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Contribution de l'aire motrice supplémentaire et du cervelet dans divers stades d'apprentissage moteur

par

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RÉSUMÉ

L'apprentissage moteur permet l'acquisition d'actes moteurs qui n'étaient pas présents à la naissance. Les données d'études évaluant les substrats neuronaux de l'apprentissage moteur suggèrent que des régions cérébrales responsables du contrôle moteur, telles que l'aire motrice supplémentaire (AMS) et le cervelet, seraient également impliqués dans l'apprentissage moteur. Afin de mettre en évidence les rôles potentiels de ces aires dans l'apprentissage moteur, le type de tâche ainsi que la phase d'apprentissage moteur seraient des variables à considérer. L'objectif principal de cette thèse était donc de découvrir le rôle essentiel de l'AMS et du cervelet dans différentes phases d'un apprentissage moteur séquentiel et d'adaptation.

Les deux études présentées ont été construites avec un design expérimental similaire. Elles évaluaient respectivement l'apprentissage d'une séquence motrice par l'entremise de la tâche de temps de réaction sérielle ainsi que l'apprentissage d'une tâche d'adaptation motrice cinématique. La stimulation transcrânienne magnétique répétée (STMr) a été utilisée afin de créer des 'lésions virtuelles' chez des participants sains lors de l'apprentissage d'une des deux tâches. Les participants ont été entraînés intensivement pendant une session de pratique et ont été testés le jour suivant. Les stimulations de STMr ont été appliquées soit au niveau de l'AMS soit au niveau du cervelet et ce au début et à la fin de la première journée d'apprentissage intensif. La performance des groupes ayant eu une stimulation magnétique a été comparée au groupe contrôle (sans stimulation).

Les résultats de l'étude portant sur l'apprentissage moteur séquentiel ont montré que l'AMS et le cervelet sont nécessaires lors de la phase initiale d'apprentissage, mais non après entraînement intensif ou le jour suivant au retest. Les résultats de l'étude portant sur l'adaptation motrice ont quant à eux montré que le cervelet jouait un rôle essentiel dans l'acquisition et le maintien de ce type d'apprentissage alors que l'AMS ne semble pas contribuer à ce type d'apprentissage.

Généralement, les résultats de ces deux études appuient des modèles d'apprentissage moteur provenant de la littérature d'imagerie fonctionnelle et soutiennent

une dissociation fonctionnelle de ces deux types d'apprentissage moteur. En outre, des résultats novateurs ont été révélé dans les deux études. L'ensemble de ces découvertes mettent en évidences la portée de l'utilisation de la STMr chez les sujets sains afin d'étudier les fonctions cognitives de l'AMS et du cervelet.

Mots-clés : aire motrice supplémentaire, cervelet, apprentissage d'une séquence motrice, apprentissage d'une adaptation motrice, stimulation transcrânienne magnétique.

ABSTRACT

Motor learning enables improvement in performance of motor acts that are not hard wired in the brain from birth. Increasing evidence on the neural substrates involved in motor learning suggests that motor execution areas such as the supplementary motor area (SMA) and cerebellum contribute to motor learning as well. However, it remains controversial to which learning phase and to what type of task these two brain regions bring a more substantial contribution to. The main objective of this thesis was to shed light on the critical role of the SMA and cerebellum to different acquisition phases of a motor sequence and an adaptation task.

The two studies presented herein had similar designs. One involved acquisition of a motor sequence through the serial reaction time task (SRTT) and the other investigated motor adaptation learning of a kinematic task. Repetitive transcranial magnetic stimulation (rTMS) was employed to create 'virtual lesions' in healthy participants while they acquired either tasks. Participants trained intensively during a first session and were re-tested the following day. Different groups of participants underwent rTMS on either the SMA or the cerebellum at the beginning and at the end of task acquisition. The performance of the stimulated groups was compared to that of a control group without magnetic stimulation.

The results of the motor sequence learning study showed that both the SMA and cerebellum are necessary in the beginning of sequence acquisition, but not after intensive training or at re-test the following day. The results of the motor adaptation experiment, revealed a critical contribution of the cerebellum in acquisition and storage of adapted movements. The SMA was not shown to contribute to this type of learning.

In general, the results of the two present studies support motor learning models from the imaging literature and bring further evidence for a functional dissociation of these two motor tasks. New findings were uncovered by employing the TMS technique in both motor sequence and adaptation experiments. These highlight the value of using interference techniques in healthy participants to study cognitive functions in the SMA and the cerebellum.

Keywords : supplementary motor area, cerebellum, motor sequence learning, motor adaptation learning, transcranial magnetic stimulation.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I : INTRODUCTION

CHAPTER II : EXPERIMENTAL STUDIES

Article 1 : Contribution of the Cerebellum to Procedural Learning of Motor Adaptation Examined Through Transcranial Magnetic Stimulation

Article 2 : The Implication of the Cerebellum and the Supplementary Motor Area in Procedural Learning of a Motor Sequence Task: a TMS Investigation

ABBREVIATION LIST

SMA	Supplementary Motor Area
Pre-SMA	Pre- Supplementary Motor Area
M1	Primary Motor Area
SRT	Serial Reaction Time
SRTT	Serial Rection Time Task
MRI	Magnetic Resonance Imaging
fMRI	Functional Magnetic Resonance Imaging
PET	Positron Emission Tomography
TMS	Transcranial Magnetic Stimulation
rTMS	Repetitive Transcranial Magnetic Stimulation
VOR	Vestibulo-ocular Reflex
EC	Eyeblink Conditioning
LTP	Long-Term Potentiation
LTD	Long-Term Depression

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PREAMBLE

What would be life without motor memory? Dressing in the morning, using the utensils for eating breakfast, walking down stairs out of one's house and driving the car to work would be a forever challenge. Indeed, the main goal of motor learning is to improve performance of those purposeful motor acts that are not hard wired in the brain from birth. The neural substrates underlying motor learning are under current investigation. A hypothesis in the literature is that brain areas regulating motor control such as the supplementary motor area and the cerebellum are also responsible for motor learning. Motor learning theories from the literature suggest that important variables determining the contribution of these areas to motor learning are the type of motor task and the level of expertise the participants perform the task at. The aim of this thesis is to study the critical role of the supplementary motor area and cerebellum in different stages of a motor sequence and a motor adaptation paradigm.

In the first chapter, motor learning will be introduced in the context of multiple memory systems, followed by a presentation of its behavioural characteristics, including its stages and the main paradigms employed to investigate it. The two main neural circuits responsible for motor control and the putative neural structures responsible for motor learning will presented. The roles of the SMA and cerebellum in motor learning will be discussed in the context of two recent learning models. Additional imaging and clinical literature concerning the implication of the SMA and cerebellum in motor execution and motor learning will be briefly reviewed. Repetitive TMS will be introduced as a new complementary technique to imaging and clinical research and the few studies that employed rTMS on the SMA or cerebellum to explore motor learning will be presented.

In the second chapter, two rTMS experimental studies will be presented, one involving motor sequence and the other, motor adaptation acquisition. These studies had similar designs. Repetitive TMS was employed to create 'virtual lesions' in healthy participants while they acquired either task. Training was intensive during a first session, followed by a re-test the second day. Different groups of participants underwent rTMS on either the SMA or the cerebellum at the beginning and at the end of intensive task training. The performance of the stimulated groups was compared to that of a control group without magnetic stimulation.

In the third chapter, a review of the findings from the two studies will be presented, followed by an integration of the results with current motor learning models and a discussion concerning the effects of rTMS in the neural substrates it affects. Then, the two experiments will be examined in the context of their strengths and limitations. Finally, several lines of research will be suggested.

CHAPTER I :

INTRODUCTION

1. Multiple memory types

The idea of multiple memory systems in the brain has received a lot of scientific attention since a report of a patient with important resection of the medial temporal lobes presented normal acquisition of a motor skill in the absence of any conscious memory of having practiced the task before (Scoville & Milner, 1957; Milner, 2005). A memory system is 'a particular neural network that mediates a specific form of mnemonic processes' (Brewer, Gabrieli, Preston, Vaidya, & Rosen, 2007). A well-accepted classification separates declarative and non-declarative memory systems (Squire, 1992; Squire & Knowlton, 1995; Squire, 2004) (see Figure 1 for a classification of the different memory types and the brain structures thought to subserve them).

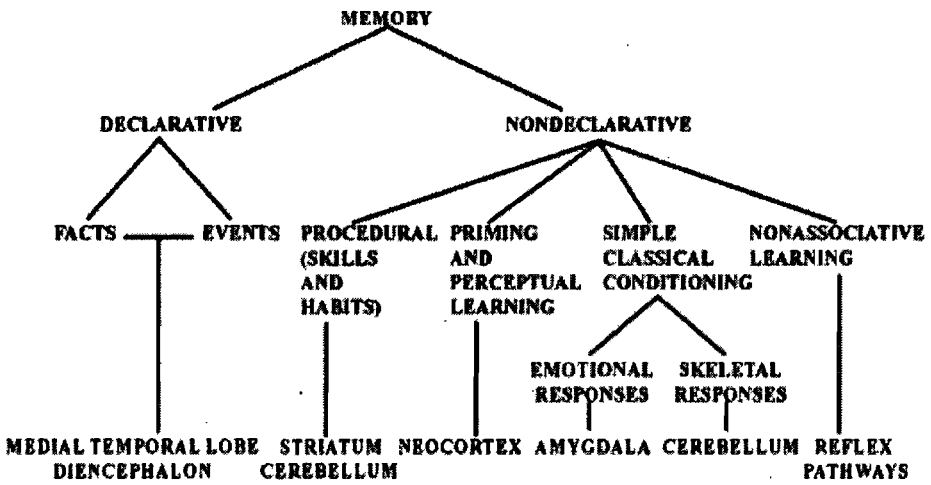

Figure 1. Taxonomy of long-term memory systems. Adapted from Squire (2004).

1.1 Declarative memory

Declarative memory consists of remembering fact or events. It involves conscious retrieval of a list of items (words, pictures, etc.) previously encoded in a single-trial fashion (Squire et al., 1996). It has been further distinguished as: semantic (i.e. memory for events or facts, which can be either true or false, such as remembering the capital of France) or episodic (i.e. memory tied to the context in which it was acquired, the time, the place and the sense that 'it happened to me', such as recalling one's lunch from yesterday). The vast majority of memory research has been devoted to the study of declarative memory. Based on evidence from human and animal research, it was uncovered that the neural substrates subserving declarative memory are dependent on structures of, and connected to the medial temporal lobe: the hippocampus, the parahippocampal gyrus, the rhinal cortex, the amygdala, the diencephalon (the dorso-median and ventro-anterior nucleus of the thalamus) and ventromedian regions of the prefrontal cortex (Squire, 1992; Squire, 2004; Petri & Mishkin, 1994; Schacter, 1987; Mishkin & Appenzeller, 1987).

1.2 Non-declarative memory

Cohen & Squire (1980) introduced the term 'nondeclarative memory' to refer to those memories that are encoded and retrieved implicitly and non-intentionally, corresponding to a facilitation effect on performance due to prior experience (Schacter, Chiu, & Ochsner, 1993; Doyon, 1997). The major component of non-declarative memory is skills or procedural memory (Robertson & Cohen, 2006). Skill learning is the gradual acquisition of an ability through repeated practice in the perceptual, motor and cognitive

domain. An accepted notion in the literature is that the striatum and cerebellum are critically involved in learning of skills (Salmon & Butters, 1995; Doyon, 1997; Doyon & Ungerleider, 2002). However, compared to declarative memory, the neural substrates of skills or procedural memory are less well-established. For instance, procedural memory is believed to be independent of medial temporal lobe structures. Yet, this idea has been recently challenged since the medial temporal lobe has been found to support procedural learning (Keele, Ivry, Mayr, Hazeltine, & Heuer, 2003). This finding points to a different conception of memory systems.

2. Another view on memory taxonomy

Another popular taxonomy distinguishes two grand memory categories based on the characteristic of awareness. In this view, one memory class is termed explicit because it involves conscious and intentional recalling of an item; while the other, implicit, because it involves facilitation of performance as a result of previous experience with a material and may not be associated with conscious recollection of having studied that material (Graf & Schacter, 1985; Schacter et al., 1993; Schacter, 1987). Equivalence between declarative memory and explicit memory and between nondeclarative and implicit memory is generally accepted in the literature (Gabrieli et al., 2003). However, if we consider a memory as 'all the information encoded during a task' (Robertson & Cohen, 2006), a procedural task may have implicit and explicit components. Several imaging studies suggest that learning a skill involves both implicit and explicit aspects and that these aspects may be reflected in different neural substrates underlying them (Willingham, Salidis, & Gabrieli, 2002).

3. Motor skill learning

3.1. Definition of Motor skill learning

Motor skill learning is defined as an improvement in time and space in the precision of movements as a result of repeated practice and interactions with the environment (Willingham, 1998). Motor skills often include a visual component, hence they are also called visuo-motor skills. In the laboratory, they are typically measured by a decrease in reaction time, number of errors, a decrease in the number of trials needed to reach a criterion or by a change in the synergy (coordination of muscles) and kinematics of movements.

3.2 Motor learning stages

Motor learning develops gradually and constantly, taking place over several training sessions (Karni, 1996). Karni et al. (1998) have proposed a model with two learning stages, one requiring practice (fast and slow learning phases) and another that does not require further training (consolidation phase). At the very beginning of the acquisition process, there is a 'fast learning' phase with considerable within-session improvement in both speed and accuracy. The participants' performance is significantly and rapidly enhanced. Within six hours post-training follows the consolidation phase. This is defined as an improvement in performance as a consequence of a latent period without practice following the fast learning phase (Karni & Sagi, 1993; Karni et al., 1998) or as resistance to interference from a competing task (Shadmehr & Brashers-Krug, 1997; Krakauer, Ghez, &

Ghilardi, 2005). Then, following repeated practice, there is the 'slow' learning stage corresponding to further improved performance. When the participants receive further training beyond the slow phase, they reach the automatic phase, during which motor skills require minimal conscious effort, they are resistant to interference by other tasks and to the passage of time (Doyon et al., 2002).

3.3 Types of motor tasks

Many daily activities involve acquiring motor skills: driving a car, knitting, typing, riding a bicycle, etc. In order to study them in the laboratory, a variety of experimental tasks have been developed. Depending on their different cognitive demands, these tasks have been divided in two broad categories: motor sequence and motor adaptation tasks (Sanes, Dimitrov, & Hallett, 1990; Hallett, 1996; Doyon, Penhune & Ungerleider, 2003). Motor sequence tasks are those that combine sequences of movements into a more precise and effective motor plan such as learning to type or play the piano (Sanes & Dogues, 2000). This grand class of motor skills has been by far the most studied in the literature. In the laboratory, the most used paradigm is the serial reaction time task (SRTT) introduced by Nissen and Bullemer (1987). On each trial of the task, a cue is presented on the center of the screen at one of four spatially distinct locations. The participant has an equal number of corresponding response keys and is instructed to press the correct keys as quickly as possible on each trial while making as few errors as possible. Cues appear in a repeating sequence. Sequence learning is measured by a decrease in reaction time to the repeating stimulus sequence or by the difference in response time between sequenced items compared

to those administered in random. Several other sequence tasks have been employed in the literature: those that incorporate timing information (Penhune & Doyon, 2002; Penhune & Doyon, 2005; Sakai, Ramnani, & Passingham, 2002), visuo-spatial information (Hikosaka et al., 1996; Sakai et al., 1998; Sakai et al., 1999) or solely motor information such as thumb-to-finger opposition (Karni et al., 1995; Karni et al., 1998). Several other paradigms, including maze tracing (van Mier, Perlmutter, & Petersen, 2004) and rotor pursuit tasks (Grafton, Woods, & Tyszka, 1994; Hatakenaka, Miyai, Mihara, Sakoda, & Kubota, 2007) have been employed to explore both the behavioral and the neural aspects of sequence acquisition (for reviews, see Rhodes et al., 2004; Ashe et al., 2006).

Motor adaptation tasks are those that compensate for environmental changes such as learning how to move the wheel in the opposite direction when driving a car backwards. Motor adaptations have been further divided into dynamic and kinematic paradigms. Kinematic adaptations are those that convert between coordinate systems (as between the position of the driving wheel and the position of the car on the street) and dynamic adaptations are those that relate 'motor commands to the motion of the system' (Wolpert, Ghahramani, & Flanagan, 2001) (adjusting the forces applied to the driving wheel and the resulting car movement, taking account the inertia of the wheel and the friction between the wheels and the pavement). A typical laboratory task to study dynamic adaptation is adapting to a unusual force-fiels in reaching movements (Thoroughman & Shadmehr, 2000; Shadmehr & Moussavi, 2000; Smith, Ghazizadeh, & Shadmehr, 2006). A kinematic adaptation laboratory task is one that converts movements based on a visual transformation, such as when the relationship between a mouse movement and the cursor on the screen is

altered (Contreras-Vidal & Kerick, 2004; Della-Maggiore & McIntosh, 2005; Graydon, Friston, Thomas, Brooks, & Menon, 2005; Krakauer, Ghez, & Ghilardi, 2005) or when the relationship between the direction of gaze and arm movement is changed through the use of wedge prisms on the eyes (Norris, Greger, Martin, & Thach, 2001; Goedert & Willingham, 2002; Richter et al., 2002).

3.4 Putative neural correlates of motor skill learning

Imaging studies have revealed many brain regions associated to motor and visuo-motor learning: the prefrontal cortex (Hazeltine, Grafton, & Ivry, 1997; Honda et al., 1998; Eliassen, Souza, & Sanes, 2001; Sakai et al., 2002; Willingham et al., 2002), the primary motor area (Karni et al., 1995; Karni et al., 1998; Grafton, Salidis, & Willingham, 2001; Penhune et al., 2005), the premotor area (Wu, Kansaku, & Hallett, 2004; Matsumura et al., 2004; Olson et al., 2006), the supplementary motor cortex (Gordon, Lee, Flament, Ugurbil, & Ebner, 1998; Honda et al., 1998; Doyon et al., 2002; van Mier et al., 2004; Heun et al., 2004), the parietal cortex (Sakai et al., 1998; Willingham et al., 2002; Olson et al., 2006; Landau & D'esposito, 2006) and subcortical regions such as the cerebellum (Flament, Ellerman, Kim, Ugurbil, & Ebner, 1996; Doyon et al., 2002; Imamizu et al., 2000; Imamizu, Kuroda, Miyauchi, Yoshioka, & Kawato, 2003; Koeneke, Lutz, Wustenberg, & Jancke, 2004; Penhune & Doyon, 2005) and the basal ganglia (Poldrack, Prabhakaran, Seger, & Gabrieli, 1999; Bischoff-Grethe, Goedert, Willingham, & Grafton, 2004; Seidler et al., 2005; Seidler, Noll, & Chintalapati, 2006). Several imaging studies have scanned participants at different stages of motor skill acquisition and have noted changes in

activation of brain areas correlated to the level of expertise (Grafton et al., 1994; Petersen, van Mier, Fiez, & Raichle, 1998; Sakai et al., 1998; Jueptner et al., 1997; Krebs et al., 1998; Nezafat, Shadmehr, & Holcomb, 2001; Hikosaka, Miyashita, Miyachi, Sakai, & Lu, 1998; Doyon et al., 2002; Floyer-Lea & Matthews, 2004; Floyer-Lea & Matthews, 2005; Poldrack et al., 2005; Landau et al., 2006). These authors argue for distinct brain structures contributing to different motor learning phases.

From a neuropsychological perspective, Willingham (1998) proposed that motor learning develops directly of motor control processes, as these become increasingly tuned to a particular task, thus functioning more efficiently. Although Willingham does not attribute a specific role to the primary motor area (M1) in his control-based learning theory, the primary motor area is being increasingly involved in motor learning. Converging evidence from neurophysiological animal and human studies have uncovered M1 activity-dependent plasticity associated with motor skill learning (for reviews, see Sanes & Donoghue, 2000; Ungerleider, Doyon, & Karni, 2002). Thus, these data point to M1's implication in early acquisition, storage and consolidation of simple motor skills.

M1 together with the premotor and the supplementary motor area of the frontal cortex have cortical connections between themselves as well as subcortical inputs. Anatomical studies have revealed that these interconnected brain regions form two distinct cortico-subcortical circuits responsible for motor control: the cortico-striato-thalamo-cortical and the cortico-cerebello-thalamo-cortical loops (Middleton & Strick, 1997) (Figure 2).

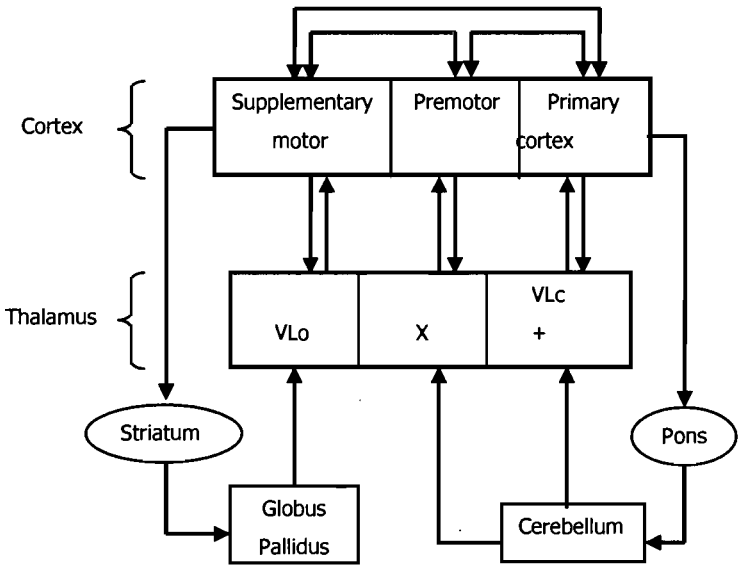

Figure 2. Cortical and subcortical input to the motor areas. Adapted from Ghez (1991). Cortical areas: SMA, supplementary motor area; PM, premotor cortex; M1, primary motor cortex. Thalamic nuclei: Vlo, ventrolateral nucleus, oral division; X, area X; VLc, ventrolateral nucleus, caudal division; VPLo, ventroposterior nucleus, oral division.

As such, the SMA and cerebellum, each part of the cortico-striatal and cortico-cerebellar circuits, respectively, might also play a role in motor learning. In the following, two learning models will be presented. The first one sheds light on the cerebellum's contribution to motor sequence and adaptation learning (Doyon & Benali, 2005) and the second one highlights the implication of the SMA to motor sequence acquisition (Hikosaka, Nakamura, Sakai, & Nakahara, 2002).

4. Models of Neural Correlates of Motor Skill Learning

4.1 Doyon & Ungerleider's proposal of motor skill acquisition

In a model of motor skill learning based on the imaging literature, Doyon and colleagues (Doyon, Penhune, & Ungerleider, 2003; Doyon & Ungerleider, 2002; Doyon &

Benali, 2005) proposed that the two cortico-subcortical circuits involved in motor control, participate in motor learning depending not only on the learning phase, but also on the type of task (motor sequence learning versus motor adaptation). The authors' hypothesis is that the early phase of motor sequence and motor adaptation acquisition is supported by both the cortico-cerebellar and the cortico-striatal networks, including other areas such as the prefrontal, parietal and limbic cortices. However, starting from the consolidation, to the slow and more to the automatic stage, the two neural circuits are thought to specialize: the striatum and cortical associated regions are believed to subserve motor sequence learning but not motor adaptation and the cerebellum and cortical associated regions are thought to support motor adaptation, but not motor sequence (Figure 3).

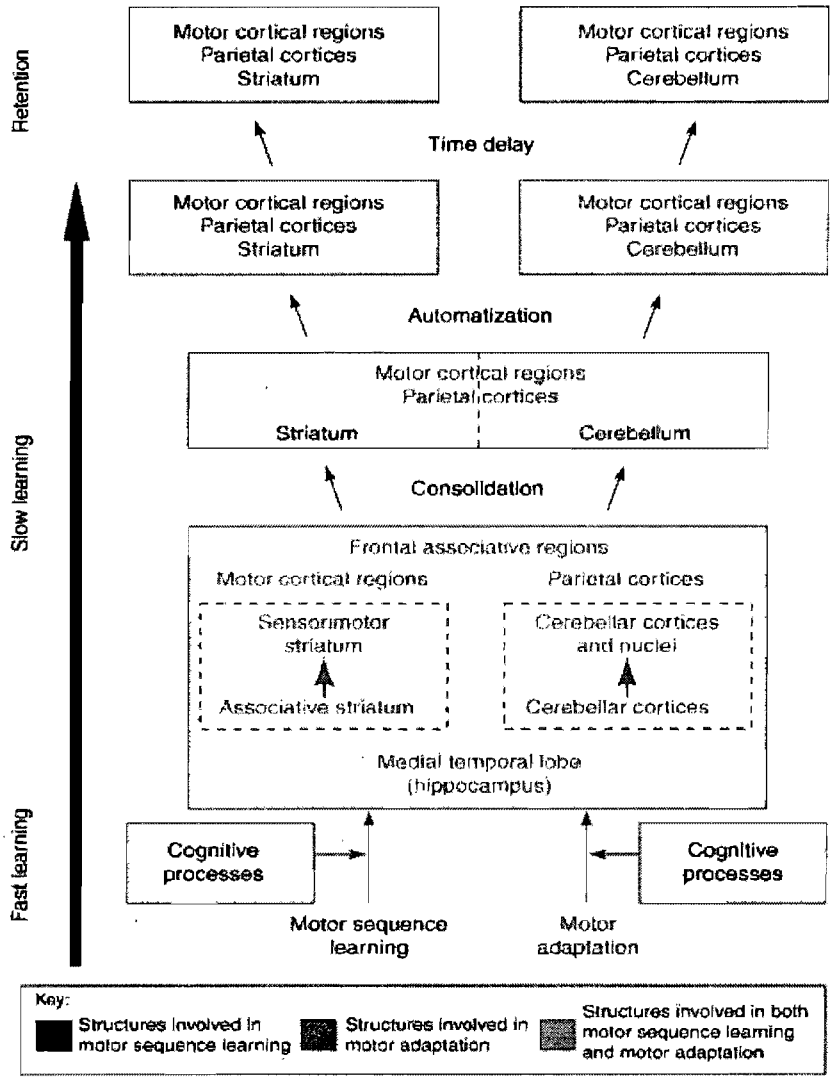

Figure 3. Model of motor learning. Reproduced with permission from Doyon & Benali (2005).

Doyon and Ungerleider's model makes testable predictions concerning the contribution of the cerebellum depending on the type of task. The cerebellum is believed to contribute only to the early, but not to the late phase of motor sequence acquisition, while, it is thought to bring an important contribution to the whole time-course of motor adaptation learning (early phase, consolidation and automatic performance of an acquired skill). This model does not make particular predictions with regards to the contribution of SMA in motor learning. Instead, the motor regions are said to participate to both tasks and to all phases of learning. Yet, the SMA has important connections to the striatum, structure that is believed to play a role in the early phase of motor adaptation, but to be involved throughout the time-course of motor sequence acquisition. In the following, we will present a framework of motor sequence acquisition in which the SMA is thought to play a central role.

4.2 Hikosaka's model of motor sequence acquisition

Hikosaka and colleagues (Hikosaka et al., 1999; Nakahara, Doya, & Hikosaka, 2001; Hikosaka, Nakamura, Sakai, & Nakahara, 2002); have proposed a model of the underlying neural substrates subserving acquisition of motor sequences based on known anatomical connections of different cortico-subcortical loops and integrating data from neurophysiological experiments in animals (recording and lesions in monkeys) and humans. They proposed that motor sequences are acquired separately by two cortico-subcortical networks working in parallel, each with its own coordinates: the spatial mechanism formed by fronto-parietal areas connected to associative regions of the

cerebellum and the basal ganglia; the motor mechanism formed by motor cortices (including M1 and SMA) connected to motor regions of the cerebellum and basal ganglia (see Figure 4). A conversion is thought to take place between spatial and motor coordinates. Spatial sequences are believed to be effector unspecific, processed explicitly, acquired rapidly and requiring high attentional demands; while motor sequences are effector-specific, processed implicitly, acquired slowly and requiring low attentional demands. Motor skill learning may be initiated by either spatial or motor mechanisms. It is thought that optimization of performance is achieved by the cerebellum that processes sensori-motor or timing errors and by the basal ganglia processing reward or likelihood values. In a case where a sequence like trial-and-error begins by being explicit and then becomes implicit, the spatial mechanism would initiate learning and guide the motor mechanisms. An implicit sequence like the SRT task, would be initiated by the motor mechanism which would guide the spatial mechanism. Because retention of a motor skill in the long–term is supported by two parallel systems, when the motor sequence mechanism is damaged, learning can be supported by the spatial system working simultaneously.

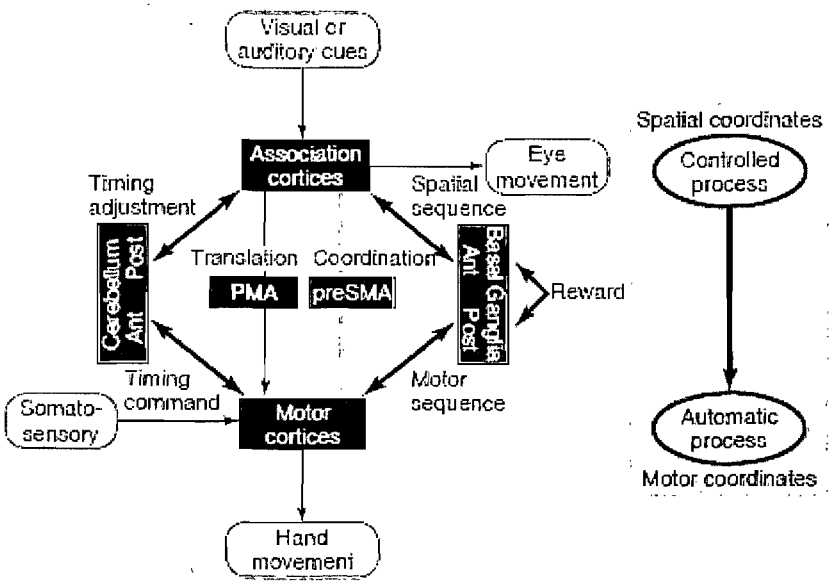

Figure 4. Model of motor learning, reproduced with permission from Hikosaka et al. (1999).

In this framework, the SMA as part of the motor network would be important for improvement of speed in early implicit sequence learning and also for the long-term retention of such skill when the performance is implicit, rapid and with low attentional demands. As for the cerebellum, its posterior part would be critical in early sequence acquisition to optimize performance of such parameters as timing. The authors believe that the anterior cerebellum (including the dentate nucleus) subserves later sequence learning.

This model does not make predictions for the other important class of motor skills, adaptations. In the following, the literature will be reviewed in the perspective of the role of the cerebellum and the SMA in motor execution and in motor learning of different tasks (with emphasis on motor sequence and adaptation studies).

5. The cerebellum

5.1 Brief functional anatomy and connectivity

The cerebellum is functionally divided in three main parts: the vestibulocerebellum, the spinocerebellum and the neocerebellum. It is now well established that each of these functional regions have a specific role in fine motor control and in coordinated movements (Timmann & Daum, 2007; Timmann & Diener, 2007). The vestibulocerebellum is implicated in eye movement and body equilibrium, the spinocerebellum controls muscle tone and adjusts ongoing movements and the neocerebellum is involved in initiation, planning and timing of movement (Ghez, 1991). We will further focus on the neocerebellum since lesions in this area, not only produce slight motor control deficits, but also learning deficits (Thack, 1996).

The neocerebellum is the largest cerebellar structure comprising the cerebellar hemispheres and the dentate nucleus. It receives information through the ponto-cerebellar fibers from all sensory modalities (pre-processed information from second-order neurons), the motor, cognitive and associative cortices (Thach, 1996). The neocerebellum is thus implicated in motor behaviour of the limbs through the cortico-ponto-cerebellar-thalamo-cortical loop. The motor cortex sends information to the neocerebellum via the cortico-ponto-cerebellar pathway and the dentate nucleus projects back to the motor cortex via the ventral lateral nucleus of the thalamus (Middleton & Strick, 1994).

5.2 Motor control in the neocerebellum

The fact that the cerebellum regulates movement control (Houk, Buckingham, & Barto, 1996) is illustrated by lesions of the hemispheres. These produce motor coordination deficits without a motor deficit as such, ipsilateral to the lesion and manifest themselves as disturbance in limb movements, intentional tremor (when the dentate nucleus is involved), decomposition of movement or dysynergy, dysmetria and dysdiadochokinesis (Timmann et al., 2007). Dysynergy is characterized by a trouble in the amplitude, direction and force of a multijoint movement. Dysmetria is an inability to control the range of movements while dysdiadochokinesis is an incapacity to perform rapid, alternating movements.

5.3 Motor learning in the cerebellum

The role of the cerebellum in motor learning was first modeled by Albus and Marr based on its unique cellular architecture (Albus, 1971; Marr, 1969). The gist of their proposal is that the cerebellum is important for response-context linkage through a mechanism of detection and correction of errors (Thach, 1997; Mauk, 1997; Boyden, Katoh, & Raymond, 2004). This is based on the fact that the sole output of the cerebellum are Purkinje cells which project to the deep nuclei and that the Purkinje cells are influenced by two different types of input. The first source of afferent input is constituted by a system of mossy fibers-granule cell contacting only one Purkinje cell. This system represents the 'context', carrying sensory and other ongoing activity of the nervous system. The second source of afferent input is the climbing fiber that has a strong synapse to one Purkinje cell.

The climbing fibers have the role of the 'error' signal, firing when a new movement is to be learned. When climbing fibers fire, they instruct the Purkinje cells to strengthen their contact with granule cells. In this manner, the correct movement is tied to the context, such that with repetitions, the context automatically evokes the correct movement.

5.4 Animal models to study motor learning in the cerebellum

Mechanisms of motor learning in the cerebellum have been extensively studied in animals with two main paradigms: the vestibulo-ocular reflex (VOR) and classical conditioning of the eyeblink (EC) (Christian & Thompson, 2003; du Lac, Raymond, Sejnowski, & Lisberger, 1995). In the VOR, when moving the head, the eyes move reflexibly in the opposite direction to stabilize the image on the retina and prevent blurred vision. The VOR can adapt when altering conditions in vision (e.g. wearing prisms) produce errors. In the EC, upon repeated association of a tone before an air puff delivered onto the eyes, the presentation of the tone alone evokes an eye blink. Lesion and recording studies in this research domain have found evidence for the Marr-Albus theory. The mossy fibers convey the context (the tone or head movements), climbing fibers convey the error signal (the air puff or image motion) and the cerebellar and vestibular nuclei carry expression of the conditioned eyeblink and VOR adaptation, respectively (for reviews see, Mauk, 1997; Lee & Thompson, 2006). Conclusions from this domain of research have revealed that the relative contribution of the cerebella's cortex versus its nuclei to motor learning might depend on the type and amount of training (Mauk, 1997). Although still a matter of present investigation (Shutoh et al., 2006), it seems that short-term acquisition of

the VOR and EC depends on the cerebellar cortex and that long-term storage involve the cerebellar and vestibular nuclei and that these are mediated by different plasticity mechanisms (for reviews, see Lee & Thompson, 2006; Boyden, Katoh & Raymond, 2004).

5.5 Motor sequence studies

Human functional imaging studies have shown activations in the cerebellum during early learning of several sequence tasks such as the SRT (Eliassen et al., 2001; Doyon et al., 2002), sequential finger-to-thumb opposition (Friston, Frith, Passingham, Liddle, & Frackowiak, 1992), sequence by trial-and-error (Toni, Krams, Turner, & Passingham, 1998; Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994), pursuit rotor task (Grafton, Woods & Tyszka, 1994) or rhythmic sequence (Sakai, Ramnani & Passingham, 2002; Penhune & Doyon, 2005). However, following practice, commensurate with better performance, either in early or later phases of sequence acquisition, several researchers have noted a decrease in the cerebellar cortex activity (Doyon, Owen, Petrides, Sziklas, & Evans, 1996; Doyon et al., 2002; Friston, Frith, Passingham, Liddle, & Frackowiak, 1992; Grafton, Woods & Tyszka, 1994; Jueptner et al., 1997; Grafton, Salidis & Willingham, 2001; vanMier, Perlmutter & Petersen, 2004; Floyer-Lea & Matthews, 2005). The observation that the cerebellar cortex is active during early sequence acquisition but that it decreases with learning is attributed, in many imaging studies, to the role of the cerebellar cortex in errors. Indeed, in a recent imaging studies on rhythmic sequence learning Penhune & Doyon (2005) have found correlations between activation in the cerebellar cortex and errors. However some authors have failed to observe activity in the cerebellum

during sequence learning (Grafton et al., 1992; Grafton et al., 1995; Rauch et al., 1995) or related activity in the cerebellum to expression of performance, but not learning (Seidler et al., 2002).

Clinical studies with patients having cerebellar lesions have uncovered important deficits in early acquisition of the SRTT. Pascual-Leone et al. (1993) have reported severe impairment in both implicit and explicit learning of the SRTT, while Molinari et al. (1997) have shown an important impairment when the task was implicit, but not when it was explicitly taught prior to the experimentation. Gomez-Beldarrain, Garcia-Monco, Rubio, & Pascual-Leone (1998) revealed that cerebellar patients had deficits in acquiring the SRTT with the hand ipsilateral to the lesion, but not with the contralateral hand. In another study, patients with cerebellar lesions were unable to learn either a spatial or a temporal sequence in the SRTT (Shin & Ivry, 2003). Interestingly, in a single experiment, cerebellar patients were impaired in late, but not in the early phase of SRTT acquisition (Doyon et al., 1997). Inconsistencies between clinical studies are possibly due to the heterogeneity of cerebellar lesions: atrophies, vascular lesions, tumors or degenerative diseases. Yet, another view is that the severe impairment in motor sequence learning of cerebellar patients is simply due to performance deficits (Hallett, 1996; Timmann & Diener, 1996). In agreement with this idea, Frings, Boenisch, Gerwig, Diener, & Timmann (2004) have revealed that cerebellar patients were not impaired in acquiring and detecting various auditory and sensory sequences with low motor demands.

Imaging and lesion studies have not yet reached a consensus regarding the role of the cerebellum in motor sequence learning. The majority of evidence points to an

implication of the cerebellum, more particularly its hemispheres, in the early fast phase of a motor sequence acquisition, but not consistently in later phases, when the task is well-learned.

5.6 Motor adaptation studies

A few imaging studies employing fMRI and PET have addressed the specific role of the cerebellum in learning of a motor adaptation task. High activation in the cerebellar hemispheres has been found during early adaptation learning of kinematic (Imamizu et al., 2000; Flament et al., 1996) and dynamic tasks (Nezafat et al., 2001). In these studies, the early activation of the lateral cerebellum was related to visuo-spatial errors on the task. The finding regarding the role of the cerebellum in the detection and correction errors is well documented in the literature (Doyon & Ungerleider, 2002; Mier, 2000; Diedrichsen, Hashambhoy, Rane, & Shadmehr, 2005). However, areas of the cerebellum were found to be active in late phases of adaptation learning (after consolidation), when errors were minimal: an area close to the posterior superior fissure (Imamizu et al., 2000), the dentate nucleus (Flament et al., 1996; Floyer-Lea et al., 2004) and the right cerebellar cortex (Krebs et al., 1998). These findings hint at the possibility that the cerebellum might not only be involved in the processing of errors and thus have a subsidiary role in early adaptation learning, but that this structure also constitutes a memory storage site of adaptation skills. Indeed, Imamizu and colleagues (Imamizu et al., 2000; Imamizu et al., 2003) have observed activity in the cerebellar hemispheres throughout adaptation learning and have attributed this activity to acquisition of 'internal models' by this structure.

Functional imaging studies show correlations of brain activity and behaviour. Thus, even if the cerebellum might be important for learning, it might not be essential for it to occur. Natural lesions to the human cerebellum were found to impair learning of several motor adaptation tasks: visuomotor adaptation to prisms (Martin, Keating, Goodkin, & Bastian, 1996; Martin, 2003; Weiner, Hallett, & Funkenstein, 1983), mirror drawing (Laforce & Doyon, 2001), adaptation of anticipatory muscle activity during catching (Lang & Bastian, 1999) and adaptation to a new force field (Maschke, Gomez, Ebner, & Konczak, 2004; Smith & Shadmehr, 2005). In the later two studies, cerebellar patients were impaired on several dependent variables when acquiring a dynamic task, results that were interpreted as a difficulty in establishing and updating internal models of limb dynamics. In the study by Smith & Shadmehr (2005), cerebellar patients (with cerebellar degeneration of several etiologies) showed deficits in the use error from a previous trial to change motor commands to the next despite intact ability to correct errors during the trial itself. Maske et al. (2004) have noted a negative correlation between the severity of progressive cerebellar ataxia and extent of learning. Their patients showed little learning-related aftereffects, no generalization of learned movements to targets outside the learned space and diminished retention 3hrs later.

In sum, the cerebellar cortex seems to be involved in the fast learning phase and in later phases of motor adaptation learning.

6. Supplementary motor area (SMA)

6.1 Brief functional anatomy and connectivity

The SMA may be anatomically divided in the pre-SMA, anterior to the coronal plane through the anterior commissure, and the SMA-proper, posterior to the plane (Rizzolatti, Luppino, & Matelli, 1998). Work on monkeys has revealed that only the SMA-proper has anatomical connections to M1 and to the spinal cord, while the pre-SMA has abundant connections to the prefrontal cortex (Luppino, Matelli, Camarda, & Rizzolatti, 1993; Wang, Shima, Sawamura, & Tanji, 2001). In humans, it was shown that the pre-SMA and SMA send projections to different parts of the striatum, SMA-proper having a similar connectivity to that of M1 (Lehericy et al., 2004). Using imaging in the monkey, and Picard & Strick (2003) observed that motor-related activity in SMA-proper was coupled to that of M1. Based on their different connectivity and function, Picard & Strick (2001) argued that the pre-SMA is resembles a prefrontal region and the SMA, a motor region. From now on, we will focus on the SMA-proper simply referred to as SMA.

Connectivity between SMA and cerebellum

The SMA and several other cortical regions project to the contralateral cerebellar hemisphere through the pontine nuclei (Ghez, 1991). The SMA although a major target of basal ganglia output through the thalamus, also receives minor dentate input from the cerebellum (Akkal, Dum & Strick, 2007; Sakai, Inase & Tanji, 2002; Sakai, Inse & Tanji, 1999).

6.2 Motor control in the SMA

The motor representation of the SMA is contralateral (Fried et al., 1991) when the task requires movements on one side, but SMA neurons have shown activity during movement selection of the two limbs (Hoshi & Tanji, 2004). Electrophysiological recording and imaging in humans suggest that the SMA is somatotopically organized (Fried et al., 1991; Fontaine, Capelle, & Duffau, 2002). Lesions in the human SMA speak of its role in initiating and planning of motor activity. Ressectioning the SMA unilaterally in humans produces the SMA syndrome characterized by severe and reversible motor and speech deficits post-operation first described by (Laplane, Talairach, Meininger, Bancaud, & Orgogozo, 1977). Motor deficits immediately after SMA removal consist of global reduction of spontaneous movements contralaterally (Krainik et al., 2001; Zentner, Hufnagel, Pechstein, Wolf, & Schramm, 1996) of variable intensity and related to the extent of SMA removal (Russell & Kelly, 2003). In addition, there have been reports of hemineglect and apraxia in the contralateral limb (Bannur & Rajshekhar, 2000). Lastly, an almost complete recovery follows within days to weeks with residual reduction in controlateral motor activity (Bannur & Rajshekhar, 2000; Duffau et al., 2003). In the long-term, the motor function is essentially completely recovered, the only permanent sequela reported are disturbance of alternating movements (Zentner et al., 1996). Laplane et al. (1977) have also suggested an implication of the SMA in sequential movements. Recording studies in monkeys have indeed shown an implication of the SMA in control of sequential movements (Tanji & Shima, 1994; Shima & Tanji, 2000; Lee & Quessy, 2003).

6.3 Motor sequence studies

SMA activation in well-learned movement sequences was noted in several PET and fMRI studies. Jueptner et al. (1997) have observed significant activity in the SMA when comparing overlearned to newly acquired trial-and-error sequences. Doyon et al. (2002) have shown an increase of SMA activity in late compared to early acquisition of the SRT task. Gordon et al. (1998) have specifically revealed strong SMA activity in well-learned movement sequences, but not in simple repetitive key presses. Poldrack et al. (2005) have implicated the SMA in well-learned sequences by showing a decrease in SMA activity when comparing well-learned SRTT trials to random ones. Recently, a functional near-infrared spectroscopy study has shown an increase in SMA activity after within-session learning of a rotor pursuit task, but not in beginning of task acquisition (Hatakenaka et al., 2007). However, activation of the SMA in late sequence learning has not been consistently observed in imaging studies. In a fMRI study, Toni et al. (1998) have revealed a modulation of SMA activity in the early phase of within-day intensive sequence acquisition. Compared to the baseline, SMA was minimally active in the first third of the experiment, significantly increased in the second third and decreased at the end of the experiment. Several fMRI studies have also shown SMA activation in early learning of different versions of the SRT task (Daselaar, Rombouts, Veltman, Raaijmakers, & Jonker, 2003; Heun et al, 2004; Landau & D'Esposito, 2007), while other imaging studies have correlated activity in the SMA during early sequence acquisition to improvement in performance. Van Mier, Perlmutter & Petersen, (2004) have uncovered early learning-related increases in the SMA correlated with the number of stops in tracing of a cut-out

maze. Floyer-Lea & Matthews (2004) noted decreases in SMA activation associated to early improvement in performance on a tracking task. The inconsistent activation in the SMA regarding several sequence learning stages might stem from the fact that imaging studies report relative levels of activations between tasks. It is posible that a significant performance improvement during motor learning corresponds to an increasing involvement of the SMA. Indeed, in imaging studies, the SMA might be found more active when comparison between relative levels of activations involve time points in learning corresponding to performance improvement.

Clinical studies regarding the role of SMA in motor learning are scarce because this area is involved in motor execution. Lesion research suggest that the SMA is critical in early and late sequence learning. In a controlled a case study of a patient with a left SMA lesion, Ackerman et al. (1996) have reported impaired acquisition of a motor sequence through the SRTT at the beginning of learning. Botez (1992) has reported anecdotal cases of loss of automatic complex voluntary movements in patients with SMA lesions: a secretary who while being able to type the right letters had considerably lost her typing rapidity; a person could not play the piano anymore, but was able to play each note separately. In the monkey, chemical inactivation of the SMA bilaterally produced errors on a sequence of movements performed from memory, even when animals were able to associate a visual cue with the different movement to make or when animals with the same lesion performed adequately other types of reaching movements (Shima & Tanji, 1998).

In sum, several studies involve the SMA in the late stage of sequence acquisition, while others suggests an implication of this structure in early sequence learning as well.

6.4 Motor adaptation studies

The SMA has been mostly implicated in sequence learning and much less in motor adaptation. Several functional imaging studies have not registered activation in the SMA during early or late learning of adapted movements (Krebs et al., 1998; Shadmehr & Holcomb, 1997). Using fMRI, Seidler, Noll & Chintalapati (2006) have noted activations in several cortical and subcortical regions associated with early learning of a kinematic adaptation task. In this study, SMA activity was associated to sensori-motor processes and thus considered subsidiary. In another recent imaging study (Imamizu, Higuchi, Toda, & Kawato, 2007), learning of a kinematic task was associated with development of an internal model in the cerebellum, while activation of the SMA was explained as a result of cerebellar output to this region.

Animal studies have not been conclusive either. Padoa-Schioppa, Li, & Bizzi 2004) have recorded the neuronal activity in the SMA as monkeys adapted to an external perturbing force field. Their results indicate that the SMA participates in movement dynamics (the forces exerted by the muscles that cause the movement) during motor preparation (before massive activity is registered in M1) and execution. However, results concerning learning of adapted movements were less conclusive since SMA cells underwent plastic changes in control sessions as well. Paz, Natan, Boraud, Bergman, & Vaadia (2005) have used recording techniques in monkeys while they adapted to new kinematic movements. They noticed plastic-related changes in the SMA during early adaptation to kinematic movements, while later learning of the same task involved plastic changes in M1.

In sum, if any, the SMA seems to have a marginal role in learning of adapted movements.

7. Critiques of human imaging and lesion studies

Human imaging techniques such as PET and fMRI have the advantage of registering whole brain activity during task execution. However, the activated brain regions are merely associated or 'correlated' with behaviour. Not only some of the activated regions may be superfluous (false positives) to the studied behaviour, but other areas that do not show activation could in principle constitute a crucial node in the computing of the studied cognitive task (false negatives). Indeed, no matter how well-controlled, imaging studies do not provide information about brain-behaviour causal relationships. Neuropsychological studies complement imaging research by providing causal links between brain and behaviour. However, clinical research has several disadvantages related to lesion's focality, localization and compensation by other brain areas. Indeed many natural lesions are not necessarily small enough or placed in the same area for all patients, thus restricting spatial resolution and reliability of group studies. Moreover, the lesion is determined by the area of irrigation of the cerebrovascular system and not by modules of brain that neuroscientists are interested in. In addition, depending on the passage of time from the appearance of the lesion to the testing time, the patient's behaviour might reflect the ability of the rest of the brain to compensate (Kolb & Whishaw, 1998). More particularly, patients with cerebellar and SMA lesions have associated motor

execution troubles that are difficult to disentangle from motor learning deficits (Gentilucci et al., 2000; Gentilucci et al., 2000; Timmann et al., 2007).

8. Advantages of TMS over imaging techniques and clinical studies

Transcranial magnetic stimulation (TMS) allows non-invasive and temporary disruption of a brain region in healthy and diseased individuals. It is a unique method to establish true functional significance of imaging studies by providing causal information about the activated brain area and the related behaviour. Compared to classical neuropsychological studies, it has the added advantage of well-controlled temporary 'virtual lesions' in healthy participants.

8.1 Presentation of the TMS technique

The TMS technique enables the induction of currents of physiological amplitude in the cortical surface of the brain. Intense pulses of current are passed through a coil that is placed above the subject's scalp. This current generates a time-varying magnetic field (1.5 to 3 Tesla) that penetrates undeflected into the subject's brain, inducing a secondary current of inverse orientation and parallel to the current from the coil (Hallett, 2000). The brain-induced current corresponds to simultaneous neuronal firing in the stimulated region and propagation of neuronal activity (Ilmoniemi, Ruohonen, & Karhu, 1999). In one mode of TMS stimulation, a single pulse or a short train of pulses induce a brief 'virtual lesion' by temporarily disrupting neural processing. In the repetitive mode, longer trains of rTMS modify cortical excitability of the stimulated area for several minutes after the end of the

stimulation period. Low frequency rTMS (≤1Hz) was shown to depress transiently cortical excitability and cause an effect lasting for a period of half the time of the stimulation period (Chen et al., 1997) while high frequency TMS (> 1Hz to 50 Hz) was shown to increase cortical excitability (Pascual-Leone, Valls-Sole, Wassermann, & Hallett, 1994).

9. Motor learning studies involving rTMS of the SMA and Cerebellum

Many recent rTMS studies have employed rTMS on M1 to explore learning of different motor tasks (Muellbacher et al., 2002; Kobayashi, Hutchinson, Theoret, Schlaug, & Pascual-Leone, 2004; Robertson, Press, & Pascual-Leone, 2005; Carey, Fregni, & Pascual-Leone, 2006; Cothros, Kohler, Dickie, Mirsattari, & Gribble, 2006; Shemmell, Riek, Tresilian, & Carson, 2007). Fewer such studies have induced behavioral effects via rTMS over the SMA or cerebellum to investigate motor control or motor learning. Gerloff, Corwell, Chen, Hallett, & Cohen et al. (1997) have applied high frequency rTMS over the SMA during performance of overlearned finger sequences and produced accuracy errors in the complex sequence only. The authors concluded to a critical role of the SMA in planning of complex overlearned movement sequences. Muri, Rivaud, Vermersch, Leger, & Pierrot-Deseilligny (1995) employed single pulse TMS and impaired acquisition of memory-guided saccades. Early sequence acquisition of the SRT task was not impaired by 5Hz rTMS over the SMA (Pascual-Leone, Wassermann, Grafman, & Hallett, 1996), while moderate practice levels of short sequences were hindered by 1Hz rTMS over the SMA (Verwey, Lammens, & van Honk, 2002). Two studies have shown motor behavioral effects by applying 1 Hz rTMS on the cerebellum: stimulation of the medial cerebellum

increased variability in self-paced finger tapping (Theoret, Haque, & Pascual-Leone, 2001) and stimulation of the lateral cerebellum increased movement times on a pegboard task (Miall & Christensen, 2004). In a single experiment, 1Hz rTMS over the lateral cerebellum hindered early acquisition of the SRT task (Torriero, Oliveri, Koch, Caltagirone, & Petrosini, 2004). None of these motor sequence learning studies employing rTMS over the SMA or cerebellum have allowed intensive training to attest the time-course of the TMS effect. To our knowledge, no study has employed rTMS over the SMA or cerebellum to investigate motor adaptation.

SYNTHESIS, GOALS AND HYPOTHESES

Imaging and clinical studies suggest that the SMA and cerebellum play a role in motor learning, besides their established role in motor control. Still a matter of debate in the literature concerns the contribution of the SMA and cerebellum to different motor learning stages and the type of tasks to which they bring a more substantial contribution to. The majority of imaging and lesion evidence reviewed thus far suggest that the cerebellum plays a particular role throughout the acquisition of adapted movements (early phase, consolidation and storage) (Doyon & Benali, 2005), while it seems to be critical only in the early phase of sequence acquisition (Molinari et al., 1997; Shin and Ivry, 2003). An important body of research points to a specific role of the SMA in learning of sequential procedures. Yet, the stage of learning to which the SMA is more important remains controversial. The Hikosaka's model of sequential procedures (1999) specifically hypothesizes an implication of the SMA in the early fast phase of sequence learning with an emphasis on performance improvement. In the same time, several imaging studies point to a role of the SMA in late stages of motor sequence acquisition (Doyon et al., 2002; Wu, Kansku & Hallett, 2004; Poldrack et al., 2005; Megumi et al., 2007). Although few studies addressed the issue of the role of the SMA in motor adaptation learning, these suggest a subsidiary role of the SMA in this type of learning.

The main goal of the present thesis was to complement imaging and lesion research via the use of rTMS to shed light on the critical role of the SMA and cerebellum in several stages of acquisition of a motor sequence and an adaptation task. Temporary 'virtual lesions' were created via 1Hz rTMS on either the SMA or cerebellum at the beginning and

at the end of within-day intensive training of either task. A re-test was conducted 24 hours later.

General hypotheses:

- The SMA and the cerebellum were expected to bring a different contribution to learning depending on the type of motor task (sequence versus adaptation).
- The implication of the SMA and the cerebellum to motor sequence or motor adaptation was expected to depend on the expertise level.

Hypotheses pertaining to motor adaptation learning:

- The cerebellum was expected to be necessary from the beginning to the end of intensive within-day training and possibly in continuing learning the second day.
- The SMA was expected to play a subsidiary role in this type of learning.

Hypotheses pertaining to motor sequence learning:

- The cerebellum was expected to be necessary at the beginning, but not at the end of within-day intensive training or in continuing learning the second day.
- The SMA was expected to be critical in well-learned motor sequence, after intensive within-day training or the second day at re-test, but not at the beginning of acquisition.

CHAPTER II : EXPERIMENTAL STUDIES

Article 1

Contribution of the Cerebellum to Procedural Learning of Motor Adaptation Examined Through Transcranial Magnetic Stimulation

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Contribution of the Cerebellum to Procedural Learning of Motor Adaptation Examined Through Transcranial Magnetic Stimulation

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ABSTRACT

Increasing evidence suggests that the cerebellum does not only contribute to motor execution but that it also plays a role in procedural learning. A type of procedural task is motor adaptation, in which one compensates for environmental changes. One view is that the cerebellum is implicated in error-correction and suggests that it contributes only to the beginning of motor adaptation, while another proposes that the cerebellum is a storage site of this type of learning and is involved throughout motor adaptation acquisition. The present study used repetitive transcranial stimulation (rTMS) in healthy participants to study the role of the cerebellum in the beginning and at the end of intensive within-day training on a kinematic adaptation task. A re-test was conducted the second day. Two different groups received rTMS on either the ipsilateral cerebellum or the contralateral SMA at two times during training: before and after intensive within-day training. Another group received no stimulation. The results showed a critical contribution of the cerebellum at the beginning of the acquisition of the internal model of the adaptation task that endured during the whole intensive within-day training and at re-test (24 hrs later). The group with rTMS on the SMA behaved as the group with no rTMS. These results support a critical role of the cerebellum in storage of adaptation skills. In conclusion, our study is the first to show a lasting behavioural effect of virtually lesioning the cerebellum in healthy participants and complements imaging and lesion studies on the involvement of the cerebellum in this type of procedural skill.

KEY WORDS : cerebellum, procedural learning, motor adaptation, transcranial magnetic stimulation

INTRODUCTION

The role of the cerebellum in the acquisition of motor skills is still disputed. While many modern theories of cerebellar function implicate the cerebellum in motor learning (Marr, 1969; Thach, 1996), others disagree with such a view (Llinas, Lang, & Welsh, 1997). For example, using functional magnetic resonance imaging (fMRI), Seidler and colleagues (2002) reported that the cerebellum is critical for the expression of an improved motor performance of sequential movements, but not for the learning *per se*.

According to recent reviews of literature, the cerebellum's role in motor learning is dependent upon the paradigm used (Bloedel, 2004; Doyon, Penhune, & Ungerleider, 2003; Doyon & Benali, 2005). At least two types of motor skills have been investigated to date: sequence skill learning, i.e. the ability to combine new sequences of behaviour into a well coordinated action plan such as learning to play the piano, and motor adaptation, which refers to continuous readjustment of motor commands to environmental changes, like learning to drive backwards (Hallett, 1996; Sanes, Dimitrov, & Hallett, 1990; Doyon et al., 2003; Doyon and Benali, 2005). Although there is ample evidence showing that the cerebellum participates in motor sequence learning in the early phase of the acquisition process (Doyon et al., 2002; Pascual-Leone et al., 1993; Shin & Ivry, 2003), evidence suggests that it also plays a particular role in the acquisition of adapted movements (Hallett, 1996). Indeed, lesions to the human cerebellum have been shown to impair motor adaptation in different experimental conditions: visuomotor adaptation to prisms (Martin, Keating, Goodkin, & Bastian, 1996; Martin, 2003; Weiner, Hallett, & Funkenstein, 1983), adaptation to mirror drawing (Laforce & Doyon, 2001), adaptation of anticipatory muscle

activity during ball catching (Lang & Bastian, 1999) and adaptation to a new force-field (Maschke, Gomez, Ebner, & Konczak, 2004; Smith & Shadmehr, 2005). For example, Smith and Shadmehr (2005) have used a target reaching task where subjects had to adapt to a force-field and compared the performance of healthy controls to those of patients with cerebellar damage or Huntington's disease. While normal controls and patients with Huntington disease were able to learn the internal model of the task as indexed by a decrease in reaching errors, movement time and path length, cerebellar patients were markedly impaired.

Several authors (Thach, 1996; Doyon and Benali, 2005) have also proposed a role of the cerebellum in motor adaptation depending on the level of skill acquisition. Indeed, neuroimaging evidence suggests that the cerebellum contributes to the different stages of motor adaptation. A few authors have reported activation in the cerebellum in the early phase of a motor adaptation task (Flament, Ellerman, Kim, Ugurbil, & Ebner, 1996; Nezafat, Shadmehr, & Holcomb, 2001; Imamizu, Kuroda, Miyauchi, Yoshioka, & Kawato, 2003), and have related this pattern of activity to the detection and correction of spatial errors. However, other imaging studies have revealed areas of activation in the cerebellum that were interpreted as being related to the development of an internal model necessary to perform adapted movements in later phases of the adaptation process. For example, the right cerebellar cortex was found to be active during within-session skilled performance (Krebs et al., 1998), the ipsilateral anterior cerebellar cortex in consolidation (Shadmehr & Holcomb, 1997) and an area close to the posterior superior fissure in the storage of internal models (Imamizu et al., 2000).

In a recent model of motor skill learning based on the imaging literature, Doyon and colleagues (2003; Doyon and Benali, 2005) have argued for a role of the cortico-cerebellar system throughout the course of motor adaptation (early learning, consolidation and automatic performance of an acquired skill). Moreover, consistent with other studies from the animal literature (Floyer-Lea & Matthews, 2004), Doyon and colleagues claim that learning-related changes take place within the cerebellum itself, where activity in the cerebellar hemispheres at the beginning of the acquisition process is then transferred to the dentate nucleus as learning progresses. Yet, imaging studies show only correlation of behaviour with brain function, and thus do not allow to determine whether an activated brain region is necessary for the studied behaviour. Lesion research complements neuroimaging data by providing causal information. However, cerebellar patients often have associated motor coordination deficits that are difficult to disentangle from impairments in motor learning (Timmann & Diener, 1996). In the present experiment, we thus aimed to investigate the causal role of the cerebellum in motor adaptation learning (the early fast learning phase and the beginning of the slow phase) in healthy participants using a 'virtual-lesion' technique, thereby avoiding the impact of motor execution deficits on motor learning of cerebellar patients.

We transiently inhibited the cerebellar cortex of healthy participants with repetitive transcranial magnetic stimulation (rTMS) at two times during the fast learning phase of motor adaptation acquisition. We then compared their results to a group of participants who had rTMS on the supplementary motor area (SMA), a motor execution area thought to play a minor role in motor adaptation, and to a group who did not receive rTMS. One Hz rTMS

trains were applied at two different times on Day 1 during learning of the motor adaptation task: before proper training began (following the first familiarisation block) and after extensive practice (when subjects started to reach asymptotic performance). Participants were then re-tested 24 hrs later (the beginning of the slow phase). We hypothesised that if the cerebellar cortex is important for the detection and the correction of errors, transient inhibition of the cerebellar cortex would impair motor adaptation only in the beginning of the acquisition process but not after extensive within-day training or at re-test (24 hrs later). However, if the cerebellar cortex is an important storage site of the internal model built during the adaptation task, we should observe a learning impairment after extensive within-day practice and at retest 24 hours later.

METHODS

Participants

Twenty healthy volunteers took part in this study. They were asked to complete a health questionnaire to rule out any health conditions contraindicated to TMS administration (such as a history of neurological or psychiatric disorders) or to the anatomical MR procedure. Participants who had extensive videogame experience were excluded from the study (such experience allowed them to almost automatically complete the task). Participants were randomly assigned to three stimulation groups: 1) the cerebellum group (6 females, 3 males), 2) the SMA group (4 females, 2 males) and 3) a group who had no stimulation (8 females, 3 males). The mean age for the entire sample was 23.4 years ±2.7 (SD) and did not significantly differ in the three groups [One-way

ANOVA $F_{(2, 17)} = .85$; $p = .45$]. Participants performed the task with their preferred/dominant hand (19 were right handed; 1 left-handed was assigned in the cerebellar group). They were recruited at the University of Montreal campus through ads. A monetary compensation was attributed for participation in the study and for transportation costs. The research protocol was accepted by the University of Montreal and the Notre-Dame hospital ethical committees. All volunteers signed an informed consent form before taking part in the study.

TMS stimulation protocol

Repeated TMS was delivered with a Magstim Rapid Transcranial Magnetic Stimulator (Magstim Company, Whitland, UK) having a maximum output of 2.0 T connected to a 7cm figure-of-eight shaped coil. Participants received a low frequency stimulation of 1 Hz rTMS for ten minutes (600 pulses) as these TMS parameters have been shown to suppress cortical excitability (Chen et al., 1997; Boroojerdi, Battaglia, Muellbacher, & Cohen, 2001; Muellbacher, Ziemann, Boroojerdi, & Hallett, 2000).

The coil was held tangentially to the scalp with the handle pointing a) superiorly along the midsaggital axis for the cerebellar stimulation; b) posteriorly and to the right from the midline for the right SMA stimulation. The stimulation sites were dependent on the hand used to perform the motor task because of their predominant anatomical connections to the effector: rTMS stimulation of the cerebellum was ipsilateral while SMA stimulation was contralateral to the dominant hand. The stimulation spot on the cerebellar cortex (lobules V & VI) or on the contralateral SMA (see Fig. 1) was marked on each individual's

MRI using anatomical landmarks from two atlases (Schmahmann, Doyon, Petrides, Evans, & Toga, 2000; Talairch & Tournoux, 1988). We used frameless sterotaxy (for more details, see **Frameless stereotaxy registration** section) to ensure precise and consistent localisation of the region to be stimulated in each participant and to monitor the head position and the position of the coil in real-time during the TMS experiment.

Insert Fig 1 about here

The SMA group was stimulated at motor threshold (MT) intensity (values ranging from 49 to 60% of total stimulator output) as determined by the level of intensity used for the primary motor area (M1). Motor threshold in the contralateral M1 was determined as the minimal intensity of stimulation capable of inducing a visible muscle twitch in the contralateral thumb in 50% of a sequence of 10 consecutive trials. This level of intensity was judged to be the highest possible to stimulate SMA without producing any muscle twitch in the effector used to perform the task. The cerebellar group was stimulated at a fixed 55% of stimulator output because the excitability of M1 is not a good predictor of the excitability of other brain areas (Robertson, Theoret, & Pascual-Leone, 2003). The latter level of intensity was adjusted to be the highest possible without being uncomfortable to neck muscles.

Frameless stereotaxy registration

We coregistered the actual position of the subject's head with MR images (see **Structural MRI** section) of the subject's brain (see Paus, 1999) for a detailed discussion of

the procedure used here). This co-registration was achieved using anatomical landmarks such as the ears, the bridge of the nose and the tragus of the two ears that are equally visible on both the subject's head and on the MR images and by employing the Brainsight image analysis and Frameless Stereotaxy software developed by Rogue Research. The 3-D location of the landmark was measured with a digitizing pen using an optical tracking system (Polaris optical tracking system by Northern Digital Inc.). The camera of the optical tracking system measured the 3-D locations of infra-red LEDs attached to the TMS coil and the subject's head.

Structural MRI

A 1.5 Tesla system was used (Magnetom Vision, Siemens Electric, Erlangen, Germany). High-resolution data were acquired via a Tl-weighted three-dimensional volume acquisition procedure using a gradient echo pulse sequence (TE = 44 ms, Flip = 12° FOV = 250 mm, Matrix = 256 x 256, Voxel size = 0.94 mm3).

Motor adaptation task: the eight-target tracking (ETT) task

The ETT is a motor adaptation task similar to that used by Flament et al. (1996) and Hadj (Hadj, Blanchet, & Doyon, 2004). During the ETT task, participants were required to manipulate a joystick in order to reach a target presented on the computer screen following an elliptical trajectory (see Fig. 2). The ETT task was used in two modes: the direct mode, where the cursor moves in the same direction as the joystick (first block only), and the adaptation mode, where the cursor's movement is opposite to that of the joystick (e.g. if the

joystick is manipulated in the upward and right direction, the cursor moves down and to the left, all other blocks).

Include Fig 2 about here

Trials began in the center of the screen with the cursor (cross-shaped) superimposed on the starting point (a full white circle of 0.75 cm in diameter). One hundred milliseconds later, a target (red dot of 1.5 cm in radius) linked to an elliptical trajectory (grey-coloured, 2.5 cm radius and 0.5 cm thick) was displayed. The targets were presented one at a time in a random fashion. There were eight possible target locations, separated by a 45-degree angle and evenly distributed on a circle measuring 10 cm in radius from the starting point.

A trial was considered a success when 90% of the cursor was located within the target and when 100 ms have elapsed on the target. During this time, the target changed color from red to green, informing the participant of their success. There was a maximum of 3s to reach the target. If the time limit was exceeded, the trial was considered a failure. Blocks were composed of 64 trials (each target location was presented an equal amount of 8 times).

The eight-target tracking task generated a multitude of measures on each trial: (a) the reaction time (RT, in milliseconds): the time between the appearance of the target and the first movement of the cursor; (b) movement time (MT, in milliseconds): the time between the first movement of the cursor and the moment when the target was reached; (c) total distance (TD, in centimetres): the distance of the cursor's path between the starting point and the successful reaching of the target; (d) success rate (SR): the percentage of correctly reached targets.

Procedure

Participants were introduced to the task with two blocks in the direct and adaptation modes, respectively. This ensured familiarisation with the task and provided a baseline measure of the subject's initial adaptation abilities. They then underwent brain stimulation (TMS-1) over the assigned brain area (cerebellum or SMA group) or had an equivalent 10 min pause (no stimulation group). Next, participants underwent intensive training (n = 12 blocks) on day 1, allowing subjects to complete the fast learning phase of motor adaptation. Subsequent to this training period, a second rTMS (TMS-2) session was performed over the same brain area. Following TMS-2, participants performed three additional blocks of the task to verify whether rTMS interfered with performance at the end of the fast learning phase. The next day (24 hours later), participants returned to the laboratory for a re-test during which they performed three blocks of the task (cf. design in Fig. 3) to attest of any rTMS-2 effects on the slow learning phase (after a period with no practice during which consolidation takes place).

Include Fig 3 about here

RESULTS

Data analysis

Analysis of variance (ANOVAs) were performed to compare the performance of the three groups at various stages of learning: **1)** at baseline (i.e. during performance of the first block of practice), **2)** following rTMS-1 application (at the beginning of the fast learning phase), **3)** during the intensive learning period, **4)** following rTMS-2 application (at the end

of the fast learning phase), and **5)** at re-test, 24 hrs later (at the beginning of the slow phase).

Local rTMS effects were tested on 2 blocks of trials (lasting 10 minutes) following each magnetic stimulation, as previous studies have shown that rTMS effects with parameters similar to ours outlast the stimulation period for a time equivalent to the duration of the rTMS trains themselves (Théorêt, Haque and Pascual-Leone, 2001; Robertson et al., 2001; Lewald et al, 2002).

All dependent variables (SR, RT, MT, TD) underwent the same statistical analyses. The same statistical approach was used for all dependent variables separately. Main group effects were further specified by post-hoc Tuckey tests. We reported Greenhouse-Geiser corrected p-values with original (uncorrected) degrees of freedom.

1) Baseline performance

After familiarisation with a block of trials in the "direct" condition, subjects underwent adaptation training. The groups' performance at baseline was assessed with one-way ANOVAs on the first adaptation block. Analyses revealed that the three subject groups did not differ ($p \geq .10$) with respect to all measures of motor behaviour [SR: $F_{(2, 19)} = .85$; RT: $F_{(2, 19)} = .02$; MT: $F_{(2, 19)} = .26$ and TD $F_{(2, 19)} = 2.69$].

2) Effects of rTMS-1 (at the begining of the fast learning phase)

The effects of rTMS on motor performance at the onset of learning were analysed by means of two-factor ANOVAs with one repeated factor at two levels, and one between

factor at three levels. The results showed a significant improvement in performance as participants reached an increasing number of targets with practice [$F_{(1, 17)}$ =14.65, p<.01] (see Fig. 4), the improvement in performance not reaching statistical significance on the other behavioural measures ($p \geq .10$). The groups' motor performance was not affected by rTMS as indexed by SR, RT and MT measures, but rTMS had an impact on the TD measure [$F_{(2, 17)} = 5.18$; $p = .02$] (see Fig. 5). Tuckey post-hoc tests revealed that performance of the no stimulation group did not differ from that of the SMA ($p = .12$), nor the cerebellar ($p = .42$) group, whereas, the cerebellar and the SMA groups showed a significant difference in performance ($p = .01$). In fact, the group that received stimulation of the cerebellum travelled a significant larger distance on the curved path than the group that was stimulated on the SMA region (see Fig. 6).

Include Fig 4, 5 and 6 about here

3) Intensive training

Two-factor ANOVAs with one repeated factor at ten levels and one between factor at three levels were then employed to measure the groups' improvement in performance over the training session. Participants from all groups significantly improved their performance ($p < .01$) as they reached increasingly more targets [SR: $F_{(9, 153)} = 14.84$] and moved faster to the target [MT: $F_{(9, 153)} = 24.14$]. The initiation time, however, did not improve over this training period [RT: $F_{(9, 153)} = 1.82$, $p = .17$].

As expected from previous work in our laboratory (Doyon, personal communication), the participants did not show any improvement in their precision to follow the path [DT: $F_{(9, 153)} = .45$, $p < .90$] because they followed the ideal curve from the

beginning of the learning process. Nevertheless, we observed a marked group effect depending on the stimulated brain region [$F_{(2, 17)} = 10.00$, $p = <.01$]. Post-hoc tests (Tuckey) revealed that performance of the cerebellar group was significantly worse (i.e. they travelled a larger distance) compared to the no-stimulation ($p = .03$) and SMA ($p < .01$) groups, respectively, while the performance of the SMA and the no-stimulation groups did not differ from each other ($p = .19$).

4) Effects of rTMS-2 (at the end of the fast learning phase)

The effects of rTMS on motor performance at the end of the fast leanring phase were analysed by means of one-factor repeated ANOVAs. The motor performance of the three groups was not affected by a second rTMS on the following indices: SR, RT and MT. Participants from all groups continued to improve on the SR measure [Block effect: $F_{(1, 17)} = 4.48$, $p = .05$] and reached an asymptote on the RT [Block effect: $F_{(1, 17)} = .31$, $p = .58$] and the MT measures [Block effect: $F_{(1, 17)} = 1.99$, $p = .18$]. We once more observed a pervasive group effect on the TD measure [$F_{(2, 17)} = 5.55$; $p=.01$]. Tuckey post-hoc tests revealed that performance of the cerebellar group was significantly different from that of the no-stimulation ($p=.02$) and SMA ($p=.03$) groups, but that the performance of the latter two did not differ significantly from each other ($p=.98$).

To further explore the effect of the second rTMS, we then used three-factor ANOVAs to compare the groups' performance on two blocks prior to TMS administration to two blocks affected by TMS. We observed no interaction between TMS administration

and the group factor on any variables. This analysis confirms that rTMS did not further impact learning at the end of the fast phase of adaptation learning.

5) Re-test, 24hrs later (at the beginning of the slow phase)

One-way ANOVA on the first block in the adaptation mode revealed that all three groups have equally ($p \geq .10$) retained the motor adaptation task [SR: $F_{(2, 17)} = .06$; RT: $F_{(2, 17)} = .04$ and MT: $F_{(2, 17)} = 1.19$]. At the level of the TD, the significant group difference after the first intensive day of training was retained the second day [$F_{(2, 17)} = 10.19$, $p<.01$]. Tuckey post-hoc tests revealed that this difference was due to the cerebellar group who still travelled a larger distance than the no-stimulation group ($p<.01$) or the SMA group ($p<.01$) who followed the ideal path (refer to Fig. 5 & 6).

No significant consolidation effect (i.e. delayed improvement), was observed on any variables (SR, RT, MT and TD) when comparing the last two blocks of trials from the first day to the first two blocks from the second day. Thus, the participants' performance from all groups remained stable. The lack of offline improvement of a motor adaptation task was equally reported by research in our lab (Morin et al. submitted, 2007) and is in line with the literature on procedural consolidation of kinematic adaptation tasks (Robertson, Pascual-Leone, & Miall, 2004).

DISCUSSION

In the present experiment, we explored the role of the cerebellum during the early fast phase of visuomotor adaptation learning (in commencing of the task and after intensive

within-day training) and at the beginning of the slow phase (re-test, 24 hours later). We have used rTMS to transiently disrupt the lateral part of the cerebellar cortex in a group of subjects and we have compared their performance to a group of subjects with rTMS on the SMA and to another group of subjects who did not receive magnetic stimulation. Repetitive TMS was applied in the beginning of adaptation learning and after intensive within-day training. Our results have shown a selective impairment in the case of the cerebellar group by comparison to the other two. The first rTMS on the cerebellum disturbed solely the subject's precision to follow the path, that is the distance travelled between the starting point and the target (i.e. spatial precision) but not performance indexed by the other dependent variables (reaction time, movement time or success rate). Moreover, rTMS on the cerebellum disturbed spatial precision from the beginning of the early phase of adaptation learning. The disturbed travelled path was maintained throughout intensive within-day training and at re-test (24 hrs later). At the end of the first learning day, a second rTMS did not further disrupt learning on any variable. At re-test, the participants from all groups retained the adaptation task (without spontaneous performance gains). These findings suggest that the cerebellum provides a necessary role to acquisition and storage of the internal model for spatial precision in adapted movements. As such, our results provide support for Doyon's model of motor skill acquisition drawn from human imaging literature (Doyon and Benali, 2005). In this account, plastic-related changes are thought to occur in the cerebellum itself, where activity in the cerebellar hemispheres at the beginning of adaptation learning is transferred to the dentate nucleus as learning progresses.

The cerebellar transfer hypothesis is endorsed by animal research in the field (Lea-Floyer & Mathews, 2004).

Our results do not exclude a role of the cerebellum in the detection and correction of spatial errors at the beginning of task acquisition as proposed by imaging research in the field (Flament et al, 1996). Nor do they preclude an involvement of the cerebellum in motor execution, even though other dependent variables (RT, MT or success rate) were not affected by rTMS application. Our results shed light on the involvement of the cerebellar hemispheres in adaptation learning (acquisition of the internal model for spatial precision).

In our experiment we have used 1 Hz rTMS to produce virtual lesions in healthy participants without having the drawbacks of cerebellar classical lesion studies. Yet our finding of impoverished precision following cerebellar stimulation alludes at a classical symptom observed in patients with cerebellar lesions called dysmetria. Dysmetria refers to execution errors in the range and direction of movements causing metric errors. When cerebellar patients are asked to move their finger from a point in space to their nose, they show unsmooth movement and tremor when they approach their nose (Ghez, 1991). In our study, we did not observe any execution errors by inhibiting the cerebellar cortex, but a motor leanring deficit manifesting itself as unsmooth following of a curved path resembling dysmetria in patients with cerebellar lesions.

Furthermore, recent research on cerebellar lesions using force-field adaptation tasks has demonstrated that these patients do not learn on dependent variables such as movement time and path length. It has also been observed that participants were not able to use error from a previous trial to adjust their movements on the next one (Smith et al., 2005).

Similarly, Maske et al. (2004) have shown that patients with hereditary cerebellar ataxia cannot adapt to a forcefield task or retain it 3 hrs later. In the present experiment, we mimicked a transient and mild version of cerebellar cortex lesions and we have revealed a impairment in visuomotor adaptation. This was indexed by a difficulty in acquiring the ideal path length early in the acquisition process, maintained despite intensive within-day training and retained 24hrs later.

Adaptation studies with natural lesions on the cerebellum have uncovered learning-related deficits on several dependent variables (RT, MT, path length, success rate). In our study, only subtle effects on the path length were observed, the other dependent variables (RT, MT, success rate) were unaffected by by rTMS on the cerebellum. It is possible that the effects restrained to spatial precision seen here are due to the fact that the total distance dependent variable is more sensitive to rTMS interference than the others. The few other studies that used rTMS over the cerebellum in the field of motor behaviour or motor learning have also observed slight behavioural effects. For example, the first study showing the feasibility of rTMS on the cerebellum uncovered an effect of rTMS on the variability of self-paced movements without affecting RT as such (Théorêt et al., 2001). Even the effects of rTMS on the motor cortex are not detectable behaviourally for simple motor tasks (Lee et al., 2006).

Research using rTMS in a virtual technique fashion over the cerebellum is rather scarce. To our knowledge, three studies employing rTMS over the cerebellum have been shown to disturb motor execution or procedural learning. Theoret, Haque, & Pascual-Leone (2001) have been the first to demonstrate the feasibility of this technique to examine

cerebellar functions. In their study, they revealed an increased variability in paced finger tapping using 1Hz rTMS for 5 min over the medial cerebellum, while stimulation on the lateral cerebellum or sham stimulation did not disturb the task. In a second article, Miall & Christensen (2004) used the same parameters of stimulation and have observed a significantly increased movement time on a pegboard task when comparing the performances of lateral cerebellar stimulation to a control group with no rTMS. Most importantly, in the third article, Torriero, Oliveri, Koch, Caltagirone, & Petrosini (2004) have used stimulation parameters similar to ours (1Hz rTMS, 10 min, 90% of MT intensity, lateral cerebellum) and have shown disturbed procedural learning on a motor sequence task (RT was maintained high on serial reaction time task) at the beginning of the acquisition process. In our study, we have also interfered with procedural learning but of a different motor task (visuo-motor adaptation) and based our evidence on a different dependent variable, the travelled distance. While they studied only the beginning of the fast learning phase and showed short lived rTMS effects on sequence learning, we studied the entire fast learning phase and the beginning of the slow learning phase (24 hrs later, at re-test). In our experiment, we observed a lasting effect following rTMS on the cerebellum during the entire studied learning period. These apparent contradictory findings point to a role of the cerebellum in motor learning that is dependent on the type and the amount of training.

The spatial precision deficit obtained in the present experiment seen after transiently disrupting the cerebellum with rTMS could be ascribed to disruptions of connections within the larger cortico-thalamo-cerebellar network. Indeed, imaging the brain after rTMS showed local effects reflecting cortical excitability at the site of stimulation, as well as distal effects reflecting connectivity of the stimulated region (Paus et al., 1999). In the present study, the TMS coil was placed over an area of the cerebellum with major anatomical connections to the motor cortex and minor connections to the SMA (Middleton & Strick, 1997). Thus, the cerebellar stimulation could have affected the motor cortex, which in turn could have contributed to the observed behavioural effect. However, SMA which has abundant connections to M1, was also stimulated with rTMS and the results of this group of participants did not differ from those who did not undergo rTMS. Therefore, it is unlikely that effect of rTMS on the cerebellum could be attributed to its connections to M1. Moreover, we expect the rTMS effect to be maximal at the stimulated site and we precisely targeted the ipsilateral cerebellum and the contralateral SMA in each subject using frameless stereotaxy.

CONCLUSION

This study is yet another example of the feasibility of 'virtual' lesions induced by 1 Hz rTMS to study diverse cognitive functions of the cerebellum in healthy participants. For the first time, the results of this experiment show that rTMS over the cerebellum in the beginning of adaptation learning interfered with procedural learning of the entire fast learning phase and at re-test, 24 hrs later. As such, our finding support the view that the

learning phase and at re-test, 24 hrs later. As such, our finding support the view that the cerebellum might be a storage site of motor adaptation learning and further elucidates the modulatory role of the cerebellar cortex in procedural memory.

Figure captions

Figure 1. SMA and the cerebellum stimulation sites.

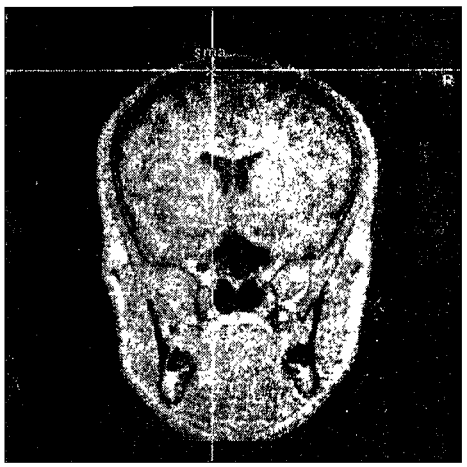
Figure 2. The eight-target tracking task.

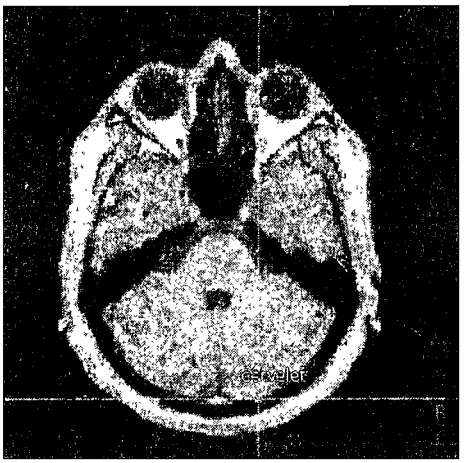
Figure 3. Timeline of the motor adaptation task acquisition.

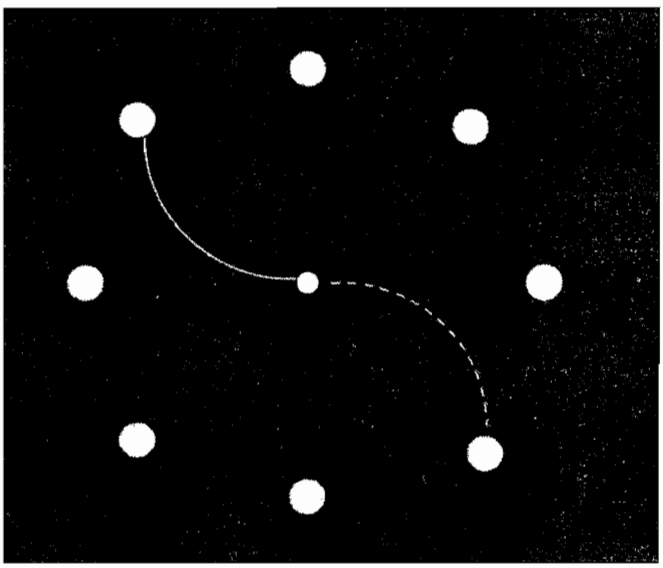
Figure 4. Percentage of correct responses (i.e. number of trials during which participants reached the target within the time limit). Illustration of the means and standard errors of the mean for each block of trials.

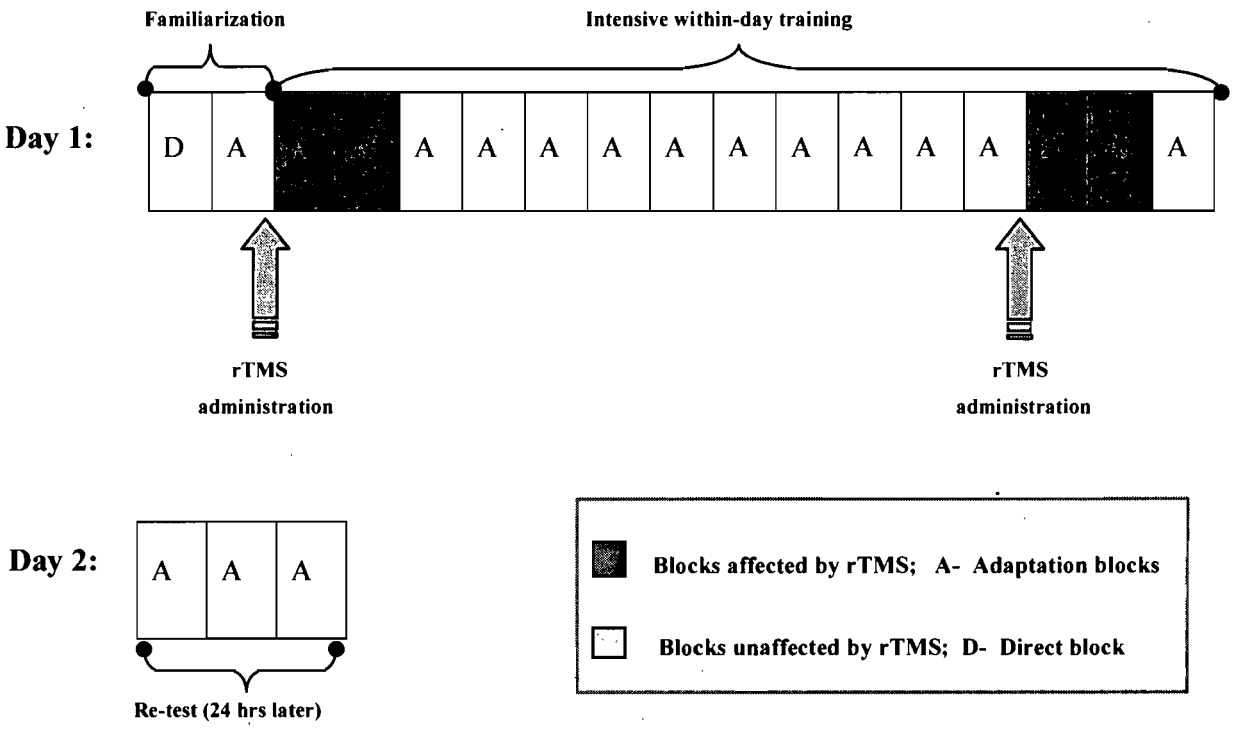
Figure 5. Total distance to reach the target during learning. Illustration of means and standard errors of the mean of individual blocks.

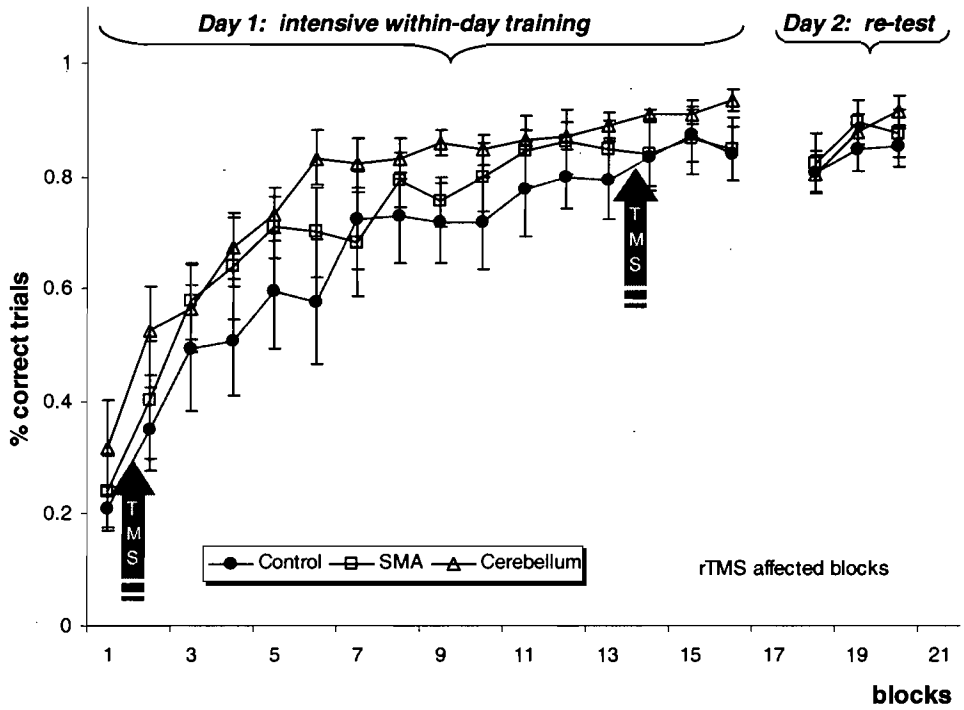
Figure 6. Illustration of typical travelled distance after intensive within-day training in a control participant (a) and in a participant stimulated on the cerebellum (b).

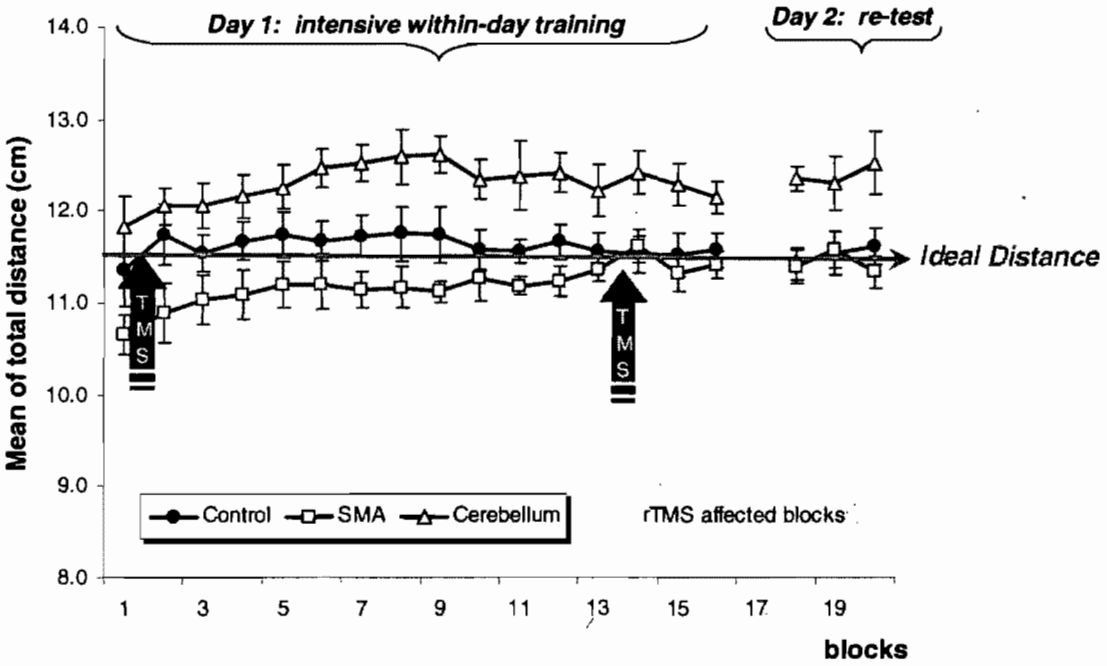




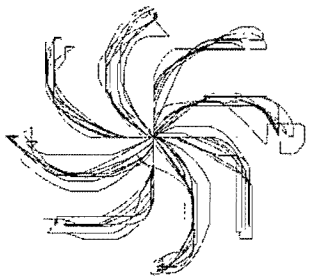



Familiarization
Intensive within-day training
Day 1:
D
A
A
A
A
A
A
A
A
A
A
A
A
rTMS
administration
rTMS
administration
Day 2:
A
A
A
Re-test (24 hrs later)
Blocks affected by rTMS; A- Adaptation blocks
Blocks unaffected by rTMS; D- Direct block

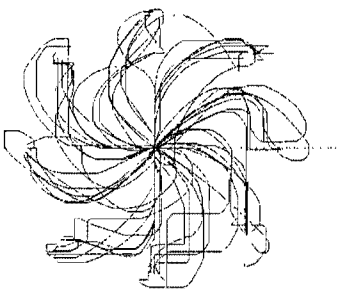

Day 1: intensive within-day training
Day 2: re-test
% correct trials
blocks
Control
SMA
Cerebellum
rTMS affected blocks
TMS
TMS


Day 1: intensive within-day training
Day 2: re-test
14.0
13.0
12.0
11.0
10.0
9.0
8.0
Mean of total distance (cm)
Ideal Distance
TMS
TMS
Control
SMA
Cerebellum
rTMS affected blocks
1
3
5
7
9
11
13
15
17
19
blocks

(a) Typical travelled distance in a control participant



(b) Typical travelled distance in a participant stimulated on the cerebellum



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Article 2

The Implication of the Cerebellum and the Supplementary Motor Area in Procedural Learning of a Motor Sequence Task: a TMS Investigation

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The Implication of the Cerebellum and the Supplementary Motor Area in Procedural Learning of a Motor Sequence Task: a TMS Investigation

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ABSTRACT

Human imaging and classical lesion studies suggest that motor execution areas such as the cerebellum and the supplementary motor area (SMA) might play a role in motor sequence learning, and that this contribution is dependent on the acquisition phase. To complement imaging and lesion studies, we have used 1 Hz rTMS, a 'virtual lesion' technique, in healthy participants. We have applied rTMS at the beginning and at the end of intensive within-day training of the serial reaction time task. A re-test was conducted 24 hrs later. Eighteen participants were assigned to three different groups: 6 participants underwent rTMS on the contralateral SMA (to the hand used), 6 participants underwent rTMS on the ipsilateral cerebellum and 6 participants served as control (no rTMS). Our results indicate that interference with either the SMA or the cerebellum impaired the beginning of early sequence acquisition, whereas interference with either brain structure after within-day intensive practice had no impact on learning or on re-test (24 hrs later). These findings bring evidence of a necessary role of the SMA and the cerebellum in the beginning of the early fast phase of sequence learning and support the view that their critical role is dependent on the learning phase the participants have achieved.

Key words : cerebellum, SMA, motor sequence learning, transcranial magnetic stimulation

INTRODUCTION

Typing and playing the piano, are only few of the many daily activities involving motor sequence learning, that is combining elements of movements into a more precise and effective motor plan. One of the most employed tasks to study motor sequence learning is the serial reaction time task (SRTT) developed by Nissen & Bullemer (1987). Briefly, the participant presses a series of keys in response to a cue, which is presented either in a sequence or in a random order. Following repetitions, participants respond increasingly faster to the sequence items, providing a measure of procedural sequence learning.

Extensive research over the last fifteen years has characterized both the behavioural aspect and the neural correlates of motor sequence acquisition. Such skill evolves from an early, fast phase, during which considerable within-session improvement is observed to a slow, late phase with incremental performance gains over several practice sessions. The late phase is strengthened by consolidation, a process of practice-independent learning, emerging after the first practice session (Karni et al., 1998; Robertson, Pascual-Leone, & Miall, 2004). Recently, the neural underpinnings of motor sequence learning have been extensivly explored in human imaging studies. During different phases of motor sequence acquisition, plastic changes have been observed in the cortico-striatal and the cortico-cerebellar systems involving motor areas of the frontal cortex, the prefrontal, the parietal and subcortical areas such as the cerebellum and the striatum (van Mier, Perlmutter, & Petersen, 2004; Seidler et al., 2005; Poldrack et al., 2005; Floyer-Lea & Matthews, 2005). Yet, there is no unifing theory of the brain regions subserving motor sequence learning.

In a model drawn from the imaging literature, Doyon and colleagues (Doyon & Ungerleider, 2002; Doyon, Penhune, & Ungerleider, 2003; Doyon & Benali, 2005) have proposed that early motor sequence acquisition is subserved by both the cortico-striatal and the cortico-cerebellar systems, while the late phase of learning is supported only by the cortico-striatal system. From a neuropsychological point of view, Willingham (1998) posits that motor memory resides in motor effector areas, as these became more tuned to a particular task. For instance, although the primary motor area (M1) is well-known for controlling volitional motor behaviour, converging evidence from neurophysiological animal and human studies have uncovered activity-dependent plasticity associated with motor skill learning, including motor sequence acquisition (for reviews, see Sanes & Donoghue, 2000; Ungerleider, Doyon, & Karni, 2002). These data point to M1's implication in the early learning phase, consolidation and storage of motor skills. The literature is less abundant concerning the supplementary motor area (SMA) and the cerebellum, two neural substrates critical for motor sequence execution, each part of the cortico-striatal and cortico-cerebellar loops, which are thought to be involved in motor sequence learning. As such, interesting matters of investigation are the plastic changes in the SMA and the cerebellum during different learning phases of same motor sequence task.

Human imaging and neurophysiological recording studies in animals have demonstrated the implication of the SMA in control of sequential movements (Gordon, Lee, Flament, Ugurbil, & Ebner, 1998; Tanji, 2001), in new sequence learning (Grafton, Hazeltine, & Ivry, 1998; Toni, Krams, Turner, & Passingham, 1998; Lee & Quessy, 2003) and in well-learned sequence performance (Poldrack et al., 2005; Honda et al., 1998;

Gordon et al., 1998; Doyon et al., 2002), but the most frequently supported hypothesis in the literature is that the SMA is essentially involved in well-learned sequence acquisition.

The cerebellar cortex has a well-known implication in regulation of motor control (Houk, Buckingham, & Barto, 1996). Imaging studies have shown a role of the cerebellum in motor sequence execution (Honda, 1998; Seidler et al., 2002), while a majority of such studies have noted a decrease in cerebellar cortex blood flow associated with better performance in motor sequences (Grafton, Woods, & Tyszka, 1994; Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Toni et al., 1998; Penhune & Doyon, 2005).

Imaging research allows associations between certain behaviours and neural substrates, while natural lesion studies are performed to determine causality between a brain area and a particular behaviour. Studies with patients having cerebellar (Pascual-Leone et al., 1993; Molinari et al., 1997; Gomez-Beldarrain, Garcia-Monco, Rubio, & Pascual-Leone, 1998; Shin & Ivry, 2003) or SMA lesions (Ackermann, Daum, Schugens, & Grodd, 1996) have uncovered deficits in early acquisition of the SRTT. However, these studies are limited by the heterogeneity of lesion distribution and the confounding plastic reorganisation following brain injury. In addition, investigation of motor learning is encumbered by the motor deficits such as akinesia and tremor, which are usually associated with these lesions. A more recent technique, complementary to imaging and natural lesions, consists of creating 'virtual' lesions in healthy participants by 1 Hz repetitive transcranial magnetic stimulation (rTMS), known to transiently suppress cortical excitability (Chen et al., 1997). Two studies have applied 1Hz rTMS over the cerebellum and have demonstrated motor execution deficits (Theoret, Haque, & Pascual-Leone, 2001;

Miall & Christensen, 2004). Another study using the same method has shown impaired sequence learning through the SRT task in the very beginning of the early acquisition phase (Torriero, Oliveri, Koch, Caltagirone, & Petrosini, 2004). However, subjects were not allowed intensive within-day training or learning into the slow phase.

Repetitive TMS was also applied on the SMA to study motor execution and learning of sequences. Gerloff, Corwell, Chen, Hallett, & Cohen (1997) used 15-20Hz over the SMA and hampered accurate performance of complex motor sequences rehearsed from memory. Verwey, Lammens, & van Honk, (2002) have applied 1Hz rTMS on the SMA and impaired moderate practice levels of the fast learning phase of six-item sequences, but have concluded that the SMA plays a role in sequence execution, but not learning. Pascual-Leone, Wassermann, Grafman, & Hallett (1996) have stimulated the SMA with 5Hz TMS during the beginning of the SRTT and did not find any effects on sequence learning at this performance level. Until now, no "virtual" lesion study has investigated the contribution of the SMA and the cerebellum to several phases of the same motor sequence acquisition task.

In the present study, we have created transient 'virtual' lesions in healthy participants with 1 Hz rTMS on either the SMA or the cerebellum to reveal their crucial role in different learning phases of the SRTT. Different groups of participants were submitted to 1 Hz rTMS on either the SMA or the cerebellar cortex. These results were compared to those of a control group who did not receive magnetic stimulation. The rTMS trains were applied at two different times during learning of the SRTT: before the fast learning phase (following the first familiarisation block) and at the end of intensive within-day practice. Participants were re-tested 24 hrs later. At the end of the experiment, we

administered a last rTMS before a random block during which there was no learning in order to account for any effect of rTMS on motor execution. We expected rTMS on either the SMA or cerebellar cortex to alter motor learning, but not motor execution (i.e. performance on the random block). In addition, if the contribution of these areas to motor sequence acquisition depended on the expertise level, then the cerebellar cortex would be necessary for the begining of the early phase, but not after intensive within-day training or in continuing learning the second day. The SMA, could preferentially contribute to intensive within-day practice and possibly to subsequent learning the second day, but not necessarily at the beginning of motor sequence acquisition.

METHOD

Participants

Eighteen healthy participants with no neurological or general medical condition took part in this study. None of the subjects were either a musician or a professional typist in order to eliminate subjects with pre-existing skills requiring highly coordinated finger dexterities. Participants were randomly assigned to three groups: a SMA group (6 participants, 1 man), a cerebellar group (6 participants, 3 men) and a control group (6 participants, 3 men). The mean age of the subjects in the three groups was 22.9 years ±2.1 (SD) and did not significantly differ in the three groups.

Participants performed the task with their dominant hand and all were right-handed. They were recruited at the University of Montreal campus through billboard ads. A monetary compensation was attributed for participation in the study and for transportation

costs. The research protocol was accepted by the University of Montreal and the Notre-Dame Hospital Ethics committees. All volunteers signed an informed consent form before taking part in the study.

TMS stimulation protocol

The rTMS was delivered with a Magstim Rapid Transcranial Magnetic Stimulator (Magstim Company, Whitland, UK) having a maximum output of 2.0 T connected to a 7cm figure-of-eight shaped coil. Participants received a low frequency stimulation of 1 Hz rTMS for ten minutes (600 pulses). The coil was held tangentially to the scalp with the handle pointing a) posteriorly and pointing to the right from the midline for right SMA stimulation; b) superiorly along the midsaggital axis for cerebellar stimulation. The stimulation sites were dependent on handedness: rTMS stimulation of SMA was contralateral to the dominant hand, while cerebellar stimulation was ipsilateral. We stimulated the SMA contralaterally and the cerebellum ipsilaterally because of their predominant anatomical connections to the effector. The stimulation spot on the cerebellar cortex (lobules V & VI) or on the contralateral SMA (e.g. Fig. 1) was marked on each individual's MRI (see **Structural MRI** section) using anatomical landmarks from two atlases (Schmahmann, Doyon, Petrides, Evans, & Toga, 2000; Talairch & Tournoux, 1988). We then used a frameless stereotaxy system to ensure precise and consistent localisation of the region to be stimulated in each participant and to monitor the head position and the position of the coil in real-time during the TMS experiment (see **Frameless stereotaxy registration** section).

Insert Fig. 1 about here

The SMA group was stimulated at motor threshold (MT) intensity. This intensity did not produce any muscle twitch during the stimulation period. MT in the contralateral M1 was determined as the minimal intensity of stimulation capable of inducing a visible muscle twitch in the contralateral thumb in 50% of a sequence of 10 consecutive trials. Previous research has demonstrated that neurophysiological and visualisation movement thresholds produce similar results (Pridmore, Fernandes Filho, Nahas, Liberatos, & George, 1998). The cerebellar group was stimulated at 55% of the stimulator output. We selected a fixed intensity to stimulate the cerebellum since the excitability of M1 is not a good predictor of the excitability of other brain areas (Robertson, Theoret, & Pascual-Leone, 2003). This intensity was adjusted to be the highest intensity possible without being uncomfortable to neck muscles.

Frameless stereotaxy registration

We coregistered the actual position of the subject's head with the MR images of the subject's brain. This co-registration was achieved by means of anatomical landmarks such as the ears, the bridge of the nose and the tragus of the two ears that are equally visible on the subject's head and on the MR images (Brainsight image analysis and Frameless Stereotaxy software by Rogue Research). The 3-D location of the landmark was measured with a digitizing pen using an optical tracking system (Polaris optical tracking system by Northern Digital Inc.). The camera of the optical tracking system measured the 3-D

locations of infra-red LEDs attached to the TMS coil and the subject's head. The procedure has previously been described in detail by (Paus, 1999).

Structural MRI

A 1.5 Tesla system was used (Magnetom Vision, Siemens Electric, Erlangen, Germany). High-resolution data were acquired via a Tl-weighted three-dimensional volume acquisition procedure using a gradient echo pulse sequence (TE = 44 ms, Flip angle = 12° FOV = 250 mm, Matrix = 256 x 256, Voxel size = 0.94 mm^3).

Motor Sequence Task

The SRT task used in this experiment is a version of the well-known paradigm introduced in 1987 by Nissen and Bullemer to study procedural sequence learning. On each trial of the task, an asterisk was presented on the center of the screen at one of four spatially distinct locations. The participant had an equal number of corresponding response keys and was instructed to press the correct keys as quickly as possible on each trial. The participant made the key presses with the fingers of his/her dominant hand (index, middle, ring and little finger). The visual cue disappeared with the subject's response and another asterisk was presented in a different position. If an incorrect button was pressed, the asterisk disappeared and no feedback was given. Unknown to the participant, the asterisks appeared in a 12-item sequence (1-2-1-3-4-2-3-1-4-3-2-4), the positions of each trial being designated as 1 to 4 from left to right (Fig. 2). The beginning and the end of each sequence was not designated in any way, such that the end of a sequence continued with the

beginning of the next one. A block of trials consisted of 10 cycles of the repeating sequence. At the beginning of the task, the subjects were unaware of the repeating nature of the sequence. However, repetitions of the blocks induced procedural learning through implicit strategies, as a gradual decrease in reaction time was apparent after a few blocks of trials. Because we allowed extensive practice with the task, all participants gained awareness of the sequence. Explicit knowledge of the task was tested at the end of the experiment by asking the participants whether they noticed a special feature to the presented stimuli and then they were asked to reproduce the sequence from memory.

Insert Fig 2 about here

Procedure

Participants were introduced to the task with a random and a sequence block. This ensured familiarisation with the task and provided a baseline measure of motor behaviour. They then underwent brain stimulation (TMS 1) over the assigned brain area (SMA or the cerebellum) or had an equivalent 10 min pause (no stimulation group). Next, participants underwent intensive within-day training (thirty blocks), allowing for the fast learning phase until they reached an asymptotic performance. Subsequent to this additional practice, a second rTMS (TMS 2) was performed on the same brain area. Following TMS 2, participants performed five additional blocks of the task. On the next day (24 hours later), participants returned to the laboratory for a re-test. They performed five blocks of the task. They then underwent a last rTMS on the same brain region and completed a final random block to test for non-specific motor behaviour effect of rTMS (cf. design in Fig. 3). Then,

in order to test for explicit knowledge, participants were asked to reproduce the sequence from memory using the response box.

Insert Fig. 3 about here

Data Analysis

Dependent variables. Data analyses were carried out on mean reaction time (RT). We first computed the median RT for each sequence and then computed the mean of these medians for a given block. The percentage of correct trials in the SRTT was not included as it is not an accurate measure of procedural skill due to extremely low error rates (less than 1-2%), even with limited experience on the task.

Independent variables. There was a between "Group" (intergroup) variable and two (intragroup) within variables: "Block" and "Day".

Statistical analyses. Separate ANOVAs were performed on each of these sections of the learning curve: **1)** at baseline **2)** on rTMS-affected blocks during early learning, **3)** on blocks during the intensive learning period, **4)** on rTMS-affected blocks during late learning, **5)** at re-test and **6)** at the end of the experiment to test for explicit knowledge and for the effects of rTMS on performance (i.e. using the last random block).

Because previous studies have shown that, with parameters similar to ours, rTMS effects outlasted the stimulation period for a time equivalent to the duration of the rTMS trains themselves (Théorêt, Haque and Pascual-Leone, 2001; Robertson et al., 2001; Lewald et al, 2002), local rTMS effects were tested on five blocks of trials (lasting approximately seven minutes) following each magnetic stimulation.

We only reported main effects if interactions were not significant. Main group effects were further specified by *post-hoc* Tuckey tests. We reported Huynh-Felt corrected p-values with original degrees of freedom. A p-value of less or equal to .05 was considered significant.

RESULTS

1) Baseline performance

The groups' motor baseline performance was assessed with a one-way ANOVA on the first random block. Analyses revealed that the three groups did not differ in terms of their motor performance at the very beginning of the acquisition process.

2) Effects of TMS during early learning

The effect of rTMS on early motor learning was evaluated using a two-factor ANOVA with one repeated measure (Block) with five levels and one between factor (Group) with three levels. We observed that the effect of rTMS on performance was distinct for our three groups [Group x Block: $F_{(8, 60)} = 2.11$, $p = .05$ HF-corrected]. Decomposition of the effect showed that the control group improved its performance [$F_{(4, 20)} = 4.09$, $p = .00$], but no improvement was observed for the stimulation groups [SMA: $F_{(4,20)} = 1.65$, $p = .23$; cerebellum: $F_{(4,20)} = .44$, $p = .68$] (see Fig. 4).

Include Fig. 4 about here

3) Intensive training

We used a two-factor ANOVA with one repeated measure (Block) with 25 levels and one between factor (Group) with three levels to evaluate the groups' performance improvement over the training session (the first five blocks following TMS were excluded). An effect of learning was observed [Block effect: $F_{(24, 360)} = 34.57$, $p < .01$] as well as a significant difference amongst groups [Group effect: $F_{(2, 15)} = 4.40$, $p = .03$]. The *post-hoc* Tukey tests showed that the overall group difference observed in the fast learning phase was in fact due only to a significant performance difference between the cerebellum and the control groups ($p = .03$). The other group comparisons were not significant: the SMA and the cerebellum ($p = .40$), the SMA and the control ($p = .27$) (see Fig. 4). We interpret the difference between the control and the cerebellar groups during the intensive training period as a spurious finding since there were no statistical differences between groups before (when the rTMS effect was present) and after this training period.

4) Effects of TMS at the end of the fast learning phase

The effect of TMS on late motor learning (the last training blocks of day 1) was assessed with a two-factor ANOVA with one repeated measure (Block) at five levels and one between factor (Group) at three levels. No significant effect of TMS (no difference among groups) was observed. All groups continued learning [Block effect: $F_{(4,60)} = 2.94$, $p = .03$]. In addition, no TMS effect was shown when comparing the five blocks before TMS administration with five blocks under TMS effect. Furthermore, analyses on the last four

blocks at the end of the first training day, showed no main or interaction effects, indicating that participants of all groups reached an asymptote at the end of the first training day.

5) Re-test (24hrs later)

First, we tested for a consolidation effect by means of a three-factor ANOVA with two repeated measures (Day at two levels and Block at five levels) and one between factor (Group) at three levels. No consolidation as such or TMS effect on consolidation was observed. However, the learning rate of all groups was different depending on the day of testing [Day X Block: $F_{(4,60)} = 8.32$, $p = .00$]. Decomposition of the interaction showed that learning (the effect of Block) at the end of the first day was significant [$F_{(4,60)} = 2.94$, $p = .05$], but smaller than the effect of learning at the beginning of day 2 [$F_{(4,60)} = 31.22$, $p = .00$].

Second, we tested for learning the second day with a two-factor ANOVA with one repeated factor at five levels (Block) and one between factor (Group) at three levels. At this learning phase, all subject groups continued learning [$F_{(4,60)} = 31.22$, $p = .00$] with no group difference or interaction between groups and learning. Finally, a one-way ANOVA was performed on a last random block to assess the effect of rTMS session on motor behavior. No such effect was observed [$F_{(2,17)} = 1.57$, $p = .24$]. At the end of the experiment, the different groups had similar explicit knowledge [$F_{(2,17)} = .34$, $p = .72$]. Participants recalled on average 65% of the correct sequence items (7.8 elements of the 12 item-sequence). In addition, subjects spent on average 149ms ±39 (SD) to respond to the sequence items, which is 69% better than their performance at the onset of learning. The

subjects also responded 15% faster to the random items at the end of the experiment compared to the beginning. Therefore, the improvement specific to the sequence was 54%.

DISCUSSION

In the present study, all subject groups (control, SMA and cerebellum) started and ended the acquistion of the SRTT at the same performance level. At the end of the experiment, the participants developed equivalent explicit knowledge of the sequence. Application of rTMS on either the SMA or the cerebellum impaired performance at the beginning of the sequence acquisition process. More specifically, the control group improved its performance on the first five blocks of the SRTT, but none of the stimulation groups did. Following intensive within-day training, when all groups had reached an asymptote, the second rTMS session on either the cerebellum or the SMA did not have any effect on performance. On the second day, all groups continued learning in the slow phase in a similar fashion (the rTMS administered at the end of the previous training day had no effect on continuing learning the second day). Briefly, our results reveal that both the SMA and the cerebellum are necessary for the early learning phase, but not after intensive training has occurred and when performance is asymptotic or in subsequent learning on day 2.

Our data support the general hypothesis that brain areas responsible for motor control also play a critical role in motor learning, and that their contribution is dependent on the expertise level. As such, our results are in agreement with the model proposed by (Doyon & Benali, 2005) drawn from the imaging literature. Firstly, the authors propose

that both the cortico-striatal and the cortico-cerebellar loops participate in the fast phase of motor sequence acquisition. The present study brought evidence that the cerebellum and the SMA (which are part of these two neuronal circuits) are crucial at this learning level. Secondly, Doyon and Benali (2005) proposed that with passage to the slow phase, the skill is subserved by the cortico-striatal only. Our data suggest that indeed the cortico-cerebellar system does not seem to be involved in a later learning stage, since the cerebellar cortex does not bring a necessary contribution at the end of the fast learning phase when the skill was well-learned. However, the present data do not shed light on the brain areas supporting motor sequence in the slow phase, since rTMS on the SMA did not either hinder learning at the end of the within-day training or subsequent learning on day 2.

The fact that applying 1Hz rTMS over the SMA or the cerebellum impaired early sequence acquisition only, but not intensive training or continuing learning the second day, could possibly be attributed to peripheral motor deficits. In fact, rTMS on the cerebellum may induce stimulation on the brainstem that influences spinal cord excitability (Gerschlager, Christensen, Bestmann, & Rothwell, 2002). In addition, recent work has shown that 1Hz rTMS over the cerebellum has a modulatory effect on the excitability of M1 contralaterally (Torriero et al., 2004; Oliveri, Koch, Torriero, & Caltagirone, 2005; Oliveri et al., 2007; Fierro et al., 2007). In a similar manner, the SMA has direct anatomical connections to the spinal cord and to the M1 (Picard & Strick, 1996). Furthermore, it was shown that stimulating the SMA with rTMS influences the cortical excitability in M1 (Matsunaga et al., 2005). However, given that rTMS on the SMA or the cerebellum did not impact performance on random blocks or at a later time, but impeded

improvement on the sequence blocks only at the beginning of training, we feel confident to attribute the observed effect to motor learning, not execution.

Similarily, we cannot exclude the possibility of transynaptic effects to our data. Imaging the brain after rTMS has shown local effects reflecting cortical excitability at the site of stimulation and distal effects reflecting connectivity of the stimulated region (Paus et al., 1999). The results obtained in the present experiment by transiently disrupting the cerebellum and the SMA with rTMS could also be ascribed to connections within the larger cortico-thalamo-cerebellar and the SMA-thalamo-basal ganglia loops. However, we expect the rTMS effect to be maximal at the stimulated site and we precisely targeted the ipsilateral cerebellum and the contralateral SMA in each subject using frameless stereotaxy. We thus consider that the cerebellum and the SMA are critical 'nodes' in the cortico-cerebellar and cortico-striatal networks subserving early sequence learning.

The main finding concerning the cerebellum is that its hemispheres are critical for the beginning of the early sequence acquisition, but not later when the sequence is well-learned. This is supported by many imaging studies in which activations in the cerebellar cortex observed during the early fast phase of learning have decreased with practice of the SRT task (Eliasen, Sousa and Sanes, 2001; Doyon et al., 2002), of a sequential finger-to-thumb opposition task (Friston et al., 1992), of a sequence by trial-and-error task (Toni et al., 1998; Jenkins et al, 1994), or of a rythmic sequence task (Penhune & Doyon, 2005). A few of these imaging studies have found correlations between activation in the cerebellar cortex and errors. Our finding is not readily compatible with a role of the cerebellar hemispheres in error-correction because our participants made few errors from the

beginning of SRTT learning. However, rTMS over the cerebellum impeded performance improvement, implying a critical role of the cerebellar hemispheres in optimization of performance at the beginning of sequence learning.

Human studies with focal lesions or degenerative diseases of the cerebellum have shown severe deficits mainly early in the acquisition of a motor sequence using the SRTT (Pascual-Leone et al, 1993; Molinari et al., 1997; Gomez-Belderrain et al., 1998; Shin & Ivry, 2003), but also in the late phase (Doyon et al., 1997). However, these studies have included heterogeneous subjects with variable lesions to the cerebellar hemispheres and/or their nuclei due to diverse pathologies (infarcts, syndromes, atrophies). The present work reproduces classical findings from neuropsychological research, with the added advantage of control over the extent, the location and without the confounding effects of plasticity. We have restricted the 'virtual' lesion to the cerebellar hemispheres and have revealed a necessary implication in the beginning of sequence acquisition, but not later when the performance is asymptotic.

We obtained the same pattern of results in regards to the contribution of the SMA in early motor sequence learning. A few imaging studies are in agreement with our finding, and have reported SMA activation in early sequence acquisition (Grafton et al., 1998; Jenkins et al., 1998; Heun, 2004), but also in the late sequence learning phase (Honda et al., 1998; Gordon et al., 1998; Doyon et al., 2002). Findings from animal research may shed light on the inconsistency found in the imaging data. While recording single cells of the monkey SMA during within-day learning of a SRT task, Lee and Quessy (2003) have revealed that many neurons involved in control of individual movements also displayed

sequence learning-related changes. This learning-related neuronal activity was observed during early sequence learning (within-day training), as in the present experiment. Another interesting observation in the study by Lee & Quessy was that the number of SMA neurons increasing their activity with practice was similar to those showing a decrease. Thus, metabolic measures might not record a change in overall regional cellular activity. The latter finding could explain why the contribution of the SMA in early sequence learning could be underestimated in several functional imaging studies.

The study of procedural sequence learning in patients with SMA lesions has been scarce, many studies being devoted to the motor functions subserved by this area (Duffau et al, 2003; Russell et al., 2003; Krainik et al., 2001). In a case study with a patient having a SMA lesion, Ackermann (1996) has shown a procedural learning deficit when acquiring the SRTT. Nakamura, Sakai & Hikosaka (1999) performed mucimol injection in the monkey to reversibly inactivate the pre-SMA and the SMA while the animals performed new and learned motor sequences. They found that, while injection in pre-SMA produced more errors in the new sequences, injection in the SMA retarded acquisition of quicker response times in the new sequences. Hence, the importance of the SMA for the improvement of reaction time in sequence learning is in line with our finding in healthy humans where temporary inhibition of the SMA with rTMS impeded early acquisition of the motor sequence as measured by a decrease in reaction time, but not after intensive within-day training when the performance was asymptotic.

On the following day, at re-test, all groups continued to learn. We did not observe a form of consolidation described in the literature as 'offline' improvements between practice

sessions (Robertson et al., 2003). Our data is consistent with a recent finding on consolidation of the SRTT in which the implicit aspect of the SRTT, indexed by the reaction time measure, did not undergo consolidation (Fischer et al., 2006).

The fact that rTMS over either the SMA or the cerebellum did not impact asymptotic performance at the end of extensive within-session performance does not necessarily mean that these structures do not contribute at all in learning at this level of performance. One possibility is that rTMS cannot interfere with reaction times this short. Another possibility is that brain regions from the motor sequence learning network such as the basal ganglia or other structures compensate to maintain this high level of performance (Doyon & Benali, 2005).

To our knowledge, this is the first study using rTMS over the cerebellum and the SMA in separate subject groups to explore different learning stages of the same motor sequence task. Other research has employed rTMS over the cerebellum or SMA to study only the early stage of sequence learning. Torriero et al. (2004) have used stimulation parameters similar to ours over the lateral cerebellum (1Hz, 10 min, an intensity of 90% of MT) and have disturbed the beginning of early SRTT acquisition, as was observed in our study. However, further learning was not allowed to attest the time-course of such effect as in the present study. In contrast to our results, rTMS over the SMA during the beginning of the SRTT acquisition did not impair learning (Pascual-Leone et al., 1996). This discrepancy may be attributed to differences in TMS parameters. The latter study employed TMS parameters known to increase cortical excitability (5Hz at 115% of subject's motor threshold), while we depressed cortical excitability (1Hz at 90% subject's

motor threshold). Verwey et al. (2002) have studied moderate practice levels of short sequences and have successfully disturbed improvement in execution of explicitly learned six-element sequences with rTMS over the SMA (1Hz, 90% intensity, during 20 min). In our study, only early sequence learning was impaired by rTMS on the SMA, while later learning (after intensive within-day training) was not. However, at the end of our training, the performance was asymptotic. Indeed, the SMA might be important for improvement in performance, independent of the learning phase the participants are at. This is consistent with the notion from the literature that the SMA's involvement in sequence learning is related to improvement in performance (Hikosaka, 1999).

In conclusion, the present study demonstrates that transient inhibition of the ipsilateral cerebellum and the contralateral SMA disrupts early motor sequence acquisition, and that these effects cannot be accounted by rTMS effects on motor control. The present results further extend our knowledge on the brain substrates implicated in procedural sequence learning, and stress the value of interference techniques in healthy subjects to complement classical lesion and imaging studies on this topic.

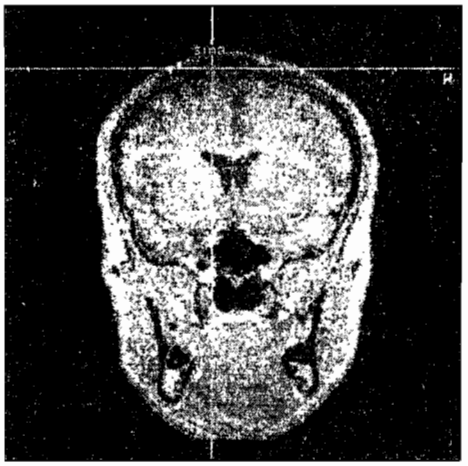
Figure captions

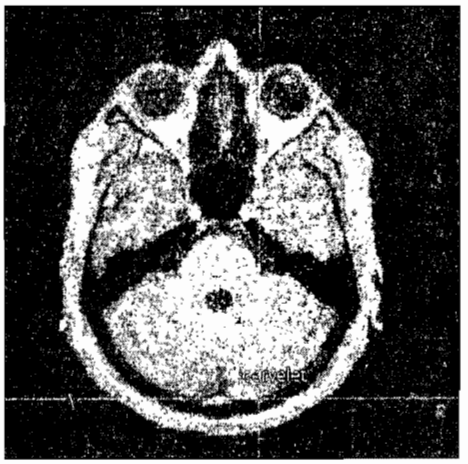
Figure 1. Coronal and transversal views of the SMA and the cerebellum stimulation sites.

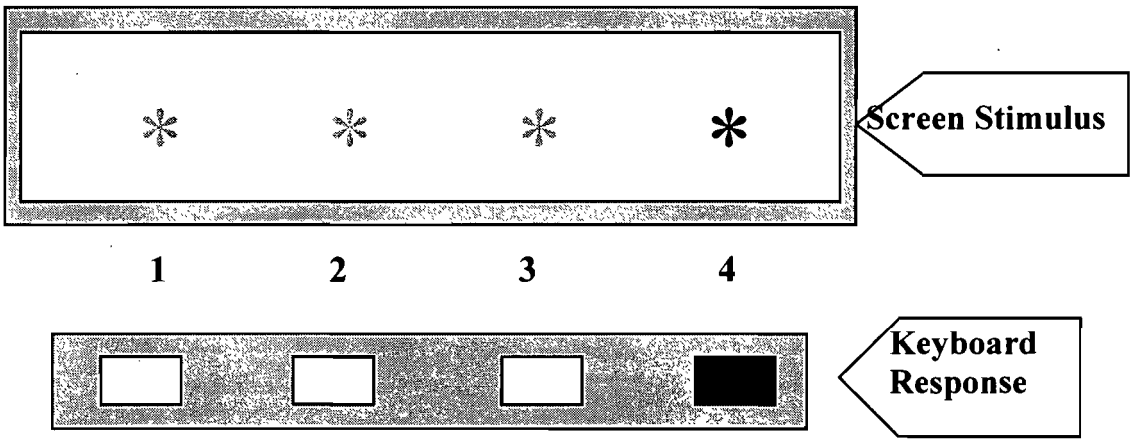
Figure 2. Serial Reaction Time Task: The participant had to push the appropriate key as fast as possible when an asterisk was presented on the screen. If an error was committed, the next stimulus appeared with no performance feedback. Unknown to the participant, the asterisks appeared in a 12-item sequence. Despite lack of awareness, the participant responded faster to the sequence items, providing a measure of implicit procedural learning.

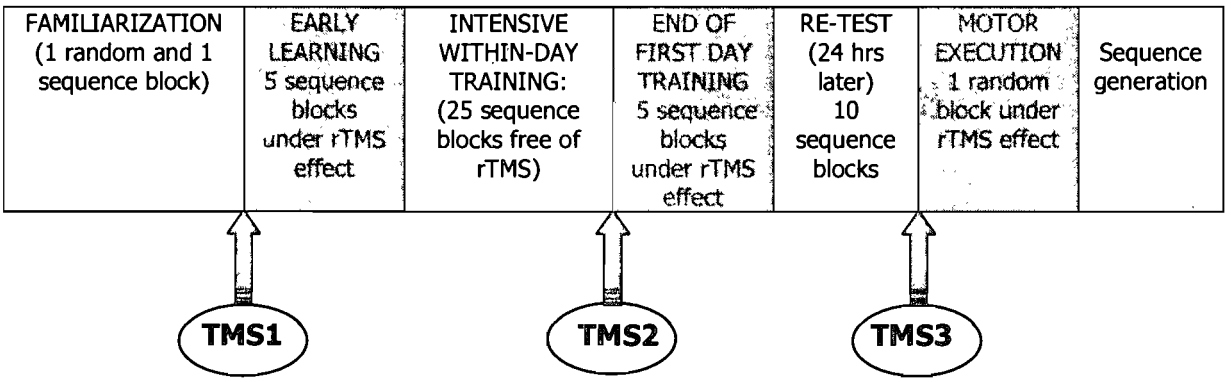
Figure 3. Timeline of the motor sequence task acquisition.

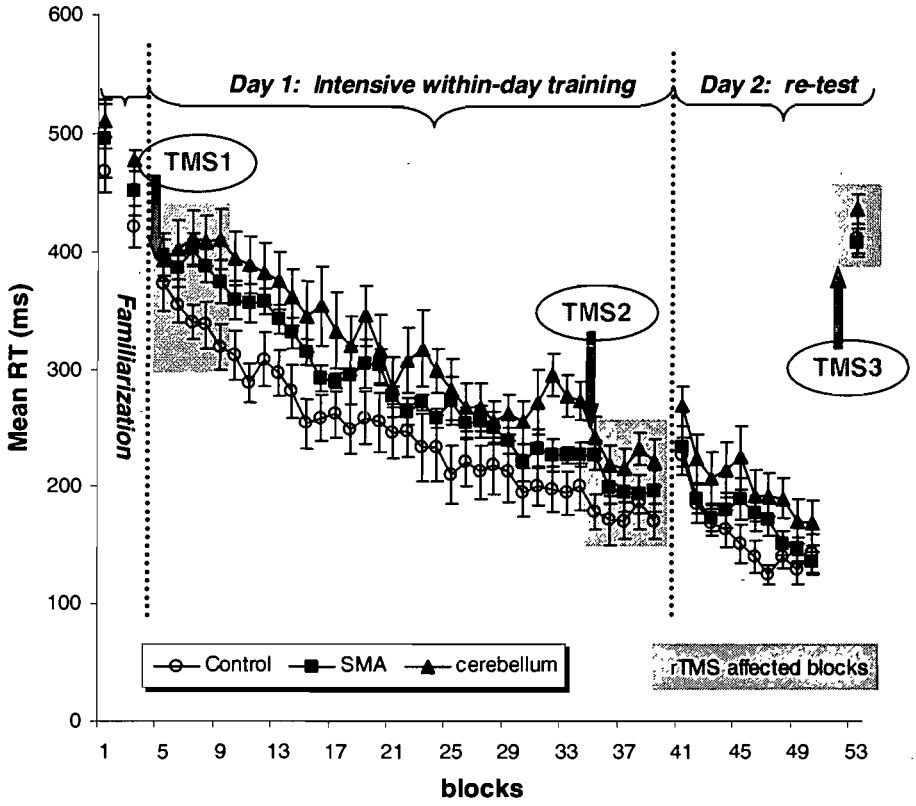
Figure 4. Mean RT and standard errors of the mean for all 45 blocks during sequence learning over the two days. Following familiarization, the first five blocks corresponding to the fast early learning phase were under TMS1 effect, the last five blocks of intensive first day training were under TMS2 effect and the last random block during which there was no learning was under the effect of TMS3. All TMS sessions were alike.






Screen Stimulus
1
2
3
4
Keyboard Response


FAMILIARIZATION (1 random and 1 sequence block)
EARLY LEARNING 5 sequence blocks under rTMS effect
INTENSIVE WITHIN-DAY TRAINING: (25 sequence blocks free of rTMS)
END OF FIRST DAY TRAINING 5 sequence blocks under rTMS effect
RE-TEST (24 hrs later) 10 sequence blocks
MOTOR EXECUTION 1 random block under rTMS effect
Sequence generation
TMS1
TMS2
TMS3


Day 1: Intensive within-day training
Day 2: re-test
TMS1
TMS2
TMS3
Familiarization
Mean RT (ms)
blocks
Control
SMA
cerebellum
rTMS affected blocks
600
500
400
300
200
100
0
1
5
9
13
17
21
25
29
33
37
41
45
49
53

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CHAPTER III :

GENERAL DISCUSSION

1. Review of goals, methods and main results

In the present work we aimed to contribute to the uncovering of the neural substrates involved in learning of motor skills. Motor skill learning was first introduced in the context of multiple memory systems. The idea that motor control areas such as the SMA and cerebellum might be responsible for motor learning was presented. The implication of these two brain regions in motor learning depending on the type of motor task and learning phase in light of two recent motor learning models from the literature was discussed. Our hypotheses were tested by conducting two experiments with a similar design investigating the acquisition of a motor sequence and of a motor adaptation task. In order to complement previous imaging and lesion studies on this topic, we employed rTMS in a 'virtual lesion' fashion to transiently disrupt the SMA or the cerebellum in different groups of healthy participants and compared their performances to a control group (without any magnetic stimulation). Repetitive TMS was applied at two periods during the acquisition of a sequence or an adaptation task: at the beginning of the fast learning phase and after intensive within-day training. Participants were also tested for subsequent learning in the slow phase on the following day. In the motor sequence experiment, both disruption of the SMA and the cerebellum hindered skill acquisition at the very beginning of the fast learning phase, but not after intensive within-day training or in subsequent learning the second day. In the motor adaptation experiment, disruption of the cerebellum compared to either rTMS on the SMA or to the control group, impaired acquisition of spatial precision without affecting other variables, at the beginning of the learning process. This effect was maintained after intensive within-day practice or on the following day. The

group that was stimulated on the SMA did not differ from the control group on any of the dependent variables.

2. Integration of findings with hypotheses from literature

First, the present findings support the view that the SMA and cerebellum contribute to motor learning, beyond their well-known role in motor control. Second, we found support for the hypothesis that the SMA and cerebellum each contribute separately to motor learning depending on the task at hand: the SMA was found to be more important for sequence, but not for adaptation acquisition, while the cerebellum was found to be involved in both tasks. Third, we contributed support for the hypothesis that the SMA and cerebellum change their role depending on the participants' expertise. Indeed, only the beginning of learning of either task was convincingly hindered by rTMS.

Our results are generally in agreement with the model of Doyon and colleagues (Doyon & Benali, 2005) drawn from the imaging literature. The model posits that early learning of either type of task is subserved by both the cortico-striatal and cortico-cerebellars circuits. We brought support for this contention in the case of sequence acquisition since one area of each circuit, was found to be critical in early learning. In the case of motor adaptation our data partially support the model since early adaptation was found to be dependent on the cerebellum only, but not on the SMA. However, other regions from the cortico-striatal network might be involved in early motor adaptation. The model postulates that following consolidation, in later phases of motor skill acquisition, the two cortico-subcortical systems specialize to subserve mainly one type of motor learning,

but not the other. Specifically, the cortico-striatal system supporting motor sequence and the cortico-cerebellar system subserves motor adaptation. In the present work, we did not find evidence of the participation of either the SMA or cerebellum to late sequence learning. However, apart from the SMA other components of the cortico-striatal loop might contribute to late motor sequence acquisition. In the case of motor adaptation, the cerebellum, but not the SMA, was found to be critically involved in late adaptation learning. Thus, the cortico-cerebellar circuit might be particularly important in late adaptation learning.

Our findings concerning the motor sequence experiment are in agreement with Hikosaka's model of sequential procedures (Hikosaka et al., 1999; Nakahara, Doya, & Hikosaka, 2001; Hikosaka, Nakamura, Sakai, & Nakahara, 2002). In this model, the SMA as part of the motor loop contributes to early, implicit sequence acquisition, to improve motor performance. Indeed, we found the SMA to bring a critical contribution to early implicit sequence acquisition. In addition, Hikosaka and colleagues proposed that the SMA makes an important contribution to well-learned sequences. Yet, in our experiment, the SMA was not found to be critical for well-learned sequences. The authors also proposed that when a component of the motor system is injured, the sequence can be maintained by the spatial system that works in parallel. This idea is directly applicable to our sequence experiment since we 'virtually lesioned' the SMA and we found that well-learned performance was maintained. This effect was probably due to compensation from connected brain regions. In addition, the fact that the cerebellar cortex is important in early sequence acquisition, but not later when the sequence is well-learned is consistent with

Hikosaka and colleagues' proposition that this structure contributes to optimization of performance by evaluating sensori-motor and timing errors. Such performance optimization would indeed be more important in early sequence learning rather than later on, when the sequence is well-learned.

3. Mechanisms of action of the rTMS technique

An important issue related to the technique employed in our studies is the effect of rTMS on the neural substrates it affects. Low frequency rTMS ($\leq$ 1 Hz) on M1 has been shown to decrease cortical excitability while high frequency rTMS (> 1 Hz) has been shown to increase it (Chen et al., 1997; Berardelli et al., 1998). Plausible mechanisms of the rTMS aftereffect are changes in excitability of cortical neurons or changes in efficacy of local cortical synapses (Touge, Gerschlager, Brown, & Rothwell, 2001). Findings from the TMS literature allow us to suppose that rTMS on SMA changes cortical excitability in a manner similar to its effects on M1 (Matsunaga et al., 2005). More importantly, behavioural effects have been observed: 1Hz rTMS applied to the SMA was shown to reduce medication-induced dyskinesia in patients with Parkinson disease (Koch et al., 2005; Brusa et al., 2006), while 5Hz rTMS increased dyskinesias (Koch et al., 2005).

The effect of rTMS on the cerebellar cortex is more questionable because of its different anatomical structure in comparison to M1. Since the demonstration of a measurable behavioural effect following rTMS on the cerebellum (Theoret, Haque, & Pascual-Leone, 2001), few studies have addressed this topic. Fierro et al. (2007) have

recently investigated the possible changes in M1 cortical excitability following 1 Hz rTMS over the cerebellum and have shown an increased intracortical facilitation on the contralateral M1. In order to explain this effect, the authors have hypothesized that rTMS depressed Purkinje cells of the cerebellar cortex. These cells have ordinarily an inhibitory effect on M1. Thus rTMS has depressed an inhibitory effect on M1, increasing its excitability.

4. Cellular mechanisms underlying motor learning in the SMA and the cerebellum

A relevant issue to understanding motor learning are the mechanisms of plasticity subserving it. In a literature review, Sanes (2003) suggested several possibilities (not mutually exclusive, but complementary) in which motor learning could be represented at the cortical level in areas such as the SMA: fundamental modification in neural-spiking properties, formation of new intrinsic or extrinsic synaptic contacts, long-term potentiation (LTP) and long-term depression (LTD) and changes in intracortical processing. In the case of the cerebellum, mechanisms such as LTD have been demonstrated in the cerebellar cortex of animals (Shutoh, Ohki, Kitazawa, Itohara, & Nagao, 2006; for a review, see Boyden, Katoh, & Raymond, 2004).

5. Strengths and limitations of the presented studies

The present studies have several strengths. First, we reproduced neuropsychological data in healthy participants by employing a new investigation technique, we demonstrated the utility of rTMS to study motor learning in two execution

areas other than M1, we extended findings from the TMS literature and we ensured precise localization of TMS administration. Indeed, the fact that we reproduced findings from patient studies with transient virtual lesions of the SMA and cerebellum stresses the value of the rTMS technique for exploring similar research questions. While many studies have employed rTMS on M1 to study motor learning, few have used rTMS to explore the role of the cerebellum and SMA in procedural learning. Our experiments serve to further extend previous data from the literature. We successfully reproduced findings concerning the participation of the cerebellum in early sequence acquisition and provided new data showing that this area is not critical to well-learned sequence performance. Another novel finding arising from our studies is that the cerebellum is critical for early visuo-motor adaptation as well. Moreover, we have demonstrated a necessary role of the SMA in early sequence learning, but not in adaptation, further specifying the functional role of this area. An added strength of our experiments is the use of frameless stereotaxy, a precise method of rTMS application in all subjects.

The present experiments have a number of limitations. These are related to the tasks themselves, the research design and the sample size. For the adaptation task, we investigated several variables, while for the sequence task we only collected one variable, the reaction time. Other variables may be manipulated in the SRT task (spatial, visual, rhythmic and motor information). Moreover, the implicit and explicit aspect of the SRTT was not controlled in our experiment such that the participants started motor learning implicitly and then developed explicit knowledge. Even though registering different variables at once implicated in this task might not be possible, a viable solution would be to

verify how manipulation of these variables influences the participation of a target neural substrate.

In our design, the participants received two rTMS sessions in succession (with one hour gap in-between) on the same brain region. A few studies have shown that the effect of rTMS outlasting the stimulation period might not wash-out in 10 or 15 minutes as previously thought, but in a time period of approximately one hour (Verwey, Lammens, & van Honk, 2002). Thus, we do not know if administering the second TMS might have had a cumulative effect. Should that have been the case, we might have accentuated the effect of the second rTMS. This idea is directly applicable to our adaptation experiment in which the first rTMS on the cerebellum had an enduring effect on spatial precision. This effect lasted until the end of within-day training when a second TMS was administered and at re-test, the second day. In this case, we can only be certain of the first TMS effect on learning. A better design for future studies would be to virtually lesion a brain region only once at a different learning level.

The present studies were conducted with a limited number of subjects (six participants per group) restraining their generalizability. However, the significant effects obtained here suggest an important effect size. Employing a small number of subjects is common practice in the TMS literature. Moreover, we diminished interindividual differences in brain anatomy by ensuring precise and consistent stimulation in each subject by stereotaxy-guided TMS in all subjects

6. Future research

In order to improve our understanding of the neural substrates subserving motor learning, we suggest: 1) better task definition, 2) focusing on stages of learning in a subject-tailored fashion, 3) employing complementary investigation techniques, 4) employing the TMS technique in different ways.

Several divergent results in the motor learning literature may be explained by differences in the motor tasks used. These tasks enclose different cognitive components (visual, spatial, sequential, adaptation or motor information) and are executed with more or less awareness. Depending on the contribution of these variables, different tasks are likely to solicit different brain substrates. While it might not be technically possible to separate all of these variables in themselves, one can collect as many dependent variables for each studied task and to investigate how each of these correlate with performance and/or with the engagement of the different brain substrates.

Since the level of expertise is an important variable determining the participation of the different neural substrates to learning, an interesting suggestion would be to define the level of expertise in a subject-tailored fashion, because each participant needs a different number of sessions to achieve asymptotic performance on a task; moreover, asymptotic performance for a single subject might correspond to a specific pattern of brain area participation.

TMS could be used in conjunction with functional imaging such as PET, fMRI and EEG, to complement these investigation methods by determining causal information between an activated region and its behavioural relevance. Several activated regions during

a motor learning experiment can be targeted via rTMS without necessarily an *a priori* hypothesis. For example, if the temporal cortex is activated during motor learning, virtually lesioning this area with rTMS will provide information about the region's functional significance for motor learning.

Furthermore, the TMS technique can be used in different ways to study motor learning. Since TMS applied on one area can reach a circuit of interconnected structures, one can target two areas of the motor network with rTMS and asses how that additional interference interacts with motor learning. Similarly, functional connectivity analysis realized on brain imaging data in our laboratory has shown that in the initial learning stage, a lot of brain regions are interconnected with each other. As motor learning progresses into the late phase, these connections become fewer and fewer (perhaps more specialized). A suggestion would be to 'virtually' lesion one brain area implicated in the circuit. This type of experiment would provide information about how the brain reorganizes connections when one node of the network is missing in a motor learning situation. Ultimately, we can gain insight of how connectivity is reorganized following brain injury.

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