induced speech arrest. Cortical sites for which electrical stimulation induced speech arrest according to Ojemann and collaborators (1989) (the circled number indicates the percentage of subjects in whom language impairments were evoked after stimulation of that site). From (Purves et al., 2005).

Magnetic stimulation has also proven effective to induce speech arrest, so that the possibility of using TMS as an alternative tool to localise speech production centres in preoperative patients has been considered (Pascual-Leone et al., 1991). However, its clinical usefulness has been challenged, mainly because of a lack of sensitivity in some studies (Jennum et al., 1994; Michelucci et al., 1994). However, with technological advances in the TMS field - more focal coils and more powerful protocols of stimulation - it has been demonstrated (Stewart et al., 2001b; Aziz-Zadeh et al., 2005) that in fact, speech arrest can be obtained in either the left or right hemisphere if the stimulation is applied over the most posterior part of the IFG, but only in the left hemisphere if stimulation is applied over its most anterior part. To explain these observations, it has been assumed that the more posterior part may correspond to a portion of the ventral premotor cortex (PMv) responsible for controlling the orofacial musculature, while the more anterior part may correspond to the posterior portion of Broca’s area i.e. the left BA44, pars opercularis, not controlling the motor aspects of speech production but rather controlling the articulatory plans for speech (Petrides et al., 2005; Devlin and Watkins, 2007). The two areas are connected (Greenlee et al., 2004).

Importantly, it is possible to rule out the possibility that speech arrest is simply due to a motor disturbance, caused for instance by a spread of the magnetic stimulation to the region controlling the orofacial musculature. Indeed, while Stewart and collaborators (2001b) actually altered with TMS the subject’s ability to speak aloud corresponding to an overt speech arrest, Aziz-Zadeh and coworkers (2005), using a syllable counting task, demonstrated that rTMS over Broca’s area also induced covert speech (internal speech) arrest. This finding corroborates the crucial role of this region in “the organization of language production” (Aziz-Zadeh et al., 2005).

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9 Concerning the determination of hemispheric language dominance, TMS over-estimated the implication of the right hemisphere in comparison with the Wada test (Devlin and Watkins, 2007; Epstein et al 2000).
2.1.2. Speaking without Broca’s area

Broca’s aphasia is untreatable per se (Varona et al., 2004; Varona, 2010). However, in the period of time following brain injury, there is generally a slight spontaneous recovery and an individualized therapy can be set up, depending on the state of the patient. Generally, following a brain lesion, a recovery of the impaired function is possible through the reorganization of neural connections, a process known as “plasticity”. However, due to the persistence of long-term deficits and to the strong influence of the localisationist view considering Broca’s area as a crucial centre for language, it has been commonly thought that in cases where the so-called “eloquent area” was lesioned, plasticity was drastically limited and principally confined to the areas in close proximity to the lesioned one (Rijntjes and Weiller, 2002). Challenging results come from clinical studies on patients suffering from low grade glioma (LGG) who have to undergo a surgical ablation of the tumour. It has been observed that, unexpectedly, the deficits subsequent to LLG surgery could be imperceptible, and even more surprisingly, even in cases with large cortical resections and even if the resection involved an “eloquent” region such as Broca’s area, many patients still benefitted from intact linguistic faculties (Duffau et al., 2002; Benzagmout et al., 2007; Wu et al., 2008; Plaza et al., 2009) (Figure 1.15).

![Figure 1.15: Tissue resection in Broca’s area.](image)

Complete resection of a low-grade glioma localised in Broca’s area. The patient has no neurological deficit pre- and post-operatively. A. Preoperative anatomical MRI showing an low-grade glioma infiltrating Broca’s area (pars opercularis et pars triangularis). B. Intraoperative view before tumour resection. C. View after resection. Electrical mapping shows a redistribution of eloquent areas, with recruitment of perilesional sites (numbered tags). The resection was performed in depth until the associative pathways of language, especially the anterior part of the inferior fronto-occipital fasciculus (50, its electrical stimulation leads to a semantic paraphasia) and the anterior part of the arcuate fasciculus (40, its electrical stimulation leads to phonological deficits). From (Bonnetblanc et al., 2006).
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Functional reorganization after LGG involving Broca’s area is possible because compensatory mechanisms probably start coincident with the tumour development and continue after the surgery (Desmurget et al., 2007). These mechanisms consist of a shift of functions to ipsilateral areas either adjacent (perilesional) (Meyer et al., 2003; Benzagmout et al., 2007) or more distant (Duffau et al., 2002) and also to contralateral regions (Holodny et al., 2002; Wu et al., 2008). Furthermore, these mechanisms could be exploited to improve the quality of life of LGG patients by using a protocol of fractionated resections (Wu et al., 2008): by proceeding with a step-by-step removal compensatory mechanisms take place progressively, so that the ultimate deficits remaining after the complete resection are drastically reduced. Altogether, the LGG literature forms a particularly interesting contribution to the understanding of brain plasticity (e.g. Bonnetblanc et al., 2006; Desmurget et al., 2007) making surgical resection feasible in eloquent region, including the Broca’s area (Lubrano et al., 2010).

In conclusion, the speech faculty can subsist without Broca’s area and the recovery from Broca’s area lesions varies according to the speed at which the lesion developed and, hence, to some extent, according to the damage’s etiology. While a lesion induced by stroke is sudden and results in severe long-lasting deficits and poor recovery mechanisms mainly limited to peri-lesional areas, progressive lesions such as LGG allow the development of compensatory mechanisms in other areas.

2.1.3. Speech production network

If data coming from functional imaging studies have unsurprisingly shown an activation of Broca’s area during speech production (e.g. Blank et al., 2002) (for reviews Munhall, 2001; Price, 2010), many other cortical areas have been found recruited as well. These include premotor and motor areas, insula and temporal cortex (Levelt, 2001; Indefrey and Levelt, 2004) as well as subcortical structures such as the basal ganglia and cerebellum (Booth et al., 2007). Additionally, speech production involves several levels of processing, from conceptualization to articulation (Levelt, 1999; Indefrey and Levelt, 2000; Munhall, 2001) and a meta-analysis of neuroimaging studies of word production (Indefrey and Levelt, 2000) demonstrated that different cortical regions could be associated with these different production processes; the activation of the left inferior frontal regions corresponding to syllabification during phonological encoding. A few years later, the same authors analyzed

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10 Syllabification is the generation of syllabic structures (e.g., syl-la-bi-fi-ca-tion).
again the data of their previous study, but in a way that allowed them to reveal the time course of activations (Indefrey and Levelt, 2004). Activation in Broca’s region appeared from 400 to 600 ms after the occurrence of a given stimulus triggering word production (Figure 1.16); this result confirms and specifies the time course of the contribution of Broca’s area during the syllabification process.

Figure 1.16: Schematic representations of meta-analysis and chronometric data.  
Left: Schematic representation of meta-analysis results for word production. Right: Time course of picture naming as estimated from chronometric data. Identical colours indicate relations between regions and functional processing components (right column). The numbers indicate the time windows (in milliseconds) during which the regions are activated in picture naming. Further regions involved in phonetic encoding and articulation are the right sensorimotor cortex, the right SMA, the left and medial right cerebellum, the left and right thalamus, and the right midbrain. A further region involved in self-monitoring is the right mid superior temporal gyrus. From (Indefrey and Levelt, 2004).

2.1.4. Speech comprehension

Broca’s area plays also a significant role in language comprehension (reviewed in Bookheimer, 2002; Vigneau et al., 2006). Some evidence is mentioned here. First, patients with lesions in Broca’s area have been found to be impaired in determining the meaning of syntactically complex sentences, typically passive sentences that exhibit a non-canonical and semantically reversible structure (“It was the cat that the dog chased”) (Caramazza and Zurif, 1976; Caplan, 2006). Second, deficits in language comprehension have been reported following the electrical stimulation of the Broca’s area and particularly in response to “complex auditory verbal instructions and visual semantic material” (Schaffler et al., 1993). Also, Broca’s area has been identified by several neuroimaging studies as being more activated during the processing of complex sentences (sentences with object relatives rather than subject relatives) (Just et al., 1996; Dapretto and Bookheimer, 1999).
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2.2. Broca’s area and language organization in the brain

The study of language has become extremely developed and concerned with multiple language-related aspects. Currently, language literature splits the discipline into several levels (Carroll, 1999):

- phonetics\textsuperscript{11}: the study of the sounds of speech, considers the sound as a physical entity and thus concerns the acoustic mechanisms involved in their production, transmission and perception;
- phonology: the study of the use of sound speech, considers the sound as a functional entity and thus concerns their linguistic relevance in a given language;
- syntax: the study of the rules for combining the units of language into sentences;
- semantics: the study of the meaning of words and sentences;
- pragmatics: the study of the social use of language for relating meaning to communicative intention.

Additionally, recent imaging studies in healthy volunteers have evidenced that, apart from classical core language areas (for reviews Vigneau et al., 2006; Hickok, 2009a; Price, 2010), language processing activates additional cortical as well as subcortical structures (Binder et al., 1997; Crosson, 1999 and Crosson et al., 2007), extending the identified cortical network of language organization (Hickok and Poeppel, 2007; Hickok, 2009a).

2.2.1. The modelisation of language processing

Hickok and collaborators (Hickok and Poeppel, 2000, 2004, 2007; Saur et al., 2008) proposed a model that includes two main pathways for language processing: 1) a dorsal pathway connecting the superior temporal and premotor regions through the AF/SLF and activated during production and 2) a ventral pathway connecting middle/inferior temporal regions and the vlPFC via the ECF, activated during comprehension (Figure 1.17).

\textsuperscript{11} Note that the distinction between phonetics and phonology is not always made.
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Two broad processing streams are depicted, a ventral stream for speech comprehension that is largely bilaterally organized and which flows into the temporal lobe, and a dorsal stream for sensory-motor integration that is left dominant and which involves structures at the parietal–temporal junction and frontal lobe. ATL: anterior temporal lobe; Aud: auditory cortex (early processing stages); BA 45/44/6: Brodmann areas 45, 44, & 6; MTG/ITG: middle temporal gyrus, inferior temporal gyrus; PM: pre-motor, dorsal portion; SMG: supramarginal gyrus; Spt: Sylvian parietal temporal region (left only); STG: superior temporal gyrus; red line: Sylvian fissure; yellow line: superior temporal sulcus (STS). From (Hickok, 2009a).

Regarding this dual stream organisation for language, it is tempting to make the analogy with the organization of other systems such as the visual system involving a dorsal “where” stream and a ventral “what” stream (see Weiller et al., 2011).

Even if to date there is no consensus, an emerging view from available data is in favour of a specialization of different anatomical areas for the different sentence processing levels. According to Sakai (Sakai, 2005), the left IFG and mainly BA44, is considered as a “grammar centre”, responsible for syntactic processing, in contrast to the more anterior part of IFG that is thought to mediate semantic processing (Figure 1.18A, 1.18B).

(A) The exact correspondences between the left (L) brain regions and linguistic factors are still under study. (B) The green region (the left pars triangularis/pars orbitalis) is selectively activated in the comprehension of sentences, whereas the red regions (the left lateral premotor cortex, the left dorsal IFG, and the left pars opercularis/pars triangularis) are specifically involved in syntactic processing and can be regarded as the grammar centre. From (Sakai, 2005).
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It is worth noting that several hypotheses have been proposed to explain the role of Broca’s area in sentence processing (see Rogalsky et al., 2008) (see also section 3.4.2.) and especially that Broca’s area could be involved in processes linked to syntactic processing such as processes for managing long distance dependencies (Grodzinsky, 2000) and working memory processes (e.g. Martin, 2003).

2.2.2. Broca’s area as the core region for syntax: origin and evidence

In the literature, even if a particular emphasis has been put onto the possible role of Broca’s area in syntactic processing (Grossman, 1980), this issue is still debated (Tyler et al., 2011). The idea that Broca’s area could have a role in syntactic processing comes originally from the clinical literature dealing with Broca’s aphasics. As described before, such patients produce “telegraphic” speech due to the fact that they generally omit function words that are essential to generate syntactically well-structured sentences (Saffran et al., 1980; Friedmann, 2006; Hillis, 2007). However Broca’s aphasics do not suffer only from production deficits; they also exhibit comprehension deficits, especially when comprehension relies on information being extracted from complex syntactic structures (Caramazza and Zurif, 1976; Schwartz et al., 1980). Syntactically complex sentences are typically passive sentences (“The cat was chased by the dog”), object-relative sentences (“The cat that the dog chased”) or even object-cleft sentences (“It was the cat that the dog chased”) that require additional grammatical processing because in such sentences, words are not in a canonical order (they are reversed relative to their assigned thematic roles). These findings have offered at the same time a new interpretation for agrammatism in aphasic patients that for many years was considered as merely a consequence of an articulatory deficit; with Caramazza and Zurif’s work, this agrammatism could rather reflect a syntactic deficit. Additionally, the fact that only Broca’s aphasics presented syntactic deficits (when compared with Wernicke’s aphasics that generate meaningless but fluent and grammatically correct sentences) has also contributed to reinforce the idea that syntax could be impaired specifically by a lesion of Broca’s area and, consequently, that this area is specialized in syntactic processing.

A great amount of additional studies on aphasic patients and later, an impressive number of imaging studies, have provided evidence that Broca’s area effectively contributes to processing syntactic information (Caplan et al., 1998; Dapretto and Bookheimer, 1999; Caplan et al., 2000; Embick et al., 2000; Friederici et al., 2000b; Hashimoto and Sakai, 2002; Suzuki and Sakai, 2003; Friederici et al., 2006b; Sahin et al., 2006; Kinno et al., 2008; Momo et al., 2008;
Iijima et al., 2009). Importantly, data from imaging studies suggest that the involvement of Broca’s area is related to the level of syntactic structure: the more syntactically complex sentences are, the higher the activation (Just et al., 1996; Caplan et al., 1998; Constable et al., 2004; Peelle et al., 2004; Friederici et al., 2006b; Meltzer et al., 2010). Event Related Potential (ERP) studies have also been of particular interest in investigating syntactic processing; amongst language-related ERP components, specific syntactic-related components have been identified:

- the P600 (e.g. Osterhout, 1997) or SPS (syntactic positive shift) (Hagoort et al., 1993), linked to syntactic abnormalities such as grammatical errors;
- the MMN (mismatch negativity), related to morphosyntactic violations (Naatanen et al., 1978; Naatanen, 2000);
- the LAN (left anterior negativity) or ELAN (early LAN) (Kluender and Kutas, 1993; Hahne and Friederici, 2002), associated with errors in local phrase structure organization.

Importantly, for the two latter ERP, the neural generator has been identified in IFG (MMN, (Pulvermuller and Shtyrov, 2003); ELAN, (Friederici et al., 2000c)). Together with clinical and imaging studies, these ERP findings have contributed to establish the syntactic function of Broca’s area (Hagoort, 2003).

The literature about the syntactic role of Broca’s area is abundant. Therefore, as it is not the topic of the present thesis, we limited this section to some critical studies. On the one hand, concerning clinical studies in Broca’s aphasics, given the facts that 1) there is not a complete correspondence between Broca’s aphasia and Broca’s area lesions (some patients suffering from “Broca’s aphasia” do not have lesions in Broca’s area and vice versa); 2) lesions are often large, generally not restricted to Broca’s area and 3) recovery and plasticity may occur, it is difficult to determine reliable causality relationships between deficits and Broca’s area dysfunctions. On the other hand, concerning functional imaging studies, given the facts that 1) they have found activations in Broca’s area for a great number of linguistic tasks apart from syntactic ones (see the following section 2.2.3.); 2) such findings provide evidence about the involvement of Broca’s area in these different processes but do not tell us anything about the causal role of this area and 3) in many studies, the task design to test syntactic processing also involved other higher cognitive functions such as working memory and semantics, the conclusions about the role of Broca’s area in syntactic computation are limited and unclear. Therefore, in the present section, we omit many clinical and functional imaging studies to prioritize recent studies providing stronger causal evidence.
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First, studies supporting such causal evidence are studies investigating focal and temporary lesions, especially interferential TMS studies. Indeed, TMS causes temporary “lesions" i.e. the stimulated area becomes dysfunctional and thus, if task performance is impaired, it indicates that this area is essential to the function tested (see the appendix at the end of the manuscript for a methodological description of TMS). For the first time, in the TMS study of Sakai and collaborators (2002), a causal link between cortical activity in Broca’s area and syntactic processing was established. Indeed, the authors have evidenced a priming effect\textsuperscript{12} for processing sentences requiring syntactic decisions (compared with those requiring semantic decisions) when Broca’s area was under TMS (Sakai et al., 2002). Another type of temporary “lesion” of Broca’s area is that caused by a transient ischemia i.e. a temporary hypoperfusion. The case of a patient with a transient ischemia in Broca’s area has enabled researchers to identify which language functions are affected by the switch from a functional to a dysfunctional Broca’s area, and by the inverse shift. The linguistic functions concerned were “production of grammatical sentences, comprehension of semantically reversible (but not non-reversible) sentences, spelling, and motor planning of speech articulation” (Davis et al., 2008). Similarly, Kinno and collaborators (2009) found that patients with tumours in Broca’s area presented syntactic impairments i.e. higher error rates in a picture-sentence matching task than matched patients with tumours located in other parts of the left frontal region, demonstrating that a focal lesion to this precise region causes syntax-specific deficits (Kinno et al., 2009).

Second, despite the limitations of functional imaging studies exposed previously, we wanted to relate the case of an outstanding one. This study employed an elegant paradigm combining different measures - functional activity, grey matter integrity and performance - in a task that consisted of listening to syntactically ambiguous or unambiguous sentences. Two groups were tested, a group of patients presenting any left hemisphere damage and a matched group of healthy subjects. Correlation analyses between the different measures in both groups demonstrated that left IFG plays an essential role within the syntactic processing network (Tyler et al., 2011). Corroborating findings arise from very recent studies (Galantucci et al., 2011; Wilson et al., 2011) in patients suffering from primary progressive aphasia\textsuperscript{13}

\textsuperscript{12} A well-known effect which reflects the influence of a stimulus over the processing of a subsequent one (e.g. Fischler and Bloom, 1980).

\textsuperscript{13} Primary progressive aphasia is a neurodegenerative disease characterized mainly by progressive language disorders. According to the linguistic impairments observed in these patients, three variants have been defined: non-fluent, semantic and logopenic. Patients diagnosed with PPA exhibit mainly left hemisphere lesions. For recent reviews, see (Gliebus, 2010; Grossman, 2010; Gorno-Tempini et al., 2011).
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(Mesulam, 1982, 2001, 2007) but importantly, these studies also demonstrated the importance of white matter tracts for syntactic processing in addition to the IFG region. Interestingly, both DTI-based studies (Galantucci et al., 2011; Wilson et al., 2011) demonstrate that damage to the dorsal AF/SLF tract results in syntactic deficits, either in comprehension or in production (Wilson et al., 2011), and affects specifically the “non-fluent” variant of patients, a result that matches with the typical agrammatism that characterizes this type of patients (e.g. Grossman, 2010). This finding is also in agreement with earlier fMRI studies which evidenced that while the ventral pathway could be involved in semantic processing (Saur et al., 2008), the dorsal pathway could be crucial for syntactic processing (Bornkessel et al., 2005). Finally, this distinction is also in accordance with the possible functional specificity of “ventrally connected” BA45 and “dorsally connected” BA44, for semantic and syntax processing respectively, recently under debate14 (Friederici, 2009; Kelly et al., 2010).

Importantly, findings that account for the involvement of Broca’s area in syntax come from studies investigating the acquisition of natural (native or additional) language (Kim et al., 1997; Bookheimer, 2002; Friederici, 2002; Kaan and Swaab, 2002; Sakai et al., 2004b; Kotz, 2009; Nauchi and Sakai, 2009; Sakai et al., 2009) or artificial languages. Studies about artificial language, mainly artificial grammar learning (AGL) work, is of particular interest. Classically, an AGL task consists of two phases: a training phase during which subjects implicitly acquire a rule from a repeating pattern in a sequence and then, after informing the subjects about the presence of a rule, a testing phase during which subjects have to use the previously learned rule to classify novel sequences as correct or not according to the rule. Classification performance reflects the implicit learning of this artificial grammar. The aim of AGL paradigms is to simulate or reproduce the process of language learning through an artificial made-up language. Functional imaging studies (Seger et al., 2000; Petersson et al., 2004; Forkstam et al., 2006) have revealed that such tasks recruit a cortical network including the prefrontal cortex, the anterior cingulate cortex, the inferior parietal cortex and parts of the occipital and temporal cortex (Fletcher et al., 1999; Seger et al., 2000; Skosnik et al., 2002; Lieberman et al., 2004; Petersson et al., 2004; Floel et al., 2009). Some of these studies put a particular emphasis on Broca’s area (Petersson et al., 2004; Forkstam et al., 2006; Floel et al., 2009). Of particular interest is the fMRI study conducted by Bahlmann and collaborators (2008). The authors compared the activations for the processing of simple structures, so-called “adjacent

14 An interesting critical discussion of diffusion imaging findings and possible solutions through resting state functional connectivity (the spontaneous activity during rest or, in other words, the residual brain activity in the absence of any task (Greicius et al., 2009)) can be found in the study of Kelly and collaborators (2010).
dependencies”, vs. center-embedded structures, so-called “hierarchical dependencies”, and found greater activations in Broca's area for the latter type (Bahlmann et al., 2008) (Figure 1.19).

![Diagram of adjacent and hierarchical dependency rules](image)

Figure 1.19: Structure of the two rule types and BOLD response on the main effect hierarchical vs. adjacent rule.

Upper part: The adjacent dependency rule was generated by simple transitions between categories of consonant-vowel syllables. The hierarchical rule was produced by embeddings between the two syllable categories. Short and long sequences were applied. Violations of the structure were situated at the 3 or 4 positions (short sequences) and at the last 4, 5, or 6 positions (long sequences). In the given example, the violations are placed at the 4 position for short sequences and at the 6 position for long sequences (bold letters).

Lower part: Brain activation pattern elicited by the contrast vector of the hierarchical dependency rule versus the adjacent dependency rule. IFG = inferior frontal gyrus; F/IT = fusiform/inferior temporal gyrus. ROI analysis of the different variables obtained in the left BA 44/6. Hierarchical rule (dark gray) shows a higher BOLD response in comparison to adjacent dependency rule (light gray) in L BA 44/6, in grammatical, short sequences (gram/short); ungrammatical, short (ungr/short); grammatical, long (gram/long); and ungrammatical, long sequences (ungr/long). From (Bahlmann et al., 2008).

A recent clinical study has shown that aphasic patients with lesions in Broca’s area, experiencing aggrammatism, also presented performance impairments in an AGL task (Christiansen et al., 2010b). Conversely, performance improvements for AGL tasks have been obtained in healthy subjects following the stimulation of Broca's area (left BA44/BA45), either by means of offline repetitive TMS (rTMS) (Udden et al., 2008) or by means of electrical stimulation (de Vries et al., 2010). For instance, in the latter study, the stimulation of Broca’s area with a transcranial direct current during the acquisition phase of an AGL paradigm
increased performance during the subsequent classification phase (de Vries et al., 2010). Very recently, Pallier and collaborators showed that the activity in Broca’s area increases proportionally with the increase of units composing the structure of a sentence, suggesting that this region contributes to the elaboration of syntactic structures assembling words into sentences (Pallier et al., 2011).

Additionally, performance in syntactic processing has been shown to depend on the integrity of the white matter arising from Broca’s area (Floel et al., 2009) and more precisely on its effective connection with MTG (Papoutsi et al., 2011), an area that has repeatedly been found co-activated with Broca’s area for AGL task (e.g. Rodd et al., 2010; Tyler et al., 2010a; Tyler et al., 2011).

However, despite the amount of evidence that supports Broca’s area as the seat of syntax, this view is not fully accepted due to some contradictory findings. First, the recent literature on aphasic patients demonstrates that damage to Broca’s area does not necessarily lead only to syntactic deficits; semantic deficits are also observed (Bushell, 1996)). This literature also demonstrates that syntactic deficits do not arise exclusively from Broca’s area lesions (Dronkers et al., 1994); another region - the anterior temporal lobe (BA38, BA21 and BA22) - is also an important candidate for the seat of syntax since it has been found involved in processing structured sentences (Kaan and Swaab, 2002; Humphries et al., 2006). Second, agrammatic Broca’s aphasics can exhibit preserved grammaticality judgement (Linebarger et al., 1983). Third, among fMRI studies on language, activation of Broca’s area is not uniquely found for tasks requiring syntactic processing (see next section) and, in syntactic processing tasks, Broca’s area seems only activated for complex material (Stromswold et al., 1996; Skipper et al., 2007). Alltogether, it suggests that Broca’s area could be rather sensitive to increases in memory or processing load during either sentence comprehension (Just et al., 1996; Kaan and Swaab, 2002) or production (Indefrey et al., 2001b; Indefrey et al., 2001a). Alternatively, Broca’s area could play a role in a very restricted aspect of syntax, namely “syntactic movement” as proposed by Grodzinsky (Grodzinsky, 2000; Santi and Grodzinsky, 2007; Grodzinsky and Santi, 2008; Santi and Grodzinsky, 2010). The “syntactic movement” hypothesis was initially formulated to account for data showing that some syntactic operations remained unimpaired in Broca’s aphasic patients (Grodzinsky and Finkel, 1998). The idea for Grodzinsky and co-workers is that Broca’s area could subserve a peculiar type of syntax: “Though likely multi-functional, the specialty of this brain region in the domain of sentence processing is syntactic movement” (Santi and Grodzinsky, 2010). Syntactic movement refers to the trace-deletion hypothesis: when words appear in a sentence in a non-canonical order, it...
means that some words are removed from their canonical position where their interpretation is directly accessible and displaced farther along in the sentence, leaving “traces” at the canonical place that substitute for the words at their new location (e.g. the active sentence “the dog catches the ball” becomes “the ball is caught (trace of the ball here) by the dog). In this example “the ball” has been moved but still benefits from its thematic role due to its trace position. However, it seems that this hypothesis could not account for all findings (Willems and Hagoort, 2009).

A related view is that of Hagoort’s team, namely “semantic unification” (Hagoort, 2003; Hagoort et al., 2004). According to this view, Broca’s area could be involved, via syntactic rule use, in unifying or merging units of information i.e. specific word meanings into general ones. Most importantly, Hagoort develop an extended model (Hagoort, 2005), in which unification follows a posterior(-dorsal; BA44 and BA45) / anterior (-ventral; BA47 and BA45) gradient for processing syntax and semantics respectively.

Finally, a third view assume a role of Broca’s area in more general (i.e., not language specific) functions typical of the prefrontal cortex (Miller, 2000) such as the selection among competitive alternatives (Thompson-Schill et al., 1999; Moss et al., 2005; Nelson et al., 2009), the resolution of syntactic as well as non-syntactic conflict (January et al., 2009), attention (Hampshire and Owen, 2006; Chong et al., 2008a) and working memory (WM) (Badre and Wagner, 2007; Rogalsky and Hickok, 2010). Indeed, considering the latter, when syntactic complexity increases the WM load also increases, so it cannot be claimed unequivocally that this area is activated by the increase in syntactic complexity as opposed to the increase in memory load linked to the processing of more complex structures. More precisely, concerning WM, either Broca’s area supports a domain general WM process (Smith and Jonides, 1999; Mecklinger et al., 2002; Ranganath et al., 2003) or it contributes to syntactic WM, i.e. to sentence processing via verbal WM (Fiebach et al., 2005; Santi and Grodzinsky, 2007). In order to disentangle these hypotheses, several attempts to dissociate WM processes from linguistic ones have been made (Fiebach et al., 2005; Makuuchi et al., 2009; Wright et al., 2011) but difficulties arise from the fact that, as mentioned, any linguistic tasks elicit language-specific processes but also more general processes depending on the cognitive demands. For instance, an fMRI study (Makuuchi et al., 2009) evaluated changes in activation following the processing of sentences for which both the levels of structural complexity and of WM load were varied. The authors showed that “the processes for structure and memory operate separately but cooperatively in the left IFG”; the activity in the left pars opercularis was only affected by the presence of hierarchical structure (the level of WM modified activations in left inferior frontal sulcus) (Makuuchi et al., 2009). In another attempt, Wright and collaborators (2011) found
that activation in the left *pars opercularis* is associated with the morphosyntactic complexity, a finding consistent with the aforementioned role of the left *pars opercularis* or BA44 in syntactic processing.

Finally, meta-analyses could bring important insight to the present issue, as for instance the work of Price (2010) who reported activation foci only from recent imaging studies dealing with different linguistic tasks. The author proposed a subdivision of linguistic labours within Broca’s area, the *pars opercularis* contributing “to sequencing by top-down constraints from prior knowledge of what event typically follows another” (Price, 2010). The author even suggested a functional subdivision of the *pars opercularis* for articulatory sequencing in its ventral part and non-language specific sequencing (see section 3.) in its dorsal part (Figure 1.20).

**Figure 1.20:** Anatomical localisation of language areas and their functions. From (Price, 2010).

### 2.2.3. Others linguistic functions of Broca’s area

Apart from syntax (*e.g.* (Ben-Shachar et al., 2003; Friederici and Kotz, 2003; Friederici et al., 2003; Heim et al., 2003)), the contributions of Broca’s region have also been described for other linguistic levels *i.e.* phonology (*e.g.* (Poldrack et al., 1999; Burton, 2001; Devlin et al., 2003; Nixon et al., 2004; Myers et al., 2009)) and semantics (*e.g.* (Poldrack et al., 1999; Devlin et al., 2003; Thompson-Schill, 2003; Hagoort et al., 2004; Rodd et al., 2005)) (for a review see (Stowe et al., 2005)).
In a recent study (Sahin et al., 2009), it has been shown that Broca’s area is not in charge of a single aspect when processing language but rather processes sequentially several types of information, namely lexical, grammatical, and phonological/articulatory ones. Sahin and collaborators used for the first time in research the intracranial electrophysiology (ICE) technique to investigate the Broca’s area activity related to grammatical speech production through local field potentials, recording with both high spatial (mm) and temporal (ms) resolutions. This method is based on the placement of electrodes in the brains of patients and recording while the patients are awake and able to answer questions. Sahin et al.’s finding was that the time course of linguistic processing could be followed in Broca’s area (Figure 1.21), i.e. this area is recruited during semantic/lexical (~ 200 ms), syntactic/grammatical (~ 320 ms), and phonological (~ 450 ms) processes (Hagoort and Levelt, 2009; Sahin et al., 2009).

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15 These findings have been highlighted in the study of Hagoort & Levelt (2009).

16 Electrode implantation has been performed during surgery with the clinical goal of determining the regions of the brain necessary for language and to spare those regions when removing seizure loci.
Figure 1.21: From intention to articulation.

Shown is an adapted version of the lexical encoding model for speech production (Levelt, 2001), specifying steps in the paradigm used by Sahin et al. Based on the visual input, a lemma is selected that specifies the syntactic features of a lexical concept. For instance, for the lemma “horse”, it specifies that it is a count noun. In addition, the morphemic codes are retrieved. For instance, when the speaker wants to produce the plural form of “horse”, the codes for both the stem and the plural suffix are retrieved. Next, the phonological codes for each morpheme are retrieved, combined, and transformed into a motor command to the articulatory system. The approximate times (in milliseconds) at which Broca’s area contributes to the different processing steps are shown. The late (i.e., at 500 to 600 ms) monophasic component observed in the temporal lobe (Sahin et al., 2009) might reflect self-monitoring of the speech output. From (Hagoort and Levelt, 2009).

Broca’s area is also involved in speech perception. To put this claim in context, it follows the finding that the perception of speech enhances the excitability of the motor cortex (Fadiga et al., 2002; Watkins et al., 2003; D’Ausilio et al., 2009). More precisely, these studies showed that when subjects perceive speech, either by listening or by observing, facilitation is obtained
in muscles corresponding to those implicated in the speech movements. Moreover, in a combined TMS/Positron emission tomography (PET) study, Watkins and Paus (2004) tried to identify the neural substrate responsible for the modulation of the motor system excitability during speech perception. To this end, they correlated the M1 excitability measure, obtained with TMS, with the simultaneous measure of cerebral blood flow, obtained with PET. The authors observed a signal increase in Broca’s area (left BA44), demonstrating that this portion of the Broca’s area could play a role in connecting speech perception and speech production together. More recent studies also account for the involvement of Broca’s area in speech perception. For instance, two fMRI studies (Fridriksson et al., 2009; Turner et al., 2009) showed a greater activation of this area when observing speech and a combined behavioural/TMS/fMRI study (Kotz et al., 2010) demonstrated that 1) the virtual lesion of the left BA44 abolished the difference of the priming effect between words and pseudo-words and 2) the activation of left BA44 was obtained for word pairs only.

The fact that Broca’s area is involved in both speech production and speech perception is not uncontentious. It reinforces the view that speech production and speech perception are tightly linked (see D’Ausilio et al., 2011) and could be viewed as an argument for the influential motor theory of speech perception (see the following section 2.2.4.).

2.2.4. The motor theory of speech perception and Broca’s area

The perception of speech includes the mechanisms of hearing, recognizing, interpreting and understanding the sounds of speech. The motor theory of speech perception hypothesizes that spoken words are processed through the intended gestures of the vocal tract that are responsible for pronouncing them rather than through the produced sound patterns (Liberman et al., 1967; Galantucci et al., 2006). Considering that the role of the motor system is twofold, a related issue is to understand how the mechanisms underlying both speech production and speech perception develop during childhood, given that speech perception is the starting point to language acquisition in infants. Broca’s area has been implicated in this facet of development since a magnetoencephalography (MEG) study demonstrated that, whereas the area is not activated by the perception of speech initially, its activation occurs in 6 month-old infants (Imada et al., 2006), possibly the time period required for the establishment of a perceptual-motor link through experience.

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17 In the control condition, a priming effect is found for word pairs but not for pseudo-word pairs. The effect found here is a priming effect for pseudo-words as well as for words, the latter remaining unaffected.
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Since this theory is closely associated with the mirror neuron system that also posits a link between perception and action, the present topic will be discussed further in section 3.3.

2.3. What about the Broca’s area homologue in the right hemisphere?

Since Pierre Paul Broca’s discovery, the left hemisphere has been considered as dominant for language (i.e., language function is lateralized to the left hemisphere). If Broca’s area (BA44/BA45 in the left hemisphere) is involved in speech production, what about its homologue in the right hemisphere? Does the right BA44/BA45 participate in language? Could this homologue be able to substitute for Broca’s area in case of a left hemisphere lesion? These questions about the role of homologous Broca’s area in the right hemisphere are tightly linked to the issue of the right hemisphere’s contribution to language.

2.3.1. Language dominance, handedness and anatomical asymmetry

Since Broca’s and Wernicke’s discoveries and research on split-brain18 patients, the left hemisphere has been considered as dominant for language and reasoning, while the right hemisphere is rather dominant in visuo-spatial processing and emotion. As a consequence, for many years, the right hemisphere was excluded from language models. The first demonstration that the right hemisphere could intervene in language was brought forth by Wada and Rasmussen in 1960 using intracarotid amobarbital injection, a procedure now known as the Wada test (Wada and Rasmussen, 2007). In 1960, Wada and Rasmussen found that language was processed in the right hemisphere in 4% of right-handed individuals, a proportion that went up to 15% in left-handed individuals. More recent studies confirmed the left-hemisphere dominance for language in a great majority (more than 90% for right-handers, slightly less for left-handers) of the healthy population (Springer et al., 1999; Knecht et al., 2000a; Knecht et al., 2000b; Knecht et al., 2002). The proportion of right-hemisphere dominance is positively correlated to the degree of left-handedness (Knecht et al., 2000b; Medina et al., 2007).

18 The corpus callosotomy carried out in patients suffering from refractory epilepsy has allowed studies that shed light on the function of each hemisphere independently (Gazzaniga, 2005).
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If these findings argue in favour of a relationship between handedness and language dominance, this is not related to linguistic faculty *per se* since in right-handers, language lateralization is not correlated to linguistic task performance (Springer et al., 1999). Individuals have comparable linguistic faculties whatever their right or left hemisphere language lateralization (Knecht et al., 2001). Furthermore, as stated in a recent review “the organization of language in right-hemisphere dominance mirrors that of left-hemisphere dominance” (Chang et al., 2011). This comparable organization - between the left hemisphere in individuals with classical left-hemispheric dominance for language and the right hemisphere in those rare individuals with right-hemispheric dominance for language - is especially true for speech production since in the latter individuals, speech arrest is obtained following stimulations in the homologous region to Broca’s area in the right hemisphere (Chang et al., 2011).

It has been proposed that language dominance should be reflected in anatomical interhemispheric asymmetry (Toga and Thompson, 2003). Historically, the first evidence in favour of such an asymmetry concerned the temporal lobe and more precisely the *planum temporale*, firstly, at the macroscopic level (Geschwind and Levitsky, 1968) and then, at the cytoarchitectonic level (Galaburda et al., 1978), this region being larger in the left hemisphere than in the right. More recent studies confirmed such asymmetries, especially studies based on a novel brain mapping technique that relies on cortical folding patterns and that allows studying the structure and development of the cerebral cortex in humans. Using such a surface-based approach, support has been provided for the presence of a left over right asymmetry near the STS (Van Essen, 2005; Hill et al., 2010) and also importantly, near the Sylvian fissure (Van Essen, 2005); asymmetries that are already present in term infants (Hill et al., 2010). Molecular correlates have also been identified through asymmetries in gene expression during development (Sun et al., 2005; Sun and Walsh, 2006). Concerning Broca’s area more specifically, even though several studies have provided evidence in favour of a left/right asymmetry (Falzi et al., 1982; Chiarelli et al., 1989; Foundas et al., 1998; Amunts et al., 1999; Amunts et al., 2003; Uylings et al., 2006) and for overviews see (Keller et al., 2009b; Amunts et al., 2010)), this point remains controversial, partly because of possible methodological bias in evaluating this morphological asymmetry (Keller et al., 2009b).

Language and motor control appear to be the most lateralized functions in the human brain. Such a link between language and motor aspects is a key element for the gestural theories about the evolution of language (see section 2.4.2.) It assumes that the dominance of the left hemisphere for language could be due to the greater control of this hemisphere for the right hand, and that these efficient hand motor circuits evolved to control linguistic abilities also.
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2.3.2. Right hemisphere contribution, and right IFG specific contribution, to language

Even if the left hemisphere is dominant for language, the fact remains that the right hemisphere also contributes to language. Clinical findings from patients suffering from right hemisphere damage show that such lesions typically cause two classes of communication impairments. The first type is indirect and affects the ability to interact with the environment, such as anosognosia (unawareness of such deficits) and hemineglect (attentional deficit). The second type is direct, causing “pragmatic disorders” that affect the capacity to use and/or understand linguistic formulae according to context (e.g. Stowe et al., 2005) or other’s intention (e.g. prosody) or to understand figurative language using figures of speech such as metaphors (e.g. Mashal et al., 2005) or jokes (e.g. Coulson and Wu, 2005). In addition, in the course of normal aging, neural resources diminish so that the right hemisphere is more extensively recruited during linguistic tasks when compared with younger individuals (Cabeza, 2002).

Concerning more precisely the right IFG involvement in language, modern imaging studies have shown that this region is activated, for instance, by processing emotional prosody (e.g. Friederici, 2001; Mitchell et al., 2003; Ross and Monnot, 2008; Rota et al., 2009) or metaphoric sentences (e.g. Bottini et al., 1994). The right homologue of Broca’s area is particularly implicated during recovery from Broca’s aphasia. From the clinical literature in Broca’s aphasics following stroke, it seems that the contribution of the right area is maximal shortly after the stroke but weakens or disappears at the chronic stage (Saur et al., 2006; Winhuisen et al., 2007; van Oers et al., 2010) and could possibly reflects non-linguistic (WM or executive) processes (van Oers et al., 2010). As mentioned, in tumour cases such as LGG around Broca’s area, the pattern of recovery is different from that for stroke since the shift of linguistic function to the right homologue region seems long-lasting (Holodny et al., 2002; Wu et al., 2008).

2.4. Is Broca’s area unique to humans?

Is Broca’s area unique to humans? If homologues could be defined in other species, do they have the same characteristics as in humans? These questions are of particular interest.

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19 It seems that the two types of prosody have different neural substrates since a right hemisphere dominance has been evidenced for processing emotional prosody (Silberman and Weingartner, 1986) while it seems that both hemispheres contribute to the processing of syntactic prosody (Luks et al., 1998).
because they are tightly linked to the language origin issue (Sherwood et al., 2003; Schenker et al., 2008; Schenker et al., 2010) and lead to another central question: did language appear suddenly in the human species or gradually during the course of evolution through the adaptation of primitive abilities, in particular gestural ones? This question about the evolutionary origin of language remains controversial and has regained interest since the discovery of mirror neurons in monkeys and of the putative equivalent system in humans (Rizzolatti and Arbib, 1998; Rizzolatti and Craighero, 2004) (but see also Toni et al., 2008).

2.4.1. Broca’s area homologue in non-human primates

Searches for a human Broca’s area homologue have been mainly conducted in non-human primates because of their phylogenetic proximity. However, establishing direct correspondences between human and non-human primate brains is nontrivial. “(...) Human, ape, and other anthropoid brains are not simply allometrically scaled versions of the same generalized design” (Rilling, 2006). Using a surface-based registration method, the comparison of the cortical organization between them has revealed a map of cortical expansion showing that both the temporo-parietal junction and the dorsolateral prefrontal cortex (DLPFC) are regions of particularly high expansion in humans (Van Essen, 2004; Van Essen and Dierker, 2007) (Figure 1.22). Therefore, establishing direct correspondences between human and non-human primate brains could be complex, since Broca’s area is part of a region of high development.

Nonetheless, homologous regions to the human BA44 and BA45 have been identified in non-human primate brains (Figure 1.23), including several species such as macaques (Macaca fascicularis, (Petrides et al., 2005) and Macaca mulatta, (Preuss and Goldman-Rakic, 1991))
and great apes (chimpanzees (*Pan troglodytes*), bonobos (*Pan paniscus*), orangutans (*Pongo pygmaeus*) and gorillas (*Gorilla gorilla*) (Cantalupo and Hopkins, 2001; Schenker et al., 2008; Schenker et al., 2010)). For instance, Petrides and collaborators (2005) conducted a study combining quantitative architectonic analyses with electrophysiological recordings of neuron activity and electrical intracortical microstimulation; the authors identified in monkeys a cortical area architectonically similar to the human BA44 and also implicated in orofacial movements (Petrides et al., 2005).

**Figure 1.23:** Photographs of a human brain and a chimpanzee brain with relevant structures marked. Images are not to scale. (A) Human brain and (B) Monkey brain. Abbreviations: ar, ascending ramus of the Sylvian fissure; cs, central sulcus; hr, horizontal ramus of the Sylvian fissure; ifs, inferior frontal sulcus; ipc, inferior precentral sulcus; los, lateral orbital sulcus; ofs, orbitofrontal sulcus; pop, *pars opercularis*; por, *pars orbitalis*; ptr, *pars triangularis*; sf, Sylvian fissure. From (Schenker et al., 2008).

It is worth noting that another nomenclature is also used for the areas 44 and 45 in the monkey, namely area F5; more precisely, the homologue of human BA44 being area F5a (Nelissen et al., 2005) (Figure 1.24).

**Figure 1.24:** Anatomy of agranular frontal and posterior parietal cortex of the macaque monkey.

The central part of the figure shows the cytoarchitectonic parcellation of the agranular frontal cortex (areas indicated with F and arabic numbers) and of the parietal lobe (areas indicated with P and progressive letters). The enlargement of the frontal region (rectangle on the left) shows the parcellation of area F5. The rectangle on the right shows the areas buried within the intraparietal sulcus. AIP, anterior intraparietal area; IP, intraparietal sulcus; LIP, lateral intraparietal area; MIP, medial intraparietal area; POs, parieto-occipital sulcus; As, superior arcuate sulcus; Ai inferior arcuate sulcus; C, central sulcus; Ca, calcarine fissure; CG, cingulate cortex; FEF, frontal eye field; L, lateral sulcus; Lu, lunate sulcus; P, principal sulcus; STS, superior temporal sulcus. From (Rizzolatti and Fabbri-Destro, 2008).
The issue about the identification of a Broca’s area in non-human primates is still a matter of controversy. This is an important question since the presence of a Broca’s area homologue in non-human primate brains leads one to suppose that its original function was not devoted to language. Furthermore, the question has regained interest since the discovery of mirror neurons (see section 3.3.), based on the fact that they could represent a substrate for language evolution (Rizzolatti and Arbib, 1998; Rizzolatti and Craighero, 2004; Pulvermuller, 2005; Galantucci et al., 2006). Broca’s area contains mirror neurons that could be the neural precursor of language, by establishing a link between action and communication.

As previously mentioned, in humans, even if controversial (Keller et al., 2009a), the BA44/45 is larger in the left hemisphere than in the right one (Amunts et al., 1999; Uylings et al., 2006). A left over right asymmetry in non-human primates is controversial too: whereas a study has evidenced such an asymmetry in three great ape species (Pan troglodytes, Pan paniscus and Gorilla gorilla) (Cantalupo and Hopkins, 2001), another study failed to replicate the finding in one of the aforementioned species (Pan troglodytes) (Schenker et al., 2010). The latter result – the absence of asymmetry in chimpanzees - suggests a possible evolutionary link between the appearance of such asymmetry in Broca’s region and the appearance of the human faculty for language.

2.4.2. Language origin and evolution

Human language is considered as the most advanced system of communication and this faculty is taken as evidence for distinguishing humans from other species. The latter use different forms of communication such as body postures, facial expressions, vocalizations, or even olfactory signals that offer a more restricted range of possibilities than oral language. How did language develop? This issue is, and has always been, controversial: did language appear suddenly, or did it evolve from another system of communication either spoken or gestural? (Corballis, 2009b, a). After considering these different questions, this section will end with the contributions of Broca’s area to the whole issue.

Notably, Ungerleider and collaborators have linked the changes, from monkeys to humans, in the neural organization for vision and working memory processes to the emergence of specific regions to support specific cognitive abilities and especially the language (Ungerleider et al., 1998). They have based their hypothesis on the organizational differences of the systems controlling visual and working memory processes between monkeys and humans. In monkeys, for the vision, it has been suggested that it is possible to distinguish two pathways: a ventral
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stream for objects (the “what” stream, involving temporal areas) and a dorsal stream for spatial locations (the “where” stream, involving parietal areas) (Mishkin and Ungerleider, 1982). Importantly, it seems that this organization also applies for working memory and that if both streams appear to be conserved in humans, some differences between the two species are found (Ungerleider et al., 1998). In addition to displacement of temporal and parietal areas in both streams (“In humans, as compared with monkeys, areas specialized for object vision in the ventral stream have a more inferior location in temporal cortex, whereas areas specialized for spatial vision in the dorsal stream have a more superior location in parietal cortex”), the authors mentioned that “whereas areas specialized for object working memory in humans and monkeys are similarly located in ventrolateral prefrontal cortex, areas specialized for spatial working memory occupy a more superior and posterior location within dorsal prefrontal cortex in humans than in monkeys”. They linked these “displacements” in both anterior and posterior perisylvian cortex to “the emergence of language over the course of brain evolution” (Ungerleider et al., 1998).

Currently, the most acknowledged hypothesis is the “gestural theory” (Hewes, 1973; Corballis, 2003; Gentilucci and Corballis, 2006), according to which language may have evolved from manual gestures. Different facts account for this theory such as the tight association between speech and gestural activity (motor representations of speech sounds, action-related language, sign language and co-speech gestures (Willems and Hagoort, 2007)) but also left hemispheric dominance, ontogenetic development (Babkin reflex, hand-mouth coordination, manual and voice prattle), and the motor theory of speech perception (Liberman et al., 1967; Liberman and Mattingly, 1985; Liberman and Whalen, 2000; Galantucci et al., 2006). As already stated, this hypothesis has regained interest since the discovery of mirror neurons since they provide a mechanism for understanding another’s actions. Therefore, human language may have evolved from this system implemented in mirror neurons, with gestures, rather than vocal sounds, being the starting point (Rizzolatti and Arbib, 1998; Gentilucci and Corballis, 2006). This theory is not universally popular (Chomsky, 1975; Bickerton, 1995). With regard to Chomsky, even if his position regarding the gestural theory has recently evolved (Hauser et al., 2002), he has always strongly claimed that something in language remains unique to humans. Importantly, he makes the proposal that there should exist a common property among the different human languages, termed “universal grammar” referring to the ability to understand/produce numerous (theoretically infinite) utterances from both a set (finite) of elements and grammatical rules (Chomsky, 1976).
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What about Broca’s area regarding all these considerations? Some results, in favour of the Chomskian hypothesis, account for a genetic origin for the language faculty and also account for the implication of Broca’s area in functional or dysfunctional language (Corballis, 2004; Cooper, 2006). Such insights come from a particular type of disorders, called specific language impairments (SLI, deficits in identifying sounds of speech, understanding sentences, using syntax, …). These deficits mimic the disorders observed in Broca’s aphasia and have been attributed to a mutation of a single gene, FOXP2 (“forkhead box protein 2”) (Lai et al., 2001; MacDermot et al., 2005). This “language gene” is expressed in Broca’s area so that a mutation of this gene can yield anatomical (Watkins et al., 2002; Liegeois et al., 2003) and functional (Vargha-Khadem et al., 1998; Liegeois et al., 2003) (Figure 1.25c) abnormalities of this area. Furthermore, across mammals, this gene is highly conserved (Figure 1.25a and 1.25b) (Vargha-Khadem et al., 2005) but the human gene presents specific regulations (Konopka et al., 2009).
Figure 1.25: A multidisciplinary perspective on language evolution.

a) Genetics - the genomic structure of human forkhead box P2 (FOXP2), showing the location of mutations that cause verbal dyspraxia, which are distinct from sites of evolutionary substitution in the human lineage (filled rectangles, coding exons; white rectangles, non-coding exons). The red bar indicates genomic regions that show evidence of a selective sweep. Exons encode polyglutamine tracts (Q40 and Q10), a zinc-finger motif (ZnF), a leucine zipper (LeuZ), the forkhead domain (FOX) and an acidic C-terminus (Acidic). s1–s3 are alternatively spliced untranslated 5’ exons. 
b) Evolution - nucleotide substitutions in the FoxP2 coding region for different lineages during primate evolution, shown as non-synonymous over synonymous substitutions (horizontal bars, nucleotide changes over time; shaded bars, amino-acid changes). 
c) Neuroimaging - humans carrying disrupted FOXP2 show functional abnormalities when carrying out a language task, even when producing verb forms mentally rather than aloud. The anomalies involve underactivation of Broca’s area and bilateral activation in multiple cortical regions. The diagram shows the group average activation in the unaffected and affected members of the KE family, which is displayed at a threshold of P<0.05, corrected for multiple comparisons (L, left hemisphere; R, right hemisphere). From (Fisher and Marcus, 2006).

However, Broca’s area is also linked to evidence in favour of evolutive theories. Broca’s area has been found involved in the system of mirror neurons (see section 3.3.) and to a number of gestural activities linked to language (Skipper et al., 2007). Very recently, it has been shown that the activation of the Broca’s area homologue in chimpanzees (Taglialatela et al., 2008) observed during the combined production of vocal signals and gestures, actually results from the production of communicative sounds by the chimpanzees rather than manual communicative gestures (Taglialatela et al., 2011). This suggests that the precursor function of Broca’s area could be vocal signalling rather than gestures (Taglialatela et al., 2011).
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3. Beyond language: the multiple roles of Broca’s area

Despite the fact that Broca’s area was the first cortical area identified and that its role in language has been repeatedly demonstrated, its exact function is still misunderstood, since it is also involved in non language-related tasks. With constant advances in brain investigation techniques, new views about Broca’s area have progressively emerged. The first advance extended its role in the language domain (section 2.) since it is now fully acknowledged that the contribution of Broca’s is not restricted to speech production as originally claimed. Indeed, it has been shown that Broca’s area is also involved in speech comprehension, as well as in different levels of processing including syntax. The second advance came from the fact that this region exhibits implications in a growing number of tasks far removed from the linguistic domain, suggesting that Broca’s area is not exclusively involved in language. Recent studies, predominantly using brain imaging, have obtained activations mapping to Broca’s area even though they were designed initially to study different cognitive functions such as calculation (e.g. Gruber et al., 2001), music (e.g. Maess et al., 2001) or motor-related functions (e.g. Koechlin and Jubault, 2006). All these facts taken from the literature underline the manifold contributions of Broca’s area (Figure 1.26), but also raise the question of the involvement of a main language centre in non-linguistic domains.

Figure 1.26: The multi-faceted Broca’s area.
3.1. Overview of its different implications

Linguistic abilities apart, a wealth of functions have been ascribed to IFG, from WM to various aspects in the action domain, encompassing the mirror neuron system (MNS) and other domains such as music and calculation. An account of such a diversity is illustrated in recent reviews (Fadiga et al., 2009; Burns and Fahy, 2010) and meta-analysis studies (Fedorenko et al., 2011; Liakakis et al., 2011). Table 1.1 lists, non-exhaustively, different tasks for which Broca’s area has been found activated. The roles played by Broca’s area in the action-related domain as well as in MNS are detailed separately, respectively, in section 3.2 and 3.3.

Table 1.1: Some implications of Broca’s area

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<thead>
<tr>
<th>Domain</th>
<th>Tasks</th>
<th>References</th>
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<tr>
<td>Memory</td>
<td>Recognition task</td>
<td>(Smith and Jonides, 1999)</td>
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<td>Delayed matching task</td>
<td>(Mecklinger et al., 2002)</td>
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<td>Rehearsal task</td>
<td>(Ranganath et al., 2003)</td>
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<td>Cognition</td>
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<td>Selection task</td>
<td>(Moss et al., 2005)</td>
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<td></td>
<td>Reasoning task</td>
<td>(Tsujii and Watanabe, 2009; Tsujii et al., 2010; Tsujii and Watanabe, 2010; Tsujii et al., 2011)</td>
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<tr>
<td>Inhibition</td>
<td>Stroop task</td>
<td>(Kemmotsu et al., 2005; January et al., 2009)</td>
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<td></td>
<td>Go/NoGo task</td>
<td>(Swick et al., 2008)</td>
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<td>Music</td>
<td>Abnormality detection task in chord sequences</td>
<td>(Maess et al., 2001; Koelsch et al., 2002; Koelsch et al., 2005; Sammler et al., 2010)</td>
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<tr>
<td></td>
<td>Harmonic / melodic / rhythmic / timbral structures processing task</td>
<td>(for a review see Koelsch, 2006)</td>
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<tr>
<td>Mathematics</td>
<td>Calculation task</td>
<td>(Gruber et al., 2001)</td>
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<tr>
<td></td>
<td>Abnormality detection task in mathematical formulae</td>
<td>(Friedrich and Friederici, 2009)</td>
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3.2. Motor-related functions

There is a growing amount of evidence that Broca’s area participates in motor-related processes (Binkofski and Buccino, 2004; Fadiga et al., 2009). Its contribution to the motor domain encompasses different aspects of motor cognition, from the active production of actions to their perception encompassing imitation and imagery. It is worth noting that some studies can be cited several times because they consist of combined tasks, for instance observation / execution of grasping movements (e.g. Grezes et al., 2003), recognition /
3.2.1. Action observation

Observing an action performed by someone else activates in the observer a set of brain areas including inferior frontal, premotor, parietal and superior temporal cortex (Grafton et al., 1996; Grafton et al., 1997; Chao and Martin, 2000; Hamilton and Grafton, 2006; Caspers et al., 2010). Even if several dozen studies have demonstrated that the observation of actions recruits IFG in humans (For reviews Turella et al., 2009a; Caspers et al., 2010), it is difficult from these studies to draw clear conclusions mainly because of the great number of factors that could vary from one study to another (shooting viewpoint, video/image content i.e. presence or absence of effectors, objects, movements and so on). For instance, Broca’s area has been implicated during observation of actions involving:

- object-hand interactions (Grafton et al., 1996; Rizzolatti et al., 1996b; Buccino et al., 2001; Perani et al., 2001; Johnson-Frey et al., 2003; Manthey et al., 2003; Buccino et al., 2004b; Iacoboni et al., 2005; Molnar-Szakacs et al., 2006; Baumgaertner et al., 2007);
- only the effector: fingers (Cochin et al., 1999; Costantini et al., 2005; Molnar-Szakacs et al., 2005), hands (Iacoboni et al., 1999), faces (Carr et al., 2003), whole body (Calvo-Merino et al., 2005; Calvo-Merino et al., 2006)
- no biological effector (Cross et al., 2009);
- no movement (i.e. static hand-object interaction or object alone) (Grafton et al., 1997; Johnson-Frey et al., 2003);
- motion via point-light display (Saygin et al., 2004).

Moreover, the line between action observation and perception, understanding and interpreting is thin. Studies looking at brain lesions, either real (Tranel et al., 2003; Saygin, 2007; Urgesi et al., 2007a) or virtual (Pobric and Hamilton, 2006; Candidi et al., 2008) that cause action perception/gesture recognition impairments have pointed to a causal link between the integrity of IFG and this ability. For instance, in a combined study, Saygin found that both the data obtained from lesion analyses in patients and fMRI data collected from healthy controls converged to a causal implication of IFG in biological action perception (Saygin, 2007). Similarly, a study has demonstrated that rTMS applied over IFG during a judgement task about the weight of a box lifted by another person impaired the performance of the task, demonstrating that left IFG is necessary to judge accurately other people's actions and therefore to understand them (Pobric and Hamilton, 2006). Another piece of evidence for
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the role of Broca’s area in action understanding, or at least in action interpretation, is that its activation has been highlighted during the observation of meaningful hand shadows that resemble moving animals (Fadiga et al., 2006) or of robotic actions (Gazzola et al., 2007). A very recent study (Molenberghs et al., 2012), using a novel method of analyzing neuroimaging data - the multi-voxel pattern analysis\(^{20}\) (Norman et al., 2006) - demonstrated the implication of the IFG (bilateral pars opercularis) when subjects observe goal directed action with the endeavour to subsequently determine the goal of the observed action. Importantly, in this study, video clips were identical across the different context conditions that determine the task of the subject while observing the action. It allows the authors to claim that IPL and PMv, including the pars opercularis, are implicated in action understanding since these areas respond differently to the observation of actions depending on the intention of the observer. Concerning clinical studies dealing with real lesions in patients, deficit in gesture recognition is a symptom encountered in some patients suffering from apraxia\(^{21}\). However it is noteworthy that, even if fMRI studies (e.g. Villarreal et al., 2008) also support the IFG contribution to gesture recognition, this view is challenged by a recent TMS study demonstrating that a virtual lesion of the left IFG only impaired gesture production, leaving gesture recognition intact (Bohlhalter et al., 2010).

3.2.2. Imitation and imagery

Several studies, especially those using functional imaging, have highlighted the role of IFG during imitation tasks of different body part movements (Iacoboni et al., 1999; Koski et al., 2002; Carr et al., 2003; Koski et al., 2003; Leslie et al., 2004; Iacoboni, 2005; Molnar-Szakacs et al., 2005; Mühlau et al., 2005) (For reviews see Turella et al., 2009a; Caspers et al., 2010). A TMS study of Heiser and collaborators has shown that a virtual lesion of the (left or right) pars opercularis impaired subject’s performances in an imitation task (Heiser et al., 2003). Additionally, other fMRI studies have demonstrated that there was a stronger response in the pars opercularis during mirror imitation comparing to congruent imitation. Notable, however, are the contradictory conclusions of two meta-analyses using the activation likelihood estimation method (Turkeltaub et al., 2002) in order to identify the cortical areas commonly

\(^{20}\) The principle of the MVPA method is to link each brain activity pattern with a particular experimental condition by means of classification algorithms, used to extract statistical regularities from fMRI data.

\(^{21}\) Apraxia is defined as the inability to carry out skilled gestures despite preserved intellectual and physical capacity and most commonly associated to left parietal brain damage (Buxbaum et al 2005; Heilman, Rothi and Valenstein 1982; Weiss et al 2008) even if lesions often extend to left frontal areas (Goldenberg et al 2007; Pazzaglia et al 2008; Tranel et al 2008 but see also Buxbaum and Kalenine 2010; Kalenine, Buxbaum and Coslett 2010).
activated by imitation (Molenberghs et al., 2009) or by imitation and action observation (Caspers et al., 2010). In the first study (Molenberghs et al., 2009), the IFG is not identified as a region involved in imitation though superior parietal lobule, inferior parietal lobule, and premotor cortex are. In the second study (Caspers et al., 2010), the comparison between the imitation and observation networks revealed that imitation activated IFG (BA44) more strongly than observation (Figure 1.27).

![Figure 1.27: Activations for action observation and action imitation.](image)

Significant results for (A) the conjunction and (B) the contrast analysis between the main categories of action observation and action imitation (colour-coding of respective contrasts within the figure). From (Caspers et al., 2010).

The involvement of Broca’s area in motor imagery has also been revealed (Binkofski et al., 2000; Gerardin et al., 2000; Grezes and Decety, 2001, 2002; Johnson et al., 2002) including mental imagery of hand grasping movements (Decety et al., 1994; Grafton et al., 1996) and mental rotation of the hand (Parsons et al., 1995).

### 3.2.3. Action production

Activation of Broca’s area has been reported during the preparation (Krams et al., 1998) and execution (Krams et al., 1998; Binkofski et al., 1999a; Binkofski et al., 1999b; Grezes et al., 2003) of hand movements, such as grasping and object manipulation. TMS studies also revealed the IFG contribution to such actions. For instance, the study of Tunik and
collaborators administered single-pulse TMS to *pars opercularis* and to *pars triangularis* during planning of goal-directed actions; only TMS to *pars opercularis* induced an increased delay of response without affecting its execution (Tunik et al., 2008).

Broca’s area has also been found involved during the execution of sign language in which classical speech sounds are replaced by visual sign patterns consisting of particular hand/arm movements and facial expressions (Knapp and Corina, 2010). It is supported by functional imaging (e.g. Horwitz et al., 2003), cortical stimulation (e.g. Corina et al., 1999) as well as lesion data (e.g. Hickok et al., 1996). For instance, lesion data demonstrated that, contrary to patients with lesions located in the right hemisphere, patients with left frontal lesion involving the BA44/BA45 region (Hickok et al., 1996), suffered from deficits in signing (see Hickok et al., 1998).

Moreover, it has been shown that the activation of Broca’s area is reduced when spoken language is accompanied by meaningful speech-associated gestures, probably because of improved comprehension (Skipper et al., 2007). Indeed, speech associated gestures or co-speech gestures *i.e.* hand and/or face movements that could accompany spoken language, convey semantic information; thus they could help to catch the meaning of the utterances they accompanied.

Additionally, Broca’s area has been found implicated in many sequencing tasks (Dominey et al., 2003; Koechlin and Jubault, 2006; Bahlmann et al., 2009) but these studies will be detailed elsewhere (section 4.).

### 3.3. A special look at the putative role of Broca’s area in the MNS

Even if, in humans, the presence of mirror neurons in Broca’s area is still a matter of debate, this constitutes an important issue. The discovery of mirror neurons is one of the most important discoveries over the last few years in the field of cognitive neuroscience, having been compared to that of DNA (deoxyribonucleic acid) in biology (Ramachandran, 2000).

#### 3.3.1. Mirror neurons in monkeys

Originally described in monkeys (di Pellegrino et al., 1992), mirror neurons are neuronal cells that discharge both when an individual performs an action and when it observes another individual doing the same action (Figure 1.28) (Gallese et al., 1996; Rizzolatti et al., 1996a). Mirror neurons owe their name from the fact that they respond by mirroring the seen behaviour in the observer’s own motor repertoire. Actually, these neurons are audio-visual
mirror neurons since they also discharge when monkeys hear the sound of an action (Kohler et al., 2002; Keysers et al., 2003).

Figure 1.28: Example of an F5 mirror neuron activity.

The neuron is selectively discharging during monkey grasping movements and during observation of a grasping movement done by the experimenter. A Lateral view of the brain with indicated the location of F5. B and C Spike recordings of the neuron in area F5 during action observation (B) and execution (C). For both conditions, the six lines represent six consecutive trials and the arrows indicate the onset of action. A arcuate sulcus, c central sulcus, ip intraparietal sulcus. From (Rizzolatti and Fabbri-Destro, 2010).

Although monkey mirror neurons were first discovered in the premotor cortex, particularly in area F5p (Matelli et al., 1985) corresponding to the posterior bank of the inferior branch of the arcuate sulcus (di Pellegrino et al., 1992; Gallese et al., 1996; Rizzolatti et al., 1996a), they have also since been identified in other brain regions: namely the rostral part of the IPL, mainly
in area PFG (Pandya and Seltzer, 1982; Fogassi et al., 2005; Fogassi and Luppino, 2005; Geyer et al., 2005; Rozzi et al., 2008) and the superior temporal sulcus (STS) (Perrett et al., 1989) (Figure 1.29).

Figure 1.29: Regions in which mirror neurons have been recorded in the macaque.

Brain has been partially inflated to reveal the sulci. Many brain regions have not yet been explored for mirror neurons in the monkey, hence the ‘?’s. IPS, intraparietal sulcus; PF/PFG, areas of the inferior parietal lobule. From (Keysers, 2009).

3.3.2. The mirror neuron system in humans

Since single-unit recordings in humans for research purposes only are unavailable, evidence for a similar MNS arises mainly from functional imaging or TMS studies (Fadiga et al., 1995; Grafton et al., 1996; Decety et al., 1997; Iacoboni et al., 1999; Blakemore and Decety, 2001; Buccino et al., 2001; Jeannerod, 2001; Rizzolatti and Craighero, 2004; Chong et al., 2008b; Gazzola and Keysers, 2009).

The first demonstration that a mirror system could also exist in the human brain is attributed to Fadiga and collaborators (Fadiga et al., 1995). Using TMS to study cortico-spinal excitability while subjects observed actions made by another individual, the authors demonstrated that the amplitude of the responses evoked by TMS in the observer’s hand was modulated by the observed actions (Fadiga et al., 1995). Several other experiments have confirmed and completed the finding that motor facilitation (MEP size increase) is induced by action observation (Strafella and Paus, 2000; Maeda et al., 2002; Fadiga et al., 2005), supporting the existence of a MNS in humans. Motor facilitation has been also demonstrated while listening to action-related sounds (Aziz-Zadeh et al., 2004) and speech sounds (Fadiga et al., 2002; Watkins et al., 2003; Watkins and Paus, 2004; D’Ausilio et al., 2009). More recent TMS experiments repeatedly showed that facilitation is congruent with respect to the movement observed and even muscle-specific (Alaerts et al., 2009; Alaerts et al., 2010b;
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Alaerts et al., 2010a; Cavallo et al., 2010; Cavallo et al., 2011). For instance, it has been shown that cortico-spinal excitability increased during the observation of a heavy object being lifting in comparison to the same action but performed with a lighter object (Alaerts et al., 2010b). This result demonstrates that cortico-spinal excitability is modulated by the level of observed grip force mirrored onto the observer’s motor system. According to the authors, the capacity to deduce an object’s weight from observation could rely on such a mapping mechanism (Alaerts et al., 2010b). Altogether, these findings suggest that the human MNS code for the precise movements forming an action.

Indirect evidence supporting the existence of MNS in humans also arises from brain imaging studies (Rizzolatti and Craighero, 2004; Fabbri-Destro and Rizzolatti, 2008; Turella et al., 2009a; Caspers et al., 2010). It has been shown that action observation recruits a network of cortical areas very similar to the monkey MNS (Aziz-Zadeh et al., 2006), namely 1) the inferior frontal /premotor region, regarded as the human homologue of the F5 region (Petrides et al., 2005; but also see Morin and Grezes, 2008), 2) the rostral part of IPL, considered as the human homologue of area PF (Fabbri-Destro and Rizzolatti, 2008) and 3) the superior temporal cortex (Grafton et al., 1996; Grafton et al., 1997; Chao and Martin, 2000; Hamilton and Grafton, 2006; Iacoboni and Dapretto, 2006; Chong et al., 2008b) (Figure 1.30).

Figure 1.30: Schematic overview of the frontoparietal MNS and its main visual input in the human brain.

An anterior area with mirror neuron properties is located in the inferior frontal cortex, encompassing the posterior IFG and adjacent PMv. A posterior area with mirror neuron properties is located in the rostral part of the IPL, and can be considered the human homologue of area PF/PFG in the macaque. The main visual input to the MNS originates from the posterior sector of the STS. Together, these three areas form a 'core circuit' for imitation. The visual input from the STS (yellow) to the MNS (red) is represented by an orange arrow. The red arrow represents the information flow from the parietal MNS, which is mostly concerned with the motoric description of the action, to the frontal MNS, which is more concerned with the goal of the action. The black arrows represent efference copies of motor imitative commands that are sent back to the STS to allow matching between the sensory predictions of imitative motor plans and the visual description of the observed action. From (Iacoboni and Dapretto, 2006).
Many attempts have been made to better characterize the human MNS by using various stimuli in action observation/execution paradigms: familiar/unfamiliar actions (Calvo-Merino et al., 2006), tool use (Stout et al., 2008), motor expertise (Calvo-Merino et al., 2005), entire model/isolated hand (Turella et al., 2009b), type of effector (Buccino et al., 2001), real/virtual hand actions (Perani et al., 2001), point light motion (Saygin et al., 2004; Ulloa and Pineda, 2007) or even biomechanically possible/impossible movements (Romani et al., 2005). From this literature, it appears that the putative human MNS exhibits different properties in comparison with the MNS originally discovered in monkeys. Whereas the monkey MNS is activated only for meaningful object/goal directed actions such as grasping an object (Rizzolatti et al., 1996a), the human MNS seems activated by a wide range of actions, even by the observation of intransitive or meaningless (non-goal directed) movements (Fadiga et al., 1995) as well as imitation (Iacoboni et al., 2005).

However, the existence of the MNS in humans is still questioned by some authors (Dinstein, 2008; Dinstein et al., 2008; Hickok, 2009b; Lingnau et al., 2009; Pascolo et al., 2009). Their criticism arises mainly from the fact that it is difficult to claim for certain that mirror neurons exists in humans because, in contrast with monkeys, the use of electrophysiological techniques is challenging. Only one single neuron recording study in humans has been made (Mukamel et al., 2010), the researchers benefiting from the surgery of patients suffering from drug-intractable epilepsy. For their recordings, the researchers used the intracranial depth electrodes that had been implanted in 21 patients to identify seizure foci before their surgical resection. Recordings were made while patients either observed or executed either grasping actions or facial gestures. The results of these direct electrophysiological measures indicate that some neurons respond both to action execution and observation (Mukamel et al., 2010). These neurons with mirror-like properties were located in the SMA and medial temporal cortex, though it should be mentioned that the sampled regions were determined solely by clinical motivations, *i.e.* locations of the electrodes were set at epileptic foci locations22, explaining the reason why no recordings from "classical" mirror areas were made (Mukamel et al., 2010). Thus, to date, the challenge to substantiate the presence of mirror neurons in human PMv/IFG and IPL regions is still relevant.

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22 In their study, Mukamel and coworkers (2010) reported that none of the epileptic foci were located neither in PMv/IFG nor in IPL and furthermore, according to the authors, these are regions from which epilepsy rarely originate.
3.3.3. About the possible roles of the MNS

It has been proposed that the MNS participates in the mechanism underlying action understanding: by recruiting action representations, the observer can simulate mentally the observed action and can therefore understand the intentions of the actor. In other words, this observation/execution matching system could constitute a link between perception and action, necessary to grasp the meaning of other’s speech during inter-individuals communication (Rizzolatti and Arbib, 1998; Rizzolatti et al., 2001; Rizzolatti and Craighero, 2004; Gallese, 2009). Many other cognitive functions have been associated, via this self-other matching mechanism, to this fronto-parietal system such as perception of other’s intention (Cattaneo et al., 2007; Rizzolatti and Sinigaglia, 2007), imitation (Iacoboni, 2005) and imitation learning (Buccino et al., 2004b; Williamson et al., 2010), and even social interaction and empathy (Rizzolatti and Craighero, 2004; Iacoboni et al., 2005; Iacoboni and Dapretto, 2006; Oberman et al., 2007a; Oberman et al., 2007b; Oberman and Ramachandran, 2007; Rizzolatti and Fabbri-Destro, 2008; Iacoboni, 2009; Bach et al., 2011).

The MNS contribution to social cognition is a critical and controversial issue. It is a critical issue because the MNS functions may have evolved from action understanding to imitation and finally, to intention and emotion comprehension (Rizzolatti and Craighero, 2004), the latter being possibly the basis for specifically human social abilities such as empathy and language (Gallese et al., 2009). It is also a controversial issue; some researchers do not entirely agree with this “theory of adaptation”, suggesting rather “associative hypothesis”. According to the latter “the mirror neuron system is a product, as well as a process, of social interaction” (Heyes, 2010). It means that it is throughout interactions with others, that is built the sensorimotor experience on which relies the MNS. Additionally, even the contribution of the MNS itself to social abilities is debated. On the one hand, for instance, a greater MNS activity has been evidenced during the observation of social or interactive/cooperative behaviours (Iacoboni et al., 2004; Newman-Norlund et al., 2007; Oberman et al., 2007a; Knutson et al., 2008; Newman-Norlund et al., 2008), suggesting that the MNS could allow mental state inferences through “experiential understanding” of the actions/emotions of others (Gallese et al., 2004). On the other hand, it has been demonstrated that cortico-spinal excitability - taken as a putative measure of the mirror system - is enhanced during either the observation of goal-directed actions or social behaviours, but to the same extent in both conditions (Donne et al., 2011). In any event, it is worth noting that MNS has no monopoly on embodiment since another system has been evidenced. This “mentalizing” system appears dedicated to the ability to mentalize observed actions, and involves other areas besides those of the MNS.
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(Keysers and Gazzola, 2007; de Lange et al., 2008; Thioux et al., 2008). Of particular interest, in a recent fMRI study Spunt and collaborators (2010) used a set of human action video clips designed to allow the manipulation of both the mentalizing or mechanizing aspects of observed actions. Their data showed that despite the fact that MNS regions were found activated, they were not influenced by the mentalizing aspect unlike areas of the mentalizing system (medial PFC, posterior cingulate cortex, and temporal regions). The authors conclude that the MNS via “embodied simulation” is not the only system involved in mentalizing during action observation (Spunt et al., 2010).

Staying with the role of the MNS in social cognition, autism, or more generally autism spectrum disorders (ASD), are of particular interest. Indeed, deficits in social and communication skills, especially in empathy, are commonly encountered in individuals with ASD (Lord et al., 2000). In order to determine the neural substrates of ASD, many comparisons have been made between healthy and autistic subjects and it is clear, especially from MRI studies, that both structural and functional abnormalities occur in ASD brains. Such abnormalities consisted, either in isolation or in combination, of dysfunctional regions (Figure 1.31), dysfunctional pathways, and/or alternative pathways (for recent reviews, Ulay and Ertugrul, 2009; Minshew and Keller, 2010; Verhoeven et al., 2010; Anagnostou and Taylor, 2011).

![Figure 1.31: The anatomy of autism.](image)

People with autism show reduced mirror neuron activity in the IFG, a part of the brain’s premotor cortex, perhaps explaining their inability to assess the intentions of others. Dysfunctions of mirror neurons in the insula and anterior cingulate cortex may cause related symptoms, such as the absence of empathy, and deficits in the angular gyrus may result in language difficulties. People with autism also have structural changes in the cerebellum and brain stem. From (Ramachandran and Oberman, 2006).
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The observation of dysfunctional areas that belong to the MNS in ASD brains is not surprising because mirror neurons appear involved in the same social functions as those impaired in ASD individuals. Such a deficiency in the MNS has been postulated to explain some of the ASD behavioural deficits, an hypothesis known as the “dysfunctional mirror neuron system hypothesis of autism” (Oberman et al., 2005; Dapretto et al., 2006; Iacoboni and Dapretto, 2006; Ramachandran and Oberman, 2006; Williams et al., 2006). Through its property to match perception of the environment to internal sensorimotor representations, the MNS contributes to action understanding and imitation (Rizzolatti et al., 2001), a possible basis for higher order behaviours including social ones. The link between MNS and ASD has been extensively investigated. For instance, Dapretto and collaborators (2006) using fMRI, have measured in ASD children the mirror activity in observation/imitation tasks of facial expressions. Their data demonstrated a negative correlation between autism severity and mirror activity in those children. The MNS/ASD link has been also investigated through mu rhythm modulations in action observation paradigms. The mu rhythm (8-12 Hz frequency band) is generated by sensorimotor cortex and recorded with electroencephalography (EEG): while at rest its amplitude is maximum, it decreases during the execution, observation, or imagination of actions, a phenomenon of neuron desynchronization known as “mu rhythm suppression”. Therefore, mu rhythm suppression has been measured to evaluate indirectly the activity of the MNS (Cochin et al., 1999; Pineda et al., 2000; Muthukumaraswamy and Johnson, 2004; Oberman et al., 2005; Pineda, 2005; Hari, 2006). Abnormal mu rhythm (reduced mu suppression) during action observation has been reported in ASD individuals with deficient empathy (Oberman et al., 2005), with the degree of autism being reflected by differential EEG signals: the more severe autism the more low signal in the beta band (12–20 Hz) (Puzzo et al., 2010).

Alongside of the impressive number of studies supporting the hypothesis of an impaired MNS in individuals with ASD (Williams et al., 2001; Nishitani et al., 2004; Williams et al., 2004; Oberman et al., 2005; Theoret et al., 2005; Villalobos et al., 2005; Dapretto et al., 2006; Hadjikhani et al., 2006; Lepage and Theoret, 2006; Williams et al., 2006; Bernier et al., 2007; Cattaneo et al., 2007; Martineau et al., 2008; Williams, 2008; Boria et al., 2009; Fabbri-Destro et al., 2009; Perry and Bentin, 2009; Welsh et al., 2009; Honaga et al., 2010; Martineau et al., 2010; Puzzo et al., 2010), it should be noted that some studies maintain the opposite point of view, claiming normal MNS function in autism (Avikainen et al., 1999; Bird et al., 2007; Hamilton et al., 2007; Dinstein et al., 2008; Leighton et al., 2008; Southgate and Hamilton, 2008; Raymaekers et al., 2009; Dinstein et al., 2010; Falck-Ytter, 2010; Fan et al., 2010).
Frontal and parietal mirror regions might have different roles: processing of action kinematics for the parietal zone, and encoding of action goals for the frontal zone (Grafton and Hamilton, 2007). Indeed, the MNS literature seems to indicate that the goal of an action is coded in the frontal part of the MNS in monkeys (Umiltà et al., 2001) as well as in humans (Iacoboni et al., 1999; Iacoboni et al., 2005; Iacoboni and Dapretto, 2006). In particular, these studies in humans have revealed that the activity within the IFG differs according the action goal. For instance, observing a given movement (i.e. grasping) performed out of context or with a cued goal (i.e. grasping to drink or grasping to clean up) generates a differential activation within the IFG. In particular, an activation increase was found in the right BA44 when observing a goal-directed grasping action (Iacoboni et al., 2005). Based on the assumption that the MNS mediates action understanding, the authors investigated fMRI activations obtained following the observation of meaningful vs. meaningless object-directed actions. They only found a double dissociation between activity and meaningfulness of actions in the parietal zone of the MNS, more precisely in SMG. According to the authors, this result refines the knowledge about the involvement of IFG in action understanding: “IFG is involved in integrating neural processes supporting actions and objects, and plays a prominent role in representing the goal-directedness of transitive actions, but not necessarily their meaningfulness” (Newman-Norlund et al., 2010a) (Figure 1.32).

![Figure 1.32: Brain areas activated by the observation of object-direct actions.](image)

Spherical ROI’s at bilateral sites showing significant BOLD signal increases (relative to Rest) during the observation of ODAs (p<0.001 uncorrected, 10 voxel extent) and located in the IPL (red and green) or IFG (blue) (All spherical ROI’s r=4 mm). Green sites in the IPL (bilateral supramarginal gyrus) were the only MNS sites that differentiated meaningful from meaningless actions. From (Newman-Norlund et al., 2010a).

Usually, subjects become faster in performing an action when they have previously seen the same action. When TMS is applied over left or right IFG, such response facilitation effect is
eliminated but only for the imitative (congruent action observation/execution) condition, whether the action was cued by a biological or non-biological stimulus. This finding contributes to reinforce the role of IFG within the human MNS in perception-action coupling rather than simply in response to biological stimuli per se (Newman-Norlund et al., 2010b). A recent study proposed that the frontal zone could be implicated in predicting others’ actions (Urgesi et al., 2010).

3.3.4. The MNS and Broca’s area

As depicted in Figure 30, the frontal MNS consists of the anterior portion of PMv together with the posterior part of IFG encompassing Broca’s area and more precisely, BA44, generally considered as the homologue of area F5 of the monkey cortex (Geyer et al., 2000; Petrides et al., 2005), where mirror neurons were originally discovered (Figure 1.33) (Rizzolatti and Arbib, 1998).

![Figure 1.33: Homologue cortical regions between the macaque (A) and human (B) cerebral cortex.](image)

The regions shown in yellow and orange, in both the monkey and human brains, indicate the primary motor and the premotor cortex, respectively. The red colored region indicates the hypothesized homologue cortical motor areas related to communication and language (monkey area F5 and human area 44, or Broca’s area). (C: central sulcus; ias: inferior arcuate sulcus; ifs: inferior frontal sulcus; L: lateral sulcus; P: principal sulcus; sas: superior arcuate sulcus; sfs: superior frontal sulcus). From (Fogassi and Ferrari, 2007).
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What evidence exists to support the existence of mirror neurons in Broca’s area? First, imaging studies have repeatedly demonstrated a common cortical network including the IFG involved both during action observation and execution (Rizzolatti et al., 1996b; Cochin et al., 1999; Buccino et al., 2001; Gazzola and Keysers, 2009). Kilner and collaborators (2009), by using an fMRI adaptation paradigm\(^{23}\), found results compatible with the existence of mirror neurons in humans (Kilner et al., 2009). Indeed, a repetition-suppression effect has been reported in the IFG when an action is executed and subsequently observed, and conversely, providing evidence that this area may contain mirror neurons. It is worth noting that several other studies have failed to confirm such findings (Dinstein et al., 2007; Chong et al., 2008b; Lingnau et al., 2009). The second piece of evidence is that TMS studies have shown that a virtual lesion of the left IFG impaired MNS-related processes such as movement imitation (Heiser et al., 2003) and action understanding (Pobric and Hamilton, 2006).

Previously, we mentioned the possible role of the MNS in social cognition. In the related literature, evidence for the implication of the IFG in social aspects has been highlighted (Iacoboni et al., 2004; Knutson et al., 2008; Newman-Norlund et al., 2008; Yamasue et al., 2008). Among these studies, some authors claimed a particular role of the right IFG (Newman-Norlund et al., 2007; Newman-Norlund et al., 2008) in processing diverse social stimuli. A virtual lesion study investigating the role of the left IFG in social perception (emotion recognition task) has showed that, following the application of rTMS over left IFG, there is both an increase in RT and no mu rhythm suppression, demonstrating the role of left IFG in social perception (Keuken et al., 2011).

We also mentioned previously that a dysfunctional MSN can lead to autistic symptoms. Importantly, an “atypical” activity in IFG (pars opercularis) has been found in ASD individuals, i.e. a decreased activation during sentence comprehension (Just et al., 2004), observation/perception or imitation of emotional face expressions (Dapretto et al., 2006; Hadjikhani et al., 2006, 2007; Bookheimer et al., 2008; Uddin et al., 2008; Greimel et al., 2010). Additionally, one of these studies (Hadjikhani et al., 2006) also evidenced a reduced cortical thickness in IFG, as already suspected (Abell et al., 1999). Other studies have also evidenced other types of abnormalities in this region in ASD patients, for instance shape abnormalities of macroscopic landmarks (Levitt et al., 2003; Nordahl et al., 2007). Importantly, in these studies, the evidenced structural abnormalities were located in the posterior part of the IFG i.e. pars opercularis.

\(^{23}\) The principle of this paradigm is that the repetition of a stimulus leads to a decrease of the strength of the response to this stimulus and thus a decrease in activation in the brain region responsible for processing this stimulus, a phenomenon known as repetition-suppression (Grill-Spector et al., 2006).
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*opercularis*/BA44, a finding confirmed recently using a different methodology (manual rather than automated techniques of analyses) and making correlations with the severity of the clinical symptoms in the ASD group (Yamasaki et al., 2010).

Surprisingly, except for one study (Hadjikhani et al., 2007), this “atypical” activity in IFG was not found in adults (Pierce et al., 2004; Ashwin et al., 2007; Bastiaansen et al., 2011). A possible explanation is related to age since Bastiaansen and collaborators have shown that IFG activity, at least for facial expression observations, is positively correlated to the age of the autistic individuals: activity increased with age and importantly, such increases were associated with social functioning improvements (Bastiaansen et al., 2011). According to Bastiaansen and collaborators, the social ability of ASD individuals might improve, with age, through an improvement of the eye gaze behaviour used to grasp the relevant information in other’s faces. Actually, their hypothesis is sustained not only by their own eye tracking data but also that of other studies (e.g. Spezio et al., 2007). Finally, an “atypical” activity in IFG has also been found in ASD individuals during observation of hand movements (Martineau et al., 2010) but for that study, an increase of activation rather than a decrease was evidenced, contrasting with the other aforementioned studies.

3.4. How to explain the multi-domain influence of Broca’s area?

Initially, the surprising contribution of a language area to non-linguistic domains was explained by a silent linguistic mediation through internal speech (Greze and Decety, 2001), but this hypothesis has rapidly fallen out of favor (Fadiga et al., 2006). New lines of investigations have arisen, seeking a way to reconcile the multiple functions of Broca’s area. To this end different hypotheses have been proposed which could be divided into two main streams.

3.4.1. Differentiation of functions within the IFG

The first idea is that Broca’s area is involved in so many tasks because it is not homogenous, but rather could be functionally segregated. This view arises from the fact that, as already mentioned, the IFG can be subdivided into several distinct sub-areas from an anatomical point of view (see section 1.2.2. and 1.3.1.). Thus the question arises as to whether these subdivisions subserve different functions. Concerning the language domain, a great number of functional imaging studies (e.g., (Fiez, 1997; Dapretto and Bookheimer, 1999; Kang et al., 1999; Poldrack et al., 1999; Binkofski et al., 2000; Bokde et al., 2001; Bookheimer, 2002;
Horwitz et al., 2003; Amunts et al., 2004; Heim et al., 2005; Heim et al., 2008, 2009; Bornkessel-Schlesewsky et al., 2010) and lesion studies (Devlin et al., 2003; Gough et al., 2005; Devlin and Watkins, 2007) indeed report functional dissociations between subregions of IFG. The posterior part of Broca’s area, i.e. the left BA44, could be the core area responsible for syntactic processing while the anterior part, i.e. the left BA45, could be in charge of semantic processing (Dapretto and Bookheimer, 1999; Friederici et al., 2000b; Grodzinsky, 2000; Heim et al., 2003; Friederici et al., 2006b; Sahin et al., 2006; Kaan, 2007) (but see also Bornkessel-Schlesewsky and Schlesewsky, 2008). However, this dichotomy is not yet the consensus since for instance 1) the involvement of BA45 has also been suggested for syntactic processing (Hashimoto and Sakai, 2002; Kinno et al., 2008; Momo et al., 2008; Iijima et al., 2009), and 2) another type of functional dissociation has been identified between BA45 and BA44 for semantic and phonological processing, respectively (Gough et al., 2005; Heim et al., 2009). In any event, this functional subdivision is supported outside the domain of language, for instance in the field of mathematical computations (Friedrich and Friederici, 2009) or of action (Koechlin and Jubault, 2006). It should be stressed that a finer subdivision within BA44 or BA45 has been proposed for both linguistic (Papoutsi et al., 2009) and non-linguistic fields (Molnar-Szakacs et al., 2005). For instance, it has been demonstrated that BA44 could be split into two distinct loci, a dorsal and a ventral one, endowed with different properties. By compiling imaging studies on imitation and action observation, Molnar-Szakacs and collaborators (2005) have isolated two functionally distinct loci within the pars opercularis. The first locus, in the dorsal part, was found activated during both action observation and imitation (mirror property) and the second locus, in the ventral part, was activated only during imitation (motor property). This finding is in accordance with the study of Amunts and collaborators (Amunts et al., 2010) who highlighted a highly probable anatomical segregation within BA44 and BA45.

Additionally, in a recent meta-analysis (Liakakis et al., 2011), the authors performed a cluster analysis based on functional neuroimaging data from studies in humans that reported activations in IFG for language tasks, but also for motor control and WM processing tasks as well as tasks involving empathy processing. Although they failed to identify a specific cluster for syntactic processing, several clusters in the left IFG were found for processing empathy, semantics, phonological processing and for WM, demonstrating “spatio-functional diversity” (Liakakis et al., 2011). It is worth noting that according to some researchers, fMRI studies are not suitable for providing a clear picture of functional specificity, since classically these studies use whole brain or region of interest (ROI) analyses at the group level and thus do not take into account inter-subject variability (Fedorenko and Kanwisher, 2009). This has led the same group to propose a new method for fMRI analyses, the individual-subjects fROI method.
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(Fedorenko et al., 2010). This proposal has encountered criticism (Grodzinsky, 2010) but is advocated by Fedorenko and Kanwisher (2011) as it allows, according to the authors, “to distinguish among conditions in a brain region’s response (...) essential for making inferences about the precise computations conducted in each brain region”. In their paper, the authors particularly stressed that this approach could be especially useful to clarify the neural basis of syntactic processing (Fedorenko et al., 2011)

Finally, it should be mentioned that this view about functional differentiation within the IFG fits well with more general models of hierarchical organization within the frontal cortex that postulate a posterior-anterior gradient for information processing: the more complex a sequence is, the more anterior recruited regions in IFG are (Fuster, 2001; Koechlin and Jubault, 2006; Badre and D'Esposito, 2007) (Figure 1.34).

![Hierarchical organization in frontal cortex](image)

Figure 1.34: Hierarchical organization in frontal cortex.

(a) The position of the DLPFC within a hierarchy of cortical areas, as described by Fuster (Fuster, 2001). (b) Levels of control represented in different sectors of frontal cortex, according to Koechlin (Koechlin et al., 2003; Koechlin and Jubault, 2006). Representations become progressively more abstract towards the rostrum. From (Botvinick, 2008).
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3.4.2. Unifying hypotheses

The second, possibly complementary main position to explain the activity of Broca’s area in so many tasks is that this area may have a function common to all these tasks. There are many proposals:

A) MNS assumption. The implication of Broca’s area in the MNS as detailed in section 3.3. constitutes a possible explanation. The rationale behind this hypothesis is that Broca’s area is involved in so many different tasks because it belongs to a network that offers a link between perceived and executed behaviours through a mirror-like mechanism (Rizzolatti and Arbib, 1998; Iacoboni et al., 1999; Chaminade et al., 2001; Carr et al., 2003). This hypothesis is based on the discovery of mirror neurons in monkeys and their putative equivalent in humans, in premotor cortex (area F5) and in Broca’s area respectively.

B) Cognitive control. This hypothesis (Novick et al., 2005; Badre and Wagner, 2007; Snyder et al., 2007) arises from the finding that IFG seems more implicated in tasks requiring higher cognitive control. According to Novick and collaborators, this cognitive process is “necessary to resolve conflicts that arise among the distinct representational subsystems necessary for language use (i.e., phonological, syntactic, and semantic subsystems)” (Novick et al., 2005). In other words, sentence processing may require cognitive control to choose the relevant representation of the sentence structure and interpretation, among others, possibly by biasing attention toward this representation. Support in favour of a role of Broca’s area in conflict resolution, either syntactic or not and thus relying on general cognitive control processes, comes from a set of studies using ambiguity resolution tasks (Mason et al., 2003; Rodd et al., 2005; Bedny et al., 2007; Mason and Just, 2007; Zempleni et al., 2007; Bedny et al., 2008; January et al., 2009; Rodd et al., 2010). Similarly, the IFG has been proposed as a “general-purpose problem solver”, which can be used to perform a wide range of goal-directed cognitive tasks (Duncan, 2001; Miller and Cohen, 2001; Fedorenko et al., 2010; Fedorenko et al., 2011).

C) Working memory. The idea that the common denominator for multimodal involvement of Broca’s area is WM (Just et al., 1996; Stromswold et al., 1996; Rogalsky et al., 2008) has arisen mainly from the fact that the processing of sentences that contain syntactic movements (those with long-distance dependencies) requires additional resources, especially of WM. Deficits regarding the processing of these types of sentences could be also explained by a
disruption of WM, so that there is an uncertainty about whether Broca’s area is responding to a specific process or rather to the greater demands on WM linked to such complex sentence structures. A second element that reinforced this hypothesis is that studies investigating the neuronal basis of WM by dedicated tasks found that Broca’s area may house some WM processes (Braver et al., 1997; Smith and Jonides, 1999; Druzgal and D’Esposito, 2001). Taken altogether, these are the reasons why WM is suspected as being the underlying cause for the involvement of Broca’s area in so many tasks. Obviously, attempts have been made to make this claim clearer, especially by dissociating the complexity effect from the WM effect as performed by a recent fMRI study (Makuuchi et al., 2009) of sentence processing that varied both aspects independently. This study demonstrated that only syntax-related aspects are represented in left BA44 (Makuuchi et al., 2009). Nonetheless, according to some authors Broca’s area and particularly the BA44 contributes to sentence processing via articulatory rehearsal\(^{24}\) (Rogalsky and Hickok, 2011). Finally, it has been proposed that the left IFG could be involved in WM mechanisms that participate in language processing rather than for syntactic integration or for general WM processes per se (Bookheimer, 2002; Fiebach et al., 2005).

D) Syntax. Broca’s area could be associated with syntactic mechanisms regardless of domain and therefore could act as a “supramodal hierarchical/syntactic processor” (Friederici et al., 2000a; Fiebach and Schubotz, 2006; Koechlin and Jubault, 2006; Tettamanti and Weniger, 2006; Bahlmann et al., 2009; Fadiga et al., 2009; Tettamanti et al., 2009; Pallier et al., 2011), an expression indirectly introduced by Grossman (1980) and first used by Tettamanti and Weniger (2006). This hypothesis comes mainly from the conjunction of the facts that Broca’s area has been repeatedly associated with syntactic processing in language studies, and that a parallel in structural organization can be drawn between language and several other behaviours. Among all these different assumptions, the last one is of particular interest and is the subject of the following section.

\(^{24}\) Articulatory rehearsal is a subcomponent of verbal WM in the proposed model of Baddeley (Baddeley, 1986) and consists of retrieving the verbal information from a phonological loop by subvocally repeating it.
4. The hypothesis of Broca’s area as a supramodal syntactic processor

Broca’s area may act as an invariant “syntax” processor for language as well as for any other sequence-based domain. Two points have contributed to the emergence of this hypothesis. First, Broca’s area plays a crucial role in syntax processing in the language domain. Recall that there are many clues in favour of a role of Broca’s area in linguistic syntax from the “telegraphic speech”, reflecting agrammatism, reported in patients with a lesion of Broca’s region (see Goodglass, 1997 for a review) to more recent studies showing that this area is involved in the learning of natural (Musso et al., 2003) or artificial (Bahlmann et al., 2008; de Vries et al., 2008; de Vries et al., 2009; Floel et al., 2009; Christiansen et al., 2010a) grammars. Second, a parallel could be drawn in terms of the structural organization between human language and some behaviours in various domains. Importantly, there are several demostrations about the implication of Broca’s area in such syntax-like functions. To summarize, according to this assumption, linguistic syntax could be considered as a particular case of hierarchically organized behaviour and since Broca’s area is known to play a significant role in grammatical/syntactic processing, it has been suggested that this area could be a center for processing all hierarchical sequences, irrespective of their nature.

4.1. Shared organizing principles

The crucial point beyond this assumption is that both human language and some non-linguistic abilities are hierarchical in structure, and that this hierarchical organization is under the control of syntactic rules that specify how to combine single units together to form structured sequences.

Since it is not trivial to consider syntax outside of the language domain, this point requires some explanation. In language, sequence organization consists in concatenating words into sentences. This organization follows grammatical rules, known as syntax, generally depicted as tree-like hierarchical structures (Figure 1.35, upper left corner). Karl Lashley (1951) was the first to propose applying the notion of syntax to non-linguistic behaviours, including goal-directed actions, and thus to consider that the language shares it structural principles with action sequences. Thus “action/motor syntax”; i.e. the syntactic organization of actions, was born. By analogy with the definition of syntax used in linguistics, the motor syntax can be regarded as the set of rules governing the combination of movements to form a structured sequence, a sine qua non condition to perform elaborate actions. In other words, complex
motor sequences can be subdivided into smaller units (individual motor acts) that need to be combined according to a defined order to form elaborate sequences and, in fine, complex behaviours, which would be unachievable using simple serial sequences (Lashley, 1951; Rosenbaum et al., 1983; Dehaene and Changeux, 1997). A behavioural sequence is thus controlled by action plans, plans that are hierarchically organized (Lashley, 1951; Rosenbaum et al., 2007). Such an organization, from the various motor commands to the ultimate goal, could be applied to almost all everyday life actions. For example, to make a cup of coffee you have to execute several intermediate actions such as to take a filter, to put coffee in the filter and water in the coffee-maker and so on, each of which involves elementary actions (reaching, grasping, etc). Many non-linguistic activities apart from actions also rely on a certain form of syntax, or of hierarchical organization: calculation and music are two other examples (Figure 1.35).

![Diagram of hierarchical structures in different domains](image)

**Figure 1.35:** Examples of hierarchical structures in different domains, all represented as tree-like structures.

Schematic structures of sequences in linguistics, motor, mathematics and musical domains showing the arrangement of lower-level components (words, motor acts, numbers and pitches respectively). Upper right corner: from (Greenfield, 1991); lower left corner: from (Friedrich and Friederici, 2009); lower right corner: from (Patel, 2003).

### 4.2. Evidence in favour of a common syntactic processor

The hypothesis started with the view that Broca’s area could process hierarchical structures crucial to both language and action. This view arises from the study of Grossman (1980) showing that agrammatic Broca’s aphasics were impaired in reproducing the models of
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tree structures\(^{25}\) (Grossman, 1980). Currently, there are several lines of evidence supporting this view in each domain separately, but also strong findings from studies crossing the linguistic and non-linguistic domains (e.g. Dominey et al., 2003; Fazio et al., 2009; Tettamanti et al., 2009).

4.2.1. Processing hierarchical sequences

In the music domain, Broca’s area has been implicated in tasks in relation to the processing of musical syntax by several functional imaging studies (Figure 1.36), fMRI (e.g. Koelsch et al., 2005) and MEG (Maess et al., 2001). Few clinical studies exist, though one with patients having lesions restricted to Broca’s area did evidence that a preserved Broca’s area is required to process musical syntax accurately (Sammler et al., 2010). It is worth noting that, even if not directly linked to musical syntactic ability, an increase in both grey matter density (Sluming et al., 2002) and volume (Abdul-Kareem et al., 2011) in Broca’s area has been highlighted in expert musicians, especially in the posterior part BA44 (Abdul-Kareem et al., 2011). Finally, to our knowledge, no TMS studies related to this issue have been performed.

![Figure 1.36: Broca’s area activation for music-syntactic processing](image)

Activation foci (small spheres) reported by functional imaging studies on music-syntactic processing using chord sequence paradigms (Koelsch et al., 2002, 2005a; Maess et al., 2001; Tillmann et al., 2003) and melodies (Janata et al., 2002a). Large yellow spheres show the mean coordinates of foci (averaged for each hemisphere across studies, coordinates refer to standard stereotaxic space). From (Koelsch, 2011).

In the action domain, different organizational levels can be extracted according the complexity of the action and, importantly, it has been suggested that this structural outline corresponds to a cortical network, also hierarchically organized along a rostro-caudal axis in the frontal lobe (Koechlin and Jubault, 2006) (Figure 1.37). This gradient, both anatomical and functional, extends from premotor areas to more anterior frontal regions encompassing

\(^{25}\) Actually, Grossman (1980) used the same hierarchical material as that used by Greenfield and Schneider (Greenfield and Schneider, 1977) (see section 4.2.2.).
Broca’s territory and is in charge of processing, respectively, simpler to more abstract behaviour (Dehaene and Changeux, 1997; Passingham et al., 2000; Fuster, 2001; Badre and D’Esposito, 2007; Badre, 2008; Badre and D’Esposito, 2009; Badre et al., 2009; Badre et al., 2010).

As mentioned earlier, the implication of the fronto-parietal human mirror neuron system in representing observed actions has been repeatedly established. Interestingly however, in a recent fMRI study (Molnar-Szakacs et al., 2006), the question of whether the activity of this network varies according to the degree of hierarchical organization of object-hand interaction movements was investigated. The varying complexity was based, according to Greenfield (Greenfield et al., 1972), on the different structures that appear successively during childhood development. Molnar-Szakacs and collaborators failed to evidence that the MNS is sensitive to the hierarchical level of action’s structure. Their explanation for the discrepant results is that their participants were adults and thus the level of complexity is not as marked as for child (Molnar-Szakacs et al., 2006). It was the first study that tried to link the MNS activity to the processing of hierarchical structure of observed object-directed action sequences. In a more recent study, Fazio and collaborators (2009) showed that adult Broca’s aphasic patients, with a lesion encompassing BA44, had difficulties in reordering pictures representing human actions whereas their performance was normal for pictures representing non-biological events (Figure 1.38). This result account for the involvement of Broca’s area is the embodiment of complex actions, suggesting that, because there is different ways to combine movements to reach a given action, we implicitly rely on a motor syntax to determine the relations between the
different sub-actions, so that it creates the adequate association between motor acts and a given action (Fazio et al., 2009).

![Figure 1.38: Sequencing biological action sequences in Broca’s aphasic patients.](image)

Left: Experimental set-up and task. (A) The videoclip is presented on the screen, (B) Four snapshots are presented at the four corners of the screen, (C) Example of physical events snapshots and (D) Example of Human Actions snapshots.

Right: Accuracy results. Histograms depict accuracy rates in aphasic patients (Aphasics) and normal subjects (Controls) for both human actions (white bars) and physical events (black bars) conditions. Whiskers indicate the standard error of the mean. Asterisks denote statistically significant differences (P<0.05) in accuracy between aphasics and controls in the human action condition. From (Fazio et al., 2009).

### 4.2.2. Learning hierarchical sequences

It is of crucial importance to understand how one learns the ability to make use of hierarchical structures. This issue has been investigated thoroughly in the linguistic as well as in the motor domain, based on the subject’s ability to acquire, even unintentionally, a set of new syntactic rules from experience. The ability has been experimentally demonstrated by means of several paradigms, especially through AGL tasks (Reber, 1967, 1989) and serial reaction time (SRT) tasks (Nissen and Bullemer, 1987).

Both the principle of the AGL paradigm and the implication of Broca’s area in AGL-related experiments have already been described in section 2.2.2. Bahlmann and coworkers have adapted their AGL paradigm previously used ((Bahlmann et al., 2008); Figure 1.19 with non-linguistic, visuo-spatial, stimuli) (Bahlmann et al., 2009) (Figure 1.39). As for linguistic stimuli, Broca’s area was found more activated in response to the hierarchical structures than to the linear ones.
Upper part: Adjacent dependency rule and hierarchical dependency rule with examples of abstract symbol sequences. The adjacent dependency rule involves the processing of local transitions between single elements. By contrast, in the hierarchical dependency rule, long-distance dependencies between non-adjacent elements must be processed. Note that during the experiment, each element was presented separately on the screen. Examples are provided for grammatical short (gram/short), ungrammatical short (ungr/short), grammatical long (gram/long), and ungrammatical long (ungr/long) sequences. Violated sequences in the example are marked with bold letters. Lower part: A ROI in BA44 was defined that comprised of the voxels with a value of at least 30% overlap in the cytoarchitectonical probability map. The BOLD response in BA44 on the hierarchical dependency rule (right bar) was significantly higher than on the adjacent dependency rule (left bar). From (Bahlmann et al., 2009).
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As mentioned, another way to test implicit learning is to use SRT tasks (Nissen and Bullemer, 1987). In such tasks, participants have to react to successive stimuli by pressing a response key as quickly and as accurately as possible. The rationale of this task is that, without informing the participants, the same structured sequence is repeated within a block and across blocks and then contrasted with a pseudorandom sequence presented at the end of the block series. As the RT decreases with practice of the structured sequence, the difference between this RT and the RT obtained for the pseudorandom sequence is regarded as evidence for procedural and implicit learning (Robertson, 2007).

SRT tasks have been found particularly useful for investigating how complex sequences are learnt. Indeed, by using these types of experiments it has been shown that the strategy consists of parsing the complex sequences into smaller parts, a process known as chunking. This term was coined by Miller in 1956 in his paper entitled “The magical number seven plus or minus two: some limits on our capacity for processing information” to explain the memory span: 5-9 chunks of information (Miller, 1956). Chunking was therefore regarded as a strategy that enhances the quantity of information stored in short-term memory. Practically, it consists of the hierarchical parsing of a long sequence into sub-sequences that are easier to memorize. For example, since a phone number is a sequence of 10 elements that exceeds the theoretical memory span of 7 elements, can be parsed into shorter sub-sequences. Each sub-sequence thus contains only 3 or 4 units and is easier to remember (Figure 1.40). Importantly, according to Miller, a chunk could refer to digits, to words, or to any other meaningful units (Miller, 1956).

Figure 1.40: Hierarchical parsing of a sequence.

Parsing of a large sequence (blue) made of x individual units (grey, x=10) into y chunks (red, y=3 or 4). This example illustrates the most probable strategy for learning/remembering a phone number.
Similarly, in motor control, chunking can be regarded as an efficient way to bypass the limitations of learning large sequences. Actually, this view was first established by Lashley (1951) since, in addition to its theoretical claims about the way human actions are structured and how they can reach such high levels of complexity, he also argued that new skills are learnt as an organization chart, by integrating successively units into larger ones. Expressed differently, a complex motor-sequence is learned by its parsing into smaller subsequences or chunks, each processed as a single memory unit. This view considering “chunking” as a strategy that accounts for the capacity to perform increasingly complex behaviours is also largely supported by more recent works (Rosenbaum et al., 1983; Sakai et al., 2003; Stocker and Hoffmann, 2004; Koch et al., 2006; Shea et al., 2006; Koch, 2007; Schneider, 2007; Agam and Sekuler, 2008; De Kleine and Verwey, 2009). Importantly, SRT studies have reinforced this theoretical claim by showing that chunking significantly contributes to enhance sequence learning (Rosenbaum et al., 1983; Koch and Hoffmann, 2000a; Sakai et al., 2003; Verwey and Eikelboom, 2003; Sakai et al., 2004a; Kirsch et al., 2009) (but see also Jimenez, 2008). These studies have also enabled investigating the formation of chunks, demonstrating for instance that the process is spontaneous (e.g. Sakai et al., 2003), but that it is possible to ensure its development by using highly structured sequences (e.g. Koch and Hoffmann, 2000a), and that practice type (Wright et al., 2004) and age (Verwey, 2010) might influence it. However, an imposed chunking can be detrimental to learning, as demonstrated by “teaching in chunks” tasks (Cunningham, 1971; Cohen and Sekuler, 2010).

Functional imaging studies using SRT paradigms have evidenced typical activity in supplementary motor area (SMA)/pre-SMA, in frontal cortex (PMC and IFG) and in the inferior parietal lobule (IPL) (Grafton et al., 1998b; Bischoff-Grethe et al., 2004; Bapi et al., 2006). However, in contrast with AGL studies, the involvement of Broca’s area in such studies has not been clearly established. Interestingly, Dominey and collaborators demonstrated that in aphasic patients, agrammatism was associated with impairments in non-linguistic cognitive sequencing tasks. This study is of particular importance since it introduces a notion closely related to the acquisition of syntactic ability: the notion of abstract structure. According to Dominey and collaborators, the abstract structure corresponds to the rules that settle on the sequence organization and that can be used to generate other sequences sharing the same organizational structure (Dominey et al., 1998; Dominey et al., 2003; Allen et al., 2010). Thus, more precisely, Dominey and coworkers (Dominey et al., 2003) established that agrammatic aphasics with Broca’s area lesions are impaired in extracting the abstract structure in SRT task involving letter sequences (Figure 1.41).
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Figure 1.41: Performance of agrammatic and control subjects on an SRT task.

The SRT task measures serial and abstract structure learning in 10 blocks of 108 trials (elements) per block. Blocks 1–6 and 8 use the repeating sequence ABCBACDEFEDE, that contains the abstract structure 123-213. Block 7 uses a random series to assay learning of serial structure. Blocks 9 and 10 use a new isomorphic sequence GBEBGEFDGFDC that shares the same abstract structure 123-213, but has a different serial structure. For such isomorphic sequences, elements corresponding to 123 are unpredictable by the abstract structure, while those corresponding to 213 are predictable, based on their fixed relations to elements 123. Reduced RTs for the unpredictable elements is due to learning of the serial structure. Additional reduction for predictable elements indicates learning of abstract structure that transfers to new isomorphic sequence blocks 9–10 for the Control but not Agrammatic patients. From (Dominey et al., 2003).

Furthermore, during child development, strategies of object manipulation, namely pairing, pot and subassembly, are acquired successively in this order. Crucially, the linguistic structures, grammatically simple, coordinate and complex, developed on the same schemata (Greenfield et al., 1972; Greenfield and Westerman, 1978). A few years later, Greenfield proposed that in addition to the fact that language and object manipulation share the same organizing principles, they could also have a neural substrate in common, Broca’s area (Greenfield, 1991). Moreover, according to Greenfield, during the course of the development a specialization may occur in Broca’s region so that the function of this area evolves from a dual one (organizing both gestures and utterances combinations) to two specialized functions, anatomically segregated, for object manipulation and speech (Figure 1.42).
Figure 1.42: Hypothesized development of neural circuits for hierarchically organized manual sequences/grammar.

In the left drawing, the absence of borders and divisions for Broca's area, as well as the position of the arrows, represents its undifferentiated character at this early stage of development. The circuits in the left drawing are hypothesized to undergo development in the approximate age range of 12 to 16 months. The left-hand portions of the circuits in the right drawing are hypothesized to undergo development in the approximate age range of two to four years. From (Greenfield, 1991).

In accordance with this hypothesis of increased specialization with development, it has been more recently demonstrated that, for instance, the increase of syntactic skills during development is associated with an activation increase in the left IFG (BA44, pars opercularis) (Nunez et al., 2011). Furthermore, in section 2.2.2., it has been held that the integrity of the connectivity between Broca’s area and parietal/temporal areas is crucial for linguistic syntactic abilities. Complementary findings have arisen from recent studies investigating syntax acquisition (Friederici et al., 2011a) and more particularly from a DTI study (Brauer et al., 2011) demonstrating that, in children, while the ventral fiber tract is well myelinated (i.e. the same degree of myelinization as in adult), the dorsal one, connecting BA44 to the temporal lobe is not yet fully mature (Figure 1.43). This result has been regarded as evidence that maturation of the syntactic circuit involving BA44 is required to reach complete syntactic ability (Friederici et al., 2011a).
Figure 1.43: Fiber tracts in 7-year-old children and adults.

Average tractography result with seeds in BA 44 and BA 45. Seeds were chosen based on the maximum functional activation in a language comprehension task. The dorsal pathway connecting BA 44 to the temporal cortex consists of the AF and the SLF. The ventral pathway connecting BA 45 to the temporal cortex involves the ECF. Figure is adapted from (Brauer et al., 2011). From (Friederici et al., 2011a).
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5. Conclusion and presentation of the experimental questions addressed in this thesis

5.1. Concluding remarks

From the literature reviewed in this introductory chapter, it appears clearly that the initial view of Broca’s area as devoted solely to speech production is outdated, challenged on many fronts. Even its status of an “eloquent” area has been questioned, since in the particular case of brain tumours that slowly degrade it, speech ability can remain unaffected suggesting that speech production can be supported by nearby cortical areas. Also, other linguistic functions have been attributed to Broca’s area, especially a role in syntax, which is still under investigation. When it was revealed that Broca’s area was implicated in non-linguistic tasks, the understanding about its function was once again deeply challenged. Even if we known now that a confusion about Broca’s area terminology has contributed to assigning to this region more functions that it should have, it remains that a pool of recent studies leave no doubt as to the non-linguistic involvement of the area, currently defined as BA44 and BA45. Broca’s area is certainly a multifaceted area. However, the problem remains of how to explain it. In light of the different assumptions discussed so far, we have focused on a particular hypothesis that views Broca’s area, and especially BA44, as a supramodal syntactic processor. In light of the previous evidence of the involvement of Broca’s area in syntax processing, it seems likely that its posterior part, BA44 is particularly important. Indeed, this part of Broca’s area has been found highlighted in the processing of hierarchical sequences in language (Friederici et al., 2006a; Friederici et al., 2006b; Bahlmann et al., 2008; Makuuchi et al., 2009), music (Koelsch, 2011), the visuo-spatial domain (Bahlmann et al., 2009) and action (Fazio et al., 2009).

5.2. Overview of this thesis

The present work is centred on the hypothesis that Broca’s area could be the core area for processing syntactic structures, especially in the action domain. In the related literature, there is a growing amount of data that substantiate this hypothesis but among them, little TMS evidence. This thesis addresses the presented issue through six TMS studies. By means of appropriately chosen TMS protocols, either off-line (continuous, cTBS) or online (repetitive, rTMS), we interfered with the function of Broca’s area (BA44 and/or BA45) in healthy participants, respectively before or during a sequence-related task.
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We studied the role of Broca’s area, successively in processing sequences that closely resemble to everyday life behaviours (study 1 and study 2), in processing more abstract sequences but that require higher levels of action control (study 3), in learning, by observation, a rule-based sequence of key-presses (study 4) as well as in learning, through physical practice, complex structured sequences either motor (study 5) or cognitive (study 6).

5.3. Important methodological information about TMS

The TMS technique has the advantage of overcoming some of the limitations encountered with the use of other methods to investigate the human brain. On one hand, functional imaging experiments showing activations i.e. transient local changes in neural activity during a given task, are useless for revealing causal relationships between changes in brain activity and their respective behavioural consequences. On the other hand, patient studies have to contend with the functional re-organization that occurs spontaneously after brain lesions. TMS enables to induce a transient “virtual lesion” of the area under stimulation: if the area is essential to a task, a behavioural effect should be observed (Pascual-Leone et al., 2000; Walsh and Cowey, 2000). In other words, TMS allows us to determine whether a cortical area is essential to the process under investigation. Since all the experiments in the present work are TMS experiments, we first want to highlight briefly the relevant features of its use. In order to grasp the methodological accounts of the TMS experiments that will be presented hereafter, an appendix is available, at the end of the manuscript, for more details about the TMS technique.

In the experiments of the present work, we only used repetitive stimulations, either through on-line (rTMS) or off-line (cTBS) protocols. We used an online protocol in studies 1, 2 and 3. Noteworthy, in the first two studies, we were interested in investigating the time period during which subjects reorganize a sequence. Therefore we used a delayed (with respect to the stimulus onset) and particularly long train of pulses in order to cover a larger time window because subjects needed several seconds to respond. An offline protocol was chosen in the other studies because in these studies we used learning paradigms so we have to determine the initial performances of subjects in a task, then applied TMS over the sites of interest, starting the learning procedure and finally testing performances again. Among the different off-line protocols we chose the cTBS because it has proven to induce long lasting effect despite a short period of application (40s) (Nyffeler et al., 2006).

Since it has been shown that the strength of stimulation increased when the induced current is perpendicular to the gyrus of the stimulated area (Thielscher et al., 2011), in our
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different studies we oriented the coil to reach this position but such position was occasionally adjusted to prevent discomfort in the subject. Also, since we have seen in section 1.2.2. and in section 1.2.3 that 1) BA44 and BA45 do not strictly correspond to, respectively, *pars opercularis* and *pars triangularis* and 2) there is a large inter-subject variability in the sulcal/gyral landmarks in this region, locating BA44/BA45 only by morphological landmarks could lead to imprecise locations. To avoid such difficulty, for all studies, we determined the coordinates of the area of interest from the literature and, through a reverse normalisation procedure, we were able to display the “ideal” site to stimulate onto the MRI image of each subject. Finally, in order to position the coil accurately over such site, we used an image-guided localisation system, so called neuronavigation system.
Chapter 2. Experimental contributions

1. Broca’s area contribution to the processing of biological action sequences

This first study was inspired by a recent study (Fazio et al., 2009), showing that aphasic patients, with a lesion encompassing Broca’s area (left BA44), were impaired in reordering pictures representing human actions (normal performance for pictures representing non-biological events). As this patient study cannot strictly correlate the observed deficit with a left BA44 dysfunction, we used TMS to bypass this limitation as this technique enables to create a focal “virtual lesion”. Therefore, we tried to pinpoint the causal contribution of the left BA44 in processing “biological”, i.e. human action, sequences. To this end, we performed virtual lesions of left BA44 in healthy volunteers executing the same task as that used by Fazio and collaborators.

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26 This section has been edited from the following article: Clerget E., Winderickx A., Fadiga L. & Olivier E. (2009). Role of Broca’s area in encoding sequential human actions: a virtual lesion study. NeuroReport, 20(16), 1496-1499.
Chapter 2. Experimental contributions

1.1. Abstract

The exact contribution of Broca’s area to motor cognition is still controversial. Here we used repetitive transcranial magnetic stimulation (rTMS, 5 Hz, 5 pulses) to interfere transiently with the function of left BA44 in 13 healthy subjects; the task consisted of reordering human actions or non-biological events based on three pictures presented on a computer screen and extracted from a video showing the entire sequence beforehand. We found that a virtual lesion of left BA44 impairs subject performance only for biological actions, and more specifically for object-oriented actions. Our finding provides evidence that Broca’s area plays a crucial role in encoding complex human movements, a process which may be crucial for understanding and/or programming actions.

1.2. Introduction

The contribution of Broca’s area to processes other than language is now widely recognized as indicated by its possible involvement in some aspects of memory, calculation (Gruber et al., 2001) and music processing (Maess et al., 2001). Moreover, Broca’s area is likely to contribute to high-level motor functions as suggested by the finding that some patients with lesions of the left inferior frontal gyrus may show apraxia (Goldenberg et al., 2007). Functional imaging studies have also reported activations of Broca’s area during various motor-related paradigm such as observation (Buccino et al., 2001), execution (Fadiga and Craighero, 2006; Koechlin and Jubault, 2006), imitation (Iacoboni, 2005) of actions and during motor imagery (Grezes and Decety, 2001).

Action observation is of particular interest because of the cytoarchitectonic similarity of Broca’s area with monkey premotor area F5, where mirror neurons have been originally found (Rizzolatti and Craighero, 2004) and because several neuroimaging studies have suggested that Broca’s area may be critically involved in this process. For instance, it has been shown that the activation of Broca’s area varied with the complexity of observed actions, indicating that this area could underlie the pragmatic encoding of observed actions in relation with their hierarchical organization (Molnar-Szakacs et al., 2006). Along the same lines, a recent study has revealed that patients with a lesion involving Broca’s area present deficits in reordering pictures showing human actions whereas this ability was preserved for non-biological events (Fazio et al., 2009). This finding further supports the hypothesis that Broca’s area could play a key role in encoding the hierarchical structure or, in other words, the motor syntax, of human actions. However, although patient studies provide useful cues about the causal relationship
between the Broca’s area lesion and the aforementioned behavioural deficits, the conclusions of such a study may be biased by the extent of the lesion and/or the possible brain reorganisation which may have occurred since that lesion.

To circumvent these limitations, we performed an interferential transcranial magnetic stimulation (TMS) experiment in healthy subjects, based on a paradigm close to that developed by Fazio and collaborators (Fazio et al., 2009). TMS was delivered over the pars opercularis of left Brodmann area (BA) 44, which corresponds to the posterior part of Broca’s region, the site where the maximum overlap between patient’s lesions was found by Fazio’s et al (Fazio et al., 2009).

1.3. Methods

Participants

Thirteen volunteers (mean age ± SD: 26.1 ± 5.4 years), right-handed as assessed by the Edinburgh handedness inventory (Oldfield, 1971), without any history of neurological disorders, participated in this experiment. They were screened for contraindications to TMS and informed about the nature of the experiment. All subjects gave their written informed consent and were paid for their participation. rTMS was administered according to current safety guidelines (Wassermann, 1998) and all procedures used in this study were approved by the Ethical Committee of the Université catholique de Louvain, in agreement with the Declaration of Helsinki.

Experimental procedure

The task consisted in reordering three pictures extracted from a video showing either a human action or a non-biological event that is an object in movement (Table 2.1). We used the same 15 biological and 10 non-biological videos as those used by Fazio and collaborators (Fazio et al., 2009) and added 5 new non–biological videos to reach the same number of biological and non-biological videos. Each video was presented four times in 4 different blocks of 30 trials each (15 biological and 15 non-biological trials pseudo-randomly distributed). The experiment was run on a personal computer connected to a touch-sensitive screen and controlled by a custom made program running under Labview. The time course of a trial is illustrated in Figure 2.1. After showing the video, three pictures extracted from it were displayed simultaneously, in a random order, on the computer screen and subjects had to point, with the right index finger, toward the picture representing the middle of the sequence. Participants were instructed to perform the task as accurately and as quickly as possible. The error rate and
reaction time (RT, the delay between the onset of the three picture display and the moment when the finger touched the screen) were automatically recorded and stored for off-line analysis. Each experiment started with a practice block followed by the four experimental blocks (two per site of stimulation, counterbalanced across subjects, see below).

Table 2.1: List the 15 biological and non-biological videos.

<table>
<thead>
<tr>
<th>Biological actions</th>
<th>Non biological sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>To open a cupboard by turning the key *</td>
<td>A bicycle falling down</td>
</tr>
<tr>
<td>To wipe out a blackboard *</td>
<td>An automatic drill</td>
</tr>
<tr>
<td>To turn one’s head and to point</td>
<td>A door closing</td>
</tr>
<tr>
<td>To serve a cup of tea *</td>
<td>An espresso machine</td>
</tr>
<tr>
<td>To open a wallet and take out a piece of paper *</td>
<td>A ball rolling down an inclined plane</td>
</tr>
<tr>
<td>To get up from the ground*</td>
<td>A lamp bubbling</td>
</tr>
<tr>
<td>To grab a bottle</td>
<td>A compact disc player</td>
</tr>
<tr>
<td>To cut a sheet of paper with a pair of scissors *</td>
<td>A moving train</td>
</tr>
<tr>
<td>To approach a wall on all fours and touching it</td>
<td>A miniature car rolling</td>
</tr>
<tr>
<td>To bow</td>
<td>A bouncing ball on the ground</td>
</tr>
<tr>
<td>To take off one’s glasses*</td>
<td>An escalator</td>
</tr>
<tr>
<td>To opening a notebook and write*</td>
<td>A Venetian blind</td>
</tr>
<tr>
<td>To climb a ladder to get a box*</td>
<td>A turning coin</td>
</tr>
<tr>
<td>To get over a scaffolding</td>
<td>A burning piece of paper</td>
</tr>
<tr>
<td>To touch the tip of one’s nose</td>
<td>A wheelchair</td>
</tr>
</tbody>
</table>

Asterisks indicate biological stimuli classified as “transitive and syntactic”.
Chapter 2. Experimental contributions

Figure 2.1: Experimental procedure and effect of left BA44 virtual lesions on reaction times.

Bottom: Time course of a trial. Each trial started with the display of a message ("ready?") that instructed the subject to put his/her right index finger on the circle displayed at the bottom of the touch screen, a position he/she had to keep until the display of the three pictures (see below). This contact of the index finger with the touch screen triggered the display of a short video clip (duration: 1-48 s) showing either a biological action or a non-biological sequence (see Table 2.1 for the full list). At the end of the video, a blank screen appeared for 500 ms, followed by 3 pictures extracted from the video clip. The subject had to point toward, and touch, the picture showing the middle of the biological action or of the non-biological sequence. When the answer was correct, the selected picture was surrounded by a green frame; in case of an incorrect response, the frame was red. The next trial started after a 1 s delay. rTMS (5 Hz, 5 pulses, shown in red) was delivered 500 ms after the display of the three pictures either over the left BA44 or over the leg representation of the primary somatosensory cortex (control).

Upper right corner: Histograms showing the mean reaction times (RT) and standard deviation (SD) in different conditions across subject (n=13) for biological and non-biological sequences. Asterisks indicated significant results (p<0.05).

Transcranial stimulation

rTMS (110% of resting motor threshold of the first dorsal interosseus muscle, 5 Hz, 5 pulses) was delivered 500 ms after the display of the three pictures over left BA44 and, as a control site, over the leg representation of the primary somatosensory cortex (S1 leg). A neuronavigation technique was used to determine and record the coil position for each subject (Noirhomme et al., 2004) (see Figure 2.2 for averaged locations and coordinates of stimulation sites).
Chapter 2. Experimental contributions

Figure 2.2: Mean location of stimulation sites.

The two stimulation sites were the pars opercularis of the inferior frontal gyrus (left BA44, red ellipse) and, as a control site, the part of the primary somatosensory cortex located near the midline, corresponding to the representation of the lower limb (S1 leg, blue ellipse).

Each ellipse is centered on the mean MNI (Montreal Neurological Institute) gathered for all subjects. The average of coordinates (mean±SD of x, y and z) for left BA44 were -59.1±2.6, 16.4±3.8, 20.6±4.7 mm and -2.7±2.4, -20.5±8.5, 74.9±5.9 mm for S1 leg. The surface of the ellipse represents the 95% confidence interval of the normalized coordinates calculated for each subject.

Statistical analysis

The effect of BA44 TMS on error rate and RT was analyzed by means of repeated measures (RM) ANOVA with SITE (left BA44 and S1 leg) and TYPE (biological and non-biological video) as within-subject factors. Post-hoc comparisons were performed using Tukey paired t-tests. This statistical analysis was performed twice; first, on all trials and second, on a subset of human action trials, as described in the Results section.

1.4. Results

When considering all biological trials together, the ANOVA\textsubscript{BA44} TYPE (2) x SITE (2) showed a main effects of the TYPE (F(1, 12) = 20.94, p<0.001) on RT and the post-hoc indicated that RT in biological trials were significantly longer than in non-biological trials. However, this analysis failed to demonstrate a main effect of SITE and a significant interaction (p>0.1).

In a second analysis, we categorized the biological actions according to the following criteria: 1) the presence of a hand-object interaction (transitive versus intransitive actions) and 2) the presence of a complex structure i.e. an action combining several individual motor acts (syntactic versus non-syntactic actions). Only the biological actions that met both criteria (i.e. transitive and syntactic actions, n=9/15, 36 trials/subjects) were taken into account in this second analysis and compared with the non-biological trials. The ANOVA\textsubscript{BA44} TYPE (2) x SITE (2) demonstrated a main effect of the TYPE (F(1, 12) = 29.72, p<0.001), as already reported.
Chapter 2. Experimental contributions

previously, but also revealed a significant interaction ($F(1, 12) = 6.60, p=0.024$) between TYPE
and SITE. A post-hoc analysis showed that TMS delivered over left BA44 led to longer RTs for
syntactic and transitive biological actions, when compared to the control condition (df=12,
p<0.001) (Figure 2.1).

Attempts to apply the same analysis to differently categorized biological trials (syntactic
only, transitive only or neither syntactic nor transitive actions) have been unsuccessful in
revealing a significant interaction. Analyses also failed to show any main effect or interaction
on error rate (all p>0.5).

1.5. Discussion

We found that a virtual lesion of left BA44 only affected the reordering task for transitive
and syntactic biological actions i.e. actions showing both a hand-object interaction and a
complex sequencing of individual motor acts. Non-transitive and non-syntactic actions and
non-biological sequences remained unaffected by left BA44 virtual lesions.

The present study differs from the study of Fazio et al. (Fazio et al., 2009) in two points.
First, we found that left BA44 virtual lesions had no consequence on response accuracy, but
only affected RTs, a finding rather common in the TMS studies (Stewart et al., 2001a). Second,
when all biological actions were taken into account, no TMS effect was observed for these
trials in comparison with non-biological trials. A possible explanation is that the present study
unveils the specific contribution of left BA44 to action recognition, thanks to the higher spatial
resolution of TMS than lesion studies. It is therefore sensible to assume that the results of
Fazio et al. (Fazio et al., 2009) were partly biased by the lesion extending to adjacent cortical
areas.

Importantly, we found that solely the processing of biological “syntactic” actions was
affected by left BA44 virtual lesions, suggesting that only the structure of biological actions is
processed by Broca’s area. This is consistent with the conclusion of a previous study using a
point light motion paradigm depicting simplified biological motions (Saygin, 2007) and showing
that only motion cues of biological actions activated the inferior frontal gyrus. Together with
our results, this finding may suggest that Broca’s area encodes both the sequence and the final
goal of biological actions from available cues. Moreover, in the context of the mirror neuron
theory (Turella et al., 2009a) we propose that Broca’s area may be critical in deciphering the
intermediate steps required to understand the final action goal. Indeed, in monkeys, mirror
neurons have been proven to match the agent's observed movements onto the observer's
motor repertoire (Gallese et al., 1996). If one admits that any observed action is automatically

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mirrored, causing the motor system to “resonate”, one could expect that not only the final goal is processed, as often claimed (Fogassi et al., 2005), but also some pragmatic details. Considering that any human action could be regarded as a combination of individual motor acts in which the order of the elements is of extreme importance to give sense to the global action, Broca’s area, in addition to its role in extracting the final goal of actions, may also encode the way this goal is achieved.

The present experiment provides new insight into the implication of Broca’s area in action observation by highlighting its role in decoding the hierarchical structure of observed actions. A recent study (Cattaneo et al., 2007) further illustrates the possible link between BA44 and the syntactic organization of actions. In this study, the authors reported that children with autism, a disorder possibly related to a dysfunction of the inferior frontal gyrus (Oberman et al., 2005), show a deficit in understanding the intentions of others during the observation of multistep-organized actions. In addition it is noteworthy that ideational apraxia, which can be defined as the disturbance of the conceptual organization of complex, sequential, actions (Rumiati et al., 2001), could result from a lesion of left the inferior frontal gyrus (Rumiati et al., 2004; Ebisch et al., 2007).

Finally, the methodological approach used in the present study should allow us to explore the possible role of the right homologue of Broca’s area in action observation and recognition. Indeed, on the one hand, brain imaging studies have often shown an activation of this area (concomitantly with Broca’s area) in such tasks (Buccino et al., 2001; Saygin, 2007), but, on the other hand, the pars opercularis of the right inferior frontal gyrus is known to be involved in distinct cognitive function such as the motor inhibition control and task switching (Aron et al., 2004). In the same way, the present paradigm should also allow us to dissociate between the distinct contribution of BA44 and BA45 (anatomically closed to BA44), in action encoding since it has been suggested that BA45 could process hierarchically higher events than BA44 (Koechlin and Jubault, 2006).

The present study further strengthens the view that the involvement of Broca’s area in language, and particularly in syntax processing, might be rooted in its premotor origin, as shown by the finding that actual or virtual lesions of this area led to deficits in pragmatic encoding of observed actions.
Chapter 2. Experimental contributions

2. Role of Broca’s area in retrieving the syntax of structured human actions\textsuperscript{27}

In study 1, we found that a left BA44 virtual lesion only impaired the reordering of biological actions when these actions were both transitive and syntactic \textit{i.e.} when they represented a sequence of individual motor acts involving a hand-object interaction. Since a dissociation between these two features of movement sequences was unachievable for methodological reasons, in a subsequent study (study 2), we aimed at testing whether the consequence of left BA44 virtual lesions, reported in study 1, could be explained by the syntactic aspect of the sequences. We also tested the other part of Broca’s area, the left BA45 as well as the right counterpart of BA44. To summarize, we used rTMS to perform virtual lesions of either left BA44, left BA45 or right BA44 in three distinct groups of healthy volunteers who had to rearrange sequences of biological or non-biological movements that consisted of either a chain of discrete actions (syntactic movements) or of a single continuous action (non-syntactic movements). Moreover, this syntactic function of Broca’s area could be viewed as a possible explanation to the deficit in gesture recognition observed in apraxic patients.

\textsuperscript{27} This section has been edited from the following article: Clerget E., Andres M., Winderickx A., Fadiga L. & Olivier E. (in preparation). Left BA44 contribution to gesture recognition and its possible implication in limb apraxia.
2.1. Abstract

A recent study on patients with limb apraxia has shown that lesions of the left inferior frontal gyrus (IFG), including Broca’s area, yield deficits in gesture recognition. Whether Broca’s area is specifically involved in this process and whether its lesion may be responsible for some of the symptoms reported in apraxic patients remain to be explored in detail.

To circumvent the unavoidable limitations of patient studies, we investigated this issue in healthy volunteers by interfering selectively with the functioning of the two Brodmann areas (BA) constituting Broca’s area (left BA44 and BA45) and its right homologue (right BA44) by using repetitive transcranial magnetic stimulation (rTMS). The task consisted in reordering pictures extracted from a short video-clip showing a movement sequence that could be either biological or non-biological and, within these two categories, either composed of a series of discrete events (“syntactic”) or non-syntactic i.e. consisting of a single continuous, linear movement.

We found that virtual lesions of the left BA44 interfered with subject performance only when they had to reorder movements that were both biological and syntactic, all other movement categories being unaltered. Interfering with the functioning of left BA45 or right BA44 had no effect on task performance, whatever the type of movements to be processed.

The present results shed new light on the contribution of Broca’s area to higher motor functions and on its possible involvement in limb apraxia. Because of its role in sequencing the different subunits of biological movements, a lesion of Broca’s area might impair the recognition, and possibly the understanding, of complex actions.

2.2. Introduction

A recent clinical study has indicated that lesions of the left inferior frontal gyrus (IFG), including both the pars opercularis (Brodmann area 44, BA44) and pars triangularis (BA45) (Amunts et al., 2010), are associated with deficits in gesture recognition (Pazzaglia et al., 2008). This finding is important because, although many functional imaging studies have suggested the possible role of left IFG in action recognition (e.g. Villarreal et al., 2008) and action understanding (e.g. Molenberghs et al., 2012), unambiguous validations are still required to ascertain the causal role of left IFG in those processes. Moreover, the exact function implemented in Broca’s area that might explain why its lesion leads to a deficit in action recognition is still unclear. Since Broca’s area is commonly regarded as a “universal” syntactic processor (Friederici et al., 2000a; Fiebach and Schubotz, 2006; Koechlin and Jubault, 2006;
Tettamanti and Weniger, 2006; Bahlmann et al., 2009; Fadiga et al., 2009; Tettamanti et al., 2009; Pallier et al., 2011), it is plausible that its lesion leads to a deficit in action recognition consequent to a difficulty in deciphering the hierarchical relationship between the different subunits of complex actions. This hypothesis predicts that a lesion of Broca’s region should alter only the recognition of actions containing a well-defined syntactic structure. On the other hand, since IFG is part of the putative mirror neuron system in humans (for a recent review see Rizzolatti and Sinigaglia, 2010), if its disruption is at the origin of the deficits in action recognition reported by Pazzaglia and co-workers, we predict that TMS applied over this region should impair action recognition, syntactically organized or not.

The finding of Pazzaglia et al. (2008) is also of significant importance in clinical neuroscience because it supports the, still debated, view that lesions of left IFG could, in some patients, lead to deficits classically associated with limb apraxia, such as pantomime of tools use (Goldenberg et al., 2007) and imitation of finger postures (Goldenberg and Karnath, 2006). Indeed, this vision appears somehow unconventional since limb apraxia - commonly defined as a disorder characterized by the inability to perform skilled gestures despite preserved intellectual and physical capacity - is still most frequently attributed to a lesion of the posterior parietal cortex (Heilman et al., 1982). However, such results gathered in patients have to be interpreted with caution because of the heterogeneity in lesion size and location, the possible involvement of the underlying white matter and the unavoidable functional reorganisation consequent to such lesions.

The aim of the present study was to investigate the role of Broca’s area in action recognition in order to gain further insight into its possible implication in limb apraxia. Transcranial magnetic stimulation (TMS) is a technique of choice to address this issue because it can be used to disturb transiently the functioning of a very small cortical area in order to pinpoint its causal role in the task at hand (Pascual-Leone et al., 2000; Sandrini et al., 2011). In particular, it has already been suggested that TMS delivered over left IFG, and more precisely over the pars opercularis, impaired action perception (Pobric and Hamilton, 2006) and action discrimination (Urgesi et al., 2007b; Urgesi et al., 2007a), although these results are still controversial (Hickok, 2009b; Kilner, 2011). Here we used repetitive TMS (rTMS) in healthy volunteers to interfere selectively with the functioning of the two cortical areas composing Broca’s area, namely left BA44 and BA45. We also aimed to explore the possible role of right BA44 in action recognition since several functional imaging studies have also shown an activation of this region in tasks involving action recognition although its exact contribution remains unclear (e.g. Schubotz and von Cramon, 2004; Hamilton and Grafton, 2008). The task we used in the present study was adapted from a recent clinical study (Fazio et al., 2009) and
Chapter 2. Experimental contributions

consisted of reordering pictures extracted from a short video-clip showing a movement sequence, which could be either biological or non-biological and, within these two categories, either composed of a series of discrete events (syntactic) or non-syntactic i.e. consisting of a single continuous movement.

We found that only left BA44 virtual lesions impaired the subject’s performance but solely when biological and syntactic movement sequences had to be processed. The present results offer a possible explanation for the deficits in pantomiming tool use found in some patients suffering from a lesion encompassing left IFG (Goldenberg et al., 2007). Indeed, by analogy with its function in language, the role of BA44 might be to compile the different movement subunits in order to build up a coherent action, reflecting the “syntax” of movements. The present study also allows us to differentiate the role of the different subparts of Broca’s area and its right homologue in movement recognition.

2.3. Methods

Participants

Twenty-four volunteers (10 women, mean age: 26 ± 6 years) were recruited and screened by a neurologist to rule out any potential contraindication for TMS (e.g. head injury, metal implants, pacemaker, epilepsy or any neurological disorder) according to the safety guidelines (Wassermann, 1998). All participants were right-handed according to the Edinburgh Inventory (Oldfield, 1971) and had normal or corrected-to-normal vision. After receiving a complete explanation about the different phases of the experiment, each participant gave his/her written informed consent. This study received the approval of the ethics committee of the Université catholique de Louvain, in agreement with the declaration of Helsinki. All subjects were compensated for their participation in this experiment.

Task

The task consisted in observing a short video clip (mean duration= 19.7 ± 9.9 s) showing different categories of movements (see below) and displayed on a 19” touch-screen monitor (ProLite T1930S, Iiyama, Tokyo, Japan) positioned 30 cm in front of the subject. Then three static pictures extracted from this video clip, and corresponding to three different phases of the movement (early, middle, late), were displayed in a random order on the screen and the subjects had to point, as fast as possible, with his/her right index finger, onto the image showing the middle stage of the movement.
Chapter 2. Experimental contributions

In the present experiment, we used forty video clips, showing either biological (n=20) or non-biological (n=20) movements and within each category, these movements could be either “syntactic” (n=10) i.e. composed of a series of three discrete events involving at least two objects or “non-syntactic” (n=10) i.e. consisting of one single continuous movement (see Table 2.2 for a full list of movements). Therefore, we ended up with four different categories of 10 video clips showing either: 1) biological and syntactic movements (BS); 2) biological and non-syntactic actions (BN); 3) non-biological and syntactic movements (NS); 4) non-biological and non-syntactic sequences (NN) (Figure 2.3). All biological actions involved a human actor performing a manual action but only his hands were visible. Only familiar objects/actions were used and, whenever possible, actions took place on a wooden table with a black background, except for those requiring a specific environment.

Table 2.2: List of the 40 video clips used in the present study.

The video clips were classified into 4 categories, namely biological and syntactic actions (BS), non biological and syntactic actions (NS), biological and non syntactic actions (BN), non biological and non syntactic actions (NN).

<table>
<thead>
<tr>
<th>Biological &amp; Syntactic actions</th>
<th>Non biological &amp; Syntactic actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>01. To clean a table</td>
<td>01. A complex of dominos falling down</td>
</tr>
<tr>
<td>02. To write a note on a post-it</td>
<td>02. A multistep photocopier</td>
</tr>
<tr>
<td>03. To replace the battery of a flashlight</td>
<td>03. A ball falling</td>
</tr>
<tr>
<td>04. To prepare a sandwich</td>
<td>04. An egg falling on the ground</td>
</tr>
<tr>
<td>05. To prepare a banana split</td>
<td>05. A balloon bursting in a flame</td>
</tr>
<tr>
<td>06. To put a CD in a CD player</td>
<td>06. A magnet attracting metallic objects</td>
</tr>
<tr>
<td>07. To turn on a notebook</td>
<td>07. A chocolate fountain functioning</td>
</tr>
<tr>
<td>08. To have a manicure</td>
<td>08. A vending machine distributing a can</td>
</tr>
<tr>
<td>09. To file a paper</td>
<td>09. A house of cards collapsing</td>
</tr>
<tr>
<td>10. To cut a star in a piece of paper and to glue it on a card</td>
<td>10. An automatic audio CD player</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Biological &amp; Non syntactic actions</th>
<th>Non biological &amp; Non syntactic actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>01. To absorb coffee with kitchen paper</td>
<td>01. A scanner functioning</td>
</tr>
<tr>
<td>02. To highlight a paragraph of a text</td>
<td>02. An automatic coffee machine</td>
</tr>
<tr>
<td>03. To pour water into a glass</td>
<td>03. Knots disentangling</td>
</tr>
<tr>
<td>04. To cut a slice of bread</td>
<td>04. A lamp making moving bubbles</td>
</tr>
<tr>
<td>05. To wipe out a whiteboard</td>
<td>05. A sweep hand of a clock moving</td>
</tr>
<tr>
<td>06. To peel an apple</td>
<td>06. A sand-glass elapsing</td>
</tr>
<tr>
<td>07. To remove a glove</td>
<td>07. A piece of paper burning</td>
</tr>
<tr>
<td>08. To measure something with a tape</td>
<td>08. An electrical teapot</td>
</tr>
<tr>
<td>09. To remove a sewing thread of clothes</td>
<td>09. A printer printing</td>
</tr>
<tr>
<td>10. To flick through a book</td>
<td>10. Ice cubes smelting</td>
</tr>
</tbody>
</table>
Chapter 2. Experimental contributions

Each trial consisted of the following events (see Figure 2.3):

- the word “Ready” was displayed at the centre of the computer screen to prompt the subject to put his/her right index finger on the starting position identified as a light green button and located at the bottom of the touch screen;
- the contact of the finger with the starting button changed its colour to dark green and triggered the presentation of a video clip;
- after a 500 ms blank screen, three static pictures extracted from the video were presented on the upper half of the touch screen;
- a rTMS train (see below) was delivered 600 ms after displaying the pictures;
- the subject had to provide, as quickly and accurately as possible, a response by pointing toward the “middle” picture with his/her right index finger;
- the selected picture was instantly surrounded by either a green or a red rectangle indicating, respectively, a correct or an incorrect response.
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Figure 2.3: Time course of a trial.

Each trial began with the display of a warning message (‘Ready?’) notifying the subject to place his/her right index finger on the green circle displayed at the bottom of the touch screen. This triggered the display of a video clip (see Table 2.2 for a list of all videos). At the end of the clip, a blank screen was displayed for 500 ms, followed by three still pictures extracted from the video clip. The participant had to point with his/her index finger onto the picture showing the middle of the sequence. When the answer was correct, the selected picture was surrounded by a green frame; in case of an incorrect response, the frame was red. The next trial started after a 1 second delay. The rTMS train (5 Hz, 6 pulses, shown in red) was delivered 600 ms after the display of the three pictures. Biological and syntactic actions (BS), non biological and syntactic actions (NS), biological and non syntactic actions (BN), non biological and non syntactic actions (NN).

All video clips were filmed with a Sony video camera (Sony Z7 HDV, Sony Corporation, Japan). Video clips were encoded by using Compressor 3.5 (Apple Inc, United States) and Mpeg StreamClip (Squared 5, Italy) on an iMac computer (Apple Inc, United States); then they were edited by using Final Cut Pro 6.5 (Apple Inc, United States) in order to synchronize the onset and offset of each video clip with the beginning and end of movements, respectively. Finally, for each video clip, 4 different series of 3 pictures (early, middle, and late stages), were
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extracted, saved as .jpeg files and de-interlaced by using Adobe Photoshop CS3 (Adobe System Inc., USA) in order to create 4 distinct trials for each video clip so that each subject can perform 4 blocks without repetitions of the same trials (see below).

Experimental design

The present study consisted of three distinct experiments designed to assess the possible contribution of left BA44 (Experiment #1), left BA45 (Experiment #2) or right BA44 (Experiment #3) in action recognition. Each experiment involved 8 different subjects and was composed of two sessions: a training session (2 blocks of 40 trials) and a TMS session (4 blocks of 40 trials), which took place on two different days. To avoid using the same video clip during the training and experimental sessions, videos from a previous experiment (Clerget et al., 2009; Fazio et al., 2009) were used to train subjects. In the TMS session, to avoid the repetition of the same trials in the 4 blocks, a different version of each trial (same video clip but 3 different pictures) was used for each block.

The experiment took place in a dimming room where the participants were seated in a comfortable armchair. The experiment was controlled by a PC running a program written in Labview (National Instruments; Austin, Texas, USA). Responses and reaction times (RT) were acquired and stored on the computer for the off-line analysis.

Neuronavigation and TMS application

The “ideal” coordinates for the three target sites were chosen on the basis of data available in the literature (Amunts et al., 2004; Anwander et al., 2007); for targeting left BA44, we used -43.5, 11.5, 16.5 mm (X, Y, Z, Montreal Neurological Institute, MNI, coordinates), -47.7, 29.2, 11.8 mm for left BA45 and 49.0, 14.0, 21.0 mm for right BA44. Since we stimulated areas in both hemispheres, as control sites, rTMS was applied over the representation of the leg in the primary somatosensory cortex (S1leg) in the corresponding hemisphere; the coordinates of the leg representation in S1 was estimated at -4.2, -24.2, 73.8 mm (left S1leg) and 4.2, -24.2, 73.8 mm (right S1leg) (Maldjian et al., 1999; Ragert et al., 2003).

Then, the MNI coordinates were reverse normalized to fit individual subject’s brain, whose MRI was gathered prior to the study. Before each experiment, the coil position was determined by using of a home-made MRI-guided neuronavigation system (Noirhomme et al., 2004; Davare et al., 2006; Davare et al., 2007a). After the experiment, the coordinates of the actual position of the coil were computed by off-line normalizing the individual actual coordinates with respect to the MNI brain atlas; these values were then averaged for all subjects. The mean coordinates of stimulated sites were -58.3±2.6, 19.2±3.4, 20.6±7.8 mm for
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left BA44 (mean±SD for x, y and z); -57.1±2.9, 34.7±1.3, 17.4±2.3 mm for left BA45; 61.4±1.2, 12.1±8.1, 24.5±7.0 mm for right BA44; these stimulated sites are shown in Figure 2.4A (Experiments #1 and #2) and in Figure 2.6A (Experiment #3). For left S1leg, the mean coordinates were -3.6±5.1, -13.0±7.8, 76.3±1.8 mm (Experiments #1 and #2, not illustrated) and 3.6±2.3, -10.9±10.7, 76.2±1.6 mm for right S1leg (Experiments #3, not illustrated).

TMS was delivered through a 70 mm external diameter figure-of-eight shaped coil with the handle pointing backward, and connected to the stimulator (Magstim Super Rapid, Magstim Company, Whitland, UK). The resting motor threshold (rMT) was measured in each subject in order to determine the intensity of stimulation for the rTMS (110% of the rMT). The rMT was defined as the lowest intensity for which ten single-pulse TMS applied over the hand representation of the primary motor cortex elicited five measurable motor evoked potentials (MEPs) of 50 µV amplitude peak to peak, in the contralateral first interosseous muscle (FDI). On average, the mean stimulation intensity (mean±SD) was 54 ± 6%, 55 ± 7% and 56 ± 5% of the stimulator maximal output, for Experiments #1 (left BA44), #2 (left BA45) and #3 (right BA44), respectively. However, it is noteworthy that, for sake of subject’s comfort, the maximal TMS intensity we used was limited at 63% of the stimulator maximal output, so that in 5 subjects (1 subject in Experiment #1, 2 in Experiment #2 and 2 in Experiment #3) the TMS intensity was slightly lower than 110% of the rMT. During each trial, 6 TMS pulses were delivered at a frequency of 5 Hz and after a 600 ms delay following the display onset of the three pictures. The long rTMS train duration (1 second) and the 600 ms delay were used to take into account the rather long RT found in this task (about 2.5 s) and to make sure to interfere with the sequence reordering process.

Data and statistical analyses

The statistical analyses were conducted both on RT and accuracy (percentage of correct responses) by using Statistica 7 (StatSoft Inc., Tulsa, Oklahoma, United States) and were performed separately for each experiment. For RT analysis, trials for which the response was incorrect (169/1280 in Experiment #1, 174/1280 in Experiment #2 and 172/1280 in Experiment #3) and trials during which technical problems occurred (3/1280 in Experiment #1, 2/1280 in Experiment #2 and 1/1280 in Experiment #3) were discarded. Altogether, 13.5% of trials were discarded in Experiment #1, 13.7% in Experiment #2 and 13.4% in Experiment #3. As our main objective was to determine whether rTMS affected the RT differently depending on the type of movements to be processed (BS, BN, NS, NN), the mean RT in the control condition (i.e. left S1leg for Experiment #1 and #2, right S1leg for Experiment #3) was compared with the mean RT gathered in the corresponding experimental condition (i.e. TMS applied over left BA44 in
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Experiment #1, left BA45 in Experiment #2 and right BA44 in Experiment #3. To that end we performed repeated measures ANOVA (ANOVA_RM) on these data with three within-subjects factors, namely SITE (control site vs left BA44 in Experiment #1, left BA45 in Experiment #2 or right BA44 in Experiment #3), BIOLOGICAL (biological vs non-biological) and SYNTACTIC (syntactic vs non-syntactic).

2.4. Results

Role of left BA44 in action recognition

An ANOVA_RM was performed on RT with SITE, BIOLOGICAL, and SYNTACTIC as within-subjects factors (Figure 2.4B). The ANOVA_RM revealed a significant main effects of BIOLOGICAL (F(1, 7) = 28.97; p = 0.001) and SYNTACTIC factors (F(1, 7) = 41.71; p < 0.001), a significant interaction between these two factors (F(1, 7) = 8.70; p = 0.02) and also a significant triple interaction (F(1, 7) = 11.02; p = 0.01). Post-hoc analyses revealed that, for the main effects of BIOLOGICAL and SYNTACTIC factors, RT for biological trials was larger than for non-biological trials and RT for syntactic trials were larger than for non-syntactic trials. For the double interaction the post-hoc tests showed that RT for biological and syntactic trials (BS) was significantly longer than RT for all the other movement categories. Finally and most importantly, as far as the triple interaction is concerned, post-hoc revealed that in the left BA44 TMS condition, RT selectively increased for the biological syntactic (BS) trials (3.35 ± 1.25 s) when compared to the control (3.04 ± 0.84 s, df = 7, p = 0.008). A comparable ANOVA_RM (ANOVA_RM with BIOLOGICAL, SYNTACTIC and SITE as within-subjects factors) performed on the percentage of correct responses failed to reveal any significant main effects or interactions (all F(1, 7) < 0.67; all p>0.10) and thus no effect of TMS on accuracy when applied over left BA44.
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Figure 2.4: Results from Experiment #1 and #2.

A. Mean location of the stimulation points normalized into the MNI system of coordinates over left BA44 (blue) and left BA45 (orange). The ellipse surface (centred on the mean coordinates of each stimulation site) indicates the 95% confidence interval.

B. Effects of virtual lesions of left BA44 (blue) and left S1leg (gray) on reaction times. The histograms showed the mean reaction times (RT) and standard error (SE) for all the participants (n=8) and for the four different conditions. Significant results (p<0.05) are indicated by an asterisk.

C. Effects of virtual lesions of left BA45 (orange) and left S1leg (gray) on reaction times. The histograms showed the mean reaction times (RT) and standard error (SE) for all the participants (n=8) and for the four different conditions.
Because the previous analysis showed that the mean RT for BS movements was significantly longer than that in the other conditions, it was important to rule out the possibility that the effect of TMS on left BA44 was not due to a higher difficulty in processing BS sequences. To do so, we computed, for the four trials based on the same video-clip, a correlation between RT in the control condition and amplitude of left BA44 virtual lesion (Figure 2.5). The amplitude of the TMS effect was expressed as a percentage of the RT increase induced by left BA44 TMS with respect to the control condition (\((\text{RT}_{\text{BA44}} - \text{RT}_{\text{control}})/\text{RT}_{\text{control}}\) x 100). This analysis did not show a significant correlation \((r = -0.0535, p=0.74)\) between the amplitude of TMS effect and trial difficulty, indicating that the specific effect of left BA44 virtual lesions on RT for the BS movements could not be due to the difficulty factor. A comparable result was obtained when performing the same analysis only on trials from the BS category \((r = 0.0398, p=0.91)\).

Figure 2.5: Correlation analysis.

Correlation analysis between the relative TMS effect induced by BA44 virtual lesions and the RTs in the control condition, for the different types of sequences. The RT was regarded as an index of trial difficulty.
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Effects of TMS applied over left BA45

In Experiment #2, an ANOVARM with SITE, BIOLOGICAL and SYNTACTIC factors as within-subjects factors performed on mean RT (Figure 2.4C) revealed a main effect of BIOLOGICAL (F(1, 7) = 44.27; p < 0.001) and SYNTACTIC factors (F(1, 7) = 50.69; p < 0.001) on RT, whereas the SITE had no significant effects (F(1, 7) = 0.24; p = 0.64). A marginal double interaction between BIOLOGICAL and SYNTACTIC factors was also found (F(1, 7) = 3.28; p = 0.11) and post-hoc corroborated that BS trials led to longer RT when compared to other movement categories (all p<0.04). No other interactions were found (all F(1, 7) < 0.69; all p > 0.43). A similar analysis performed on the percentage of correct responses failed to reveal any significant main effects or interactions (all F(1, 7) < 0.47; all p > 0.21).

Right BA44 virtual lesions

Following right BA44 virtual lesions (Experiment #3), an ANOVARM showed a significant main effect of BIOLOGICAL (F(1, 7) = 111.88; p < 0.001) and SYNTACTIC factors (F(1, 7) = 129.19; p < 0.001) and a significant interaction between these two factors (F(1, 7) = 36.03; p < 0.001) (Figure 2.6B). The post-hoc test confirmed that BS trial led to longer RT than other trial categories (df = 7, all p < 0.001). The main effect SITE was not significant (F(1, 7) = 1.30; p = 0.29) and no triple interaction was found (F(1, 7) = 0.19; p = 0.68). As far as the percentage of correct responses is concerned, ANOVARM failed to unveil any significant main effect or interaction (all F(1, 7) < 0.56; all p > 0.19).
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Figure 2.6: Results from Experiment #3.
A. Mean location of the stimulation points, normalized into the MNI system of coordinates over right BA44 (red). The ellipse surface (centred on the mean coordinates of each stimulation site) indicates the 95% confidence interval.
B. Virtual lesions effects over right BA44 (red) and over right S1leg (gray) on RT. The histograms showed the mean RT and standard error (SE) for all the participants (n=8) and for the four different conditions.

2.5. Discussion

The aim of the present study was to investigate the role of Broca’s area in action recognition and to elucidate its possible involvement in limb apraxia. In particular, we wanted to distinguish between the contribution of the two areas constituting Broca’s area – BA44 and BA45 – to this process since a functional dissociation between both parts has already been proposed for action observation and imitation (Molnar-Szakacs et al., 2005). In addition, we aimed to investigate the possible role of right BA44 in action recognition since the exact function of the right homologue of Broca’s area remains controversial mainly due to frequent bilateral activations in this region (e.g. Buccino et al., 2001). The present results indicate that a virtual lesion of left BA44 impaired action recognition only when subjects had to process
movement sequences that were both biological and syntactically organised; TMS applied over left BA44 had no effect when subjects had to process other movement categories, including biological but non-syntactic movements. We also found that interfering with the functioning of left BA45 had no effect on task performance, irrespective of the type of movements to be processed; similarly, a virtual lesion of right BA44 left subject performance unaltered.

The present study corroborates the view that left BA44, the caudal part of Broca’s area, plays an important role in arranging biological actions (Fazio et al., 2009) but it goes further by showing that this is true only when biological movements are composed of several subunits which are hierarchically organized (Clerget et al., 2009). Several studies have already suggested the contribution of Broca’s area to the recognition of biological actions as part of the MNS (Rizzolatti and Arbib, 1998; Buccino et al., 2001; Buccino et al., 2004a; Saygin et al., 2004; Clerget et al., 2009; Fazio et al., 2009) but the present study allows us to establish that only the posterior part of Broca’s area (pars opercularis, BA44) is involved in this function, thanks to the relatively high spatial resolution of TMS with respect to the size of human brain (Ro et al., 1999; Walsh and Cowey, 2000; Bolognini and Ro, 2010). It is worth noting that clinical studies have been unable to locate this function with such a precision (Goldenberg et al., 2007; Pazzaglia et al., 2008; Fazio et al., 2009) and that functional imaging studies do not allow us to determine the causal relation between a given brain region activation and its contribution to the task at hand. This further corroborates the unique place of TMS in cognitive neurosciences (Walsh and Rushworth, 1999; Walsh and Cowey, 2000; Bolognini and Ro, 2010; Sandrini et al., 2011).

The finding that only the recognition of a specific category of movements relies on the integrity of left BA44, namely the so-called ‘syntactic’ actions, is also of interest. In the present study, we defined an action as “syntactic” when being composed of several distinct subunits that have to be arranged in a precise manner to lead to a comprehensible and meaningful action. It is therefore tempting to conclude that the contribution of BA44 to action observation, recognition and/or understanding reported in a myriad of previous studies (for a recent review see Caspers et al., 2010) relies on its role in deciphering the hierarchical relationship between the different subunits of complex actions. This explanation is compatible with the view that Broca’s area, as a whole, is generally regarded as a universal syntactic processor (Grossman, 1980; Greenfield, 1991; Patel, 2003; Koechlin and Jubault, 2006; Tettamanti and Weniger, 2006; Bahlmann et al., 2009; Fadiga et al., 2009; Tettamanti et al., 2009). The absence of syntactic structure in the “linear” biological movements would explain why they remained insensitive to left BA44 virtual lesions. This result fits with the results of a study using video clips depicting biological actions (i.e. object-directed hand actions) vs
“inanimate motion events” and in which the authors found that the processing of biological actions activates predominantly the left BA44 (Baumgaertner et al., 2007). The present results also corroborate the view that processing non-biological stimuli relies on distinct brain areas, although the processing of structured sequences may involved the same primary neural network, then further complemented by additional areas, depending on the nature - biological or non-biological - of the stimuli to be processed (Schubotz and von Cramon, 2004).

Because Broca’s area corresponds, at least partly, to the homologue of area F5 in monkeys (Rizzolatti et al., 2002), from which mirror neurons were recorded (Gallese et al., 1996; Rizzolatti et al., 1996a), and because many functional imaging studies in humans have shown an activation of Broca’s area during action observation, this brain region, as part of the MNS, has been regarded as essential for action understanding (Rizzolatti and Sinigaglia, 2010). The mirror neurons theory posits that human beings understand another person’s goals by mapping observed actions into their own motor repertoire and if, the observed action contains recognizable parts, which have already been used by the observer, it may facilitate both the understanding and imitation of this action. The task we used in the present study does not allow us to determine whether a virtual lesion of BA44 also alters the understanding of the goal of actions shown in the video clips but because recognizing an action is a necessary condition to understand it, endowing Broca’s area with an additional function is unnecessary to explain the present results. The link between action understanding and Broca’s area may therefore be grounded in the key role played by this area in processing syntactic aspects of complex actions.

The lack of contribution of the anterior part of Broca’s area (pars triangularis, BA45) to action recognition is not really surprising since few studies have highlighted a role of this part of Broca’s area in related task (Grafton et al., 1996; Rizzolatti et al., 1996b). As previously mentioned, the literature on syntax processing rather focuses on BA44. Indeed, this part of Broca’s area is a core area both for syntactic processing (e.g. Bahlmann et al., 2009) and in the MNS (e.g. Rizzolatti et al., 1996b). Alternatively, it is possible that our action sequences are too simple to involve BA45. Indeed, some authors (Koechlin and Jubault, 2006) have proposed a model of hierarchical control for sequence processing in which more complex sequences activate more anterior region in Broca’s area i.e. BA45. However it has been shown that even if during action observation the mirror activity in frontal areas is correlated to the “motoric complexity” of the observed actions, there is no activation of BA45 related to this increasing complexity (Molnar-Szakacs et al., 2006). Therefore, the present results support the view that Broca’s area is, from a histological point of view, a multifaceted cortical region (Amunts et al.,
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2010), probably supporting manifold functions (Nishitani et al., 2005; Grodzinsky and Amunts, 2006; Fadiga et al., 2009).

As already mentioned in the Introduction, Pazzaglia et al. (2008) recently suggested that left IFG lesions might be responsible for deficits in action recognition described in patients with limb apraxia. Although this claim remains somehow odd because limb apraxia has been associated for ages with lesions of the posterior parietal cortex (Heilman et al., 1982), a possible involvement of Broca’s area in limb apraxia has already been suggested occasionally (Goldenberg and Karnath, 2006; Goldenberg et al., 2007). Because of its role in sequencing the different subunits of complex actions, and therefore possibly in recognising actions, a lesion encompassing Broca’s area may indeed yield deficits in some tests traditionally used in clinical neurology and neuropsychology to assess limb apraxia. Amongst those, pantomiming tool use is regarded as very sensitive for limb apraxia (Dovern et al., 2012). One possible explanation for a deficit in such a task following a left IFG lesion, encompassing BA44, is the difficulty, in those patients, in organising the elements necessary to build up a coherent sequence of actions, reflecting the “syntax” of movements. Lacking this access to syntactically-related knowledge following a Broca’s area lesion might therefore be detrimental when pantomiming tool use (Goldenberg et al., 2007) but be unnoticeable when patients had to perform the real action involving the same tool because using the actual tool is probably less demanding for the system (Goldenberg et al., 2007) and may rely on a great redundancy in the neural circuits responsible for naturalistic actions (Schwartz and Buxbaum, 1997). Another task in which patients with left IFG lesions have been found impaired is the imitation of finger postures, the imitation of hand postures being preserved but altered after parietal lesions (Goldenberg and Karnath, 2006). It has been suggested that imitation of finger postures is performed by matching the demonstrated finger position to a known hand shape stored in the motor system and associated with the use of a precise object or tool (Goldenberg and Karnath, 2006). Although this explanation cannot probably account for deficits in all meaningless gestures, it can indeed explain why, on average, patients with a left IFG lesion are less proficient in this task.

Finally, the present study failed to attribute any causal function in action recognition to the right homolog of Broca’s area (right BA44). The exact role of the homolog of Broca’s area in the right hemisphere remains puzzling (Nishitani et al., 2005) since many functional imaging studies have shown its activation in action observation and motor imagery (Binkofski and Buccino, 2004; Schubotz and von Cramon, 2004) but also when executing hierarchically
structured motor sequence (Koechlin and Jubault, 2006) while other rather suggested a specific role of the left BA44 (Bahlmann et al., 2009). It is quite possible that its contribution to syntactic processing is subsidiary and that its lesion is naturally compensated by left BA44. Performing a bilateral BA44 virtual lesion could answer this question by showing a supplementary deficit in action recognition, a technique which has proved to be useful to address the issue of the lateralization of higher motor and cognitive functions (Davare et al., 2007b).

The present study shed new light on the contribution of Broca’s area to higher motor functions and on its possible involvement in limb apraxia by demonstrating the involvement of left BA44 in sequencing complex actions.
3. Contribution of Broca’s area to complex sequence initiation

In the two previous studies we used videos and pictures of real action sequences. In the present study, we wanted to determine if Broca’s area could be involved in processing more abstract sequences like key-presses sequences, easier to manage in experimental paradigms. The present study is based on a key article (Koechlin and Jubault, 2006) in which the authors, based on the fMRI data they obtained, proposed a model associating the different parts of Broca’s region with successively higher levels of action control. In other words, their theoretical model proposes an anatomo-functional gradient: BA6 is in charge of the processing of the units that constitute action sequences, BA44 is involved in the selection of hierarchically lower elements and BA45 is involved in processing hierarchically higher motor plans. To bring causal support to this model, the original tasks of Koechlin and Jubault were used but with some changes to adapt them to a TMS protocol.

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28 This section is a modified version of an article, in preparation, with identical title by Clerget E. & Olivier E.
3.1. Abstract

While the involvement of Broca’s area into many non-linguistic functions is now widely accepted, its precise role remains a matter of debate. A recent functional imaging (fMRI) study (Koechlin and Jubault, 2006) has shown activations in Broca’s area while processing hierarchically structured movement sequences and, importantly, the location of these activations varied according to the hierarchical level to be processed: the anterior and posterior parts of Broca’s being specifically activated while processing, respectively, high and low-order hierarchical elements. That study yielded a stimulating hypothesis that Broca’s area contributes, with the dorsal premotor cortex (PMd), to a network organized along a rostro-caudal axis and processing the different hierarchical levels along the same rostro-caudal gradient. However, because fMRI data are, by definition, correlative, the causal relationship between changes in brain activity and their respective behavioural significance cannot be ascertained.

To circumvent this unavoidable limitation of fMRI, we used transcranial magnetic stimulation (TMS) to interfere transiently with the function of three areas in the left hemisphere that corresponded to activation peaks reported by Koechlin and Jubault (2006), namely the anterior and posterior parts of Broca’s area and part of the premotor cortex. Subjects had to perform two different tasks, adapted from those used by Koechlin and Jubault (2006), which differed by the hierarchical organisation of the key-press sequence. We found that the performance of the lower-order hierarchical task (Experiment #1) was unaffected when TMS was applied over any of these three cortical areas. In contrast, we found a specific contribution of the anterior part of Broca’s area to the initiation of sequence in the higher-order hierarchical task (Experiment #2).

The present study demonstrated the critical role of Broca’s area in processing structured sequences and, in particular, of its anterior part, which appears crucial when initiating complex sequences. This finding highlights the role of Broca’s area in processing hierarchically organized movements.

3.2. Introduction

While Broca’s area is still predominantly known for its key contribution to language, its involvement in other non-linguistic functions is now acknowledged, though still puzzling. One stimulating assumption is that Broca’s area could act as a “supramodal hierarchical processor” (Grossman, 1980; Tettamanti and Weniger, 2006), able to process any kind of hierarchically
organised behaviours, including language. This hypothesis is rooted in the involvement of Broca’s area in processing syntax and syntax-like aspects of various non-linguistic domains (Fadiga et al., 2009). Furthermore, much evidence tends to show that, within Broca’s area, only the posterior part (Brodmann area 44, BA44) is the area responsible for processing syntax (Bahlmann et al., 2008; Bahlmann et al., 2009; Fazio et al., 2009). However, this view is still highly controversial since some studies have attributed a similar function to the anterior part of Broca’s area (BA45) (Santi and Grodzinsky, 2007; Friedrich and Friederici, 2009; Tyler et al., 2010b; Pallier et al., 2011).

A possible explanation for this discrepancy is that, both BA44 and BA45 are involved in processing different hierarchical levels. Support in favour of this hypothesis comes from functional imaging studies having investigated the syntactic complexity in both linguistic (e.g. Friederici et al., 2006b) and non-linguistic tasks (e.g. Koechlin and Jubault, 2006). These studies have demonstrated that the activity in Broca’s area depends on the degree of complexity of the syntactic sequences to be processed. Interestingly, in the study of Koechlin and Jubault (2006), the authors proposed a model in which the anterior (BA45) and posterior (BA44) parts of Broca’s area process different levels of hierarchy in movement sequences. More precisely, the control of hierarchically organized sequences is performed by distinct cortical regions distributed along a rostro-caudal axis: the more rostral the brain regions are, the higher the hierarchical level they are able to process. This view is in line with numerous studies on the functional organization of the frontal cortex, including the Broca’s area (Koechlin et al., 2003; Badre and D’Esposito, 2007; Botvinick, 2007; Koechlin and Summerfield, 2007; Badre, 2008; Botvinick, 2008; Badre and D’Esposito, 2009; Badre et al., 2010). These studies posit that the frontal lobe, mainly responsible for cognitive control, is organised in such a way that the most rostral areas process the most complex levels of action/behaviours organisation. However, if the study of Koechlin and Jubault allows us to identify which areas are active when processing hierarchically structured motor behaviours, it cannot determine whether those areas are indeed causally involved in this process.

To address this issue, we adapted the tasks designed by Koechlin and Jubault (2006) to investigate the contribution of Broca’s area to sequence execution with distinct hierarchical levels by taking advantage of the Transcranial Magnetic Stimulation (TMS) technique. Hence, the cortical sites chosen to apply TMS were the areas in the left hemisphere in which Koechlin and Jubault (2006) reported activation peaks, namely the anterior and posterior parts of Broca’s area, and a site located in the premotor cortex chosen for its known involvement in action selection.
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3.3. Methods

Experiment #1. Simple task

Participants

Seven subjects (27±4 years) participated in Experiment #1. They were all right-handed, as assessed by the Edinburgh handedness inventory (Oldfield, 1971), had normal, or corrected to normal, vision and no neurological disease history; none of them was under the influence of medication, alcohol or drug. Each subject was seen by a neurologist to rule out potential risk of adverse reactions to TMS, based on the Transcranial magnetic stimulation Adult Safety Screen (TASS; (Keel et al., 2001)). All subjects gave their written informed consent and were compensated for their participation. The Ethics Committee of the Université catholique de Louvain has approved all experimental procedures.

Task

The task used in Experiment #1 (see Figure 2.7A and Figure 2.8A) corresponded to the “simple task” designed by Koechlin and Jubault (2006). It consisted of executing a pre-learned sequence of key-press movements performed either with the left (L), the right (R) or both index fingers (LR). The sequence of key-press was always the same in this experiment, namely LR, LR, R, R, L. A green square displayed on the centre of a computer screen indicated the beginning of a new sequence and that subjects had to generate the first movement (LR) of the sequence; this green square was named the initiation (INIT) cue and was displayed for 500 ms, like all the other cues. The next movements of the sequence (LR, R, R) were triggered by a so-called intermediate (INTER) cue represented, randomly, either by a blue or a yellow square, and displayed at an interval varying between 2500 and 4000 ms, incremented by steps of 500 ms. The end of the sequence was indicated by the presentation of a red square, so-called the termination (TERM) cue, used to trigger the last movement (L); the TERM cue could either be displayed after the completion of a full sequence (endogenous termination) or, unpredictably, during the course of a sequence (exogenous termination). The proportion of endogenous and exogenous terminations was identical and the three possible cases of exogenous termination (after the INIT cue or after the first or the second INTER cue) were evenly distributed. In order to gather a baseline between each sequence, the subjects performed LR movement(s) triggered by so-called baseline (BL) cues, which were either blue or yellow squares; one to four BL cues were presented between each sequence, at a variable delay (2500 to 4000 ms, step of 500 ms).
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Overall, one trial consisted of one (complete or aborted) sequence followed by 1 to 4 baseline movements, its duration varied approximately between 9 and 27 s.

Experimental procedure

The experiment was divided into three sessions, distributed over one week. The first session was a training session in which the subjects repeated the task until their performance reached 95% of correct trials. This training session usually consisted in 4 blocks of 15 trials. The two following sessions were TMS sessions (see below), each of them being composed of 4 blocks of 15 trials. During each block, TMS was applied over one of the four stimulation sites investigated in the present study (see below). The block order was counterbalanced across subjects but was kept constant for each subject across sessions.

Transcranial magnetic stimulation

The TMS was delivered through a 70 mm figure-of-eight coil connected to a Rapid Magstim model 200 stimulator (Magstim Company, Whitland, UK). During the first training session, the resting motor threshold (rMT) for the hand representation of left primary motor cortex was evaluated for each subject. The rMT was defined as the minimum TMS intensity necessary to induce 50µV peak-to-peak motor evoked potentials (MEPs) in the first dorsal interosseus muscle in about 5 out of 10 trials. The TMS intensity was set at 110% of individual rMT. Pulses were delivered at 10 Hz during 200 ms and were synchronized on the cue onset (3 pulses at 0, 100, 200 ms with respect to the cue onset). To comply with the safety guideline (Wassermann, 1998), only 2 trains of rTMS were delivered in each trial, one synchronized with the onset of either an INIT, INTER or TERM cue, and the second one with the onset of a BL cue; each trial was organised in such a way that rTMS was never delivered on two successive cues and were therefore separated by at least 5 s.

Localisation of stimulation sites

The coil position was precisely determined by means of a neuronavigation technique (Davare et al., 2007b; Clerget et al., 2009; Clerget et al., 2011; Clerget et al., 2012) of the stimulation sites onto individual anatomical magnetic resonance images previously gathered for each subject. Three sites were targeted, namely, a premotor site (“PMd”) and two others sites located in Broca’s area, i.e. a posterior site (“PB”) and an anterior site (“AB”). The leg representation of the left primary motor cortex was used as a control site (“control”). In each subject, the PMd, PB and AB sites were localised by using a “probabilistic” approach (for further details, see Sparing et al., 2008). To localise PMd (target coordinate: [-25, 4, 72 mm],
Montreal Neurological Institute (MNI) system of coordinates), we averaged the coordinates of three TMS experiments (Davare et al., 2006; O'Shea et al., 2007a; O'Shea et al., 2007b). To target PB (target coordinate: [-44, 2, 39 mm]) and AB (target coordinate: [-44, 24, 19 mm]) we used the coordinates of the activation peaks reported by (Koechlin and Jubault, 2006).

These sites were transformed to the individual subject’s brain coordinate “native” space using a reverse normalization procedure and were displayed on the MR image of each subject. Then, the accurate positioning of the coil onto the scalp was ensured by using a home-made neuronavigation program (Noirhomme et al., 2004). The location of the control site in M1 was determined in each subject by searching the point that produced an observable movement of the right leg.

Finally we performed an off-line normalization of individual coordinates of the TMS sites with respect to the MNI brain atlas. The normalization procedure was performed by normalizing each individual (native) head image to the standard MNI brain template by mean of an iterative algorithm that searches for the optimal projection of a given brain onto the MNI. Details about these neuronavigation and normalization procedures are available elsewhere (Noirhomme et al., 2004; Davare et al., 2006; Davare et al., 2007b). For the two TMS sessions, the mean coordinates (mean±SD for each coordinates x, y and z, n=14) of the four stimulated area were as follows: control (-4±5, -24±22, 74±6 mm); PMd (-28±4, 10±2, 65±4 mm); PB (-55±3, 10±8, 43±3 mm) and AB (-56±3, 27±5, 23±4 mm) (Figure 2.7B).

Data acquisition and statistical analysis

The experiment was implemented with Matlab (The Mathworks, Inc.) running on a personal computer. Reaction times (RT) were defined as the delay between the cue onset and the moment the subject presses the key; they were computed separately for each cue. For each subject and condition, the relative TMS effects were computed as follows: (RT\textsubscript{TMS} - RT\textsubscript{noTMS})/( RT\textsubscript{noTMS}) * 100. Then, the relative TMS effect on the RT gathered in the control condition was subtracted from the relative TMS effects gathered for PMd, PB and AB in order to isolate the “specific effect” of TMS; this value was expressed in percentage. Incorrect trials (2.45 ± 1.28%) and trials in which RT was larger than the mean individual value ± 2 standard deviations for each subject were discarded (4.71 ± 1.25%) from the subsequent analyses.

Data were analyzed by using repeated measures ANOVA (ANOVA\textsubscript{RM}). To determine the subject performance in control (no-TMS) trials, we performed an ANOVA\textsubscript{RM} on RT with the CUE (BL, INIT, INTER, TERM) as a within factor. The unspecific TMS effect on RT was analysed by mean of a one-way ANOVA\textsubscript{RM} with TMS (no TMS, TMS) as a within factor. Finally, to demonstrate a specific TMS effect on RT for the different cues and stimulation sites, we
performed a two-way ANOVA with the following factors: SITE (PMd, PB and AB) and CUE (BL, INIT, INTER, TERM).

When appropriate, post-hoc comparisons were performed using a Tukey test, except for the analysis of the specific effect of TMS for which we used a Dunnett test, the most appropriate test to reveal significant differences between the BL cue and every other cues as this test is favoured when the mean has to be compared with a standard reference.

Experiment #2. Superordinate task

The aim of Experiment #2 was to address the same issue as in Experiment #1 but in a more complex task, named “superordinate task” by (Koechlin and Jubault, 2006). Because both Experiments #1 and #2 were identical in many aspects (TMS application, stimulation sites, data acquisition, analyses,…), only the distinctive points of Experiment #2 will be described in the following sections.

Participants

Sixteen subjects took part in Experiment #2, five of them having participated in Experiment #1. Two subjects were excluded from the subsequent analyses because either the mean RT (subject #2) or error rate (subject #13) was larger than the mean group value ±2 SD. The mean age of the 14 remaining subjects was 27±4 years.

Task

In the “complex task” (Figure 2.8B and Figure 2.8C), squares were replaced by three letters (A, B, or C) while both the timing and colour code were kept the same as in Experiment #1: green and red letters indicated, respectively, the INIT and TERM cues; blue and yellow letters were either INTER or BL cues. The subjects had to perform the same key-press task but, in contrast to Experiment #1, the sequence of responses differed across trials depending on three different rules (r1, r2, r3). Thus, in Experiment #2, subjects learned a rule instead of a sequence of movements (Figure 2.8B). The order according to which these three rules had to be applied was the same in each trial (r1, r1, r2, r2, r3), but, because the movement triggered by a given cue was different in each rule, this procedure ensured that each trial was different in terms of movement sequence. Indeed, in the r1 rule, “A” indicated a right index key press whereas “B” and “C” a left index response. In the r2 rule, “B” indicated a right key press, “A” and “C” a left key press and in r3, “C” was associated with a right finger response, “A” and “B” with a left one (Figure 2.8C). In a given block of 15 trials, letters were pseudo-randomly chosen
so that the proportion of left and right responses was equal. In response to BL cues, participants were instructed to apply always the r1 rule.

As in Experiment #1, incorrect trials (3 ± 2%) or trials with RT longer than the individual mean ± 2 SD were discarded (4.79 ± 0.74%) from analyses.

Data acquisition and statistical analysis

For Experiment #2, an additional ANOVA_{RM} was performed on significant “specific TMS effect” (see the Results section) in order to investigate the hand contribution to these effects.

Figure 2.7: Trial time course and stimulated sites.

A. Time course of stimuli presentation for one trial.
   A first stimulus (a square, as illustrated, in Experiment #1, or a letter in Experiment #2) appears during 500 ms and is followed by a waiting period of different possible durations (2500, 3000, 3500 or 4000 ms) until a second stimulus appears and so on. For each train of stimulation, the TMS pulses occurred at 0, 100, 200 ms after the onset of the cue. Per trial, one train of stimulation (rTMS_j) was delivered in the baseline and one (rTMS_j) in the sequence; arrows indicate the different possible onsets for delivering both trains of stimulation (rTMS_1 and rTMS_2).

B. Location of the four stimulated sites
   The stimulated sites are depicted as coloured ellipses on two different brain views. Each ellipse is centred on the mean MNI coordinates of the corresponding stimulation point (control in red, PMd in green, PB in pink and AB in orange). The surface of each ellipse shows the 95% confidence interval of the normalized coordinates calculated for each subject in both experiments.
Chapter 2. Experimental contributions

Figure 2.8: Stimulus-response mapping in both tasks.

Green and red stimuli are instructive cues, indicating respectively the initiation (INIT cue) and the termination (TERM cue) of the sequence. INTER and BL cues could be either blue or yellow but since these colours don’t have any signification for subjects, they are shown in blue/yellow to simplify. On a computer keyboard, the left ‘ctrl’ key is associated to the index finger of the left hand and the right ‘ctrl’ key is associated to the index finger of the right hand.

A. For the simple task (Experiment #1)

In this task, the stimuli are squares. There are three possible responses, namely ‘L’ (a left key-press), ‘R’ (a right key-press) and ‘LR’ (double simultaneous key-presses). As illustrated, each cue is directly associated with a key-press.

B. For the superordinate task (Experiment #2)

In this task, the stimuli are letters (A, B or C), represented by a X symbol. Each cue is associated with one specific rule among 3 (for instance: the INIT cue with the rule r1).

C. The 3 rules (r1, r2 and r3) determining which key-press (‘L’ (a left key-press) or ‘R’ (a right key-press)) should be performed in response to each letters (A, B and C).

3.4. Results

Experiment #1. Simple task

As reported in the original fMRI study (Koechlin and Jubault, 2006), we found a main effect of the cue in control (no-TMS) trials ($F_{3,18}=6.779$, $p<0.003$) (Figure 2.9A). Post-hoc comparisons showed that RT in response to the INIT cue was significantly longer (311±35 ms, mean±SD, n=7) than RT to the INTER cues (286±23 ms, $t_{18}=0.006$) and TERM cues (289±22 ms, $t_{18}=0.014$).
This corroborates the fact that the initiation of a new sequence is more demanding than processing the following cues.

We also found a main effect of TMS on RT ($F_{1,6}=141.34$, $p<0.001$) unveiling the well-known unspecific effect of TMS. Indeed, post-hoc comparisons showed that in the TMS conditions, RT (236±33 ms) was about 20% shorter than in the no-TMS conditions (296±27 ms, $t_{6}<0.001$).

In this simple task, we failed to found a specific effect of TMS on RT as ANOVA$_{rm}$ did not show a main effect of the SITE or CUE, or an interaction (all $F<1.2$, all $p>0.3$).

**Experiment #2. Superordinate task**

As in Experiment #1, we found a main effect of the CUE in no-TMS trials ($F_{3,39}=16.03$, $p<0.001$) (Figure 2.9B). Post-hoc comparisons analysis confirmed that RT in response to the INIT cues (624±147 ms, mean±SD, n=14) was significantly longer than RT to the BL (586±116 ms, $t_{39}=0.002$) and INTER cues (563±105 ms, $t_{39}<0.001$). In contrast to Experiment #1, we also found that RT for the TERM cues (615±131 ms) was significantly longer than responses to BL cues (586±116 ms, $t_{39}=0.027$) and to INTER cues (563±105 ms, $t_{39}<0.001$).

The unspecific effect of TMS was confirmed by showing a main effect of TMS on RT ($F_{1,13}=36.33$, $p<0.001$). Post-hoc comparisons indicated that, in the TMS conditions, the RT (559±119 ms) decreased by about 5% when compared with the no-TMS conditions (587±117 ms) ($t_{13}<0.001$).

The specific effects of virtual lesions of PMd, PB and AB on RT are shown in Figure 2.10: positive values indicate that TMS led to an increase in RT whereas negative values indicate a decrease in RT. The ANOVA$_{rm}$ evidenced a significant SITE x CUE interaction ($F_{6,78}=2.53$, $p=0.027$). Post-hoc comparisons showed that virtual lesions of PMd significantly decreased the RT for responses performed to INTER cues ($t_{78}=0.036$). In contrast, we found that a virtual lesion of AB impaired this task by yielding a significant increase in RT for responses to the INIT cues ($t_{78}=0.038$). A virtual lesion of PB had no effect in this complex task.

In order to investigate further these effects, we performed an additional ANOVA$_{rm}$ taking into account the hand factor. This analysis showed a main effect of HAND ($F_{1,9}=5.42$, $p=0.045$, indicating that the specific TMS effect was larger for the responses performed with the right index than with the left index ($t_{9}=0.045$). This ANOVA$_{rm}$ also demonstrated a SITE x CUE x HAND interaction ($F_{6,54}=3.06$, $p=0.012$). When focusing on the significant effects, the post-hoc comparisons indicated that the decrease in RT found after a virtual lesion of PMd for INTER cues resulted from a decrease in RT of responses performed with the right index finger, the left index responses being indistinguishable from the baseline. The TMS effect found for the INIT cues after AB virtual lesions was similar for both hands ($t_{54}=0.72$).
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Figure 2.9: Reaction times for each type of cue in trials without magnetic stimulation.

Mean ± SEM reaction times across subjects of trials corresponding to response to baseline (BL) cues and to the three different cues in the sequence: INIT for initiation cues, INTER for intermediate cues and TERM for termination cues.

A. For the simple task (Experiment #1), RT were longer for INIT cues than INTER and TERM cues.
B. For the superordinate task (Experiment #2), RT were longer for INIT and TERM cues than BL and INTER cues.
Figure 2.10: Specific effect of TMS (Experiment #2).

For each cue, the TMS effect obtained in the control site was subtracted from the TMS effect obtained in all three sites of interest. In this way we obtained the specific effects of virtual lesions of PMd, PB and AB on RT as depicted here. A positive value indicates that TMS led to an increase in RT whereas negative values indicate a shorter RT. Significant specific effects were found following PMd and AB virtual lesions, respectively a decrease in RT for the INTER cues and an increase in RT for the INIT cues.

3.5. Discussion

In the present study, we aimed to determine whether the anterior and posterior parts of Broca’s area are causally involved in processing hierarchically structured motor sequences and if so, whether their contribution can be discriminated. To this end, we applied TMS over three cortical areas including the anterior and posterior parts of Broca’s area in which activation peaks have been found by Koechlin and Jubault (2006) in their original fMRI study, suggesting a rostro-caudal organisation in the frontal cortex for processing hierarchically structured motor sequences. The effect of a virtual lesion of these brain regions, as induced by TMS, was investigated during the execution of two motor sequences characterized by different levels of hierarchical organization. Importantly, in both tasks, it was possible to analyse the responses to each category of elements constituting the motor sequence and, especially, the INIT cue since it triggers the sequence performance.

The most important result of the present study is that the anterior part of Broca’s area plays a causal role in initiating complex sequences as shown by the finding that that only a virtual lesion of this region disrupted the processing of the INIT cue, as shown by a specific
increase in RT for those cues. Importantly, this finding only concerns Experiment #2 in which complex rules had to be applied to generate the correct sequence. This result is in accordance with our predictions and the model of Koechlin and Jubault (2006) postulating a rostro-caudal organization in the frontal lobe in which more anterior regions process higher-level hierarchical behaviours.

However, this model also makes some predictions that we failed to validate. First, according to this model, the processing of the TERM cue should be also disrupted following left AB virtual lesion in the complex task. Indeed, in the fMRI study of Koechlin and Jubault (2006), activation in the aforementioned areas was found at “chunks boundaries” (i.e. for the INIT and TERM cues). According to these authors, the anterior part of Broca’s area (typically pars triangularis, BA45) is involved “in selecting/inhibiting superordinate action chunks through top-down interactions that initiate and terminate successive selections of superordinate chunk components”, which is thought to occur in the posterior part of Broca’s area (i.e., simple action chunks). The present study (Experiment #2) failed to confirm this prediction since virtual lesions of AB had no effect on RT for the TERM cues. This indicates that the anterior part of Broca’s area, despite its involvement in inhibiting action chunks, is not crucial to that process. Second, the model also predicts that more posterior regions process subordinate elements within sequential behaviours. Therefore, the prediction based on the Koechlin and Jubault’s model is that TMS application over PB should yield an increase in RT for the INIT / TERM cues in Experiment #1. Again, the present study failed to confirm this prediction since virtual lesions of PB had no effect on RT for INIT / TERM cues in Experiment #1. To explain this lack of effect it is important to keep in mind that the task in Experiment #1 consists in performing a pre-learned sequence of motor responses and thus subjects can always anticipate their response to the forthcoming cue; this problem is even amplified by the fact that the responses for the baseline cues always corresponded to the same motor response ‘LR’, which is the same response as for the two first elements of the sequence, including the INIT cue. However, the analysis of no-TMS trials showed an increase in RT for the INIT cues, a finding which confirms that the initiation of a sequence requires additional resources (Koechlin and Jubault, 2006) and validates the task we used in the present study. Therefore the lack of effects could originate from the fact that since TMS application occurred at the cue onset, it seems possible that when the TMS pulses occur, the response (‘LR’) is already initiated since at this time, choosing the ‘LR’ response cannot be an error and the initiation of the whole sequence can be slightly delayed. In comparison, in the complex task, because the sequence of motor responses is unknown, subjects never known in advance which motor response they will have to perform so that when the green INIT cue appears, subjects cannot anticipate. Another possible
explanation for the discrepancy between this prediction and present results is the exact location of the PB stimulation site. As shown in Figure 2.8B, while the location of the AB site fits well with BA45, the anterior part of Broca’s area, the PB site is located slightly dorsally with respect to the inferior frontal sulcus, and therefore near the upper limit of the left BA44, even if this location has been chosen based on the coordinates of activations reported in the fMRI study of Koechlin and Jubault (2006). In previous TMS studies dealing with action sequences processing, we targeted successfully a site more ventral within BA44 (Clerget et al., 2009; Clerget et al., 2011; Clerget et al., 2012). Finally, it should be mentioned that in the paper of Koechlin and Jubault (2006), brain activity has been detected bilaterally for all sites as in other studies dealing with syntax in Broca’s area about non-linguistic tasks (for instance, Maess et al., 2001). Thus, it seems possible that because of this bilateral activation, since we limited our TMS investigations to the left hemisphere, areas in the right hemisphere may have compensated the dysfunction of their left homologue. Answering the question as to whether distinct behavioural deficits could be obtained following a simultaneous stimulation of both the left and right hemisphere would reinforce the model of Koechlin and Jubault and hence, is an attractive question for future investigations.

In addition to the impairment for the INIT cues following AB virtual lesion, we also observed a facilitation after PMd virtual lesions for the INTER cues. When the laterality of the response was taken into account, we found that the effect - a decrease in RT - found following the TMS application over the left PMd for INTER cues was mainly explained by a RT decrease for right hand responses, the left hand response RT remaining indistinguishable from baseline. In contrast, the effect - an increase in RT - found for the INIT cues after a virtual lesion of AB was similar for both hands. The location of the PMd site corresponds to the dorsal part of the premotor cortex, a region known to play a role in action selection (Schluter et al., 1998; Davare et al., 2006; O’Shea et al., 2007a; O’Shea et al., 2007b). However from previous studies, the left PMd appears dominant, as concluded for instance by Schluter and collaborators, since the TMS application over the left PMd affected performances in an action selection task for both the right and the left hand whereas the stimulation of the right PMd only impaired contralateral responses. It is worth noting that a somewhat similar enhancement of performance in an action selection task following the TMS application over left PMd has been previously evidenced following 1 Hz stimulation and for both hands (Ward et al., 2010). Interestingly, our effect was only found for INTER cues. This could be explained by the fact that changes in rule selection occurred during the appearance of these INTER cues even if the selection of motor responses occurs for any cues in the complex task.
Chapter 2. Experimental contributions

The tasks used in the present experiments investigated two levels of hierarchical organisation of action, integrating both simple and superordinate chunks. In the Introduction, we pointed out that studies exploring syntax-related tasks conclude to the contribution of posterior part of Broca’s area *i.e.* BA44, but sometimes its anterior part, *i.e.* BA45. The hypothesis that there exists a gradient in Broca’s area for controlling different hierarchical levels allows us to reconcile these discrepant findings and could be viewed as a working hypothesis to interpret the contribution of the different parts of Broca’s area across domains. Determining the neural basis that sustain the control of chunks is of particular interest since the learning of complex sequences is tightly linking to the chunking strategy *i.e.* the integration of sequential elements into successively higher-order clusters, or chunks, that *in fine* compose the structure of the sequence. Functional imaging (Bahlmann et al., 2009; Tettamanti et al., 2009), clinical (Dominey et al., 2003) and TMS (Clerget et al., 2012) data corroborated this view. However, as noted by Friederici and collaborators (2011b), in the language domain, the syntactic ability remains mainly confined to the posterior part of Broca’s area. More precisely, in a recent publication of Friederici and coworkers (Friederici et al., 2011b), the authors reviewed several of their recent work and proposed “a model of the prefrontal cortex assuming two systems of processing complex hierarchy: one system determined by cognitive control for which the posterior-to-anterior gradient applies active in the case of processing hierarchically structured mathematical formulae, and one system which is confined to the posterior parts of the prefrontal cortex processing complex syntactic hierarchies in language efficiently”. In other words, Friederici and collaborators suggested that the complexity gradient of hierarchical control could be modulated by another gradient running from highly automatic, less demanding to less automatic, high demanding resources, respectively, in the posterior and anterior parts of Broca’s area. The sequence used in the complex task of the present experiment appears as difficult (less automatic, high demanding) as, for instance, the hierarchically structured mathematical formulae used in Friederici and coworkers (Friederici et al., 2011b), explaining the implication of the anterior part of Broca’s area.
4. Investigating the role of Broca’s area in learning a structured key-presses sequence

Applying accurately syntactic rules is important to achieve well-formed action sequences but a pre-requisite is the learning of such rules. In the light of the literature, it appears possible that Broca’s area is also involved when learning structured sequence. Therefore, from this study, we aimed at investigating the role played by this area in implementing structural rules during sequence learning. To address this last issue, we first used an off-line TMS protocol to interfere with the function of the left BA44 in healthy participants (and of the vertex in a control group) just before they learned, by observation, a sequence of key-presses structured according to different timing rules.

\[29\] This chapter has been edited from the following article: Clerget E., Badets A., Duque J. & Olivier E. (2011). Role of Broca’s area in motor sequence programming: a cTBS study. *NeuroReport*, 22(18), 965-969.
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4.1. Abstract

Besides language, the contribution of Broca’s area to motor cognition is now widely accepted. Here we investigated the role of its posterior part (left Brodmann area (BA) 44) in learning of a motor sequence by altering its functioning with a continuous theta-burst transcranial magnetic stimulation (cTBS) in 12 healthy subjects before they learned the sequence by observation. Twelve control subjects underwent the same experiment with cTBS applied over the vertex. Although cTBS over BA44 did not impair sequence learning, it significantly increased the response latency as measured during the retention test, performed 24 hours later. This finding suggests that Broca's area might be critically involved in organizing, and/or storing, the individual components of the motor sequence before its execution.

4.2. Introduction

Reaching a consensus about the role of Broca's area has become increasingly difficult because of the large number of tasks and processes which seem to rely on this brain region. Indeed, since the pioneering work of Paul Broca (Broca, 1861a), much evidence indicates that speech production is not the exclusive function of this area and that it may also play a role in others aspects of language (Vigneau et al., 2006). Additionally, several studies have suggested that this area also contributes to non-linguistic tasks such as calculation, music and action (Fadiga et al., 2009).

The most prevalent hypothesis for explaining the ubiquitous role of Broca's area is its possible role in the syntactic processing, i.e. the rule-based hierarchical organization, of sequences irrespective of their nature (Grossman, 1980; Tettamanti et al., 2009). In this context, several studies have confirmed the contribution of Broca's area to the acquisition of syntax in artificial language (Bahlmann et al., 2008; Udden et al., 2008) and, because the contribution of Broca's area to language syntax is necessarily more recent in the course of evolution than that to movement control, it has been proposed that the foundation for the involvement of Broca's area in language should be found in motor behaviour (Greenfield, 1991). Accordingly, the role of Broca's area in syntax processing has been shown for non-linguistic sequences (Koechlin and Jubault, 2006; Bahlmann et al., 2009) and recently (Clerget et al., 2012) we found that altering the function of its posterior part, the left BA44, in healthy subjects before they performed a serial reaction time task (SRTT) impaired the implicit learning of a complex structured sequence, suggesting a role of Broca's area in high-hierarchical level processing.
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However, the results gathered in SRTT may be difficult to generalize to other motor tasks (Robertson, 2007) and Broca’s area may play other function(s) in motor control, such as retrieving learned sequences, as shown by observations made in patients with brain lesions encompassing Broca’s area (Maas et al., 2008). The aim of the present study was to gain further insight into the role of Broca’s area in motor cognition, by determining its possible role in learning and retrieving a key-press motor sequence. To do so, we applied cTBS over left BA44 to alter its functioning before participants learned the motor sequence. Furthermore, to avoid any possible side-effect of left BA44 inhibition on movement execution during the training session, learning was performed by observation. Learning was evaluated 24 hours later, during a retention test.

4.3. Methods

Participants

Twenty-four naive subjects participated in the present study (six women; 25.1±3.5 years old). All participants were right-handed as assessed by the Edinburgh handedness inventory (Oldfield, 1971); they had normal, or corrected to normal, vision and were exempt of neurological history, influence of medication, drug or alcohol. All participants were screened by a neurologist to rule out potential risk of adverse reactions to TMS (Keel et al., 2001). The study was carried out according to the Declaration of Helsinki and was approved by the Ethics Committee of the Université catholique de Louvain. Written informed consents were obtained from all subjects. Each subject was compensated for her/his participation.

Task and apparatus

The participants seated on a chair at a distance of 50 cm in front of a 21 inches CRT computer screen. A Matlab program (The Mathworks, Inc.) controlled the experimental procedure; data were saved on a personal computer for off-line analyses. The task consisted of pressing sequentially, with the right index finger, four keys (2, 4, 8 and 6) on the numeric keypad of a computer keyboard Figure 2.11A); this sequence had to be performed in a given time (target total movement time (TMT_target) = 900 ms). In addition, the participants had to comply with 3 relative intermediate movement times (IMT_target) between two consecutive key presses. The 3 intermediate movement times were, respectively, 22 % (between keys 2 and 4, IMT1_target), 45 % (between keys 4 and 8, IMT2_target), and 33 % (between keys 8 and 6, IMT3_target) of the TMT_target. The idea behind this task was to use a sequence characterized by a complex rule-based structure based on both an imposed sequence duration and imposed timings
between key-presses, this rule-based structure being reminiscent of the syntactic organisation of a sequence. The instructions emphasized that the subjects should match the TMT$_{\text{target}}$ and the three IMT$_{\text{target}}$ as accurately as possible. Each trial started with an auditory cue warning the participant to prepare the sequence but, she/he was free to start whenever she/he felt ready; because there was no constraint to respond as fast as possible, we called the delay between this auditory cue and the first key-press the response latency (RL).

**Experimental procedure**

The experiment was divided into four phases: 1) a pre-training phase, 2) cTBS application, 3) a training phase by observation and finally 4) a post-training phase performed 24 hours later and consisting of a retention test (Figure 2.11B). Before the pre-training test, the task was explained to the participants; the TMT$_{\text{target}}$ and the three IMT$_{\text{target}}$ values were displayed on a piece of paper near the subjects until the end of the training phase. Note that subjects only executed the task during the pre-training and retention tests. During the training phase, participants watched a video showing one of the experimenters performing the task (see below).

The participants started the experiment with the pre-training test during which they performed 15 trials with the right index, without any feedback. Then, each subject was randomly assigned either to the left BA44 group (n=12) or to the vertex (control) group (n=12). After cTBS application (see below), subjects were asked to remain relaxed for five minutes. Then, during the training phase, participants had to learn the sequence by watching a video showing the right hand of one of the experimenters performing 75 trials (movement parameters: TMT: 940±242 ms, IMT1: 23±4 %, IMT2: 45±6 %, IMT3: 32±4 %, mean±SD). The hand of the model was filmed from above his right shoulder in order to provide to the participants with the same view as if they were performing the task themselves. At the end of each trial, the video included a verbal feedback about the model performance (TMT in ms and the 3 IMT in percentages). Participants were asked to remain relaxed while watching the video.

Twenty-four hours later, in the post-training phase, subjects underwent a retention test consisting of 15 trials performed with the right index, without any feedback. Trials in which the participants made errors in the sequence order were repeated.

**Transcranial magnetic stimulation**

The cTBS train was delivered by means of a Super Rapid Magstim Stimulator (Magstim Company, Whitland, UK) connected to a 70 mm figure of eight coil. The cTBS protocol (Huang et al., 2005) consisted of a series of rTMS trains (3 pulses at 50 Hz) repeated every 200 ms for
40 s (600 pulses) at an intensity of 80% of resting motor threshold gathered for the first dorsal interosseus muscle. For each participant, an MRI-guided neuronavigation tool (Noirhomme et al., 2004) enables to position accurately the coil and to record the location of the stimulated point (Figure 2.11B). The actual coordinates, expressed in Montreal Neurological Institute (MNI) space, of left BA44 stimulation site were -59±2, 17±6, 23±5 mm; this site fits well with the location of the pars opercularis of the inferior frontal gyrus (Amunts et al., 2004). The actual coordinates of the vertex were 0±5, -16±6, 77±4 mm (mean±SD of x, y and z) (Figure 2.11B).

Data analysis

For all trials performed in the pre- and post-training phases, we measured the response latency (RL), the total movement time (TMT) and the three intermediate movement times (IMT1, IMT2, IMT3).

For the analysis of TMT and IMT, we computed error indexes (eTMT and eIMT). The relative error in TMT (eTMT) was calculated as follows:

\[ e_{TMT} = \frac{|TMT - TMT_{target}|}{TMT_{target}} \times 100. \]

Similarly, the relative error in IMT (eIMT) was calculated as follows:

\[ e_{IMT} = \left( \frac{|IMT1 - IMT1_{target}|}{IMT1_{target}} \times 100 \right) + \left( \frac{|IMT2 - IMT2_{target}|}{IMT2_{target}} \times 100 \right) + \left( \frac{|IMT3 - IMT3_{target}|}{IMT3_{target}} \times 100 \right). \]

Each variable (eTMT, eIMT and RL) was analyzed by using repeated-measures ANOVA (ANOVA_{RM}) with GROUP (control vs. left BA44) as between-subject factor and TEST (Pre-training test vs. Retention test) as within-subject factor. When appropriate, Tukey post-hoc tests were performed. In order to fulfil the normality condition, all these statistical analyses were conducted on log-transformed data. However, for the sake of clarity, non-transformed data are illustrated in Figure 2.11C). All statistical analyses were performed with Statistica (StatSoft Inc., Tulsa, OK, USA).

4.4. Results

First, we investigated whether the training by observation yielded sequence learning. For eTMT and eIMT variables, we performed repeated measures ANOVA (ANOVA_{RM}) with GROUP (control vs left BA44) as between-subjects factor and TEST (Pre-training test vs Retention test) as within-subjects factor. These analyses showed a main effect of TEST for both eTMT (F(1,22)=22.2; p<0.001) and eIMT (F(1,22)=14.7; p<0.001) but no main effect of GROUP and no
interaction between these factors (all $F(1,22)<0.21$; all $p>0.64$). Post-hoc tests confirmed that both eTMT and eIMT decreased significantly in the retention test when compared with the pre-training values (respectively, $p<0.001$ and $p=0.001$). These findings indicate that subjects from both groups learned the sequence equally well.

The same analysis performed on the RL revealed a main effect of TEST ($F(1,22)=11.5$, $p=0.003$) and importantly, an interaction between GROUP and TEST on RL ($F(1,22)=4.1; p=0.05$). Post-hoc tests revealed a significant increase in RL in the retention test when compared with the pre-training test ($p=0.005$) only in the left BA44 group (Figure 2.11C).
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Figure 2.11: Task description (A), experimental procedure (B) and results (C).

A. Schematic view of the task.

The task consisted in performing four sequential key-presses (2, 4, 8 and 6) on a computer keyboard with the right index finger. Subjects had to perform this sequence in 900 ms (TMT target) and had to conform to three distinct intermediate movement times (IMT target: 22 %, 45 % and 33 % of the TMT target for each segment, respectively).

B. Summary of the experimental procedure.

The experiment was divided into 5 distinct phases 1) a pre-training phase in which subjects had to perform the task 15 times without feedback in order to determine a baseline performance before training. 2) a cTBS phase during which cTBS was applied either over left BA44 (experimental group, n=12) or over the vertex (control group, n=12). The figure shows the location of the mean stimulation points and the actual coordinates (mean of x, y and z) after a normalization into the MNI system; the ellipse surface indicates the 95 % confidence interval. 3) All subjects underwent a training by observing a model performing the task they performed during the pre-training. 4) A 24 hour delay. 5) A post-training consisting in a retention test, identical to the pre-training test (15 trials).

C. Results.

Mean response latency (RL) in the pre-training test (white histogram) and in the retention (black histogram) for the left BA44 and control group.
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4.5. Discussion

The goal of the present study was to determine the possible role of the posterior part of Broca’s area in learning and/or preparing a motor sequence. We found that cTBS applied over left BA44 in healthy participants had no effect on learning by observation, which was indistinguishable from that of the control group. However, we found that cTBS applied over left BA44 led to a significant increase, when compared with controls, in the RL gathered 24 hours later, in the retention test.

The absence of effect of left BA44 cTBS on learning cannot be explained by a lack of efficacy of this TMS protocol since we have already used it successfully in a previous experiment to alter the function of the same area (Clerget et al., 2012). The most likely explanation for this absence of TMS effects on sequence learning is that this sequence was too simple and/or that this task did not rely on processes controlled by left BA44, possibly because the relative timings between key-presses was not treated as syntax. According to Dominey and collaborators (Dominey et al., 2003), a sequence is characterized by different structures, namely a serial, a temporal and an abstract structure. The serial structure is the order of the elements within the sequence; the abstract structure can be regarded as the "generative rules that describe relations between repeating elements within a sequence" and the temporal structure is “the durations of elements (and the possible pauses that separate them), and intuitively corresponds to the familiar notion of rhythm” (Dominey et al., 2003). The authors demonstrated a specific deficit for abstract structure learning and a lack of impairment in the temporal structure in patients with aphasia following a lesion encroaching the Broca’s area. Therefore, together with the present results, this suggests that processing the temporal aspect of motor sequences is not controlled by the left BA44; this view is also in accordance with the conclusion of Koechlin and Jubault (Koechlin and Jubault, 2006).

Interestingly, however, we found that inhibiting left BA44 before learning the motor sequence led to an increase in RL gathered in the retention test, performed 24 hours later. It is important to remind that in the present study there was no time constraint on participants and that the instructions clearly indicated that they should start the task whenever they felt ready to do so. Because there is no evidence in the literature that an actual (Maas et al., 2008), or a virtual, lesion (Davare et al., 2006; Tunik et al., 2008) of the inferior frontal gyrus alters RL in simple motor tasks, we suggest that this increase in RL resulting from a left BA44 inhibition is specific to motor sequences and unveils a disruption of the central sequence programming. Indeed, according to the two-stage model of Klapp (2003), preparing a sequence of movements involves two separate programming processes: 1) a first process responsible for
organizing the internal (INT) structure of each individual unit of movements and for storing it in a buffer; INT depends on the unit complexity and is completed before movement initiation; the "study time" provides an estimate of the INT process duration; 2) a second process consists of reading the motor buffer and reordering the movement units on-line into the appropriate sequence (SEQ); SEQ is therefore proportional to the sequence length (number of units) and cannot be programmed before the imperative signal.

Although the present protocol did not allow us to calculate separately the duration of the INT and SEQ processes, our results are reminiscent of those of Maas and collaborators (Maas et al., 2008) showing that patients with an apraxia of speech (AOS) take longer to complete the INT process, as evidenced by a specific increase in the "study time" whereas the duration of the SEQ process was normal (Immink and Wright, 1998, 2001); this finding applies to both speech and non-speech (finger) movements. Maas and collaborators concluded that AOS reflects an effector-independent disruption of the pre-programming (INT) processing. Because AOS results from a left hemisphere stroke (Maas et al., 2008) likely to encroach the Broca’s area (Hillis et al., 2004), it is tempting to establish similitude between the results of Maas and ours. Indeed, in the present study, the larger amount of time needed to organize the individual units of the sequence when no time pressure was imposed could result from an increase in study time comparable to that observed in AOS. In the present task, each of the 4 key-presses is a unit of the sequence whose complexity is reflected by the temporal relationship between each key-press. Exactly as for AOS patients described by Maas et al. (Maas et al., 2008), we found that subjects in whom cTBs was applied over left BA44 were still able to learn and program the sequence correctly but that they required more pre-programming time to do so. This suggests that Broca's area may be responsible for encoding the properties of the individual units of sequential movements and/or for storing them in a motor buffer possibly as a single component, the two mechanisms thought to occur during the pre-programming, INT, process.

The present study indicates that processing syntax is probably not the only contribution of Broca's area to movements control but that it may also have a more elementary role in encoding and preparing the properties of each individual element of sequences, a function that might be a prerequisite to process syntax. Future studies will benefit from using a controlled self-select paradigm (Immink and Wright, 2001), allowing a separate measure of the INT and SEQ processing times. Indeed, the present study allows us to make very specific predictions about the consequence of a virtual lesion of BA44 on INT and SEQ processes occurring during the preparation of sequential movements. This will be necessary to gain further insight into the role of Broca's area in motor control.
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5. Role of Broca’s area in chunking during implicit sequence learning

In study 4, we investigated the role of Broca’s area in the explicit learning of a structured, but relatively simple, motor sequence. What about the involvement of Broca’s area when subjects are unaware 1) that they have to learn a sequence and 2) that such sequence is structured? It is an interesting question since it has been shown that during implicit sequence learning involving complex sequences, subjects generally generated some kind of rules that enable to establish relationships between the elements of the to-be-learned sequence; it is called “chunking” and in fine, it enables to structure such sequence, in other words, to build a rule-based sequence. Considering the role of Broca’s area in syntax-related processes, it seems plausible that this area could play a role in chunking during implicit sequence learning. Investigating this facet of sequence learning is the goal of the present study.

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5.1. Abstract

Complex actions can be regarded as a concatenation of simple motor acts, arranged according to specific rules. Because the caudal part of the Broca’s region (left Brodmann area 44, BA44) is involved in processing hierarchically organized behaviours, we aimed to test the hypothesis that this area may also play a role in learning structured motor sequences.

To address this issue, we investigated the inhibitory effects of a continuous theta-burst transcranial magnetic stimulation (cTBS) applied over left BA44 in healthy subjects, just before they performed a serial reaction time task (SRTT). SRTT has been widely used to study motor skill learning and is also of interest because, for complex structured sequences, subjects spontaneously organize them into smaller sub-sequences, referred to as chunks. As a control, cTBS was applied over the vertex in another group which underwent the same experiment.

Control subjects showed both a general-practice learning effect, evidenced by a progressive decrease in reaction time (RT) across blocks, and a sequence-specific learning effect, demonstrated by a significant RT increase in a pseudorandom sequence. In contrast, when cTBS was applied over left BA44, subjects lacked both the general-practice and sequence-specific learning effects. However, surprisingly, their chunking pattern was preserved and remained indistinguishable from controls.

The present study indicates that left BA44 plays a role in motor sequence learning, but without being involved in elementary chunking. This dissociation between chunking and sequence learning could be explained if we postulate that left BA44 intervenes in high-hierarchical level processing, possibly to integrate elementary chunks together.

5.2. Introduction

The ability to arrange, learn over practice, and then perform structured sequences is critical in most behaviours, such as language, music, but also in skilful movements, that make humans so distinctive (Fadiga et al., 2009). This ability has been regarded as an ultimate factor of the human cognitive development (Greenfield, 1991; Keele and Curran, 1996; Conway and Christiansen, 2001; Corballis, 2003) and is mostly noticeable in language. Indeed, words composing sentences are not arranged randomly but have to comply with a precise hierarchical organization, based on grammatical rules - or syntax - allowing the production of meaningful sentences. Similarly, complex actions, which also result from merging several simpler units, also critically depend on the ability to arrange them into the appropriate order, according to certain rules. Recently it has been suggested that language and action may share
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the same syntactic processor, possibly located in Broca’s area, which could act irrespective of the domain (language, action, music, calculation...) and whatever the nature of the sequences (perceptual, motor or cognitive) (e.g. Dominey et al., 2003; Koechlin and Jubault, 2006; Tettamanti and Weniger, 2006; Bahlmann et al., 2008; Bahlmann et al., 2009; Clerget et al., 2009; Fadiga et al., 2009; Fazio et al., 2009).

Whether Broca’s area is also involved in learning new rules has been addressed in linguistics by using artificial grammar learning (AGL) tasks, taking advantage of subjects’ ability to detect and acquire implicitly a set of new syntactic rules from experience (Reber, 1967, 1989). AGL tasks are typically divided into two phases: an acquisition phase, consisting of learning implicitly a new syntactic rule and a classification phase, in which subjects have to detect violations of the newly acquired rule. Functional neuroimaging studies have shown that AGL tasks activate a large network of brain areas including the prefrontal cortex, anterior cingulate cortex, inferior parietal cortex and regions in the occipital and temporal cortices (Fletcher et al., 1999; Seger et al., 2000; Skosnik et al., 2002; Lieberman et al., 2004; Petersson et al., 2004; Forkstam et al., 2006). In addition, some of these studies have reported an activation of Broca’s area in AGL tasks, suggesting that it may be involved in extracting artificial rules from different types of sequences, an ability which could underlie the acquisition of natural languages (Lieberman et al., 2004; Petersson et al., 2004; Forkstam et al., 2006). This view about the role of Broca’s area is further supported by clinical studies showing that patients with a lesion of this brain region, resulting in an agrammatic aphasia, have difficulties in performing AGL tasks (Dominey et al., 2003; Christiansen et al., 2010b). Additional evidence for the role of Broca’s area in acquiring new syntactic rules comes from studies showing that the application of either transcranial direct current stimulation (de Vries et al., 2009) or off-line repetitive TMS (transcranial magnetic stimulation) (Udden et al., 2008) over the Broca’s area during the acquisition phase of AGL tasks, enhances the subject’s performance in the classification phase. Finally Floel and collaborators (2009), using diffusion tensor imaging, have shown that the ability to extract grammatical rules depend on the integrity of the white matter fibre tracts originating from Broca’s area.

However, whether Broca’s area is also involved in learning complex actions requiring syntactical processing remains puzzling. By analogy with AGL tasks, SRTT could be used to address this issue since subjects learn a motor sequence which, without subject’s knowing, is repeated several times in consecutive blocks (Nissen and Bullemer, 1987). This procedure leads to a gradual decrease in response time across blocks, regarded as evidence for implicit learning. More importantly, because learning such a task relies, under certain circumstances, on the segmentation of the main sequence into several sub-sequences, known as "chunking"
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(Miller, 1956), SRTT seems appropriate to investigate, in combination with the TMS technique, the neural correlates of hierarchical/syntactic processing in the motor domain. Previous investigations in Broca's aphasic patients have shown that such patients with left perisylvian lesions are still able to perform SRTT but, in these studies, no particular attention was paid to the chunking strategy used by participants (Goschke et al., 2001; Dominey et al., 2003).

Our working hypothesis is that Broca's area is involved in this chunking process, a view further supported by the results of some neuroimaging studies showing an activation of Broca's area during SRTT (Bischoff-Grethe et al., 2004; Bapi et al., 2006). Although this activation is clearly marginal when compared with that of the other cortical and sub-cortical structures (Hazeltine et al., 1997; Grafton et al., 1998a; Willingham et al., 2002; Schendan et al., 2003; Bischoff-Grethe et al., 2004; Poldrack et al., 2005; Seidler et al., 2005; Bapi et al., 2006), it is possible that the recruitment of Broca's area varies during the course of the learning process, leading to a rather weak activation when averaged over a long period of time (Bapi et al., 2006). If this holds true, it is clear that functional imaging is not the most appropriate approach to determine the possible involvement of Broca's area in SRTT. In addition, functional neuroimaging studies do not allow us to determine the causal contribution of a given area to the process under investigation (Walsh and Cowey, 2000; Bolognini and Ro, 2010). One way to circumscribe these limitations is to investigate the consequences of a transient inhibition of Broca's area, as induced by cTBS, on SRTT performance.

In the present study we investigated implicit learning in SRTT in two groups of subjects, in which cTBS was applied either over the caudal part of Broca's area (left BA44) or over the vertex (control group). We assessed the learning effects classically reported in SRTT (Nissen and Bullemer, 1987; Robertson, 2007) and we also quantified the chunking strategy used by the participants. Our prediction was that an inhibition of left BA44 will impair the chunking pattern, leading to a deficit in sequence learning.

5.3. Methods

Subjects

Seventeen healthy volunteers (9 women, age 20-42, mean age 27 years) participated in the experiment. They were all right-handed as assessed by the Edinburgh Inventory (Oldfield, 1971) and were pseudo-randomly assigned to either the left BA44 group (left BA44 stimulation, n=8) or to the control group (vertex stimulation, n=9). All participants had normal neurological functions and met the safety criteria for TMS (Wassermann, 1998). The procedure was approved by the Ethic Committee of the Université catholique de Louvain.
Task and experimental procedure

We used a classical SRTT (Nissen and Bulleme r, 1987) in which subjects had to learn implicitly a motor sequence by associating four possible visual cues to a particular finger movement. In this task, the visual cues are, without subject’s knowing, presented in a fixed order, which is also repeated several times during a given block. This procedure led to a gradual decrease in RT across blocks, which is typically regarded as evidence for implicit motor skill learning.

In the present study, the visual cue was a white rectangle (9.55° wide and 13.37° high) displayed on a 21” computer screen (ViewSonic P227f, ViewSonic Corporation, Taiwan) at one out of four positions arranged horizontally. Each screen position, designated as 1-4 from left to right, corresponded to a given response button on a computer keyboard (F5-F8) and, therefore, to the movement of a particular finger (fingers II-V) of the right hand (see Figure 2.12A). Each visual cue was displayed until a key was pressed and the subsequent cue was displayed after a response-stimulus interval (RSI) of 250 ms, whatever the response was correct or not (see Figure 2.12B); this short RSI was chosen because it has proven to enhance sequence learning (Destrebecqz and Cleeremans, 2001; Soetens et al., 2004). Participants were told to keep each finger on the appropriate response button during the block and to respond to each cue presentation as quickly and as accurately as possible. When an error occurred or when the RT was longer than 1000 ms, the screen background became red for 50 ms. At the end of each block, the subjects received a feedback about their speed (mean RT for the correct trials) and accuracy (number of correct responses in the block); those two values were displayed on the screen.

The experiment was controlled by a PC running a program written in Matlab (The Mathworks Inc., USA). In order to minimize the measurement errors in RT due to the timing uncertainty of the operating system (Windows, Microsoft, USA), we built a device to detect, with millisecond accuracy, the display of each frame on the computer screen and the subsequent key-press.

In order to maintain a high level of motivation throughout the whole experiment (Wachter et al., 2009), the participants were told that they will be rewarded proportionally to their performance. To do so, at the end of each block, a score was calculated based on the difference between the mean RT in block #1 and mean RT in the current block (1 point/ms); this score was also affected by the number of errors (-0.5 point/error). However, irrespective of their actual scores, all subjects received the same amount of money at the end of the experiment.
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Finally, after the experiment, participants were informed about the existence of a repeated sequence in the blocks and they were asked to reproduce the sequence, or part of it, by pressing the response buttons (see below).

Figure 2.12: Stimulus-response mapping (A) and time course of a block (B).

(A) Each cue position displayed on the computer screen corresponded to a response button on a keyboard (F5-F8) and, therefore, to the movement of a given finger.

(B) Each block started with a waiting period of 3000 ms during which the four possible cue locations were indicated by empty rectangles. Then, a visual cue appeared (filled rectangle) and the subjects had to press the corresponding response key as quickly as possible. The cue was displayed until a key was pressed and then the next cue was displayed after a response-stimulus interval (RSI) of 250 ms.

Experimental design

The whole experiment contained eight blocks, seven blocks (#1-6 and #8) consisting of 5 repetitions of the same structured sequence of 20 elements and one block (#7) consisting of 5 repetitions of a 20-element pseudorandom sequence. In blocks #1-6 and #8, subjects were presented with the following sequence: 31422413421321234, in which 1, 2, 3 and 4 refer, respectively, to the four visual cues, from left to right. The order of the elements of the sequence was carefully determined so that it could be chunked as follows: the first eight items
could be chunked into 2 sub-sequences (3142-2413), with the second one being the reverse of the first one. The six following items were two n-2 repetitions (424-131) and the six last trials consisted of 2 triplets of contiguous digits (321-234).

This chunking pattern was corroborated by the results of a pilot study performed on 7 subjects, showing that they actually chunked the sequence accordingly (3142-2413-424-131-321-234). In block #7, subjects were exposed to a pseudorandom sequence (34233124134124213241), also repeated 5 times; this pseudorandom sequence matched the structured sequence in terms of element frequency and number of transitions.

Transcranial Magnetic Stimulation

In order to compare the initial performance of the 2 groups of participants (left BA44 and control), block #1 was performed before TMS application. TMS was delivered through a 70 mm outer diameter figure-of-eight coil connected to a stimulator (Super Rapid, Magstim Company, Whitland, UK). About 5 minutes after cTBS application, the subjects started to perform the next block (block #2). Since the rest of the experiment consisted of 7 blocks of about 2 minutes each, the remaining experiment duration was largely shorter than the estimated cTBS effect period, namely about 30 minutes (Huang et al., 2005; Nyffeler et al., 2006).

In the present experiment, we used the original cTBS protocol (3 TMS pulses delivered at 50 Hz every 200 ms for a duration of 40 s, pulse number=600, (Huang et al., 2005)); the stimulation intensity was set at 80 % of the resting motor threshold (rMT). In order to determine the rMT for each participant, single pulses were applied over the hand representation in the left primary motor cortex (M1) while the motor-evoked potentials (MEPs) were recorded from the contralateral first dorsal interosseous muscle. The coil was positioned tangentially to the scalp, the handle oriented backward, 45° lateral from the interhemispheric scissure. After the optimal coil position was found, we searched for the minimum TMS intensity necessary to produce 50 µV peak-to-peak MEPs in 5 out of 10 stimulations (Rossini et al., 1994).

Based on information available in the literature, the x, y and z coordinates of the two target stimulation sites were defined as follows: -43, 11 and 16 mm for left BA44 (Amunts et al., 2004; Anwander et al., 2007) and 0, -15 and 74 mm for the vertex (Okamoto et al., 2004) (Montreal Neurological Institute, MNI, system). In order to localise these targets onto each individual brain scan, a reverse normalization procedure was performed to obtain the corresponding locations expressed in individual subject's coordinates and to shift them on the scalp surface. For positioning the coil during the experiment, the actual localisation of the stimulation sites was determined by using a home-made neuronavigation program.
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(Noirhomme et al., 2004). Finally, the mean coordinates of the actual stimulation sites in all subjects were computed (mean±SD of x, y and z; MNI system of coordinates: -58±4, 14±7, 22±6 mm for left BA44 and -2±4, -13±6, 74±3 mm for the vertex); stimulation sites are illustrated in Figure 2.13.

![Mean normalized stimulation sites](image)

**Figure 2.13: Mean normalized stimulation sites.**

The centre of the ellipses indicates the mean coordinates of the stimulation sites for the vertex (green, n=8) and left BA44 (red, n=8), respectively. The ellipse surface indicates the 95% confidence interval. The coordinates (mean±SD of x, y and z; MNI coordinates) of the stimulation sites were -2±4, -13±6, 74±3 mm for the vertex and -58±4, 14±7, 22±6 mm for left BA44.

**Data analysis**

The RT was defined as the delay between the display of the visual cue and the subsequent key-press. RT from error trials (either wrong responses or no response within 1 second) were discarded from analysis (mean error rates ±SD: control group: 4.55±1.45 %; left BA44 group: 3.69±1.47 %). The following RT values were also discarded from analysis: RT exceeding the mean RT of each subject ± 2 SD (control group: 4.70±0.79 % of the trials; left BA44 group: 4.87±0.33 %) and the RT of the first trial of each block (8 trials/subject). Furthermore, one subject from the control group was discarded from the analysis because his mean RT was larger than the mean + 2 SD value of the rest of the group. For the remaining trials, mean RT was computed for each condition and for each subjects. As described elsewhere (Koch et al., 2006), we focused our analysis on the three following parameters:

1. The RT change across blocks #2 to #6, which is a measure of the general-practice learning reflecting both the learning of the mapping between the cue position and appropriate finger response.

2. The difference between the mean RT for block #7 (pseudorandom sequence) and the average RT for blocks #6 and #8, which is regarded as a measure of the sequence-specific learning (e.g. Nissen and Bullemer, 1987; Koch and Hoffmann, 2000a; Goschke et al., 2001; Dominey et al., 2003; Robertson, 2007; Jimenez, 2008). We added data from block #8 in this analysis to rule out any possible unspecific effect of fatigue.
3. The RT variation for each item with respect to its position in the sequence, which allowed us to determine the chunking strategy used by the participants. Indeed, chunks can be identified because the RT to items belonging to the same chunk should be different. Indeed, when compared with the first element of a chunk, the subsequent elements of that chunk should, because they become more predictable, lead to a decrease in RT (Rosenbaum et al., 1983; Koch and Hoffmann, 2000a).

Finally, as already mentioned, at the end of the experiment, participants were informed about the presence of a 20 item structured sequence repeated 5 times in blocks #1 to #6 and in block #8. Participants were then asked to recall the sequence as accurately as possible, by pressing the correct response buttons. In this free recall test, we scored both the length of the reported sequence and the number of elements correctly placed in that sequence. The latter is considered as an indicator of explicit knowledge of the sequence.

Statistical analysis

First, to compare the initial performance between the two groups of subjects (control group vs left BA44 group), we performed a one-way analysis of variance (ANOVA) on RT for block #1 (Statistica, StatSoft Inc., USA).

General-practice and sequence-specific learning effects were analyzed by using repeated measures ANOVA (ANOVA\textsubscript{RM}) with GROUP (control group vs left BA44 group) as between-subjects factor and BLOCK as within-subjects factor. According to the various issues we wanted to investigate, the factor BLOCK was composed as follows: to assess learning from block #1 to block #2, the BLOCK factor had 2 levels (block #1 vs #2); when analyzing general-practice learning, the BLOCK factor had 5 levels (block #2 to #6); to address the issue of the sequence-specific learning, the BLOCK factor had 2 levels (block #7 vs blocks #6+8). To analyze the chunking pattern at the end of learning, we performed an ANOVA\textsubscript{RM} with GROUP as between-subjects factor and POSITION (1, 2, 3, ..., 20) as within-subjects factor on averaged data from blocks #6+8. Additionally, in order to characterize the progressive emergence of the chunks across blocks, we also performed an ANOVA\textsubscript{RM} with GROUP as between-subjects factor and POSITION (1, 2, 3, ..., 20) as within-subjects factor for each block separately, and we determined, in each block, the number of significant chunk(s). Then, the number of significant chunks was plotted against the block number. Lastly, to evaluate the effectiveness of chunks on motor performance, for blocks #6+8, we computed the RT benefit for items INSIDE chunks (RT\textsubscript{in}) when compared with items OUTSIDE chunks (RT\textsubscript{out}). When appropriate, a Fisher’s least significant difference (LSD) post-hoc test (p<0.05) was performed.
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Finally, a one-way ANOVA was performed on the data of the free recall test (the number of elements recalled and the length of the correct sequence).

5.4. Results

In order to ensure that the initial performance of subjects from both groups was identical, we compared the mean RT gathered for block #1. The mean RT for block #1 was 411±68 ms (mean±SD, n=8) in the control group and 395±40 ms (n=8) in the left BA44 group (Figure 2.14A). A one-way ANOVA with GROUP (control group vs left BA44 group) as between-subjects factor confirmed that the two groups were not different in terms of RT (F(1,14) = 0.33, p = 0.57).

General-practice learning

First, an ANOVA with BLOCK (block #1 vs #2) as within-subjects factor and GROUP as between-subjects factor showed a main effect of BLOCK (F(1,14) = 39.93, p < 0.01) but no main effect of GROUP (F(1,14) = 0.46, p = 0.51) on RT and no interaction between these factors (F(1,14) = 0.02, p = 0.89), indicating that the performance increase from block #1 to block #2 was comparable in both groups (Figure 2.14A). The absence of difference between groups was even more remarkable when RT was expressed in relative value with respect to block #1 RT (Figure 2.14B).

Then to assess the general-practice learning across blocks, we performed an ANOVA with BLOCK (block #2 to #6) as within-subjects factor and GROUP as between-subjects factor. This analysis revealed a significant main effect of BLOCK (F(4,56) = 4.33, p < 0.01) but no main effect of GROUP (F(1,14) < 0.01, p = 0.98) on RT; it also showed a significant interaction between BLOCK and GROUP (F(4,56) = 2.81, p = 0.03). A post-hoc test indicated that, only for the control group, RT for block #2 was statistically different from RT in all other blocks (#3-6) (Figure 2.14A), all p < 0.03 and RT in block #3 was different from that in block #6 (not illustrated, p = 0.02). These results show that, whereas control subjects gradually improved their performance with practice, subjects in the left BA44 group failed to do so (all p > 0.33). This difference in the evolution of performance between both groups was even more noticeable when the RT change across blocks was expressed in relative value with respect to block #1 RT (Figure 2.14B). However, because the analysis of RT changes across blocks does not distinguish between the improvement due to general-practice and the gain in performance due to distinct sequence learning, we evaluated independently the sequence-specific learning.
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Figure 2.14: General-practice and sequence-specific learning.

(A) Mean RT (in ms) across blocks for the control (green squares) and left BA44 (red circles) groups. From blocks #1 to #6 and in block #8, the same structured sequence was presented 5 times in each block whereas, in block #7, it was replaced by a pseudorandom sequence.

(B) RT change (in % with respect to RT in block #1) across blocks for both groups (same color code as in (A)). The RT change for a given block was calculated as follows: RT change = \( \frac{[\text{RT}_{\text{block}} - \text{RT}_{\text{block1}}]}{\text{RT}_{\text{block1}}} \) * 100.

In (A) and (B), the gray rectangle symbolizes cTBS application between block #1 and #2.

Sequence-specific learning

The sequence-specific learning was quantified, in the usual manner, by contrasting the RT gathered in the pseudorandom sequence (block #7) with the averaged RT of blocks #6+8. Indeed, block #7 can be used to discriminate between the gain in performance due to general-practice from the gain due to sequence-specific learning because, at the end of the training session (blocks #6 to #8), the visuomotor association between cues and finger movements is supposed to be stable, and the presentation of a pseudorandom sequence should yield an increase in RT, unveiling the sequence-specific learning.
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An ANOVA with BLOCK (blocks #6+8 vs block #7) as within-subjects factor and GROUP as between-subjects factor revealed no main effect of GROUP (F(1,14) = 0.007, p = 0.94) but a trend for the BLOCK factor (F(1,14) = 3.75, p = 0.07). However, we found a significant interaction between BLOCK and GROUP (F(1,14) = 4.49, p = 0.05) and a post-hoc analysis showed that, in the control group only, RT in block #7 was significantly larger than RT for blocks #6+8 (p=0.01, Figure 2.14A and 2.14B). These results indicate that only the control subjects exhibited a sequence-specific learning whereas the performance of subject in the left BA44 group remained unaffected by the presentation of a pseudorandom sequence.

Chunking pattern

To investigate how the subjects actually chunked the sequence independently of its intrinsic structure, we computed the mean RT for each item of the sequence (20 items/sequence) for each block (5 sequences/block) and for each group. Because the chunking pattern is supposed to be more robust - and therefore more detectable - at the end of the experiment, we started our analyses on averaged results from blocks #6+8 (Figure 2.15); another reason for incorporating block 8 data in this analysis was to control for any possible unspecific effect of fatigue. An ANOVA on these averaged RT was performed with POSITION (1, 2, 3, ..., 20) as within-subjects factor and GROUP (control group vs left BA44 group) as between-subjects factor. This analysis did not show a main GROUP effect (F(1,14) = 0.13, p = 0.72) nor an interaction (F(1,19) = 0.61, p = 0.90) but revealed a significant main effect of POSITION (F(1, 19) = 14.21, p<0.001) indicating that the RT varied as a function of the item position in the sequence. Because a chunk is characterized 1) by a longer RT for the first item and 2) by a significant decrease in RT for the subsequent item(s) belonging to the same chunk (Rosenbaum et al., 1983; Koch and Hoffmann, 2000a), we only concentrated on results from post-hoc analyses showing a significant effect of position for adjacent items (item n vs n+1); when a significant difference between RT was found for a given pair of neighboring items, we then tested the difference between the RT for item n vs n+2, and so on. By using this approach, we found 5 chunks in blocks #6+8 starting at positions #1, #3, #6, #11 and #18 and for which at least the subsequent item showed a significant decrease in RT (all p<0.008); three out of these five chunks were triplets (see Figure 2.15). This finding indicates that subjects used a chunking strategy different from that we expected on the basis of the results of our pilot study (see Methods). However, more importantly, an ANOVA showed neither a main effect of group nor a group x position interaction, indicating that subjects from both groups used the same chunking strategy.
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Figure 2.15: Chunking pattern in blocks #6 and #8.

Mean RT for each item position (n=20) within the structured sequence in the control group (green squares) and the left BA44 group (red circles) for blocks #6 and #8. Each data point corresponds therefore to the average of 10 responses (2 blocks x 5 sequences) per subject (n=8). Items falling inside a chunk (statistically significant difference in RT between item n and item n+1 and, sometimes, n+2) are indicated by darker colors; lighter colors indicate items situated outside a chunk. Along the x axis, for each item position (from 1 to 20), the actual item number (from 1 to 4) is also indicated (white number in black circles).

In order to investigate the progressive emergence of chunks across blocks, we applied the same analysis we used for blocks #6+8 to each individual block. These ANOVA$_{RM}$ confirmed the main effect of POSITION on RT (all F(1, 19) > 5.82, all p<0.001) and, as previously, we identified the chunks based on the post-hoc analysis results. Figure 2.16A shows the number of significant chunks for each block, clearly illustrating the progressive emergence of chunks across blocks, starting with one chunk in block #1 to reach a plateau of 5 chunks in blocks #5, #6 and #8. This analysis did not show the presence of chunks in block #7. This increase in chunk numbers across blocks was best fitted with a sigmoid function (R$^2$ = 0.99, Figure 2.16A).

To characterize further the chunking pattern, we investigated the emergence of the different chunks across blocks (Figure 2.16B). Interestingly, in both groups, the first chunk that appeared was the first 2-element chunk (items #1-2, dark blue), already present in block #1, then the last 3-element chunk (items #18-20, light blue, block #3), followed by the two others chunk (items #3-4 and #6-8, respectively, orange and purple, block #4) and finally the last 3-element (items #11-13, pink, block #5).
These results corroborate the well-known observation that the chunking process builds up gradually with practice (Sakai et al., 2003; Sakai et al., 2004a) and show that the first chunks to emerge were those at the extremities of the sequence.

Figure 2.16: Chunking pattern emergence across blocks.

(A) Number of chunks across blocks. The number of chunks was determined in each block as shown in Figure 2.15 (see Methods for details). The progressive chunk increase across blocks was best fitted with a 4-parameter sigmoid ($R^2 = 0.9989$). Only one sigmoid was computed since the ANOVA with group as between-subjects factor and position (1, 2, 3, ..., 20) as within-subjects factor did not reveal a main effect of group nor an interaction between group and position. Since block #7 consisted of a pseudorandom sequence, no chunk was present in this block and data from block #7 were not incorporated in this analysis; this is symbolized by a dashed line between blocks #6 and #8.

(B) Evolution of the chunk formation across blocks. Block number is represented along the y axis. Each dot along the x axis represents one item position in the sequence. Colored dots connected to each other indicate that they belong to the same chunk; grey dots designate items outside a chunk. The five chunks (items #1-2, #3-4, #6-8, #11-13 and #18-20) are depicted by different colors (respectively: dark blue, orange, purple, pink and light blue). The first two chunks to appear were those including items situated at the two extremities of the structured sequence. There was no chunk in the pseudorandom block (block #7). Along the x axis, for each item position (from 1 to 20), the actual item number (from 1 to 4) is also indicated (white number in black circles).
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Finally, to evaluate the benefit of the chunking strategy, for blocks #6+8, we performed an ANOVARM with GROUP (control group vs left BA44 group) as between-subjects factor and ITEM (IN vs OUTside chunks) as within-subjects factor. This analysis showed a main effect of ITEM on RT ($F(1,14) = 32.12, p < 0.001$) but no main effect of GROUP and no interaction between factors. The post-hoc analysis indicated that RT$_{IN}$ (mean±SD: 332±44 ms) was significantly shorter than RT$_{OUT}$ (mean±SD: 355±47 ms, $p < 0.001$), showing that chunking had a global benefit on subject performance.

Free recall test

As mentioned in the Methods, at the end of the experiment, each subject was asked to recall and perform the sequence. A one-way ANOVA failed to show any difference for the factor GROUP (control group vs left BA44 group) both for the length of the reported sequence (5.9±2.3 elements for the left BA44 group and 6.1±2.8 for the control group; $F(1, 14) = 0.04$, $p=0.85$) and for the number of elements correctly positioned in that sequence (2.5±1.9 for the left BA44 group and 3.5±1.8 for the control group; $F(1, 14) = 1.22$, $p=0.29$). These results indicate that all subjects, irrespective of their group, remained largely unaware of the existence of a structured sequence repeated across blocks.

5.5. Discussion

The aim of the present study was to determine whether the caudal part of Broca's area (left BA44) plays a role in learning motor sequences, in particular when such a learning requires to process the hierarchical relationship between different subcomponents of the sequence. To address this issue we used cTBS to inhibit temporarily left BA44 in healthy subjects before they performed a SRTT. We used this task because, besides allowing us to investigate implicit motor skill learning (Nissen and Bullemer, 1987; Robertson, 2007), it also constraints subjects to organize spontaneously complex sequences into several sub-sequences - or chunks - characterized by a simple hierarchical relationship. This process has been proved critical for learning difficult sequences since the presence of a relational pattern between the items of the sequence improves sequence-specific learning (Koch and Hoffmann, 2000a; Sakai et al., 2003; Kirsch et al., 2009). Because it has been shown that Broca's area is involved in processing hierarchically organized behaviours, we hypothesized that a transient inhibition of this area would impair the chunking process, and therefore affect sequence learning.
Accordingly, we found that a temporary inhibition of left BA44 altered implicit motor learning. In contrast to the result gathered in the control group, when cTBS was applied over left BA44, subjects failed to show a decrease in RT across blocks, a parameter usually regarded as a measure of general-practice learning (Nissen and Bullemer, 1987; Koch and Hoffmann, 2000a; Goschke et al., 2001; Dominey et al., 2003; Robertson, 2007; Jimenez, 2008). A more specific measurement of sequence learning is typically obtained by contrasting the RT gathered for the blocks containing the structured sequence - when the general-practice learning has reached a plateau - against the RT gathered in a block containing a pseudorandom sequence. The present study shows that, following a transient inhibition of left BA44 induced by cTBS at the beginning of SRTT, this contrast failed to reveal a difference between RT; this lack of sequence-specific learning differs from what we found in control subjects and from results reported in the literature in patient with left perisylvian lesion (Goschke et al., 2001; Dominey et al., 2003). Because the results of the recall test performed at the end of the experiment showed that subjects from both groups were equally unaware of the existence of a structured sequence, the hypothesis that an inhibition of left BA44 may have modified the level of explicitness of the sequence can be ruled out. In addition, the present results clearly indicated that the consequence of a momentary inhibition of left BA44 on both general-practice and sequence-specific learning cannot be explained by a difference in initial performance between groups (see Figure 2.14A) or an effect of cTBS on the performance improvement occurring between blocks #1 and #2 (see Figure 2.14B). Altogether, the present results suggest that left BA44 is causally involved in implicit skill motor learning as investigated with SRTT.

However, the present study failed to support our working hypothesis that the Broca's area makes a significant contribution to the chunking process. Indeed, since it is well known that Broca's area plays a role in processing hierarchically organized behaviours (Dominey et al., 2003; Koechlin and Jubault, 2006; Tettamanti and Weniger, 2006; Fadiga et al., 2009), it was sensible to assume that its temporary inhibition could actually impact the chunking strategy, and therefore sequence-specific learning. The chunking process consists of splitting a large sequence into smaller sub-sequences of consecutive items, easier to memorize. Chunking was originally defined to account for the memory span (Miller, 1956) and is regarded as a strategy to enhance the amount of information stored in short-term memory. Importantly, according to Miller, a chunk could refer to either digits, words, or any other meaningful units. In the context of motor control, the chunking process can be regarded as a way to split complex actions into simpler units or motor acts, each one being executable as individual motor program, which can be merged together to form a meaningful action (Lashley, 1951; Rosenbaum et al., 1983;
Rhodes et al., 2004). In the present study, surprisingly, we found that inhibitory cTBS applied over left BA44 altered both general-practice and sequence-specific learning but left the chunking strategy unchanged and indistinguishable from that observed in control subjects.

Before discussing further this dissociation between chunking and learning, it is worth mentioning that our results are at odds with two previous studies performed in frontal aphasic patients (Goschke et al., 2001; Dominey et al., 2003). Indeed these two studies showed that these patients still present both general-practice and sequence-specific learning. Several factors can explained this discrepancy. First, in these two aforementioned studies, the lesion location was not clearly documented. Secondly, it cannot be excluded that, following a stroke, a significant reorganization process had occurred, which might have contributed to a partial recovery. Finally, this discrepancy could also be explained by a difference in task difficulty. Indeed Goschke and collaborators (2001) used a sequence of 10 elements, repeated 40 times (Experiment #1) and a 8-element sequence, repeated 60 times (Experiment #2); Dominey and collaborators (2003) used a 12-element sequence, repeated 45 times. In contrast, in the present study, we used a longer sequence (20 elements) repeated only 30 times.

Nevertheless, the dissociation between chunking and sequence learning we reported in the present study is somehow puzzling because several authors have suggested a causal relationship between learning in various tasks, including SRTT, and chunking process (Rosenbaum et al., 1983; Koch and Hoffmann, 2000a; Sakai et al., 2003; Verwey and Eikelboom, 2003; De Kleine and Verwey, 2009; Kirsch et al., 2009). In contrast, the present study indicates that chunking, or at least the low-level chunking investigated in the present study, is not a sufficient condition to learn a motor sequence. However, importantly, to the best of our knowledge, it is noteworthy that this is the first time the chunking strategy was quantified so accurately a posteriori (however see Koch and Hoffmann, 2000a; Miyapuram et al., 2006; Jimenez, 2008) and correlated with learning performance.

One possible explanation for this dissociation between chunking and sequence learning is that chunking is actually a by-product of sequence recurrences, not causally related to learning. If this holds true, chunking should be regarded as a null operation in terms of RT because the benefit for late positions in the chunk should be cancelled out by the transition cost between chunks. Our results clearly show that is not the case and that, globally, the RT for items inside chunks was shorter than for items outside chunks.

Therefore we have to resolve this apparent paradox that, following a left BA44 inhibition, subjects show a deficit in sequence-specific learning, whereas their chunking pattern remained indistinguishable from controls, suggesting that the Broca's area is responsible for another - necessary - mechanism underlying sequence-specific learning. One possibility is that when
learning a complex motor sequence, several chunking processes occur at distinct hierarchical levels, from low-order levels, i.e. the concatenation of two or three successive items into elementary chunks, to high-order levels, i.e. the integration of these elementary chunks into a higher-order sequence (Dehaene and Changeux, 1997; Koechlin and Jubault, 2006). It is therefore reasonable to postulate that the learning deficit we found following left BA44 inhibition could have resulted from an impairment in the higher-order chunking process. This interpretation is consistent with a study by Koch and collaborators (2000a) showing a difference in learning two sequences having the same elementary chunks but differing in their higher-order organization; they found that the RT decrease across blocks was larger and that the RT difference between the last block of the repeated sequence and the pseudorandom sequence was greater for the sequence containing high-order chunks, leading to a more efficient sequence-specific learning (Koch and Hoffmann, 2000a). This finding indicates that the learning of a motor sequence is less effective when the elementary chunks are present but shuffled and presented in a new order that breaks the higher relationship between them (Koch and Hoffmann, 2000a); this conclusion is also supported by a more recent study (Sakai et al., 2003). Together with the present results, these findings suggest that Broca’s area may be responsible for processing chunks at a higher hierarchical level. However, this would definitely require further investigations by using more complex sequences, structured according to certain rules, the task for the subjects being to learn the sequence explicitly by discovering these rules. For instance, this task could be inspired by the explicit m x n visuomotor sequence learning task (Hikosaka et al., 1995) that has been used to evidence the chunking strategy in complex sequences (Sakai et al., 2003; Miyapuram et al., 2006).

Our conclusion about the contribution of left BA44 to motor skill learning is consistent with the current view that Broca’s area is involved in integrating elementary components into higher-order hierarchical sequences (Gelfand and Bookheimer, 2003; Petersson et al., 2004; Koechlin and Jubault, 2006). Indeed, Koechlin and collaborators have suggested that a prefrontal network, including BA44 and BA45, processes hierarchically structured behaviours (Koechlin et al., 2003; Koechlin and Jubault, 2006). The finding that left BA44 is causally involved in learning implicitly motor sequences is reminiscent of the involvement of Broca’s area in learning artificial rules as tested in AGL paradigms (Petersson et al., 2004; Forkstam et al., 2006; Bahlmann et al., 2008; Udden et al., 2008; de Vries et al., 2009; Floel et al., 2009; Christiansen et al., 2010b). Indeed, these studies provided evidence that Broca’s area, besides its well-known contribution to syntactic processing of “natural” language (Gough et al., 2005; Grodzinsky and Friederici, 2006), also plays a role in detecting and using new artificial rules. More precisely, Broca’s area could be involved in the abstraction process that enables to
comply with sequences of different nature. Consistently with this view, a patient study has shown that agrammatic aphasics with Broca's area lesions are impaired in extracting the abstract structure in both linguistic and non-linguistic sequences during learning (Dominey et al., 2003).

Alternatively, another possible explanation for the dissociation between chunking and sequence learning we reported in the present study is that a left BA44 inhibition leads to deficit in motor performance rather than in learning. However, this hypothesis predicts that, in the two groups, the RT profile across blocks should be exactly the same with a typical RT increase for the pseudorandom block (block#7) and that the only difference between groups should be an upward shift of the RT profile for the left BA44 group when compared with the controls. Because we failed to observe a main group effect in the general-practice learning analysis, the present results do not support this hypothesis although previous studies have indicated that left BA44 may play a role in controlling the motor performance. Indeed, it has been shown that, in patients suffering from an apraxia of speech (AOS) consequent to a left hemisphere stroke, presumably involving a lesion of Broca's area (Hillis et al., 2004), the so-called "study time" (Immink and Wright, 1998, 2001) was significantly increased in tasks involving either finger or speech sequences (Maas et al., 2008). In the present study, the study time could not be computed since SRTT does not allow us to measure this parameter, but if we assume that Broca's area plays a role in arranging the different elements of the sequence before its execution, its temporary inhibition should lead to longer response time in SRTT. This conclusion is consistent with a recent TMS study in which we demonstrated an increase in preparation time induced by left BA44 inhibition in subjects learning explicitly a motor sequence by observation (Clerget et al., 2011). However, this hypothesis about an increase in study time following left BA44 inhibition cannot account for the lack of RT increase in the pseudorandom block. Therefore, although plausible, this explanation does not render null and void our conclusion that left BA44 is involved in sequence-specific learning.

As mentioned in the Introduction, several neuroimaging and TMS studies have already tried to identify the different cortical and subcortical centers involved in SRTT. However, the respective contribution of the different structures found activated in these tasks remains puzzling for several reasons. Firstly, the results from the literature are still largely discrepant. For instance, whereas a rTMS study has shown that the contralateral dorso-lateral prefrontal cortex plays a key role in learning motor sequences (Pascual-Leone et al., 1996), others studies have failed to reproduce these results (Koch et al., 2006; Wilkinson et al., 2010). Similarly, for the SMA, although this area has been found activated in SRTT (Grafton et al., 1995; Hazeltine et al., 1997; Grafton et al., 1998a; Seidler et al., 2005), its causal involvement in learning has
been questioned because of a lack of confirmation from TMS studies (Pascual-Leone et al., 1996; Wilkinson et al., 2010). Secondly, it is now clear that distinct networks are recruited during the different stages of learning (Toni et al., 1998; Toni et al., 2001; Doyon and Benali, 2005; Press et al., 2005; Bapi et al., 2006) and that a given network can show plasticity during learning (Steele and Penhune, 2010). As far as the BA44 activation in SRTT is concerned, functional imaging studies indicate that its activation, if any, is very weak (Bischoff-Grethe et al., 2004; Bapi et al., 2006). One possible explanation for this finding is that these studies did not use hierarchically structured sequences soliciting the contribution of Broca’s area; alternatively, it has been suggested that the frontal regions, including the inferior frontal gyrus, could be mainly involved in the early phase of the learning process (Toni et al., 2001; Doyon and Benali, 2005; Grol et al., 2007) when the different chunking levels have to be implemented together.

Amongst the other structures found activated in SRTT, it has been suggested that the basal ganglia are involved in the chunking process as demonstrated in both animals (Berridge and Whishaw, 1992; Cromwell and Berridge, 1996; Aldridge and Berridge, 1998; Graybiel, 1998; Jog et al., 1999; Levesque et al., 2007) and humans (Boyd et al., 2009; Tremblay et al., 2010). A similar assumption has been made for the hippocampus since its lesion impairs associative learning in SRTT (Curran, 1997), a finding confirmed in rodents (Ergorul and Eichenbaum, 2006). Further experiments will be required to bring together these results but it could be assumed that, whereas subcortical structures could be responsible for low-level chunking, left BA44 may play a critical role in higher-order chunking processes.
6. Broca’s area and high level chunking\textsuperscript{31}

The data obtained in the previous experiment were somewhat contradictory. Indeed we found that, when compared with control subjects, subjects having experienced a left BA44 virtual lesion are impaired in learning a sequence whereas they “chunked” the sequence as control subjects did. We assumed that Broca’s area could be actually involved in chunking but only in the higher-level of chunking. Our experimental strategy to verify such a hypothesis was to conduct another study (study 6) in which subjects again had to learn a complex structured sequence, but this time we used a highly structured sequence so that its chunking could be done at several levels. Also we used an explicit learning (by trial and error) paradigm in order to favour chunking.

\textsuperscript{31} This chapter has been edited from an article in preparation entitled: “Broca’s area processes high-level chunks during sequence learning: a cTBS study” by Clerget E., Van Bever V. & Olivier E.
6.1. Abstract

When memorizing a sequence, as a phone number, we automatically used a strategy known as chunking, which consists of arranging that sequence into small subunits, easier to remember. While chunking has been extensively investigated from a behavioural point of view, its neural basis remains largely unknown. Recently, it has been suggested that the prefrontal cortex, and Broca’s area in particular, plays a role in this process. The aim of the present study was to determine whether this area makes a specific contribution to high-level chunking when learning a complex structured sequence. To do so we used transcranial magnetic stimulation (TMS) in participants learning, by trial-and-error, a 16 elements sequence. In each trial, the participants had to choose between two digits, one being the target (a digit belonging to the sequence); depending on the position of the correct digit on the screen (left or right), the participant had to press a left or right response-key. A visual feedback indicated whether he/she selected the correct digit, which can then be remembered for the next sequence repetition. TMS was delivered at the beginning of the experiment, as a continuous theta burst stimulation (cTBS), either over left BA44 (caudal part of Broca’s area) or over the vertex (control group). All participants (n=22) learned the motor sequence and they all used a chunking strategy but only results from subjects who used the “expected” chunking strategy were analysed. We found that a virtual lesion of left BA44 (n=8) does not alter sequence learning when compared to control (n=8). However, the two groups differed in terms of high-level chunk processing, which took more time in the left BA44 group than in controls. These results corroborate the view that Broca’s area (BA44) intervenes in processing hierarchical structures of motor sequences, but only for higher-level elements.

6.2. Introduction

Chunking is originally and mainly known as a mnemonic strategy (Miller, 1956), commonly used when we have to remember a long string of digits, as a phone number for instance. In that case, instead of trying to remember each number individually, we break it down into several clusters, so-called “chunks”. The underlying principle is that, since the number of items we can store in our working memory is finite (7 ± 2, in general (Miller, 1956)), chunking a long sequence into subunits decreases the memory load i.e. the number of items that we have to store in memory. Indeed, each time a chunk is generated, it becomes a new unit of information. Chunking is a key concept because it can be expanded beyond the memory context. Indeed, chunking can be viewed as a broader strategy, underpinning the human
ability to carry out complex behaviours, from expert motor skills to daily actions, through the flexible concatenation of discrete movements (Rosenbaum et al., 1983; Koch and Hoffmann, 2000a; Sakai et al., 2003; Verwey and Eikelboom, 2003; De Kleine and Verwey, 2009; Kirsch et al., 2009).

Chunking has been confirmed and investigated extensively under various experimental conditions by using learning tasks (Koch and Hoffmann, 2000a; Sakai et al., 2003; Miyapuram et al., 2006), such as serial reaction time tasks (SRTT, (Nissen and Bullemer, 1987)). Classically, in SRTT participants are asked to make a key-press in response to a visual stimulus by applying a simple mapping rule, which associates the stimulus location with a response key. Across trials, and without the participant’s knowledge, the presentation of stimuli follows a constant and pre-determined order, which is repeated several times in a block and for several blocks. Across successive repetitions of the sequence, RT typically decreases, indicating that subjects implicitly learn the sequence. The RT decrease is evaluated as a “general-practice” learning measure. Usually, after a given number of blocks, a block containing a random sequence is presented to subjects; this change leads to a RT increase, allowing us to quantify the “sequence-specific” learning. Actually, it has been established that learning gradually improves as chunking develops, until the sequence’s structure is progressively integrated into manageable units (Koch and Hoffmann, 2000a; Sakai et al., 2003; Sakai et al., 2004a; Miyapuram et al., 2006; Jimenez, 2008; Clerget et al., 2012). From a practical viewpoint, a chunk is identifiable by a decrease in RT from the first to subsequent elements. The explanation for this characteristic RT pattern is that processing the first element of a chunk also involves the pre-processing of the complete chunk (Sternberg et al., 1978; Koch and Hoffmann, 2000b; Koechlin and Jubault, 2006).

The neural correlates of chunking during sequence learning are not yet fully elucidated. Regions underlying the chunking process are likely to overlap, at least partly, with those involved in learning processes, namely cortical and sub-cortical structures, such as the posterior parietal cortex, supplementary motor area (SMA) and pre-SMA, prefrontal cortex (PFC) mainly dorsolateral (DLPFC), and basal ganglia (Ashe et al., 2006; Penhune and Steele, 2011; Pammi et al., 2012). In the present study, we focused on the DLPFC and more precisely on Broca’s area (left BA44 and BA45, (Amunts et al., 2010)). Indeed, because its known contribution to processing hierarchical/syntactical structures in various domains (for a recent review see Fadiga et al., 2009), Broca’s area appears as a good candidate for monitoring the chunking process. However, in a previous SRTT experiment (Clerget et al., 2012), in which we used transcranial magnetic stimulation (TMS) to perform a virtual lesion of left BA44, we showed that while such a virtual lesion deteriorates the implicit learning of the sequence,
surprisingly, it leaves unimpaired the chunking strategy. In order to explain this discrepancy between unaffected chunking but poorer sequence learning, we hypothesized that “left BA 44 intervenes in high hierarchical level processing, possibly to integrate elementary chunks together” (Clerget et al., 2012). Indeed, when learning a complex motor sequence, chunking might occur at several hierarchical levels, from low-order levels (i.e., the concatenation of two or three successive items into elementary chunks) to high-order levels (i.e. the integration of these elementary chunks into a higher-order sequence) (Dehaene and Changeux, 1997; Koechlin and Jubault, 2006). In our previous experiment (Clerget et al., 2012), it was impossible to verify this assumption because of the experimental design we used. Therefore, the goal of the present study was to test this assumption by using a more appropriate experimental design. To carry out this new experiment, we relied upon an explicit learning task developed by Kühn, Koch, von Cramon and Schubotz (2010), which is characterized by the fact that different levels of chunking can be identified. Using this task should allow us to investigate the formation of chunks at different hierarchical levels and, to determine the impact of a virtual lesion of left BA44 on chunks of these different levels.

6.3. Methods

Subjects

Twenty-two healthy subjects (10 women; mean age = 25±5 year) participated in the present experiment. All participants were right-handed as assessed by the Edinburgh Inventory (Oldfield, 1971), had normal or corrected to normal vision and were free of neurological history. Before subject’s enrolment, potential risks of adverse reactions to TMS were evaluated according to the Transcranial Magnetic Stimulation Adult Safety Screen (Keel et al., 2001) and supervised by a neurologist. Participants were asked to be free of any substance (medication, alcohol or drug). Written informed consents were obtained from each subject. The experiment was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the Université catholique de Louvain. Subjects were compensated with monetary rewards for their participation.

Experimental setup and design

The experiment took place in a quiet and dim room. The volunteers sat in a comfortable armchair in front of a PC connected to a standard keyboard. The task was controlled through a home-made program written in Matlab (The Mathworks Inc.). In order to avoid inaccuracy in RT recordings due to possible processing delays attributable to the Windows operating system,
we used a homemade device, the Psychophysics Synchronisation Box that allows us to detect a key-press with one microsecond accuracy.

Main task

Subjects were told that the task consists in discovering by trial-and-error, and memorizing, a 16-element sequence composed of four different digits (1, 2, 3 and 4) so that they should be able to recall it at the end of the experiment (see below). The sequence (3232232341411414) was repeated 5 times in each block (8 blocks, from block #2 to block #9). This sequence was designed so that it could be chunked as follow: a) the sequence could be split into 2 subunits characterized by the same structure (32322323-41411414); b) each subunit could then be divided into 2 smaller subunits which mirrored each other (3232-2323 and 4141-1414); c) each could be viewed as a repetition of a pair (32-32, 23-23 and 41-41, 14-14) and d) each pair consisted of alternating elements (3-2, 2-3 and so on) (Figure 2.17). Therefore, its structure can be described at several organizational levels, namely (from the highest to the lowest): level 1 composed of two chunks of 8 elements, so called “8-element chunks”, level 2 with two “4-element chunks” and level 3 with four “2-element chunks”. A fourth level was composed of the remaining elements of the sequence which can not be integrated into another lower-level chunk; thus level 4 is constituted by each of the second elements of the “2-element chunks” from level 3 i.e. 8 elements (Figure 2.17). It is worth noting that, since those levels are nested into each other, an item is only ascribed to the higher level it belongs.

Importantly, subjects were informed neither about the exact sequence structure nor about the best strategy to be used to memorize it.

In order to determine whether subjects learnt the sequence properly and which strategy they used to do so, at the end of the experiment, subjects underwent a memory test consisting of writing down the sequence and explaining the way they remembered it. The appropriate response, if subjects used the chunking strategy described above, was the following sequence: 32-32-23-23-41-41-14-14. In addition, their chunking strategy should be apparent in their reaction time pattern (see the “Data and statistical analyses” section).
Chapter 2. Experimental contributions

Figure 2.17: Description of the sequence’s structure.

The illustration depicts the progressive break down of the sequence as we designed it. Each item of the sequence is indicated on a gray circle. The scheme of the break down consists of splitting into two parts the sequence, several times, in order to obtain sub-sequences or chunks of decreasing size i.e., 8, 4 and finally 2-element chunks, corresponding to different organizational levels namely, level 1 (in red), level 2 (in blue) and level 3 (in green), respectively. Level 4 (in yellow) is composed of all the second elements of the 2-element chunks of level 3.

**Trial design**

In each trial (Figure 2.18B), two rectangles (9.55° width and 13.37° height, white contrasted against a black background) were presented simultaneously at either side of the centre of the computer screen. Each rectangle surrounded one digit from 1 to 4. One of these two digits was the target (i.e. an element of the sequence) whereas the other was a distractor. The “target” succession across trials followed the order of the elements in the sequence but the “target” location, on the left or right side of the screen, was pseudo-randomly determined. The stimuli remained displayed until the subject response, but within a 5000 ms limit.

Subjects received the instruction to respond to the stimulus presentation as quickly as possible, by pressing one of two response keys on a keyboard with either their index or their middle finger of their right hand. The stimulus-response mapping was congruent: the left response key was associated with an index finger response and corresponded to a left stimulus choice while the right response key was associated with the middle finger and matched the right-sided stimuli. Immediately after their response, subject received a visual feedback for 250 ms: the surrounding rectangle turns green in case of a correct response whereas in case of an incorrect response, a red mask covered the entire rectangle (Figure 2.18B). The subject was therefore able to infer which digit belongs to the sequence to be learned. Then the next pair of stimulus was displayed 500 ms after the response or after the 5000 ms delay if no response was given during this time period. After 16 consecutive trials, a fixation cross appeared for 2000 ms indicating a new repetition of the sequence within the current block.
A. Stimulation sites.
Stimulation sites are shown as ellipses, in pink for both the vertex (upper part) and in purple for the left BA44 (lower part). For each ellipse, its center indicates the mean coordinates and its surface the 95% confidence interval.

B. Time course within a block.
Each block and each of the 5 repetitions of the sequence within a block start with the display of a fixation cross for 3000 ms. Then, the first trial (n=1) begins with the display of a first pair of stimuli followed by the subject’s response towards one of the two presented digits; immediately after his response, subject receives a feedback consisting of a red mask for an incorrect response and a green rectangle surrounding the target digit for a correct one. The same time course occurs for 16 trials that represent the 16 digits of the sequence and for 5 repetitions of these trials within a block.
Experimental contributions

Control task

As a control condition, subjects were required to execute a simple RT task. The number of trials within a block (n = 16 x 5) and the trial design were the same as in the main task. Changes were threefold, i.e., a substitution of the 4 digits with 4 letters (A, B, C and D), an absence of structured sequence in the letter presentation and the subject’s task: for each pair of letters, subjects always had to select the same target, the B letter. This control condition was tested once before (block #1) the 8 blocks of the main task in order to accustom subjects to the experimental setup and to ensure equivalent performance level between both groups, and once after (block #10), to control for a possible unspecific effect of fatigue.

Transcranial Magnetic Stimulation

TMS was delivered by means of a Magstim Super Rapid stimulator (Magstim Company, Whitland, UK) via a 70 mm diameter figure-of-eight coil, and administrated following the continuous theta-burst stimulation (cTBS) protocol originally described by Huang and collaborators (2005): 3 pulses were delivered at 50 Hz every 200 ms for a total duration of 40 s, leading to a total number of pulse of 600; the stimulation intensity was set at 80 % of the resting motor threshold (rMT) of each subject. In order to determine the rMT, at the beginning of the experiment, single pulses were applied over the hand representation of the left primary motor cortex (M1) while the motor-evoked potential (MEP) from the right first dorsal interosseous muscle were monitored. After the optimal position of the coil was found, we determined the minimum intensity necessary to produce a 50 μV peak-to-peak MEP in 5 out of 10 stimulations (Rossini et al., 1994).

The x, y and z coordinates (Montreal Neurological Institute, MNI, system) of the two target stimulation sites were: -43, 11 and 16 mm for left BA44 and 0, -15 and 74 mm for the vertex, based on information available in the literature (respectively, (Amunts et al., 2004; Anwander et al., 2007) and (Okamoto et al., 2004)). To position the coil adequately on the scalp surface, the target sites were first localised onto each individual brain scan through a reverse normalization procedure and second the coil position was monitored by using a home-made neuronavigation program (Noirhomme et al., 2004). Off-line, we computed the mean actual coordinates of the stimulation sites in all subjects (Figure 2.18A). These coordinates were (mean±SD of x, y and z; MNI system of coordinates): -58±5, 16±6, 22±6 mm for left BA44 and -4±1, -11±4, 74±2 mm for the vertex.
Chapter 2. Experimental contributions

Data and statistical analyses

Among the 22 subjects enrolled in this experiment, data from 5 subjects (4 in the control group and 1 in the left BA44 group) were not included in analyses since the strategy used by these subjects differed from the expected, and the most encountered, one (32-32-23-41-41-14-14, as described in the “Main task” section). Indeed, these subjects reported, at the end of the experiment, that they learned and remembered the sequence as follows: 323-22-323-414-11-414. Furthermore, an additional subject from the control group was discarded from the analysis because the number of correct sequences (n=10) he performed was lesser than the mean - 2 SD value of the rest of the group (27.9±5.1 sequences).

Analyses were performed on reaction time (RT), a variable defined as the elapsed time between the stimulus presentation and the subsequent key-press response. First, in order to characterize the time course of learning, the mean RT was computed for each of the 10 blocks. Second, we focused on the chunking pattern by computing, for all correct sequences: 1) the mean RT for each item with respect to its position in the sequence (Clerget et al., 2012), 2) the mean RT for items grouped according to the level of organization they represent. In addition to the latter, we computed corrected RT by means of a Z-score (Z = (RT-mean(RT_block#1))/sd(RT_block#1)) in order to take into account the difference in performances during block #1.

The statistical analyses consisted of analyses of variance (ANOVA) (Statistica, StatSoft Inc., USA). All ANOVA were performed with GROUP (control group vs. left BA44 group) as between-subjects factor but with different within-subjects factors that varied according to the analyses at hand. First, two separate one-way ANOVA were performed on RT gathered for block #1 and block #10. Second, a repeated-measures ANOVA (ANOVA_RM) was performed on RT with BLOCK (#2 to #9) as within-subjects factor. Third, two other ANOVA_RM were performed on RT for all correct sequences with either POSITION (#1 to #16) or LEVEL (#1 to #4) as within-subjects factors. Then, another ANOVA_RM was performed on Z-score for all correct sequences with LEVEL (#1 to #4) as within-subjects factors. Finally, one way ANOVA were also performed both on the total number of sequence correctly executed across blocks and on the number of repetitions that subjects took to discover entirely the sequence for the first time. When appropriate, a Fisher’s least significant difference (LSD) post-hoc test (p<0.05) was performed.
6.4. Results

First, we contrasted the RT obtained for the control task in both groups (block #1 and #10). A one way ANOVA revealed no main effect of GROUP (block #1: F(1,14) = 4.54, p = 0.06 and block #10: F(1,14) = 5.11, p = 0.06), indicating that subjects from both groups had similar RT performance at the beginning and at the end of the experiment.

Then, we compared the mean RT gathered for the different experimental blocks (Block #2 to #9) in the main task (Figure 2.19). An ANOVA on RT for block #2 to #9 revealed no main effect of GROUP (F(1,14) = 3.48, p = 0.08) but a significant main effect of BLOCK (F(9,14) = 28.47, p < 0.001) and a significant interaction (F(9,14) = 2.17, p = 0.04). As illustrated in Figure 2.19, post-hoc tests showed that 1) for both groups, RT in block #2 was statistically different from RT in all other blocks (blocks #3 to #9) (all p < .003) and 2) for the control group, RT in block #3 was significantly different from RT in blocks #6 to #9 (all p < .02) whereas for the left BA44 group, RT in block #3 and in block #4 were different from that in blocks #6 to #9 (all p < .02). These results indicate that all subjects gradually improved their performance with practice but that subjects in the control group reached a plateau slightly faster (block #4) than subjects in the left BA44 group (block #5).

![Figure 2.19: Sequence learning.](image)

Mean RT (in ms) across blocks for the control (pink circles) and left BA44 (purple circles) groups. In blocks #1 and #10, subjects executed the control task while in blocks #2 to #9, they performed the main task i.e. discovering and learning by trial-and-error a structured sequence repeated five times in each of the 8 blocks.
Chapter 2. Experimental contributions

Second, we compared the mean RT gathered for each item position in the sequence (from 1 to 16) (Figure 2.20). An ANOVA with GROUP (control vs. left BA44 group) as between-subjects factor and POSITION (#1 to #16) as within-subject factor revealed no main effect of GROUP (F(1,14)= 4.14, p=0.06) but a significant main effect of POSITION (F(15,210)= 17.55, p<0.01) and an significant interaction (F(15,210)= 2.07, p=0.01). Post-hocs enable to reveal six chunks starting from positions #1, #5, #9, #11, #13 and #15 (all p < 0.05) for the control group and five chunks starting from positions #1, #9, #11, #13 and #15 (all p < .05) for the left BA44 group.

![Figure 2.20: Chunking pattern by position.](image)

Mean RT (in ms) for each item position (from #1 to #16) in the sequence for the control group (pink circles) and the left BA44 group (purple circles) for all correct sequences across block #2 to #9. Only statistically significant chunks (significant difference in RT between item n and item n + 1) are indicated by linking adjacent positions. Along the x axis, for each item position, both the actual item number (from 1 to 4) and level (from 1 to 4) are also indicated (the item number is included in circles those color is function of the organizational level; the color code is the same as indicated in Figure 2.17).

Then, we compared the mean RT gathered for items belonging to the same levels to the sequence’s structure (from 1 to 4) (Figure 2.21A and 2.21B). An ANOVA with GROUP (control group vs. left BA44 group) as between-subjects factor and LEVEL (#1 to #4) revealed no main effect of GROUP (F(1,14)= 3.85, p=0.07) but a significant main effect of LEVEL (F(3,42)= 43.44, p<0.001) and a significant interaction (F(3,42)= 3.88, p=0.02). Post-hocs demonstrated that, for the control group, the RT for level 1 was significantly larger than the RT for level 2 (p= 0.03) and similarly, the latter is significantly higher than RT for level 3 (p= 0.05). This is in accordance
Chapter 2. Experimental contributions

with our expectations that RT is function of the chunking level concerned and the higher the
level, the longer the RT is. In contrast, in the left BA44 group, only the RT for level 1 was
different from the RT for all the other levels. Finally, only RT in level 1 differed between both
groups (p= 0.05, not shown). Similar results were obtained on Z-score. Indeed, an ANOVA_RM
with GROUP (control group vs. left BA44 group) as between-subjects factor and LEVEL (#1 to #4)
revealed no main effect of GROUP (F(1,14)= 0.76, p= 0.40) but a significant main effect of LEVEL
(F(3,42)= 46.05, p<0.001) and a significant interaction (F(3,42)= 3.66, p=0.02). Post-hocs lead to
the same conclusions as before (all p<0.05).

Figure 2.21: Chunking pattern by level.
A. Mean RT (in ms) for each organizational level (from #1 to #4) in the sequence for the control group and the
left BA 44 group for all correct sequences across block #2 to #9.
B. Same as in A. for Z-scores.
Finally, a one-way ANOVA with GROUP (control vs. left BA44 group) as between-subjects factor were performed on the total number of sequence correctly executed across blocks. This analysis reveals that both groups did not differ from each other in terms of learning performance (29±5 correct sequences for the control group and 25±4 for the left BA44 group on a total of 40 repetitions, F(1,14)= 2,30, p=0,15). A similar analysis performed on the number of sequence repetitions that subjects needed to learn the sequence (i.e. the number of repetitions before the subjects performed a correct sequence for the first time) (5±3 and 7±4, respectively for the control and left BA44 groups) showed that the two groups did not differ significantly from each other (F(1,14)= 1,45, p=0,25).

6.5. Discussion

The aim of the present study was to test the hypothesis that Broca’s area may be responsible for processing high-order chunks during sequence learning, a hypothesis that derives from our previous study showing that a virtual lesion of left BA44 worsens the implicit learning of a motor sequence while leaving unimpaired the chunking strategy (Clerget et al., 2011). To test this hypothesis, we used a highly structured sequence that allowed us to study its chunking at different hierarchical levels. Our main finding is that a left BA44 virtual lesion induces an increased in RT for the processing of stimuli that constitute the higher level of hierarchical organization of the learned sequence. This result is in accordance with our predictions and with the hypothesis that the posterior part of Broca’s area is crucially involved in high hierarchical level processing (Clerget et al., 2012). This finding indicates that the role of Broca’s area might be to combine low-level chunks together, so that the chunked sequence is recalled and assembled as a coherent unit.

First, we would like to contrast the present results with that of our previous study (Clerget et al., 2012). Indeed, even if these two studies differ markedly in relation to the task used, i.e. implicit learning SRTT task vs. explicit learning by trial-and-error, for the previous and current studies, respectively, some interesting comparisons can be made. First, in our previous study (Clerget et al., 2012), we found that a virtual lesion of the left BA44 impairs exclusively the so-called “sequence-specific learning”, leaving the “general practice learning” unimpaired. In the present study, since the experimental design has been modified in order to address a different issue, we were unable to quantify the sequence-specific learning. Nevertheless, our results tend to show similar learning performances in both groups in relation to either RT or to the total number of sequence correctly executed across blocks or the number of sequence
repetitions that subjects needed to learn the sequence. Only a slight difference between
groups was found for the block at which subjects reached a plateau in RT performance,
suggesting that all subjects gradually improved their performance with practice but that 
subjects in the control group reached a plateau slightly earlier than subjects in the left BA44 
group. Altogether, the present results corroborate the view that a lesion of left BA44 does not 
affect general practice sequence learning (Goschke et al., 2001; Dominey et al., 2003; Clerget 
et al., 2011) but that the consequences of such a lesion are more subtle. Second, as previously 
demonstrated, we found that subjects organize the motor sequence into smaller sub-
sequences, referred to as chunks. We identified chunks quantitatively by estimating the 
significant differences between RT for contiguous elements in the sequence. The novelty here 
is that we showed that a chunk can be embedded inside another higher-level chunk so that it 
should be reflected in RT differences for the sequence elements depending on the chunking 
level they belong to. Such a prediction was confirmed since differences in RT according to the 
position of items in the sequence (Figure 2.20) and, in fine, according to the chunking level 
(Figure 2.21). However for the latter, for the control group, while RT in level 1 is significantly 
increased in comparison with RT in level 2 and RT in level 2 is significantly increased in 
comparison with RT in level 3, we failed to establish a similar relationship between RT in level 3 
and RT in level 4. Actually, as mentioned in the Methods section, level 4 is made of the 
residual elements of the 2-element chunks (level 3) and as it could be seen in Figure 2.21, the 
chunks starting from position #3 and #7 in the first half of the sequence were not revealed by 
the statistical analyses, possibly because this portion of the sequence has been overlearned. 
Indeed, it should be remembered that the task constrained subjects to discover progressively 
the sequence and thus to learn the sequence from its first elements to the last ones. Thus, it 
could explain why RT in level 3 has not been found different from RT in level 4.

The methodology used in the present research is pioneering from several aspects. First, as 
in Koch and Hoffmann (2000), our subjects were directed towards a precise chunking pattern 
according to the way we designed the sequence to be learnt (repetition and inversion of 
recurrent digits) but in our experiment, the sequence of stimuli does not correspond to a given 
sequence of movements. Indeed, in the present study, the chunking pattern is independent 
from the “sequence” of motor responses (left or right key-presses). By constraining the 
chunking pattern, we maximize the probability that most subjects used the same chunking 
strategy, by contrast with individual chunking strategies observed when using unstructured 
sequences (Sakai et al., 2003; Verwey and Eikelboom, 2003; Verwey et al., 2010). Second, we 
used a sequence that is structured at several hierarchical levels. Previous studies have
established that learning efficiency depends on the sequence organization: learning is more efficient for a sequence containing high-order chunks than for the same sequence containing only elementary chunks (i.e. the order of elementary chunks could be shuffled so that the higher relationship between them, that define high-order chunks, is inoperative) (Koch and Hoffmann, 2000a; Sakai et al., 2003; Miyapuram et al., 2006). However, in these studies, the chunking strategy was not systematically investigated. Recently, researchers have tried to vary the structure of complex visuo-motor sequences (Pammi et al., 2012). Actually, using a particular sequence learning paradigm (the mxn task; Bapi et al., 2000; Hikosaka et al., 1995), it was possible to vary the structure along two dimensions, importantly, without modifying the total length of the sequence (Pammi et al., 2012). They found that RT performances were better for the 2×12 condition than for the 4×6 one, meaning that the chunking pattern influences learning: memorizing a large number (n=12) of small chunks (m=2) improves learning better than memorizing a small number (n=6) of larger chunks (m=4). Importantly, the authors also acquired functional magnetic imaging (fMRI) data during this experiment. They revealed a selective involvement of the fronto-parietal network in the 2×12 condition, and interestingly, a bilateral activation of BA44/BA45 at early stage of sequence learning. This result suggests that Broca’s area, and possibly its right homologue, plays a crucial role in managing the best pattern of chunking by controlling the development of a maximum of relationships between elements and between chunks of elements in order to yield a well-structured sequence, easier to memorize. To the best of our knowledge, this was the first fMRI study investigating the specific activation pattern related to chunking development. Further TMS experiments are required to decipher the respective contributions of the different areas of the fronto-parietal network evidenced in the aforementioned fMRI study, encompassing Broca’s area and its right homologue, in the chunking process during sequence learning.
Chapter 3. Discussion

In this thesis, we were interested in deciphering the involvement of Broca’s area in motor cognition and, more specifically, its possible contribution as a “syntactic processor”. To introduce this research, in Chapter 1, we reviewed the available literature that demonstrates the role of Broca’s area in linguistic syntax, but also its involvement in many non-linguistic tasks, raising the question of the exact function of this “language area”. We considered an attractive hypothesis which suggests that Broca’s area may process syntax in general, the linguistic syntax being simply one paradigmatic example - but probably the most remarkable one - of this ability to process hierarchically organized sequences. In fact, processing syntax-like features is probably critical in many behaviours, which are most often sequentially organized, ranging from learned/instinctive motor sequences executed by animals to complex skills of humans. These properties or rules allow the hierarchical combination of individual movement “units” into meaningful actions. Despite the fact that more and more studies tend to support this “syntax” hypothesis, causal evidence are still sporadic. In order to shed light on this issue, we performed several TMS studies, which are presented in detail in Chapter 2. The main findings and conclusion of these studies are summarized in the following section.

1. Summary of our main findings

In the two first studies (study 1 and study 2), by using a reordering task involving “real” action sequences, we found that a virtual lesion of Broca’s area (left BA44) impairs subject’s performance. This effect was only observed for movements that were both biological and syntactic, all other movement categories being unaffected, challenging to some extent the view that the left BA44 contribution to the processing of biological actions is exclusively due to its involvement as part of the putative MNS in humans. The third study (study 3) aimed to investigate the contribution of Broca’s area to the processing of more abstract sequences that varied in terms of hierarchical organization. We found a specific contribution of Broca’s area (left BA45) to the initiation of complex key-presses sequences that required applying higher-level rules.

Then, we intended to determine whether Broca’s area, throughout its contribution to syntax processing, is involved in learning structured sequences. This issue has been addressed by using different sequence learning tasks and paradigms. In study 4, we found that a virtual lesion of Broca’s area (BA44) induced before a learning motor task, leads to an increased response latency when this task was tested during a retention test occurring 24 hours later; this finding indicates that subjects needed more time to initiate the execution of the learned
motor sequence. Since this sequence was structured with different timing rules and that subjects were not impaired in learning these rules, it suggests that Broca’s area plays a role in organizing and/or storing the different elements of a motor sequence before its execution. In study 5, we demonstrated the key role of Broca’s area when learning of a more complex motor sequence. In this study, we also highlighted a peculiar learning strategy that occurs when learning long sequences, the so-called chunking strategy, consisting in organizing the sequence structure into smaller sub-units, so-called “chunks”, to memorize it more easily. However, contrary to what was expected, we failed to evidence any influence of Broca’s area (BA44) virtual lesion on this strategy. A last study (study 6) allowed us to establish that Broca’s area is actually specifically involved in high-level chunking process, i.e. chunks formed by combining low-level chunks. The conclusions of these studies contribute to strengthen the view that Broca’s area, and more precisely its posterior part (BA44), plays a crucial role in sequence learning, especially when it requires high-level chunking.

The following table (table 3.1.) summarized the main findings of the different studies as well as the different features that distinguish them.

**Table 3.1: Summary table.**

<table>
<thead>
<tr>
<th>Study number</th>
<th>TMS protocol</th>
<th>Stimulated area(s)</th>
<th>Paradigm / task</th>
<th>Type of sequence</th>
<th>Main findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>Online - rTMS</td>
<td>Left BA44</td>
<td>Pictures reordering task</td>
<td>« real » biological vs non-biological action sequences</td>
<td>Impairment (increased RT) for biological (transitive and syntactic) trials</td>
</tr>
<tr>
<td>Study 2</td>
<td>Online - rTMS</td>
<td>Left BA44, Left BA45, Right BA44</td>
<td>Pictures reordering task</td>
<td>« real » biological vs non-biological &amp; syntactic vs non-syntactic action sequences</td>
<td>Impairment (increased RT) for biological &amp; syntactic trials following TMS over the left BA44</td>
</tr>
<tr>
<td>Study 3</td>
<td>Online - rTMS</td>
<td>AB (~BA45), PB (~BA44), PM (~BA6)</td>
<td>Simple and superordinate tasks</td>
<td>Motor (key-presses) and cognitive (categorization rules) sequences</td>
<td>Impairment (increased RT) in the superordinate task for trials that initiate the sequence following TMS over the AB site</td>
</tr>
<tr>
<td>Study 4</td>
<td>Offline - cTBS</td>
<td>Left BA44</td>
<td>Explicit learning task by observation</td>
<td>Motor sequence</td>
<td>Impairment (increased response latency) for executing the learned sequence in the retention test (post learning)</td>
</tr>
<tr>
<td>Study 5</td>
<td>Offline - cTBS</td>
<td>Left BA44</td>
<td>Implicit learning task - SRTT</td>
<td>Motor sequence</td>
<td>Impairment in sequence-specific learning</td>
</tr>
<tr>
<td>Study 6</td>
<td>Offline - cTBS</td>
<td>Left BA44</td>
<td>Explicit learning task by trial and error</td>
<td>Cognitive sequence</td>
<td>Impairment (increased RT) for the first trial of high-level chunks</td>
</tr>
</tbody>
</table>
2. Conclusions, important issues and perspectives

In this thesis, we investigated the implication of Broca’s area in both the offline (learning/building syntax) and the online (applying/retrieving syntax) processing of more or less complex sequences, from abstract to more “ecological” ones. The results of those investigations allow to confirm and precise the role of Broca’s area in hierarchical processing. On the one hand, our data indicate that, when learning either implicitly or explicitly a structured sequence, Broca’s area is responsible for managing the formation of the sequence structure, especially by processing high-level chunking. On the other hand, our data also indicate that Broca’s area is in charge of retrieving and implementing the structure of a sequence during its execution. Altogether, Broca’s area may ensure a coherent connection between the elements of a sequence, when this sequence has to be learned, built or executed and, importantly, whatever the type of sequence. Hence, our results fit with an essential contribution of Broca’s area to motor cognition, the pivotal element being its ability to process hierarchies.

However, since our investigations involved different protocols, tasks and types of sequence, we wanted to discuss some apparent discrepancies between those findings. First, in one of our study (study 3) we evidenced a role of the anterior part of Broca’s area (BA45) in processing complex sequence. In the only other study in which we have tested BA45 (study 2), we have shown that interfering with this region had no effect on reordering action sequences, even biological and syntactic ones. Since such lack of effects appears at odd with the conclusion of the study 3, how to reconcile both findings? In a recent fMRI study, (Friedrich and Friederici, 2009), the authors aimed to investigate the brain regions involved in processing hierarchical vs. non-hierarchical structured mathematical formulae. They found that processing hierarchical formulae activated a region in left IFG, BA45, and an adjacent area, BA47. The authors pointed out that this cortical region (BA45/BA47) is located more anteriorly than expected and interpreted its activation as “coming from deductive mental operations during the application of the syntactic rules underlying the formation of hierarchical formulae” (Friedrich and Friederici, 2009). A similar explanation could apply to our data. Indeed, in the study in which the role of BA45 has been highlighted, the complex task required the application of rules that associate a motor response to a stimulus but the application of these rules themselves depend on the position of the stimulus in the sequence. Thus, we can assume that an impairment in this task following a virtual lesion of BA45 could reflect higher order cognitive processes not directly related to syntactic processing. It is worth noting that some authors posit that the language and calculation domains do not share similar syntactic
computations. Varley and collaborators found that agrammatic aphasic patients are not impaired in mathematical syntactic processing (Varley et al., 2005) suggesting indeed a dissociation between the syntactic processes in the domain of mathematics and those in the language domain. They formulated the hypothesis is that “language grammar might provide a “bootstrapping” template to facilitate the use of other hierarchical and generative systems such as mathematics”. Friederici and collaborators even proposed a dual system: one system for processing “hierarchically structured mathematical formulae” and one system for processing “complex syntactic hierarchies in language” (Friederici et al., 2011b). This view is not really at odd with the idea that different parts of Broca’s area are specifically involved depending on the complexity of the syntactic processes required to resolve a task; the level of syntactic complexity being different from a domain to another (see Friederici et al., 2011b).

Second, in two studies (study 1 and 2), we used biological vs. non-biological and syntactic vs. non-syntactic stimuli. These two complementary studies revealed that a lesion of the left BA44 only impaired biological and syntactic action sequences. However in all other studies we did not use such kind of sequences. Therefore the question arises about the reason why only sequences depicting biological and syntactic actions were impaired. A possible answer comes from the property of non-biological syntactic stimuli: they were multi-step events triggered automatically without human intervention. Thus, if we consider that, to reorder action sequences, subjects have to implement the representation of the syntactic rules that linked the elements of the sequence together, we can postulate that reordering non-biological sequences did not recruit left BA44 because in this case, retrieving the sequence’s structure does not require an embodiment process (non-biological action cannot be mapped onto the motor repertoire of subjects). In all other studies presented in this thesis, subjects had to use or build syntactic rules and to rely on their representations to learn, retrieve or execute the sequence at hand.

The above discussion also emphasizes the fact that, even if our findings argue for a role of Broca’s area, and more precisely of the left BA44, in processing action syntax, we are aware that it could not explain, on its own, all the different implications of Broca’s area, for instance in the action domain. Our findings that the biological nature of action sequences is decisive concerning their processing by Broca’s area indicate that several functions may overlap in Broca’s area, for instance the “mirror” and “syntax” ones. It suggests also that the frequently reported Broca’s area contribution in action observation/recognition might be partly explained by its implication as a syntactic processor. Support for this view comes from studies with patients suffering from ASD (Cattaneo et al., 2007; Fabbri-Destro et al., 2009).
Cattaneo and collaborators (2007), ASD and healthy children were asked to execute or observe someone executing a grasp directed towards a piece of food either for eating it or for placing it into a recipient (Figure 3.1, part a). In all cases, the activity of a muscle responsible for mouth movements, the mylohyoid (MH) muscle, was recorded. The authors observed significant differences between ASD and healthy children. First, for the execution condition: in the healthy children, for the grasping action for eating (Figure 3.1, part b, left), the MH activity occurs early and increases through the reaching and grasping phases. On the contrary, for the same action and same goal, in ASD children, the MH activation occurs late, just before they bring to their mouth the piece of food (Figure 3.1, part c, left). Second, for the observation condition: a MH activity occurs in healthy children when they observed the “grasping for eating” action. In contrast, in ASD children, in the same condition, no MH activity was recorded (Figure 3.1, parts b and c, right). The authors interpreted these data in those terms: “children with ASD have a severe impairment in motor organization that includes a deficit in chaining motor acts into intentional actions. Thus, during action observation the intentional motor chains are not activated” (Rizzolatti and Sinigaglia, 2010). Such a MNS dysfunction caused by an impaired organization of chained action has found further support (Fabbri-Destro et al., 2009) and, since ASD is a disorder possibly related to a dysfunction of the IFG (Oberman et al., 2005), it accounts for a syntactic perception of action, possibly under Broca’s area control.
Figure 3.1: The mirror mechanism and autism.

In this study (Cattaneo et al., 2007), children with ASD were asked to grasp a piece of food either for eating or for placing in a container (see the figure, part a) and, in another set-up, to observe an experimenter performing these actions. The activity of the mylohyoid (MH) muscle, which is involved in mouth opening, was recorded to produce an electromyograph (EMG). From (Rizzolatti and Sinigaglia, 2010).
Finally, our studies raised new issues that would be interesting to investigate. First, in several of our studies we observed that a virtual lesion of the left BA44 increases the amount of time needed to initiate or perform the task at hand. These results could be related to an increase in preparation time, also called “study time” as observed in AOS patients, suggesting that Broca’s area may be particularly involved during a pre-programming period in encoding and/or storing information relating to the sequence’s structure. Elaborating a protocol that allows us to compute separately the study time will help to confirm this assumption. Second, part of our results in favour of a role of Broca’s area in sequencing action enable us to make the connection with some deficits observed in apraxic patients who make sequential errors in their action (for instance, putting the toothbrush in the mouth before adding toothpaste). Additionally isolated evidence has been accumulated in our laboratory in favour a role of the middle temporal gyrus in conceptual tool use (Pelgrims et al., 2011). Altogether, these data account for a dissociation between “conceptual apraxia” to refer to conceptual errors in tool use and “ideational apraxia” to describe deficits concerning the sequential organization of gestures (Ochipa and Gonzalez Rothi, 2000). Thus, it would be interesting to confirm this dissociation in a single TMS experiment, by submitting subjects to a stimulation over the middle temporal gyrus or over the left BA44 in two different tasks, a conceptual and a sequential ones.

The therapeutic applications from the present work are very limited. In connection with action observation paradigms that are used as treatment to improve recovery after brain lesions (Mulder, 2007; Bellelli et al., 2010; Franceschini et al., 2010; Garrison et al., 2010), some patients, especially patients suffering from “ideational apraxia” may profit to be tested and trained with some of our tasks using video and/or pictures of biological and syntactic action sequences. Another therapeutic issue concerns our sequence learning paradigms involving the development chunking strategy since chunking during motor sequence learning has been studied in various clinical context: dyslexia (De Kleine and Verwey, 2009), Parkinson’s disease (Siegert et al., 2006) and Alzheimer’s disease (Huntley et al., 2011). Therefore, by

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32 In a clinical study on patients suffering from AOS (Maas et al., 2008), a deficit possibly related to a lesion of Broca’s area (Hillis et al., 2004), the authors found that these patients exhibit a specific increase in the “study time” for both speech and non-speech (finger) movements, suggesting that AOS reflects an effector-independent disruption of the pre-programming processing of sequence.

33 Some apraxic patients show deficits in selecting the appropriate object in order to achieve a given goal and in using tools, because of conceptual errors (for instance, using a toothbrush as a comb). Also, these patients fail to give details about how to use tools whereas they succeed in naming them.

34 The rationale is that action observation enhances recovery through the reactivation of areas through the action observation/execution network.

35 Chunking is a dopamine-dependent process in animals (Tremblay et al., 2009) and in humans (Tremblay et al., 2010).
training patients to use chunking, memorizing might be improved, especially for patients suffering from Alzheimer’s disease at its early stage (Huntley et al., 2011).

To conclude, the idea that Broca’s area is involved in hierarchical processing is an attractive hypothesis since “(...) hierarchies (...) are at the core of many complex systems” (Pumain, 2006). Etymologically, “hierarchy” is composed of two Greek words: “hieros”, sacred and “arkhē”, power. In its original meaning, the word “hierarchy” refers to the ecclesiastical organization but this term was then applied to any type of organized structures. Our behaviours are hierarchised but so are many fields such as computer science, biology, geography, society, religions and so on. Even the human needs are subjected to hierarchisation, as proposed by Maslow (1943): people needed to search for fulfilling more and more superior needs. The Maslow’s hierarchy of needs originally36 comprised five different levels, most often displayed as a pyramid (Figure 3.2) those base represents basic needs (e.g. food) and pinnacle, higher needs (e.g. personal accomplishment).

![Figure 3.2: Maslow’s classic hierarchy of needs.](image)

Hierarchy of fundamental human motives. This figure integrates ideas from life-history development with Maslow’s classic hierarchy. From (Kenrick et al., 2010).

Hierarchical processing may add to the set of cognitive control functions, known to be critical for many cognitive and social skills, success in life (school and job successes, marital harmony, ...) (see Diamond, 2011). Interestingly, a functional imaging study in humans has identified different brain activations according to the social status i.e. “when a person moves up or down in a pecking order -- or simply views perceived social superiors or inferiors” (Zink et

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36 An updated view with add-ons, mainly reproductive goals, has been proposed (Kenrick et al., 2010) even if disputable (Peterson and Park, 2010).
al., 2008). It means that different kinds of hierarchy are represented in the brain and that hierarchy is also found in the way the brain function.

Closer to the theme of the present work, a growing number of studies stress the hierarchical organisation of the brain (Fuster, 2001), and especially the frontal cortex, including Broca’s area (Koechlin et al., 2003; Badre and D’Esposito, 2007; Botvinick, 2007; Koechlin and Summerfield, 2007; Badre, 2008; Botvinick, 2008; Badre and D’Esposito, 2009; Badre et al., 2010). However, our findings rather account for a central role of the posterior part of Broca’s area in hierarchical processing. Further investigations are needed to determine whether the neural basis of hierarchical processing is centred on left BA44 or whether this area is only a fragment of a larger frontal network hierarchically organized.
References


Alaerts K, Swinnen SP, Wenderoth N (2010a) Observing how others lift light or heavy objects: which visual cues mediate the encoding of muscular force in the primary motor cortex? Neuropsychologia 48:2082-2090.


Miller GA (1956) The magical number seven plus or minus two: some limits on our capacity for processing information. Psychol Rev 63:81-97.


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Penhune VB, Steele CJ (2011) Parallel contributions of cerebellar, striatal and M1 mechanisms to motor sequence learning. Behav Brain Res.


Ramachandran VS (2000) Mirror neurons and imitation learning as the driving force behind "the great leap forward" in human evolution. In: Edge Foundation.


Wernicke C (1874) [Der aphasische Symtomencomplex. Eine psychologische Studie auf anatomischer Basis]. Breslau: Cohn und Weigert.


Appendix: Transcranial Magnetic Stimulation (TMS)

The first demonstration that the human cortex could be stimulated non-invasively and painlessly\(^{37}\) with magnetic fields has been made by Barker and collaborators (Barker et al., 1985) (Figure A.1, middle). Since that time, the use of the TMS technique in neuroscience research has become more and more widespread and sophisticated.

![Figure A.1: The TMS apparatus in development.](image)


Right: The Magstim Super Rapid Stimulator\(^{38}\) used in our experiments.

1. Principle

TMS consists of applying magnetic pulses through a coil, connected to a stimulator (Figure A.1, right) and positioned onto the scalp of the subject/patient. A TMS pulse is a rapidly changing current that flows through the coil and that produces a relatively focal magnetic field \((10^{-5} \text{ s}, \text{max } 2T)\) (Hallett, 2007). Actually, the stimulation is not a direct magnetic effect but rather an electrical one. Indeed, according to the Faraday’s principle\(^{38}\), from the magnetic field is generated a current flow that diffuses easily through the different cortical layers. The current flow in the brain is in the opposite direction than the current in the coil and its exact properties are determined by the shape of the coil (Figure A.2).

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\(^{37}\) Unlike “transcranial electrical stimulation”, a previous method to stimulate non-invasively the brain by means of scalp electrodes (Merton and Morton, 1980).

\(^{38}\) “A rapid variation of a magnetic field induces an electrical field” (Faraday 1831).
Figure A.2: Stimulating coils and induced current in the brain.

When a strong, rapid current is passed through a stimulating coil (top left and middle), a rapidly changing magnetic field is produced, which induces current into the brain (bottom left and middle). The current induced is in the opposite direction than the current circulating into the coil. From (Ridding and Rothwell, 2007b, a) at left/middle and from (Bolognini and Ro, 2010) at right.

2. Use and application procedure

TMS has a high resolution both temporally (millisecond accuracy) and spatially (one centimetre-level accuracy) and can be applied over most cortical areas. The TMS application is generally monitored by using image-guided/neuronavigation tools. One limitation on the use of TMS is in stimulating some parts of the cortex not easily accessible such as the temporal or prefrontal cortices, tissue in the sulci, or deep structures such as the basal ganglia. Indeed, a pulse given through a classical figure-of-eight coil at scalp level and at an intensity of the stimulator output corresponding to 120% of the hand rMT, induces a magnetic field that reaches 1.5 cm in depth (Roth et al., 2007). Actually, another class of coils, H-coils, have been designed to enable deeper stimulation (at 5 cm depth); the drawback is that such stimulation is less focal than the classical one (Roth et al., 2007). Moreover, H-coils have been proven effective for therapeutic applications, for instance as an alternative treatment to classical rTMS for severe depression (Rosenberg et al., 2010). TMS as therapeutic treatment is also possible for other brain disorders such as aphasia (Martin et al., 2009), epilepsy (Sun et al., 2011), psychiatric disorder (Jaafari et al., 2011), and migraine (Lipton et al., 2010). It should be mentioned, however, that the results from clinical trials can diverge; for instance, the treatment of depression has sometimes been proven helpful (Fitzgerald et al., 2003; Rosenberg et al., 2010) but sometimes not (Loo et al., 2003). Additionally, TMS could be used as a clinical diagnostic tool for evaluating the integrity of neural circuits following possible damage, for example in spinal cord injuries, strokes or other conditions such as motor neuron diseases (Rossini and Rossi, 2007).
Although TMS has the capacity to treat disorders, the method is not without side-effects. The main known risk linked to TMS application is seizure induction by a spread of activation beyond the stimulation site. However, two types of guidelines enable the safe use of rTMS (Rossi et al., 2009; Nyffeler and Muri, 2010), 1) exclusion criteria in order to reject subjects with brain disorders or conditions (e.g., medications, alcohol or fatigue) that, alone or in combination, would increase the risk of seizures; 2) guidelines for selecting the TMS parameters (intensity, duration and frequency) for which seizures are very unlikely to occur (Wassermann, 1998). Other minor side effects concern for example mood changes, local pain or headaches. Paradoxically, it should be noted that TMS could be a particularly interesting tool for suppressing seizures, especially for inoperable patients with refractory epilepsy (Theodore, 2003; Fregni et al., 2006). In a review of the literature, the risk of inducing seizures with rTMS in epileptic patients has been evaluated and estimated to be almost as low as in nonepileptics, justifying the research on the use of rTMS as a therapeutic tool (Bae et al., 2007). More recent work corroborates this view (e.g. Joo et al., 2007; Rotenberg et al., 2009; Sun et al., 2011) (but see also Bae et al., 2011).

To conduct a TMS experiment, several factors need to be considered (Bolognini and Ro, 2010):

1) Determining the coil location and positioning it: there are several ways, used alone or in combination, to place the coil accurately over the brain site of interest: neuronavigation, EEG 10-20, anatomical reference or functional measures;

2) Determining the intensity of stimulation: individually determined, through rMT measurements (e.g. 120% of rMT), or predetermined for all subjects (e.g. 65% of the maximum output of the stimulator);

3) Delivering TMS: stimulations can be administered according to different types of protocols as illustrated on the schematic diagram (Figure A.3). The best protocol should be chosen according to the experimental questions to answer.
3. Physiological basis

The induced effect on brain areas below the coil varies depending on the TMS settings. Single-pulse TMS provokes the depolarization of neurons and, in fine, the propagation of an action potential. Applied on M1, over the hand representation, such a stimulation produces activity in the corresponding hand muscle, the so-called motor evoked potential (MEP), which can be recorded by means of electromyography (EMG); this is the technique used to assess changes in cortico-spinal excitability (Fadiga et al., 1995; Hallett, 2007). Applied over the occipital cortex, “phosphenes”, namely flashes that are perceived by the subject/patient, are induced.
With the repetition of pulses in so called repetitive TMS (rTMS), longer-lasting effects are produced, outlasting the time of the stimulation application. rTMS is known to induce “virtual lesions” (Pascual-Leone et al., 1999) reflecting an inhibitory effect evidenced by performance impairments. Indeed, rTMS application can worsen performance leading to either longer RT or to more frequent errors; usually minor changes in RT occur and virtual lesions rarely cause the subjects to make errors in the way that permanent lesions do (Walsh and Cowey, 2000). The evaluation of these behavioural impairments allows conclusions about the causal implication of the area stimulated in performing a particular task. It is worth noting that these effects depend on the stimulation frequency and duration (a higher stimulation frequency and/or a longer TMS application normally lead to more pronounced effects). It is worth noting that two types of controls, a control task and a control site, are generally necessary to rule out nonspecific effects of TMS, the principle being to demonstrate a behavioural effect only in the experimental task and only for the area under investigation.

Actually, rTMS is able to increase or to decrease cortico-spinal excitability depending on the TMS settings, mainly frequency and intensity. The mechanism of these excitability changes following repeated TMS pulses is still unclear (Miniussi et al., 2010) but it is widely believed to arise from changes in synaptic activity akin to long-term potentiation (LTP) and long-term depression (LTD) (Fitzgerald et al., 2006). LTP and LTD mechanisms have been described in rodents, following the electrical stimulation of the hippocampus (Malenka and Bear, 2004). LTP is the term used to designate the synaptic enhancement that follows high-frequency electrical stimulation. Conversely, LTD designates the decrease in synaptic strength following low-frequency stimulation. Therefore, high-frequency TMS (> 5Hz), is thought to increase excitability (except theta-burst stimulation, see following paragraphs) while low-frequency TMS is thought to have the opposite effect.

Alternative/complementary explanations concerning the physiological mechanisms underlying rTMS effects have been proposed. The induced electric field may introduce noise by adding neural random activity to the ongoing activity induced by the task (Walsh and Cowey, 2000; Moliadze et al., 2003; Ruzzoli et al., 2010). Stimulation consists of the activation of a mixture of excitatory and inhibitory neurons (Figure A.4) and may induce inhibition by increasing GABA levels that subsequently suppress activity (Mantovani et al., 2006; Allen et al., 2007; Harris et al., 2008). rTMS effects may also arise from the desynchronisation of the pool of neurons in a network crucial to perform a task (Pasley et al., 2009). The rTMS effect could

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39 It is worth noting that considering a frequency <1Hz as inhibitory and a frequency >10 Hz as excitatory is a simplistic view (see variable effects obtained with a 10Hz TMS application in (Duque et al., 2012)).
be influenced by the ongoing activity in the stimulated area (Siebner et al., 2009; Miniussi et al., 2010; Sandrini et al., 2011). For instance, a combined TMS-direct electrophysiological recording-optical imaging study in the cat (Allen et al., 2007) has demonstrated that the TMS effect varies according to the task: during a visual task, they observed a suppression of the neural activity for 5 min that they did not observe during spontaneous activity, where the effect was rather enhanced activity (Allen et al., 2007). Furthermore, based on the state-depedant effect of TMS, the “TMS adaptation” protocol has been proposed (Silvanto et al., 2008). In such a paradigm, the state of the cortex is modulated using adaptation to a stimulus so that if the subsequent application of TMS facilitates stimulus processing, it means that the cortical region is implicated in encoding the adapted stimulus.

![Schematic representation of the human cerebral cortex.](image)

**Figure A.4: Schematic representation of the human cerebral cortex.**

The magnetic coil, represented as a figure-of-eight device, is placed on top of the cerebral cortex and pulses a magnetic field that induces electrical currents across the six layers of the cerebral cortex (indicated by numbers at left). The excitatory cells (green with blue axons) and the inhibitory cells (gray with black axons) have the potential to be activated at the level of their axons, which contain the highest density of ion channels. The incoming axons from other cortical areas and the thalamus (indicated in red) are also activated. The end result of the magnetic pulse is the synaptic activation of a chain of neurons, which generate feed-forward and feedback loops of excitation and inhibition. From (Huerta and Volpe, 2009).
We said previously that rTMS delivered at a frequency higher than 5 Hz has an excitatory effect. An exception is the TBS protocol. As shown in Table A.1, the main advantage of this method is that using both a lower intensity and shorter time of stimulation in comparison with rTMS, long-lasting effects - up to 1 hour after stimulation - can be obtained. Such an effect far outweighs what can be induced by conventional rTMS. Originally, Huang and coworkers (2005) designed 3 patterns of TBS, so-called continuous (cTBS), intermittent (iTBS) and intermediate (imTBS), that they applied over the hand representation of M1. Whereas the latter was reavealed to be inefficient, the two first patterns lead to opposite effects: while cTBS had an inhibitory effect on cortico-spinal excitability (decrease in MEP size), iTBS rather caused a facilitory effect (increase in MEP size) (Huang et al., 2005) (Figure A.5). TBS requires specific parameters i.e. 3 pulses at 50 Hz (20 ms interval), the so-called bursts, the latter being repeated at 5 Hz (200 ms interval) either continuously for 40.04 s or intermittently per group of 10 bursts repeated every 10 s for 191.84 s. For both paradigm types, pulses are delivered at an intensity of 80% of aMT (at least in the original protocol) and the total number of pulses equals 600 (Table A.1).

Table A.1: Comparison of the parameters and the effects of different conventional patterns of rTMS and TBS delivered over the human motor cortex.

<table>
<thead>
<tr>
<th>Type of effect</th>
<th>Conventional rTMS</th>
<th>TBS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Excitatory&lt;sup&gt;a&lt;/sup&gt; (5-20 Hz)</td>
<td>Inhibitory&lt;sup&gt;b&lt;/sup&gt; (0.9-1 Hz)</td>
</tr>
<tr>
<td>Number of pulses</td>
<td>20-50</td>
<td>600</td>
</tr>
<tr>
<td>Intensity</td>
<td>180-810</td>
<td>80% AMT</td>
</tr>
<tr>
<td>Duration of after-effects</td>
<td>3-4 min</td>
<td>15 min</td>
</tr>
</tbody>
</table>

The parameters in each modality come from the first reports of the particular TMS experiments

- a (Pascual-Leone et al., 1994)
- b (Chen et al., 1997)
- c (Wassermann et al., 1996)
- d and e (Huang et al., 2005).

From (Cardenas-Morales et al., 2010)

RMT Resting motor threshold; AMT Active motor threshold

The typical effects following cTBS or iTBS are variable. For instance, even when applied over parts of M1 not involved in hand representation, it has been shown that TBS produces differential effects on MEP size (Martin et al., 2006). Additionally, TBS is currently being extended to various non-motor areas and it seems that again, the effects obtained are less clear than those observed previously for motor areas. This uncertainty about whether the findings from TBS over M1 could be transferred to TBS applied over non-motor regions is all the more prominent given that the original protocol has sometimes undergone significant modifications of its parameters. Concerning cTBS, the protocol has been used successfully several times and the inhibitory effect has been found for instance following FEF stimulation (Nyffeler et al., 2006), in line with the decrease in hemodynamic activity evidenced following
cTBS over DLPFC (Tupak et al., 2011). Concerning iTBS, it seems that the excitatory effect is not systematically reproduced, for instance when applied over the DLPFC (Grossheinrich et al., 2009). Finally, if we consider the disadvantage of TBS, it appears that TBS protocols have no specific risk of side effects; to date, only one case of seizure has been reported following cTBS with an intensity > 90% rMT (Oberman and Pascual-Leone, 2009). Vagal reactions have been described following either cTBS and iTBS (Grossheinrich et al., 2009).

The mechanisms of action of TBS are still obscure. Changes in cortico-spinal excitability could either be mediated by particular receptors such as the N-methyl-D-aspartate receptor (NMDA-r) and GABA receptor (GABA-r) or, alternatively, result from change in the expression of particular genes. These different possible explanations are detailed and discussed elsewhere (Cardenas-Morales et al., 2010).

**Figure A.5: Schematic summary of iTBS/cTBS effects.**

Cortico-spinal excitability can be evaluated by comparing motor-evoked potentials (MEPs) recorded from the peripheral muscle in response to a single pulse to the primary motor cortex. These responses can then be obtained both at baseline and following continuous or intermittent theta burst stimulation (TBS), producing a measure of local cortical plasticity. TBS involves applying bursts of high frequency magnetic stimulation (three pulses at 50 Hz) repeated at intervals of 200 ms. After TBS is applied to the motor cortex in an intermittent fashion (iTBS), single pulse TMS-induced MEPs show increased amplitude for a period of 20–30 min, whereas continuous TBS (cTBS) leads to a suppression of the TMS-induced MEPs for approximately the same amount of time (Huang et al., 2005). Post-TBS enhancement (following iTBS) or suppression (after cTBS) of the cortical activity is considered an index of LTP and LTD-like induction of plasticity in the targeted brain area. From (Oberman et al., 2010).