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Neural substrates and functional connectivity associated with sleep-dependent and independent consolidation of new motor skills

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Résumé

La mémoire n'est pas un processus unitaire et est souvent divisée en deux catégories majeures: la mémoire déclarative (pour les faits) et procédurale (pour les habitudes et habiletés motrices). Pour perdurer, une trace mnésique doit passer par la consolidation, un processus par lequel elle devient plus robuste et moins susceptible à l'interférence. Le sommeil est connu comme jouant un rôle clé pour permettre le processus de consolidation, particulièrement pour la mémoire déclarative. Depuis plusieurs années cependant, son rôle est aussi reconnu pour la mémoire procédurale. Il est par contre intéressant de noter que ce ne sont pas tous les types de mémoire procédurale qui requiert le sommeil afin d'être consolidée. Entre autres, le sommeil semble nécessaire pour consolider un apprentissage de séquences motrices (s'apparentant à l'apprentissage du piano), mais pas un apprentissage d'adaptation visuomotrice (tel qu'apprendre à rouler à bicyclette). Parallèlement, l'apprentissage à long terme de ces deux types d'habiletés semble également sous-tendu par des circuits neuronaux distincts; c'est-à-dire un réseau cortico-striatal et cortico-cérébelleux respectivement. Toutefois, l'implication de ces réseaux dans le processus de consolidation comme tel demeure incertain. Le but de cette thèse est donc de mieux comprendre le rôle du sommeil, en contrôlant pour le simple passage du temps, dans la consolidation de ces deux types d'apprentissage, à l'aide de l'imagerie par résonnance magnétique fonctionnelle et d'analyses de connectivité cérébrale. Nos résultats comportementaux supportent l'idée que seul l'apprentissage séquentiel requiert le sommeil pour déclencher le processus de consolidation. Nous suggérons de plus que le putamen est fortement associé à ce processus. En revanche, les performances d'un apprentissage visuomoteur s'améliorent indépendamment du sommeil et sont de plus corrélées à une plus grande activation du cervelet. Finalement, en explorant l'effet du sommeil sur la connectivité cérébrale, nos résultats démontrent qu'en fait, un système cortico-striatal semble être plus intégré suite à la consolidation.

C’est-à-dire que l’interaction au sein des régions du système est plus forte lorsque la consolidation a eu lieu, après une nuit de sommeil. En opposition, le simple passage du temps semble nuire à l’intégration de ce réseau cortico-striatal. En somme, nous avons pu élargir les connaissances quant au rôle du sommeil pour la mémoire procédurale, notamment en démontrant que ce ne sont pas tous les types d’apprentissages qui requièrent le sommeil pour amorcer le processus de consolidation. D’ailleurs, nous avons également démontré que cette dissociation de l’effet du sommeil est également reflétée par l’implication de deux réseaux cérébraux distincts. À savoir, un réseau cortico-striatal et un réseau cortico-cérébelleux pour la consolidation respective de l’apprentissage de séquence et d’adaptation visuomotrice. Enfin, nous suggérons que la consolidation durant le sommeil permet de protéger et favoriser une meilleure cohésion au sein du réseau cortico-striatal associé à notre tâche; un phénomène qui, s’il est retrouvé avec d’autres types d’apprentissage, pourrait être considéré comme un nouveau marqueur de la consolidation.

Mots-clés : consolidation, apprentissage moteur, sommeil, connectivité fonctionnelle, IRMf, séquence, adaptation motrice, mémoire.

Abstract

Memory in humans is generally divided into two broad categories: declarative (for facts and events) and procedural (for skills and motor abilities). To persist, memories undergo a process referred to as consolidation, where a fresh, initially labile memory trace becomes more robust and stable. Sleep is known to play an important role in declarative memory consolidation, and in the past decade, there has been increasing evidence for a role of sleep in the consolidation of procedural memory as well. Interestingly, however, the beneficial effects of sleep do not seem to be homogenous. Motor sequence learning consolidation, in particular, has been found to be particularly sensitive to sleep effects, while the consolidation of motor adaptation has not. Moreover, neuroimaging research, has demonstrated that the long term retention of these two types of motor abilities rely on different neuronal networks, namely the cortico-striatal and cortico-cerebellar systems, respectively. Yet the implication of these networks in the consolidation of these two types of motor memory remains unclear. The aim of the present doctoral thesis was thus to determine the influence of sleep, while controlling for the simple passage of daytime, on the consolidation of a motor sequence learning task vs. a motor adaptation task. We further aimed to bring new insights into the underlying brain regions involved in consolidating these two forms of motor skills.

Consistent with previous research, we found off-line improvements in performance for motor adaptation learning, independent of whether participants had a night of sleep or remained awake during daytime. Furthermore, these improvements were correlated with activity in the cerebellum. In contrast, we found that off-line increases in performance in motor sequence learning were evident after a night of sleep but not over the day; and the putamen was strongly associated with this sleep-dependent consolidation process. Finally, while measuring brain changes in connectivity associated with the latter process, we observed that sleep-dependent consolidation is reflected by an increased level of integration within the cortico-striatal system, but not in other functional networks. Conversely, the simple passage of daytime in the wake state seems to result in decreased cortico-striatal integration. In sum our results highlight that not all motor memories undergo sleep-dependent consolidation. We demonstrated that these different paths to consolidation are also reflected by distinct underlying neuronal systems, namely a cortico-striatal and cortico-cerebellar network associated with the consolidation of motor sequence and motor adaptation learning respectively. Furthermore, we propose that consolidation of motor sequences during sleep protects and favors cohesion within the cortico-striatal system, a phenomenon that, if replicated in other types of memories, may be considered as a new marker of sleep-dependent consolidation.

Keywords: consolidation, motor learning, sleep, functional connectivity, fMRI, motor sequence, motor adaptation, memory.

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BG	Basal Ganglia
BOLD	Bold Oxygen level dependent
CB	Cerebellum
EEG	Electroencephalogram
FDR	False Discovery Rate
FEW	Family Wise Error
fMRI	Functional Magnetic Resonnance Imaging
FTT	Finger Tapping Task
HD	Huntington’s Disease
Hz	Hertz
ICA	Independent Component Analysis
LTP	Long Term Potentiation
M1	Primary Motor Cortex
MA	Motor adaptation
MSL	Motor Sequence Learning
NREM	Non Rapide Eye Movement
PET	Positron Emission Tomography
PFC	Prefrontal Cortex
PPI	Psychophysiological Interaction
PMv	Premotor ventral area
REM	Rapide Eye Movement
ROI	Region of Interest
SMA	Supplementary Motor Area
SRT	Serial Reaction Time Task
SVC	Small volume Correction
SWS	Slow Wave Sleep
S1	Primary Sensory Cortex

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Chapter 1: Theoretical Background

1.1. Memory

1.1.1. Historical Perspective

A plethora of studies in patient populations have contributed tremendously to our understanding of memory, and have allowed the scientific community to recognize that memory is not a unitary process. For example, great insights have been gained through work with H.M, who is one of the most famous patient in the memory literature. H.M suffered from severe epilepsy and underwent a bilateral resection of the hippocampus and surrounding areas of the medial temporal lobe in order to remove epileptic foci. This surgery was successful in treating epilepsy, but resulted in what they called at that time “loss of recent memories” (Scoville and Milner, 1957). The patient could maintain some information on the very short term, but as soon as his attention was drawn away, the memory slipped from his mind. He presented an apparent loss of the ability to form new long-term memories, i.e. he suffered from complete anterograde amnesia. Hence H.M was clearly impaired on tasks requiring explicit recall and recognition of events or action. Research with H.M. and others with similar brain damage has revealed that although they had no “recent memories”, such patients could still learn some new motor tasks, and demonstrate improvement in performance from one day to the other, while having no recollection of having even seen the tasks (Milner, 1962 as cited in Cohen and Squire, 1980). For example, H.M improved on a mirror-tracing task, where the goal is to draw within two contour lines of a star, while only looking at his hand and the paper in a mirror. After 3 days of practice, H.M was able to draw within the two lines with great precision, reducing his error score and time required for completion. Yet he would always claim that it was his first time at doing this task. This patient marked a clear dissociation between the “knowing how and knowing what” (Cohen and Squire, 1980), i.e. between the ability to learn and the inability to remember the event. Since then, multiple studies have shown that amnesic patients have memory impairments that could extend to words, digits, paragraphs, faces, names, maze routes, spatial layouts, geometric shapes, nonsense patterns, nonsense syllables, public and personal events and more (Cohen, 1984 as cited in Eichenbaum and Cohen, 2001). Nonetheless the patients' performance did not differ from that of healthy control subjects on a variety of motor, perceptual and cognitive skills. The landmark studies on the patient H.M and other similar cases of amnesic patients have thus marked the beginning of an era, in which different forms of memory became tied to distinct brain structures (Eichenbaum and Cohen, 2001).

Apart from amnesic patients, other clinical groups with various types of lesions have also been studied on different memory tasks in order to better understand the correspondence between memory functions and brain structures. Notably, patients with Huntington's disease (HD) have been tested. HD is a genetic neurological disorder, presenting functional abnormalities within the striatum, subcortical structures of the brain, as well as in the frontal and temporal regions. The expression of the disease is characterized by movement disorders and a decline in mental abilities. Research on these patients has revealed that, as opposed to H.M, they are still able to recognize a list of words they learned, but have difficulties learning new skills, like mirror reading (Martone et al., 1984) and motor sequences (Knopman and Nissen, 1991). Similarly, research on patients with Parkinson's disease, characterised by insufficient formation and action of dopamine in the striatum, has also shown that they experience deficits in executing sequential movements, expressed as difficulties in switching from the first movement to the second one (Benecke et al., 1987). These findings, paired with the ones from amnesic patients, have demonstrated the existence of a double dissociation between the types of memories and the brain areas responsible for their retention in the early days of neuropsychology.

1.1.2. Memory Systems Organisation

The observation of parallel memory systems in patients led to a classification of different memory types that are thought to rely on dissociated cerebral structures (Squire and Zola, 1996). The major distinction within the memory system suggested by Squire & Zola, is between the declarative and non-declarative memory. According to this nomenclature, declarative memory is defined as the capacity for conscious recollection of facts and events. It is known to depend upon the hippocampal system and related structures, as shown by the results in patient H.M and numerous studies in animals (rodents and monkeys). By contrast, non-declarative (non-conscious) memory includes procedural memory (skill and habit learning capacities) as well as other phenomenon related to performance rather than to the recollection of events. Such abilities do not need to access any conscious memory content in order to be expressed (Squire et al., 1993), and are mainly thought to be dependent upon a more extended network including the motor cortical regions, the striatum and the cerebellum, but not the hippocampus. Some investigators have reduced declarative knowledge to the term explicit knowledge (i.e., one that reaches our consciousness), and non-declarative memory to implicit memory (i.e., one that does not reach the level of consciousness). Yet, today, it is clear that habits can be learned using explicit or implicit forms of memory.

Because the present thesis project concerns motor skill learning, however, emphasis will thus be put on procedural types of memory for the rest of this essay, while other types of non-declarative memories will not be discussed.

1.2. Procedural Memory

In everyday life, we use a variety of motor skills that are acquired gradually through interactions with our environment. Skills have been defined as procedures for operating in the world, that may or may not reach consciousness (Squire et al., 1993). More specifically, Willingham (1998) refers to motor skill learning as “the increasing spatial and temporal accuracy of movements with practice.” To study the cognitive processes and the neural substrates mediating our ability to learn such skilled behaviors in the laboratory, investigators have used multiple experimental paradigms, which could be segregated into two categories: the first measures the acquisition of distinct movements into coherent, successive series of actions (motor sequence learning), such as playing piano. This type of learning encompasses distinct motor movements executed in a specific spatially distributed sequence, which can be learned explicitly or implicitly. The second category is motor adaptation, the ability to compensate for environmental changes, such as riding a bicycle. This type of learning is naturally done implicitly and has been shown to be inefficient if done explicitly (Mazzoni and Krakauer, 2006).

1.2.1. Motor learning

Changes in performance during motor skill learning are known to evolve slowly, requiring many repetitions for improvement to occur. Such learning process has been described as going through two steps (Karni and Sagi, 1993, Karni, 1996). First, a within-session fast learning phase in which there is a rapid and large increase in performance, followed by a slow learning phase during which more practice is needed, usually over multiple sessions, in order to see continued improvement. Above the different expression of behavioral performance with learning, it has also been shown that different brain structures are involved in the distinct learning phases (see Ungerleider, 1995, Censor et al., 2012).

1.2.2. Behavioural Paradigms

There are several ways of testing learning for both motor sequence and motor adaptation abilities (see Figure 1). One way to measure Motor Sequence Learning (MSL) is to use the “finger opposition task” (Karni et al., 1995), in which the thumb opposes the other fingers of the same hand in a given sequence. A second approach is to use the “finger tapping task (FTT)” (e.g. Walker et al., 2002), in which subjects have to employ a response box to produce the sequence by pressing on one of the four corresponding finger/buttons of the box. Both of these methods can be used in a “speed test” (Karni, 1996, Walker et al., 2002), i.e., by requiring subjects to do as many sequences as possible in a certain amount of time (usually blocks of 30s), hence the number of sequences executed per blocks is measured. As opposed to having fixed 30s blocks, another possibility is to employ a test where the amount of sequences per blocks of practice stays identical, and thus controlled for, from one block to the other. This approach consequently emphasizes the speed to execute one sequence and accuracy levels vary very little with a 5 element sequence. The latter approach is the one favored for the present thesis, as the task will be executed in the MR system.

The FTT can be used for testing explicitly learned motor sequences, i.e. telling the subject what the actual sequence is beforehand. In order to test for implicit sequence learning, the serial reaction time task (SRT) is usually employed (Robertson et al., 2004, Press et al., 2005). In that case, the response box is visually reproduced on a screen, and the buttons to be pressed are indicated one after the other on the screen. Unbeknownst to the participant, the button presses correspond to a specific sequence of movement. Reaction time for each button press is measured and is shown to decrease with learning, suggesting participants can anticipate the next position of the cue and that the sequential pattern is learned.

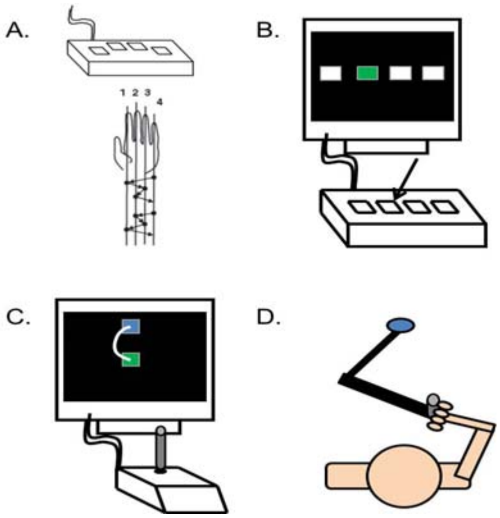

Figure 1: apparatus for testing MSL and MA.

A. when the sequence is learned explicitly, in the FTT, one simply has to execute the learned sequence on a response box. B. if the sequence is learned implicitly, like in the SRT, participants have to press on the key that corresponds to the square which lights up on the screen, based on the similar spatial disposition. C. is an example of kinematic motor adaptation in which the participants uses a joystick to move a cursor on the screen. D. is an example of dynamic motor adaptation, for which the participants uses a manipulandum, on which force is usually applied, to reach a target.

By contrast, for Motor Adaptation (MA) type of learning, investigators have used two broad categories of tests, one is called ‘dynamic adaptation’, where the use of force field disturbs the ongoing movement of the subject who is holding a robotic arm requiring some adaptation to maintain his trajectory (Shadmehr and Holcomb, 1997). The second category named ‘kinematic adaptation’ includes visuomotor adaptation, for which the visual input does not correspond to what you would expect. The latter form of learning can be tested in different ways using either the mirror tracing task described earlier, a prism adaptation paradigm in which the visual reality of the participant is distorted, or a rotation task for which the subject has to reach a target with a cursor on a screen, but the relation between the cursor and the movement is deviated by a certain angle (for example, if the rotation is of 90 degrees, moving the joystick up will cause the cursor on the screen to move right). One thus needs multiple trials in order to reach a target without making mistakes. The measures of performance typically used for this type of learning are the errors made in the movement trajectory, as well as the time taken to reach the target. Training participants on such abilities thus allows the researcher to measure the motor adaptation to a new visuomotor map.

1.2.3. Neural Correlates Mediating Motor Skill Learning

Motor sequence learning

The advent of neuroimaging methods, like functional magnetic resonance imaging (fMRI), which allows indirect measurement of the amplitude of brain activity using the blood oxygenation level dependent (BOLD) signal, has allowed researchers to visualize the brain structures involved in the learning process of new motor skilled behaviors. Regarding motor sequence learning in particular, Karni & al. (1995) observed a within-session fast learning phase, also referred to as early learning; followed by a slow learning phase, also referred to as late learning. Interestingly, the former was accompanied by primary motor cortex (M1) activity reduction. This was interpreted as reflecting a focus in the cells that best represented the movement executed. Yet, after multiple sessions of practice, these authors found increased activity in M1 that was specific to the learned sequence of movement. The observed change led them to think that the participants were in a different learning phase (slow learning phase), and indicated the possibility that additional cells were recruited into a critical network specific to the learned sequence of movement (Karni et al., 1995, Ungerleider, 1995).

Since then, numerous studies have been conducted in an effort to better describe the brain regions involved in early learning of motor skills. Findings regarding motor sequence learning suggest a clear role of the striatum, and the putamen in particular (Grafton et al., 1992, Jueptner et al., 1997, Doyon et al., 2002, Doyon et al., 2003, Floyer-Lea and Matthews, 2005, Lehericy et al., 2005) in the first learning episode. These structures were involved in the fast learning phase, together with the cerebellar cortex, premotor areas, anterior cingulate cortex (Jenkins et al., 1994, Doyon et al., 2002, Steele and Penhune, 2010), pre-SMA (Steele and Penhune, 2010) and the dorsal prefrontal cortex (Jenkins et al., 1994, Jueptner et al., 1997). Interestingly, some studies were even more specific and found the rostral part of the striatum to be involved in early learning, as opposed to the late learning phase. Indeed, after 5 and 14 days of practice, activity in the putamen remained, but was more prominent in the posterior part of the structure (Lehericy et al., 2005). These functional results are coherent with the well described anatomical organization of striato-cortical loops: the rostral part of the putamen is the associative compartment and receives input from the pre-SMA, an area that itself receives input from the frontal and parietal areas (Lehericy et al., 2004). On the other hand, the more caudal-posterior, sensorimotor, part of the putamen receives input from the SMA proper, an area receiving itself input from M1 and S1 (see Figure 2).

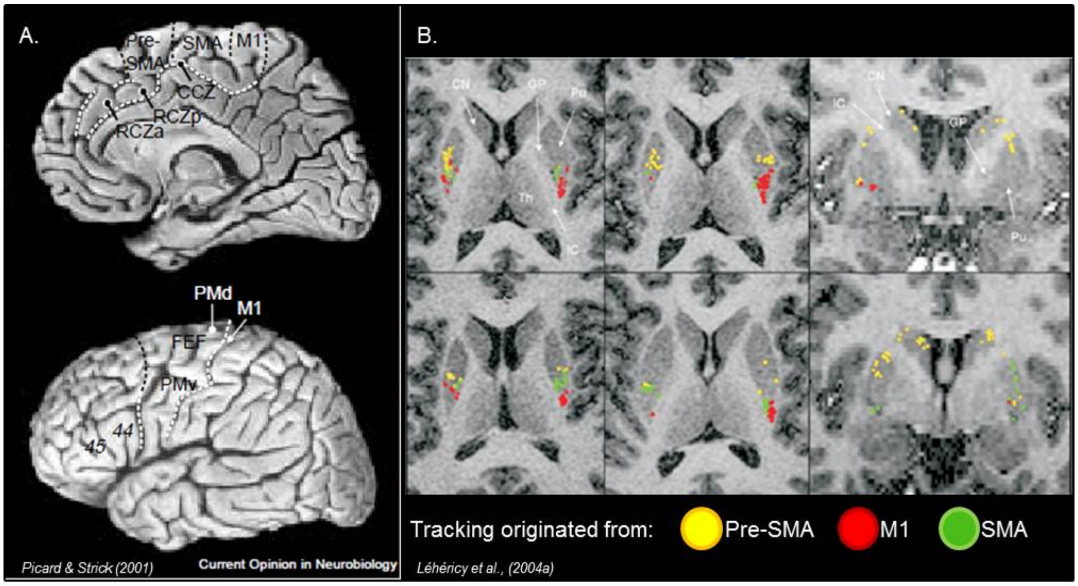

Figure 2 : Anatomy

A. Figure adapted from *Picard & Strick (2001)* nicely demonstrates the boarders of the different cortical motor areas of the lateral and medial wall of the brain. B. Figure adapted from *Léhéricy et al., (2004a)* presents the cortico-striatal tracts using diffusion tensor imaging for an individual subject. Tracks originating from M1 and SMA were directed to the posterior part of the putamen. Tracks originating from the Pre-SMA were located rostral to SMA and M1 tracks.

Although the activity of the putamen persists in the late learning phase, not all regions of the brain initially active remain so. Instead, dynamic changes take place over time (see Dayan and Cohen, 2011 for a review). An already learned sequence (be it a couple of hours or days of practice) is associated with activity in the putamen, SMA (Jenkins et al., 1994, Doyon et al., 2002), M1 (Karni et al., 1995, Floyer-Lea and Matthews, 2005, Steele and Penhune, 2010) and the dentate nuclei of the cerebellum (Doyon et al., 2002). The inferior parietal cortex was also involved both in early learning and late learning phases, sometimes as increased or decreased activation, suggesting the activity of that region to be more sensitive to the specific type of protocol used. For example the parietal cortex has been associated with explicit awareness of the sequence (Grafton & IVry 1995), and with a more abstract representation of the sequence, i.e. involved in the goal of the action rather than the specific movements of the sequence (Grafton et al., 1998). In addition to these motor related regions, the hippocampus is also increasingly found to be involved in motor sequence learning (Schendan et al., 2003, Gheysen et al., 2010, Rose et al., 2011, Albouy et al., 2012a) and is thought to interact with the striatum during the early learning phase (Albouy et al., 2008). Note that the dorsal prefrontal cortex does not seem to be active in the late learning phase. This would support the idea that learning has become more automatic and that less monitoring is needed to execute the task. Others have segregated different learning components from initial learning of a sequence until the 5th day of training. The authors found that activity in the putamen, together with the hippocampus, was specifically related to the improvement in accuracy of a complex sequence; as opposed to the timing of the key presses, which was associated with lobule VIII of the cerebellum (Steele and Penhune, 2010, Penhune and Steele, 2012).

Motor adaptation learning

Learning-related modulation of brain activity has also been observed during motor adaptation. Based on studies in animals and patients, the cerebellum is now thought to be a key structure in this type of motor learning. Indeed, patients with Parkinson’s and Huntington’s diseases who suffer from damage to the cortico-striatal network, but who have an intact cerebellum, show no impairment in visuomotor adaptation learning, as tested with mirror-tracing task or prism adaptation (Agostino et al., 1996, Gabrieli et al., 1997). Yet, patients with damage to the cerebellum have difficulty or are impaired at learning such types of skills (Martin et al., 1996). Besides, in the primates, it was found that the discharge of purkinje cells of the cerebellar cortex conveyed information of both the beginning of a reaching movement, as well as the relative error at the end of the movement (Kitazawa et al., 1998). Thus these results suggest that the cerebellum is a good candidate structure for allowing the acquisition of an internal model of the body as one learns a new tool (Imamizu et al., 2000, Penhune and Steele, 2012).

The fast learning phase of motor adaptation has been studied in humans and has been associated with activity in S1, the contralateral putamen, thalamus, medial occipital gyrus and the dorsolateral PFC at the beginning of the training (Shadmehr and Holcomb, 1997).

Yet, the specific visuomotor transformation ability seems to be associated with activity in the ipsilateral posterior (Ghilardi et al., 2000, Krakauer et al., 2004) and inferior parietal regions (Ghilardi et al., 2000), as well as with the right ventral premotor cortex and lateral cerebellum (Krakauer et al., 2004). The lateral cerebellum would be responsible for learning the prediction of visual sensory consequences of the motor command executed in order to adapt to the environmental change (Miall et al., 2007, Izawa et al., 2012). Interestingly, 5.5 hours following initial learning of a motor adaptation task, the recall is associated with activity in the contralateral dorsal premotor and posterior parietal cortex, as well as the ipsilateral anterior cerebellar cortex (lobule VI). The latter pattern of results came along with a decrease of activity in the PFC and the putamen (Shadmehr and Holcomb, 1997). These results were suggested to represent a shift in the representation of the internal model developed for the skill. The authors put forward the importance of the cerebellar cortex (lobule VI) for the storage and maintenance of the motor memory. Yet it is also suggested that the cerebellum is part of a more extended motor network which would serve to store the representation of the learned skill (Penhune and Steele, 2012).

1.2.4. Motor Learning models

Based on the numerous functional neuroimaging studies described above and in line with pre-existing neuroanatomical models demonstrating the existence of dinstict cortico-striato-cortical and cortico-cerebello-cortical loops (Alexander and Crutcher, 1990, see Middleton and Strick, 2000 for a review), different theoretical models have been proposed.

Doyon and colleagues (Doyon et al., 2002, Doyon and Benali, 2005b) have proposed that representational changes within the cortico-striatal and cortico-cerebellar systems depend not only on the stage of learning, but also on the type of motor task involved, i.e. whether subjects are required to learn a new sequence of movements or to adapt to environmental perturbations (see Figure 3). They proposed that in the fast (early) learning phase, both motor sequence and motor adaptation tasks recruit similar cerebral structures: the striatum, cerebellum, motor cortical regions (e.g., premotor cortex, SMA, pre-SMA, anterior cingulate), as well as prefrontal and parietal areas. During this phase, dynamic interactions between these structures are thought to be critical for establishing the motor routines necessary to learn the skilled motor behavior. When a task is well learned, however, the subject has achieved asymptotic performance and the execution has become more automatic. The neural representation of the new motor skill is then believed to be distributed in a network of structures that involves the cortico-cerebellar or the cortico-striatal circuit for motor adaptation and motor sequence learning respectively. Indeed, Doyon and Ungerleider (2002) suggested that for motor adaptation, at this stage, the striatum is no longer necessary for the retention and execution of the acquired skill; regions representing the skill are now involving the cerebellum and related cortical regions. By contrast, a reverse pattern of plasticity is thought to occur in motor sequence learning, such that with extended practice, the cerebellum is no longer essential, and the long-lasting retention of the skill is now believed to involve representational changes in the striatum and associated motor cortical regions.

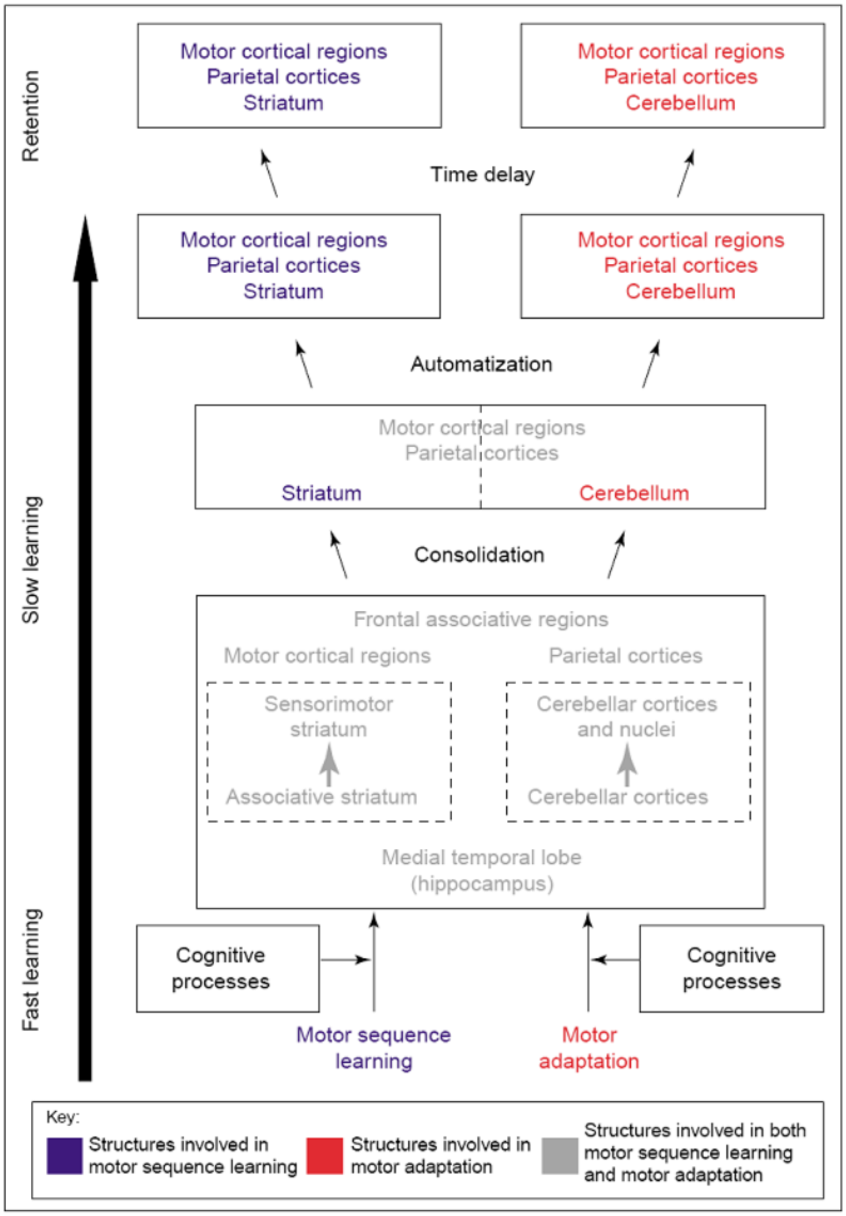

Figure 3 : Learning model proposed by Doyon and collaborators

Other models have focused on the respective role of the two cortico-striatal and cortico-cerebellar loops, based on findings from motor sequence learning studies as well as from other cognitive domains. Doya (1999) has developed learning algorithms which will not be described in details in the present thesis, but will be briefly summarised. This model suggests that the cerebellum is responsible for “supervised learning”, namely by correcting erroneous reaching motor commands. This error-based learning allowing the construction of an internal model of the body and the environment, hence improves performance of motor control. The role of the cerebellum in MSL specifically, however, is not specified. The basal ganglia would be responsible for "reinforcement learning", which is based on reward. Doya suggests that the basal ganglia, by means of the dopaminergic neurons, encodes and learn present rewards in order to predict future rewards, making it possible to eventually select the motor action with the highest expected reward. He proposes that in the fast learning phase, the sequence is represented at the cerebral level with visuospatial coordinates i.e. the spatial location of the elements of the sequence are learned. With practice, there is a switch to the use of motor coordinates in the slow learning phase of the learned sequence. This switch would take place from the PFC, preSMA and the anterior striatum to SMA and the body of the striatum (Doya, 2000).

Similarly, Hikosaka et al. (1999) have proposed that spatial and motor coordinates of the sequence are processed in parallel. As above, the spatial information conveys the visual location of the target, while the motor coordinates are the actual movements executed to reach the target. The authors further suggested that distinct cortico-subcortical loops are responsible for these two types of information processes. The loop responsible for the spatial aspect of the sequence would comprise association areas such as the PFC and the anterior portion of the basal ganglia (especially the head of the caudate), while the loop responsible for the motor aspect of the sequence would correspond to the premotor cortex (especially SMA), the putamen and the dentate nuclei of Cerebellum. As Doya suggested (1999), Hikosaka also proposes that the acquisition of the spatial aspect of the sequence would take place earlier than the motor property of the sequence.

1.2.5. Connectivity Changes in Motor Skill Learning

When using conventional activation detection types of analysis, one often looks at functional segregation. Doing so, the goal is to target a particular area of the brain associated with a specific cognitive process. Yet, analyses of neural activity that are based on functional specialization provide only a limited account of the neuronal substrate of the process investigated (Lee et al., 2003). An alternative and complementary approach is to investigate the integration of functionally specialised areas via functional connectivity, namely by quantifying the interaction amongst different brain areas. Changes in brain functioning reflected by different patterns of interactions between areas of the brain, are mediated by *functional* or *effective* connectivity (Friston 1994, Friston, 2011). Both of these approaches are briefly described below.

Functional Connectivity

What is Functional Connectivity

Functional connectivity is a great theoretical and methodological approach for measuring spatial relationship between brain regions as well as their temporal correlation. Functional connectivity between two regions is defined as the temporal correlation of a neurophysiological index, usually the extracted time course, measured between different brain areas (Friston et al., 1993a). Thus, this method allows to detect which areas of the brain are activated at the same time across the experimental session. These analyses are generally multivariate, such that the analysis of a voxel takes into account the activity of the nearest voxel. This is in opposition to standard activation detection type of fMRI analysis looking for the most active area of the brain using univariate analysis, namely analysing each voxel separately, as if they were independent. In functional connectivity, the assumption is that if the time courses of two brain regions covary, these two brain regions are most possibly exchanging information. Thus, an increase in functional connectivity between these structures would suggest that brain regions are interacting in a more synchronous and integrated fashion. Greater integration within a network thus means that the brain regions forming that network work together with more cohesion. Two major approaches have been used to study functional connectivity. One is hypothesis driven and the other is data-driven. In the former, one can explore the connectivity changes from one specific region of interest, with other distant areas (psychophysiological interaction, PPI) (Friston et al., 1997) or use multiple seeds to form a network. In that case, one determines a task- specific set of regions of interest that are known to be involved in motor learning before analysing the data. In the data-driven approach, the networks are formed based on mathematical as opposed to functionally relevant criteria. In that case, no functional constraints are applied to the data and it has the advantage that no a priori knowledge is necessary; i.e. one does not need to choose specific seeds (brain regions) prior to the analysis. A data-driven mathematical approach that has been used extensively in the past years is the independent component analysis (ICA). Briefly, ICA is a “blind source separation” algorithm that decomposes the registered signal into spatial and temporal components (networks), which are statistically independent from each other (McKeown et al., 1998, see also Boly et al., 2008). This means that the activity of one spatial map (component) cannot predict the activity of another given spatial map. In that way, the relevant maps related to brain activity, are automatically separated from noise into different components. The investigator then chooses the components he is willing to discuss or use for further analysis.

There has been an emergence of studies in which researchers examined functional connectivity patterns, generally with the use of ICA, with participants remaining simply at rest in the MRI. These studies are said to explore “resting-state” because no specific cognitive task is executed. Studying resting-state gave rise to the awareness that spontaneous fluctuations in the brain are organised in a functionally coherent manner (for a review, see Fox and Raichle, 2007). These systems generally include a motor network, visual network, fronto-parietal, executive, ventral attentional networks and the default-mode, one that systematically decreases as one engages in an externally driven task (Beckmann et al., 2005, Damoiseaux et al., 2006) (see Figure 4).

These so-called resting state functional networks are, however, also known to be stable during active sate, i.e. during execution of a task (Calhoun et al., 2008, Smith et al., 2009) in addition to being stable across rest periods. Particularly, the default mode has been shown to remain during light sleep (Horovitz et al., 2008), non-rapid eye movement (NREM) sleep

(Dang-Vu et al., 2008) and coma (Boly et al., 2008). Apart from this impressive stability across studies, the degree of connectivity within networks, however, seems to vary with age (Damoiseaux et al., 2008), neurodegenerative diseases (Filippini et al., 2009, Wu et al., 2009) and interestingly so, with learning (Albert et al., 2009b, Lewis et al., 2009b).

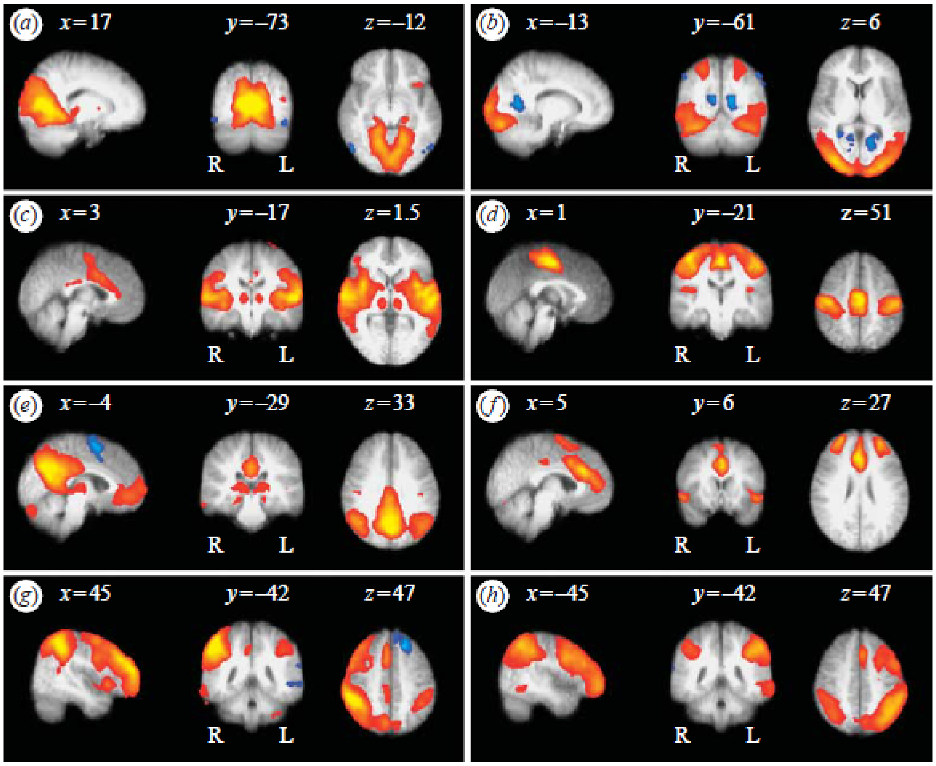

Figure 4 : Resting-state networks

Figure adapted from Beckmann et al., (2005) demonstrating the different resting state networks found using principal independent component analysis. A. medial visual cortical areas. B. Lateral visual cortical areas. C. Auditory system. D. sensory-motor system. E. Visuo-spatial system F. Executive control. G. and H. Dorsal visual stream each one lateralized

Functional connectivity with motor sequence learning

With the availability of the different motor learning models, as well as the studies characterizing the neural correlates of motor learning, methodological tools using functional connectivity allowed to better understand the brain plasticity taking place with MSL. Some studies using standard activation detection types of analysis also used PPI as a way to quantify changes in connectivity between specific ROIs. It is suggested that across multiple days of MSL, connectivity between M1 and the cerebellum lobule VII-VIII increases as synchronization of performance on the sequence also increases. During training, the hippocampus also shows competitive interaction (negative correlation) with the putamen (Albouy et al., 2008) or caudate nucleus (Albouy et al., 2013b), which, in the latter case predicted gains in performance following a night of sleep. Quantifying connectivity changes within or between networks, as opposed to between one specific brain region and the rest of the brain, has the advantage of uncovering brain communication at the systemic level. For example, Sun et al., (2007) demonstrated that when practicing a new sequence, greater inter- and intra-hemispheric integration takes place within a motor network during the early as compared to the late learning phases of learning (Sun et al., 2007). The results also revealed greater connectivity between frontal and motor cortical regions for the early vs. late phases of learning. In contrast, when executing an already learned sequence of movements, no change in functional connectivity was observed across the session. These results thus suggested enhanced inter-hemispheric coupling within a motor network only during the early stages of learning. Another study using a similar model free approach with motor sequence vs random trials, found that two independent components correlated with the task (Tamas et al., 2008). One network, comprising fronto-parietal and cerebellar regions, correlated with both types of trials; whereas a second network, including posterior parietal and premotor regions, was exclusively present during the sequence trials. Moreover activity in the latter network correlated with the amount of learning across the session. These results demonstrated how a data-driven approach was successful in identifying a motor network specific to sequence learning, as opposed to random trials, during a first learning phase. Furthermore, it suggests that premotor and posterior parietal brain regions interact with each other during early sequence learning.

Others have explored the dynamic changes occurring with motor sequence learning using a hypothesis-driven approach. Coynel et al., 2010 selected multiple seeds, based on previous fMRI results (Lehericy et al., 2005), to form a sensorimotor and an associative motor network, aiming to measure the interaction between these two motor systems as sequence learning progresses. The findings first revealed greater overall integration, i.e. greater cohesion, within and between networks during the first learning episode of a new sequence as compared to an overlearned and automatized one. Second, across 28 days of training, an overlearned sequence was associated with a lower level of integration, mainly because of a decrease in integration within the associative network. A relatively high level of integration between the two motor systems remained with learning (Coynel et al., 2010). These results thus suggest that with learning, there is a decrease of integration between higher order brain regions, which are part of the associative network; yet the interaction between this system and the sensorimotor network remains necessary.

In sum, it seems that during initial training sessions on motor sequence learning, there is a global increase or maintenance in connectivity observable between motor brain regions. This is followed, after multiple training sessions, by a decreased connectivity within a network comprising higher order brain regions such as the premotor cortex, Pre-SMA and the parietal cortex (Coynel et al., 2010).

Few studies examined functional connectivity changes with long term motor adaptation learning. In one of them, the authors used regions of the brain for which activity changed across learning, as seeds for their functional connectivity analysis (Della-Maggiore and McIntosh, 2005). They reported an increase in functional connectivity after 7 days of practice on a kinematic motor adaptation task between the bilateral anterior cerebellum, left (contralateral) middle temporal gyrus, cingulate gyrus and right putamen. Dynamic cerebral changes associated with motor adaptation was also studied using the initial post-learning rest period. Using a seed based approach; Vahdat et al (2011) dissociated changes in connectivity associated with the perceptual vs. motor aspect of motor adaptation. Changes associated with the perceptual function included increased connectivity between the second sensory cortex and frontal motor areas (PMv, SMA) as well as between the prefrontal cortex, cerebellar anterior cortex (lobule VI) and superior parietal lobule. With motor learning specifically, they found increased connectivity between cerebellar cortex adjacent to posterior-superior fissure (lobule VI, Crus I) and left M1 and SMA, as well as between cerebellar cortex and the superior parietal lobule (Vahdat et al., 2011). Increased connectivity was also observed in the post-learning resting state period, using a data-driven approach (Albert et al., 2009b). An experimental group was assigned to a visuomotor adaptation tracking task and a control group was assigned to the same task with no rotations. They found increased connectivity strength in the frontoparietal network only in the group that learned the motor adaptation, while no change in connectivity was apparent in the control group. Furthermore, a cerebellar network, which was only present in the experimental group, also showed increased connectivity following learning. These results suggest that changes in resting state activity were induced by MA learning. Furthermore, they propose that increased connectivity in a fronto-parietal circuit as well as the cerebellum might reflect the on-going “off-line” processing of information gained from earlier learning. These results bring insights to a hypothesis suggesting that these intrinsic networks observable at rest might contribute to the off-line processing and consolidation of memory (Miall and Robertson, 2006, Albert et al., 2009a).

Effective connectivity

Another mathematical approach used in fMRI studies is effective connectivity, which allows to examine the actual influence a neuronal system exerts over another. Two major methods are often used: Dynamic causal modelling (DCM) and Structural equation modelling (SEM) (Friston, 1994). The major advantage of these methods is that in contrast to correlational relationship between two brain regions, as measured in functional connectivity, these methods offer causal relationship. Yet, these types of approaches are also limited by the need of specific and strong functional and anatomical *a priori* knowledge. Furthermore, the consequence is that the findings will highly depend on the definition of the model (e.g. the choice of ROIs) chosen.

Some authors have explored effective connectivity during the course of MSL while looking at 6 regions of interest: M1, cerebellum, dorsal premotor cortex, basal ganglia, SMA, and the

PFC. The inter-regional connectivity were measured from day 1, to 2 weeks and 4 weeks of motor learning. Importantly, the findings revealed that connectivity from the cerebellum to M1 decreased across training, while connectivity from the BG to M1 increased. This is in accord with Doyon et al., 2002's model which predicts less involvement of the CB (but more of the BG) as the sequence becomes more automatic. Other interesting findings included a gradual reduction of the connection from PFC to M1, as well as strengthening of the connections from the BG to SMA and from SMA to the premotor cortex. These results were interpreted as being the reflection of a decreased need for attentional resources, an increased effectiveness in sequence control and in motor planning respectively (Ma et al., 2010).

The implication of an extended cortico-striatal network has also been studied during initial learning of a motor adaptation task. The findings suggest increases in effective connectivity as learning progresses, between the calcarine fissure and the middle temporal gyrus; and from there to the anterior striatum and the dorsal precentral gyrus. Afferents from the inferior frontal sulcus to the anterior striatum also showed increased connectivity with learning. In contrast, the authors reported significant decreases between the frontal cortical regions (Toni et al., 2002). These findings corroborate previously described imaging studies suggesting a role of the striatum in early learning of associative visuomotor learning. Yet, the findings are limited by the approach used (effective connectivity) in the sense that only the regions included by the authors in the model can be discussed. For example, it would have been interesting to explore also the regions of the cerebellum and compare its involvement at this point in learning with other motor brain regions.

These changes in the recruitment of the motor network, in terms of activation or connectivity, are thought to support learning. Yet in order to better understand long-term retention and learning, one has to understand the process of consolidation.

1.3. Memory Consolidation

For memories to be stored, be it declarative or procedural, consolidation is required. Consolidation is a process of brain plasticity whereby a fragile memory trace becomes more robust and less susceptible to interference. It was primarily noticed with declarative memory and a first theory was brought up by Müller & Pilzecker (1900) who observed that the ability to recall recently acquired verbal information deteriorated as a function of the interpolation of other tasks (John, 1967). Time-dependent effects of consolidation were also observed with the introduction of electroconvulsive shock (ECS) used to treat depression. It was demonstrated that ECS abolishes retention of a list of words paired associate learned prior to therapy, and that the severity of impairments was an inverse function of the time lapse between initial learning and ECS (Zubin and Barrea, 1941; as cited in John, 1967). These studies revealed that after a certain laps of time, the memory is stored and less susceptible to interference, i.e. consolidated. How that process occurred was unknown, but today the mechanisms responsible for consolidation can be viewed at a cellular level as well as at a system's level (Dudai, 2004).

1.3.1. Levels of Consolidation

Synaptic Consolidation

Consolidation at the cellular level (synaptic consolidation) can be seen in two forms: short-term and long term. In the short run, the memory is thought to be formed through little stimulation and to remain in a labile state. On the contrary, long term memory is believed to be formed after multiple repetitions of a stimulus, which result in the synthesis of new proteins, as reflected, for example, by synaptic growth or synaptic remodelling (for a review, see Kandel and Squire, 2000, Dudai, 2004). This process occurs mostly at the local level, i.e. in the same structure that was used for the encoding of the memory.

Systemic Consolidation

Consolidation at the systems level comes from the indication, among others, that H.M could no longer consolidate new declarative memories, yet he still had some memories from his childhood, indicating that the memory once encoded in the hippocampus, must have "migrated" somewhere else in the cortex. Thus the first model of system consolidation involved the hippocampal formation. According to this view, the stabilization of the memory trace is assumed to involve synaptic consolidation locally, achieved in minutes to hours. In parallel, or as a consequence of it, the process of system consolidation is initiated and is characterized by a shift of the representation of the information retained, from the medial temporal lobe to the neocortex (McClelland et al., 1995, Dudai, 2002, 2004). Although it is not clear how the change in neural representation occurs, the memory transformation theory emphasises the dynamic nature of the memory trace (Winocur and Moscovitch, 2011). The authors suggest that the hippocampus is the storing site of a memory as long as it is context-dependent. For each memory retrieval, a new trace is added and serves to reinforce and strengthen the memory. With time, an abstract representation of the memory is formed (schema) and is thought to be represented neocortically.

In the procedural memory domain, it has been shown that functional reorganization, particularly a shift in the motor representation, takes place among a motor cortical and subcortical network (Karni, 1995, Ungerleider et al., 2002, Doyon et al., 2003, Doyon and Benali, 2005b, Floyer-Lea and Matthews, 2005, Lehericy et al., 2005), but as noted by Dudai (2004), the protocol used in the majority of the studies involve learning across time, thus changes observed across the multiple testing sessions could be the reflection of practice, and not necessarily consolidation per se. Dissociating the learning process from the consolidation process, if possible, is quite a challenge as they certainly co-occur. Nevertheless, as it will be discussed in the next sections, it is known that consolidation continues offline following an initial training session, without further practice. This offline period leads sometimes to the stabilisation of memory, which reduces its susceptibility to interference. Alternatively, consolidation is reflected by offline gains in performance observable in a subsequent training session (see Dayan and Cohen, 2011, Censor et al., 2012).

1.3.2. Memory Consolidation and Sleep

Sleep is an active process despite the absence of consciousness and multiple studies have linked sleep to memory. Indeed, it has been shown that sleep deprivation affects behavior, that post-learning sleep architecture changes following learning and that one can observe behavioral benefits following post-learning sleep, as opposed to post-learning wakefulness (Maquet, 2001, Peigneux et al., 2001). There is also ample work showing that sleep facilitates the consolidation and long-term retention of new memories (see Diekelmann et al., 2009 for a review). Yet, the involvement of sleep, and the specific sleep characteristics found to optimize the memory consolidation process, is not yet well understood because it is thought to depend on multiple factors (see Figure 5); for example the type of learning and material used.

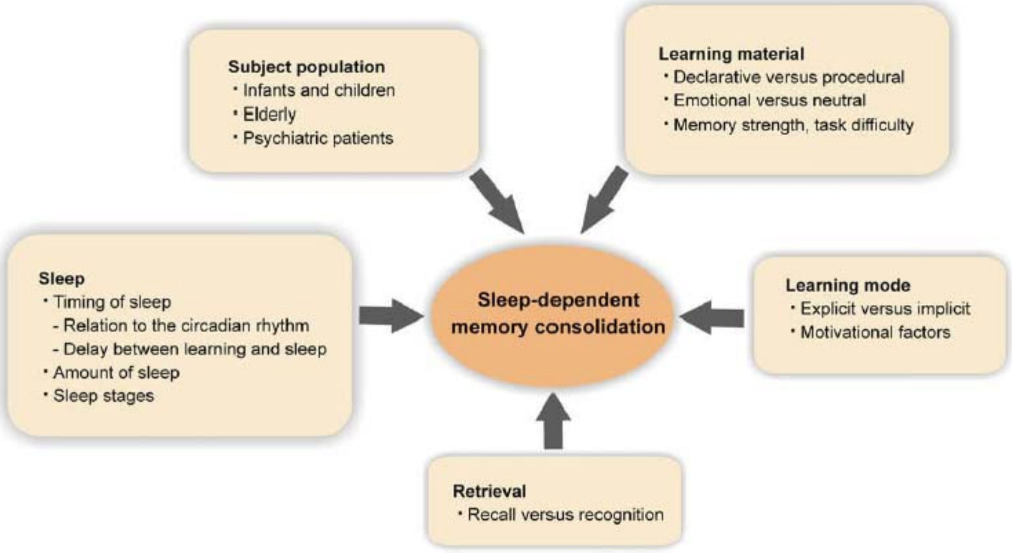

Figure 5 : Factors influencing sleep-dependent memory consolidation – from Diecklman et al., (2009)

Both declarative and procedural memories have been shown to benefit from sleep. In declarative memory, for example, a better retention of pairs of words at cued recall has been found after sleep as opposed to the same time spent awake. Similar gains in being able to recall greater information following sleep has been found with the learning of object locations, short stories and wordlists (see Diekelmann et al., 2009). More recently it has been suggested that the particular role of sleep could be in the form of a triage of relevant vs. irrelevant information to be retained. Namely, sleep would produce specific enhancement of memory for declarative information that was cued to be remembered, but not for others cued to be forgotten (see Stickgold and Walker, 2013). In procedural memory, enhanced performance on different adaptations of the serial reaction time task, finger tapping task and visual discrimination tasks has been observed specifically following sleep.

1.3.3. Motor Memory Consolidation

Generally speaking, consolidation is thought to occur between the fast and slow learning phases of motor learning and to last several hours (Karni and Bertini, 1997). Several investigators have reported that sleep plays a critical role in the consolidation of motor sequence learning in particular (Karni et al., 1998, Fischer et al., 2002, Walker et al., 2002). The important concept being that time, and sometimes sleep, is needed for the memory to become “fixed” and eventually resistant to interference, even in the long term after several weeks without practicing (Nezafat et al., 2001, Penhune and Doyon, 2002, Della-Maggiore and McIntosh, 2005). Nevertheless it is now acknowledged that consolidation is not a one-time process and that reactivation of the memory trace might render it *de novo* labile, and again susceptible to interference until reconsolidation of the trace has occurred (Nader et al., 2000, Nader, 2003, Marin et al., 2010), findings that have inspired Hollywood movies like “Eternal Sunshine of a Spotless Mind”.

Paradigms for testing Motor Memory Consolidation

In the field of skill learning, there are 2 ways that are often used to test for the presence of consolidation at a behavioural level. The first type of protocol described here is called “interference”, in which subjects are tested using an A_1-B-A_2 paradigm, where A is the first task learned and B is a similar, but different task. Retrograde interference can be measured by looking at the disruptive effect of task B on consolidation of A (i.e., the motor task originally learned) by manipulating the time interval between A_1 and B. Consolidation processes are measured by retesting performance of A (A_2) after the interfering episode. Using this procedure, it is possible to identify the critical time window that enables (or not) consolidation to take place. Differently, anterograde interference can be examined through the effect of A on the learning of the interfering task B. Although this type of protocol has been used to measure consolidation in motor sequence learning (Korman et al., 2007) it has more often been applied to measure consolidation of adaptation learning (see Shadmehr and Holcomb, 1997, Caithness et al., 2004, Krakauer et al., 2005). A second type of protocol to test for the occurrence of consolidation is to use a parametric (test-retest) design. This is done by using multiple testing sessions separated by different laps of time, while quantifying differences in behavioural performance as time goes by. This paradigm has often been used to test for the role of sleep, this so with one of two approaches: 1) the effect of post-training sleep deprivation vs. regular sleep or (2) the effects of sleep vs. the simple passage of daytime on the expression of memory consolidation by measuring performance on a delayed retest session. Using a test-retest paradigm, two phenomena have been observed when sufficient time is given for consolidation to occur: *savings*, defined as a faster rate of relearning at retest (mostly seen in motor adaptation tasks), and *gains* in performance, described as a sudden increase in performance, following a latent period of time without further practice.

Consolidation as reflected at the behavioral level

In a pioneering study, Walker et al. (2002) used a computerized version of the finger opposition task to demonstrate that spontaneous gains in performance occurred specifically after sleep, but not after the simple passage of time. There were 17-20% improvements in speed after a night of sleep (with no changes in accuracy), but only 2% improvement after an equivalent latent period during daytime (see Figure 6). Interestingly, they verified that the absence of gain during daytime was not due to interference, as subjects were asked to wear mittens during the whole day, hence minimizing hand movements during the day. Complementary findings from Fischer et al. (2002) revealed that gains in performance are similar after both night-sleep and day-sleep, on both speed and accuracy measures, suggesting no significant circadian, or time of day, influence on the consolidation of motor sequences. These first studies thus suggest an important role of sleep in the off-line consolidation process of motor sequence learning, which is reflected as off-line gains in performance.

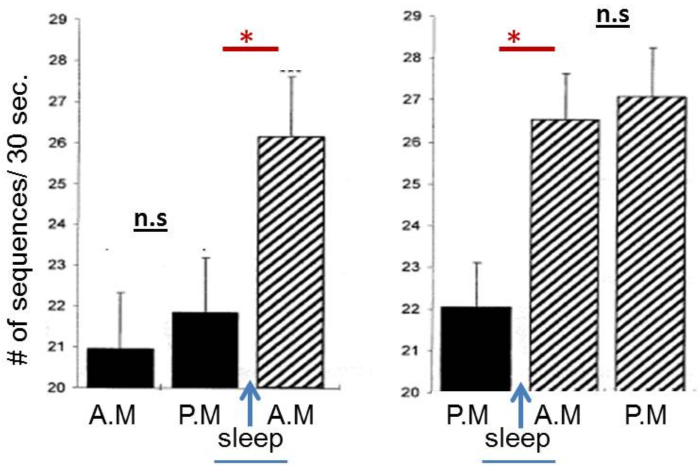

Figure 6 : Sleep-dependent consolidation at the behavioral level

Figure adapted from Walker et al., (2002). The Y axis represents the number of sequences executed within a 30 second period. The graph on the left demonstrates that when starting the training in the morning, only a period of sleep allows significant improvements in performance. The graph on the right demonstrates the same phenomenon but having participants trained in the evening.

Since then, our group has replicated the findings suggesting a benefit of sleep specifically for MSL (Doyon et al., 2009b). Furthermore it is now known that daytime naps are also sufficient to elicit similar gains in performance (Nishida and Walker, 2007). Yet, different factors are found to influence the contribution of sleep to consolidation. For example, the benefits of sleep are thought to be more important if the sequence is more complex (Kuriyama et al., 2004) or if the subject is a fast learner as opposed to a slow one (Albouy et al., 2008). Expectation to be retested is also known to favor greater sleep-dependent consolidation. Indeed, a group that learned the MSL task while expecting to be retested the following morning, performed better than a group which did not expect to be retested (Wilhelm et al., 2011).

Yet, post-training sleep does not appear to be necessary for the consolidation of all types of motor sequences, as daytime alone is sufficient to elicit off-line gains in performance when learning occurs implicitly, i.e. without being aware of the sequence (Robertson et al., 2004). These results suggest that consolidation of implicit and explicit learning of MSL seem to rely on distinct mechanisms. Yet, it is also possible to segregate MSL based on the different memory processes involved, as opposed to a segregation based on the level of consciousness (Cohen et al., 2005). Findings reveal that the spatial representation of the sequence, namely what is effector independent, is the specific process which consolidates during sleep. In opposition, the effector-dependent or motoric representation aspect of the learned sequence requires only time for consolidation to occur (Cohen et al., 2005, Witt et al., 2010, Albouy et al., 2013a).

As mentioned earlier, some researchers have explored consolidation as reflected by a resistance to interference. In these studies, consolidation is also often expressed as stabilisation, before giving rise to gains in performance (enhancement). This has been shown when a learned sequence is no longer susceptible to interference. Korman and colleagues (2007) have demonstrated that training on a second interfering sequence at 2 but not 8 hours, following the initial learning, did prevent the expression of delayed gains observed at 24-hours after training (Korman et al., 2007). These results suggested that 8 hours were necessary to stabilise the memory trace for it to undergo sleep-dependent consolidation. Similarly, Walker et al., (2003) also suggested that if a second sequence (B) was learned right after the original one (A), retest the following day showed significant gains in performance speed, but not accuracy. In contrast, when the B sequence was learned 6 hours after A, post-sleep gains were observed in both speed and accuracy measures, suggesting that 6 hours were sufficient to stabilise the memory. Yet, it is not clear why the interference was only reflected on the accuracy measure as this characteristic has not consistently been shown to change with sleep (Walker et al., 2002, Morin et al., 2008, Doyon et al., 2009b).

The effect of interference on motor adaptation is also very well documented (Brashers-Krug et al., 1996, Shadmehr and Holcomb, 1997, Krakauer et al., 2000, Krakauer et al., 2005, Krakauer and Shadmehr, 2006) (but see Caithness et al., 2004). The results of these studies suggest that, when using an ABA paradigm, learning task B prevents savings (a faster relearning rate) of task A if learned after 5 minutes, but not 24 hours (Krakauer et al., 2005). Similarly, others have shown that if a task B is learned right after A, no gains in performance are apparent the following day, whereas if 4 hours is allowed between A and B, the participants demonstrate better performance on the retest of A the following day (Brashers-Krug et al., 1996). These results thus suggest that 4 hours would be sufficient for the memory trace to be consolidated, as shown by no susceptibility to interference.

Importantly, because savings or gains in performance have been observed multiple times following 4-6 hours of daytime, the off-line consolidation of motor adaptation does not seem to be dependent on sleep. Yet Huber and colleagues have found otherwise. They used a visuomotor adaptation task where subjects learned anti-clockwise rotations of 15 degrees, then 30, 45 and 60 degrees. When two groups were retested after a night of sleep or the passage of daytime, increase on the accuracy measure was only observed after a night of sleep (Huber et al., 2004). Yet, more recently Doyon and colleagues have corroborated studies suggesting no specific effect of sleep (Doyon et al., 2009). They used 3 groups of participants executing the MA task: the first one napped during daytime, the second one slept during night time and the last one remained awake during daytime. The results between the test and retest sessions revealed no interaction between the groups, but significant delayed gains in performance in all groups, suggesting that sleep did not play a significant role. The apparent discrepancy in the results might be due to methodological differences. For example, motor adaptation in Doyon et al's study was assessed using a joystick and not a robotic arm, and only one single rotation was learned (180°), as opposed to Huber's study in which multiple rotations were learned. In addition, the behavioral measures used in these two studies were different, which could also account for the divergence of the results. Indeed, Albouy et al. (2012) examined the effect of sleep on 7 different behavioral parameters. They found a protective effect of sleep, as reflected by a stabilisation of performance on most behavioral measures, except for the directional error in reaching a target. The error in the trajectory executed was found to decrease following both sleep and the simple passage of time, suggesting that time was sufficient for at least some consolidation to occur. Thus the role of daytime vs. sleep in the consolidation process for motor adaptation certainly differs from the one of motor sequence learning. The differential pattern of results between the two types of motor learning points out to the heterogeneity of the effect of sleep on motor memory consolidation. For this reason it is an opportunity to compare, using the same protocol, the effect of sleep vs. the simple passage of day time on the consolidation of both of these types of learning.

In order to better appreciate neuroscientific findings on the neural correlates associated with the behavioral changes described above, I will first describe some of the basic characteristics of sleep and the mechanisms susceptible to play a role in consolidation.

1.4. Sleep: Influences on the Consolidation Process

1.4.1. Sleep Architecture

Sleep can be divided into two states, REM and NREM (Figure 7). NREM sleep comprises 4 stages characterized by different levels of neural synchronization, from lower synchronization in lighter sleep stages (1 and 2) to higher synchronization in deeper stages 3 and 4 (slow-wave sleep; SWS). Multiple electroencephalogram (EEG) rhythms define NREM sleep: the transition of alpha waves, present during wakefulness (8 to 13 Hz), to theta waves (4-7 Hz) in stage 1; sleep spindles (12-15 Hz) and “K-complex” in stage 2; and finally SWS is characterized by delta waves (1-4 Hz), that are prominent in stages 3 and 4. REM sleep, by contrast, is characterized by EEG activation that is unsynchronized, closer to what is found during wake states. Muscle atonia and episodic burst of rapid eye movements are also characteristics of REM sleep. Although the organisation of these stages is quite stable across individuals and across one night of sleep, SWS sleep is more prominent at the beginning of the night and increases as a function of previous wakefulness. SWS gradually decreases with sleep, while REM sleep becomes more prominent at the end of the night.

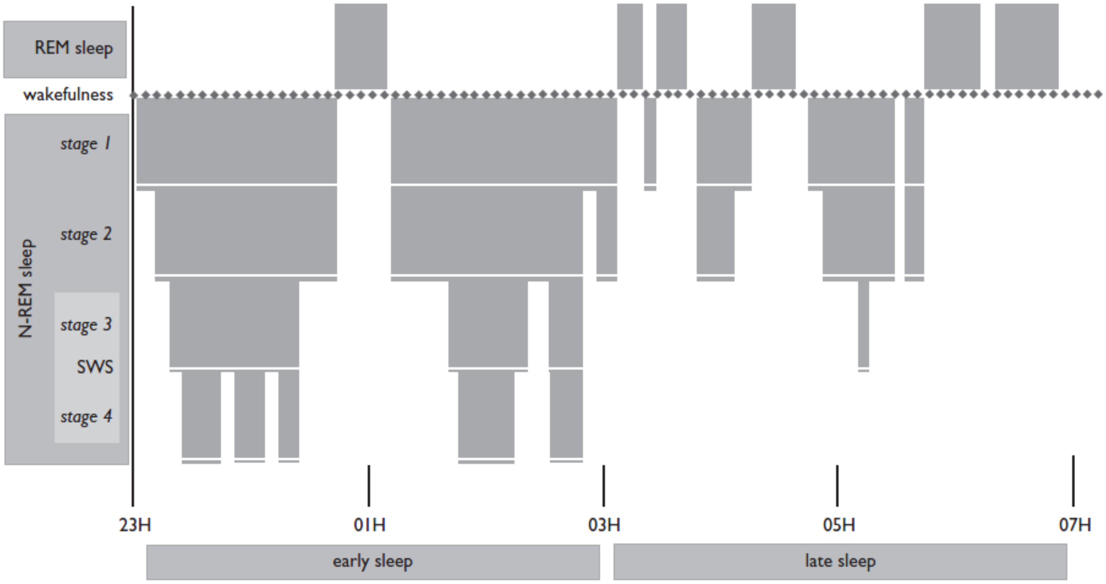

Figure 7 : Sleep architecture – adapted from Peigneux et al. (2001)

1.4.2. Physiological mechanisms associated with sleep

If it is mostly agreed upon that sleep does contribute to consolidation, how it does so is however unclear. Animal studies have shown that patterns of activation present during the behavioral learning period, were reactivated during sleep (see Maquet, 2001). For example, the firing sequences of place cells from the hippocampus during a spatial learning task were reactivated during the post-learning sleep period, more precisely during slow wave sleep (Lee and Wilson, 2002). In humans, critical motor areas that were active during daytime SRT learning, were found to be reactivated during REM sleep using PET (Maquet et al., 2000a). Furthermore, these changes in activity were paired with post-sleep behavioral improvement. These results thus suggested the possibility that memory traces were reprocessed during REM sleep. Maquet suggested, based on the animal and human literature of sleep and memory, that sleep was a privileged period for memory consolidation because it allows the reactivations of neuronal ensembles to occur (*Replay hypothesis*) (Maquet, 2001). Since then, accumulating evidence of cell replay has been observed not only in the hippocampus, but also in the striatum, prefronal cortex and the primary visual cortex (see Buhry et al., 2011 for a review). It is believed that some sleep characteristics during SWS (sharp-waves/ripples, brief high-frequency bursts) could induce long term potentiation (LTP) and thus result in neuronal plasticity and consolidation. Reactivation of the regions previously engaged during learning, in the post-learning sleep period, is also reported in humans. Multiple regions, including the cuneus, striatum and hippocampus demonstrated reactivation during sleep period that followed an SRT task or spatial learning task (Peigneux et al., 2003, Peigneux et al., 2006) .

Another, non-mutually exclusive, mechanism that could explain the benefit of sleep on consolidation is the *synaptic homeostasis hypothesis* (Tononi and Cirelli, 2003). The latter focuses on a general restorative role for sleep that can result in post-sleep higher performances. Their argument is based on the idea that daytime experiences, including learning, results in greater synaptic weight, because of the LTP-like changes in the brain. Their hypothesis states that slow-wave activity would increase as a function of the synaptic potentiation that has occurred during previous wakefulness, promoting a downscaling of synapses (a decrease in synaptic strength). This process would be restorative in the sense that neuronal synaptic weight would progressively return to baseline level, achieving *synaptic homeostasis*. The synaptic weight from accurate learning being stronger than the one coming from errors, the assumption is that the restorative effect of sleep would increase the difference between the two types of signal, so that synapses contributing to errors would cease to interfere following sleep, giving rise to post-sleep increases in performances. Support for this hypothesis, in humans, come from a study actually showing increases in SWS following the learning of a visuomotor task. Additionally, the participants presented gains in accuracy which were not observed for a control group spending the day awake (Huber et al., 2004).

1.4.3. Role of sleep for motor memory consolidation

To better understand the role of sleep in memory consolidation, numerous studies have tried to identify particular sleep characteristics or a sleep stage that would be responsible for consolidation. The findings in the declarative memory domain have suggested a role for SWS, particularly for episodic memory and of REM for semantic memory (see Rauchs et al., 2005 for a review). The first studies in procedural memory consolidation have suggested a role for REM sleep (see Peigneux et al., 2001), yet the numerous studies that explored the post-learning sleep changes following motor learning have since led to a great variety in the results. Indeed, using explicit MSL, the amount of stage 2 (Walker et al., 2002) or of NREM sleep (Robertson, 2004) increased in the post-learning sleep period and correlated with overnight improvement. Spindles, which are more frequent in stage 2 of NREM sleep, have also been associated with higher post-sleep performances (Korman et al., 2007, Nishida and Walker, 2007, Albouy et al., 2013a). The number and duration of spindles (Morin et al., 2008), as well as the density of spindles (Barakat et al., 2010) in the post-learning sleep period have been found to correlate with overnight gains in performance. Higher expectancy to be retested on MSL the following morning was also linked to an increased proportion of sleep stage 4 and an increased number of spindles during SWS (Wilhelm et al., 2011). Our group has also shown that the amplitude of spindles in the post-learning sleep period was correlated with 1) overnight gains in performance, and 2) changes in activity, the following morning, in the putamen/globus pallidus, the cerebellum, S1 and M1, among other structures. These results thus suggested a predictive role of the amplitude of spindles in the off-line consolidation process of MSL (Barakat et al., 2012b). Yet other studies have found correlations instead with the time spent in REM-sleep, using an explicit MSL task (Fischer et al., 2002, Cohen et al., 2005). The importance of the post-learning REM-sleep period is also supported by findings showing that critical motor areas activated during execution of the task, are reactivated during that period (Maquet et al., 2000a). The latter study was actually the first one in humans to support the hypothesis that regions important for consolidation are replayed during sleep. It is important to note however that this study tested the implication of

REM sleep in consolidation and did not aim at comparing the implication of REM vs. NREM sleep. Finally, slow wave sleep has also been associated with motor memory consolidation. SWS is more prominent in stages 3 and 4, and were found to increase following a visuomotor adaptation task, but not following a control task. Taken together, it is hard up to now to reach consensus as to what stages of sleep are critical for motor memory consolidation (Rauchs et al., 2005). It has to be kept in mind that both sleep and memory are heterogenous entities (Peigneux et al., 2001). Indeed, the acquisition of one motor skill depends on multiple processes and so its consolidation might depend on more than one sleep characteristic. In general, however, the results mentioned above (see Rauchs et al., 2005 for a review) support a beneficial role of REM and NREM sleep, particularly stage 2 and sleep spindles (Fogel and Smith, 2011), for the consolidation of motor skills.

Because the role of sleep in the consolidation of motor skill is now acknowledged and better understood, some studies have started to examine brain activity changes occurring before and after sleep in order to better define the memory consolidation process. These studies are reviewed in the next section.

1.4.4. Neuronal reorganization associated with the motor consolidation process

Although there is now great consensus over the neural networks underlying the course of learning of a motor sequence or a motor adaptation learning task (Hikosaka et al., 1999, Imamizu et al., 2000, Doyon et al., 2003, Imamizu et al., 2003, Lehericy et al., 2005), less is known about the structures involved in the consolidation process following initial training.

Two studies have previously investigated sleep-related cerebral changes following MSL. One has used a sleep-deprivation paradigm (Fischer et al., 2005), while the other one compared post-sleep vs. post-wake states (Walker et al., 2005). Yet the results from these two studies are very different. Fischer et al., only found decreased activity in the right premotor cortex when retesting, two days later, a group that had a regular night of sleep following learning, vs. one that was sleep-deprived. The authors decided to test for immediate effects of sleep, thus additional experiments compared the first post-training nights of one group that slept and the other that was sleep deprived. Results demonstrated that the group that was allowed to sleep, and thus to consolidate, presented a decrease of activity in the right lateral premotor cortex, SMA, superior parietal areas (BA 7) and the cerebellum. These results were interpreted as a decreased need for conscious self-monitoring of finger movements following consolidation. In contrast, Walker et al., 2005 mainly found increases in activity related to consolidation. They compared a group that slept and a group that remained awake following a first training, but the scanning session only took place during the retest session, using an auditory paced condition allowing to control for the amount of movements. The results yielded increases of activity, for the sleep group compared with the day group, in the contralateral primary motor cortex, anterior medial prefrontal areas, right hippocampus, right ventral striatum and regions of the ipsilateral cerebellar cortex (lobules VI and VII). There were also decreased activity in the sleep group compared to the day group in bilateral parietal cortices (BA 40, 39), insular cortex and ipsilateral temporal pole (Walker et al., 2005). In summary, the results from the two studies that specifically measured the role of sleep vs. time present no overlap in the mechanisms that would be involved in the consolidation process. Furthermore, the two paradigms were very different from one another, each of them presenting their advantages and disadvantages. Consequently, it is thus difficult to draw any conclusion as to the neural networks involved in sleep-dependent consolidation of MSL.

Cerebral correlates of implicit motor sequence learning were explored by Albouy et al. (2008) with the use of an occulomotor sequence learning task. They used multiple delayed retest sessions across the day and following nocturnal sleep. Their findings revealed that for fast learners, there was significant activity in the hippocampus during initial learning, which correlated with the amount of gains in performance observed in a 24hr delayed retest session. Importantly, following the night of sleep, the areas involved in the learned, as opposed to the unlearned sequence, included the hippocampus together with the ventral striatum (Figure 8), suggesting the importance of these structures for consolidation. Yet more recently, the same group of authors used sleep deprivation as control, and found for the latter group an increased activity in the cingulate cortex and the putamen three days following training. While the group that was allowed to sleep showed increased activity in the hippocampus and medial prefrontal cortex. These results thus suggested that activity in the striato-cortical network may not depend on the first post-learning sleep period (Albouy et al., 2013b).

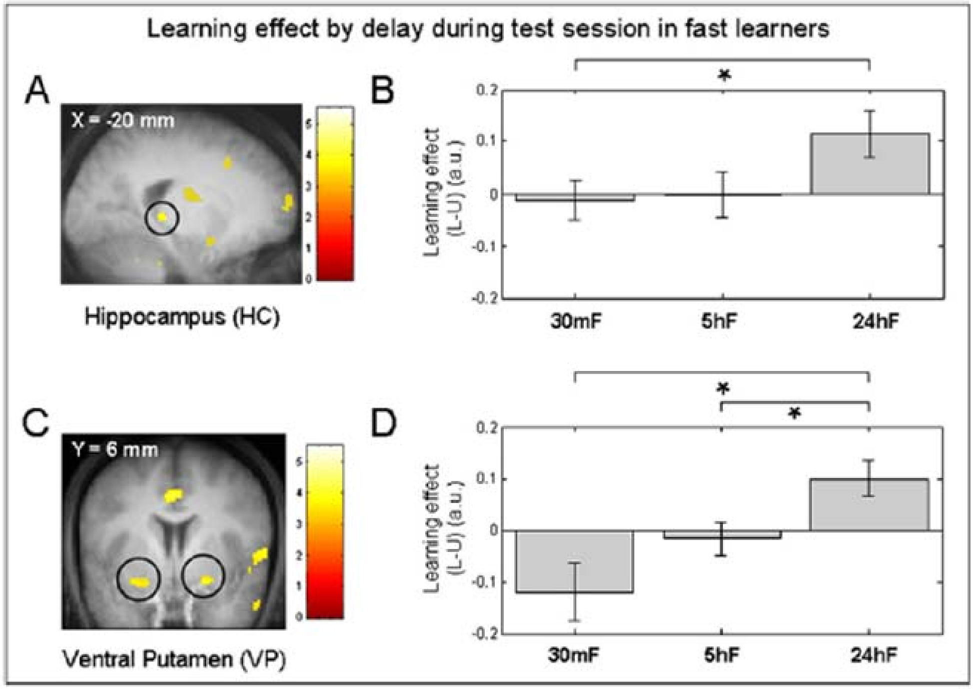

Figure 8 : Activity of the hippocampus and striatum with learning

From Albouy et al., (2008). Functional contrast above is the interaction between learning and the differed delays of fast learners. A and B represent activity in the left posterior hippocampus which was significantly larger after a 24hr delay as compared with a 30 min. delayed retest. C and D represent activity in bilateral ventral putamen which was significantly larger at 24 hr delay as compared with the 30 and 5 min. delayed retest.

Looking at motor adaptation consolidation, time alone has been shown to trigger activity changes associated with the consolidation process. Indeed, 5.5 hours after initial training, changes in activity in the contralateral PMd, contralateral posterior parietal cortex (BA 7) and ipsilateral cerebellum using PET were observed. In contrast, a delayed retest on a new type of adaptation did not show similar changes (Shadmehr and Holcomb, 1997). Others who examined the consolidation process of MA emphasized the role of the ipsilateral parietal cortex. Huber et al., (2004) compared the post-learning sleep period of a reaching task which required or not motor adaptation. Using EEG during the first 2 hours of NREM sleep, the authors reported increased slow wave activity over the right parietal electrodes (BA 40 and 7) in the first 30 minutes of NREM sleep. Furthermore, this physiological change over sleep correlated with behavioral improvements the following morning. They concluded that the results confirmed a role of sleep in the local plastic changes associated with learning, and suggested that slow oscillations might help synaptic consolidation at the local level. These results are quite convincing, yet because consolidation also happens during daytime for this type of learning, one wonders if the changes related to post-sleep or post-wake are the same.

In conclusion, there is no consensus up to now amongst the few studies that have attempted to characterize the neural substrates involved in the motor consolidation process. Studies using sleep deprivation paradigm were used and require testing three days following initial training for fatigue to dissipate. Thus no study has directly compared post-learning changes associated with sleep vs. the same amount of time spent awake, in order to clearly attribute any changes to sleep *per se*. Furthermore, no qualitative comparison between the motor adaptation and the motor sequence tasks has ever been made at the functional imaging level, which in that case would provide a comparison between a sleep dependent and possibly sleep independent motor consolidation process.

Connectivity Changes in Motor Skill Consolidation

Changes in functional connectivity, specifically related to the consolidation process that takes place during sleep (for MSL) and during the passage of time (for MA), have barely been investigated. One group of researchers explored connectivity changes in post-learning REM sleep period using an implicit SRT task and found that the dorsal premotor cortex, SMA and the posterior parietal cortex showed enhanced functional relationship during post-learning REM sleep period. Although we know, based on studies using standard approaches, that the activity in cortico-striatal areas changes with motor sequence learning, evidence of changes at the connectivity level in a cortico-striatal system, in association with consolidation are presently lacking (Dayan and Cohen, 2011).

Aim and hypothesis

In summary, although the neural correlates of motor learning in the short or long term are starting to be well characterized, the neural changes associated with the consolidation process *per se* remain unclear. More specifically, we know that consolidation depends on sleep for some types of learning, but not others, suggesting that different types of mechanisms are in place. The aim of the present thesis project was to bring new insights into the neural correlates and the dynamics of functional networks associated with the consolidation of motor skills, particularly motor sequence learning, which is known to be sleep-dependent.

The first article of this thesis will more precisely address the neural correlates of sleep-dependent and sleep-independent motor consolidation for two types of motor learning: motor sequence learning and motor adaptation. We aimed to do so by directly comparing a state where behavioral asymptotic performance is reached, with either the post sleep state or after the simple passage of daytime. For this purpose, fMRI was used to compare activation maps between a Sleep group and a Wake group. For both groups, there was a 12-hour delay between the test and re-test sessions, which included either sleep and polysomnographic measures for one group or the simple passage of daytime while staying awake in the laboratory for the other group. This procedure was the same for both motor sequence learning and motor adaptation. Based on animal research (Doya, 1999, Hikosaka et al., 1999) and on the model based on humans studies (Doyon and Benali, 2005b) described above, it was hypothesised that the consolidation of the motor sequence task involved sleep-dependent changes in the cortico-striatal network, more specifically in the putamen, as this structure has been found to be a key structure in multiple learning studies, (Doyon et al., 2003, Lehericy et al., 2005). Furthermore, it was predicted that the consolidation of the motor adaptation task would produce changes in the cortico-cerebellar network, independent of group belonging.

For the second paper of the thesis, we aimed to characterize the dynamic changes in connectivity taking place following the sleep-dependent consolidation process of motor sequence learning. Indeed, we firstly aimed at extending the findings deriving from article one, and explore the influence of sleep at the level of functional integration within networks. Importantly, because previous studies using functional connectivity mostly used specific seed regions, based on a priori knowledge to explore motor sequence consolidation; a general view of the systems associated with the latter process and the way they are influenced by sleep is presently lacking. We used a novel bayesian method which has been proposed to quantify changes in connectivity. This original approach combines both data-driven (Perlbarg et al., 2007) and *a priori* knowledge of the predicted networks of interest. A multivariate measure of integration, quantifying the level of brain interaction via a measure of statistical dependence between the regions of a system, is available in Netbrainwork toolbox. In that manner, using the same data as described for article 1, we were able to quantify changes of integration within networks before and after either sleep or the simple passage of daytime. This was done firstly in a network for which we chose our own regions of interest, i.e. by selecting motor cortical and subcortical regions known to contribute to motor sequence learning and/or consolidation. Secondly, we tested how spatially specific the results were at the systems level by using a data-driven approach.

Chapter II: Research articles

2. Article 1 “Brain plasticity related to the consolidation of motor sequence learning and motor adaptation”

Statement of contribution for all authors

Julie Carrier, Abdallah Hadj Tahar, Avi Karni, Leslie G Ungerleider, Habib Benali and Julien Doyon participated in the design of the experiment and elaboration of the objectives and hypotheses.

Karen Debas participated in the acquisition of the data, did a literature review, analysed behavioral and functional imaging data for the two types of tasks and wrote the manuscript.

Pierre Orban and **Gilles Vandewalle** participated in the fMRI analyses.

Ovidiu Lungu participated in the behavioral statistical analyses.

Marc Barakat and Pierre Bellec edited the manuscript

Julien Doyon, as supervisor, participated in all the steps enumerated above.

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Title: Brain plasticity related to the consolidation of motor sequence learning and motor adaptation

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Abstract

This study aimed to investigate, through fMRI, the neuronal substrates associated with the consolidation process of two motor skills: motor sequence learning (MSL) and motor adaptation (MA). Four groups of young healthy individuals were either assigned to: a) a Night/sleep condition in which they were scanned while practicing a finger sequence learning task or an eight-target adaptation pointing task in the evening (test), and were scanned again 12 hrs later in the morning (retest), or b) a Day/awake condition in which they were scanned on the MSL or the MA tasks in the morning, and were re-scanned 12 hours later in the evening. As expected and consistent with the behavioral results, the functional data revealed increased test-retest changes of activity in the striatum for the Night/Sleep group as compared to the Day/awake group in the MSL task. By contrast, the results of the MA task did not show any difference in test-retest activity between the Night/sleep and Day/awake groups. When the two MA task groups were combined, however, increased test-retest activity was found in Lobule VI of the cerebellar cortex. Together, these findings highlight the presence of both functional and structural dissociations reflecting the off-line consolidation processes of MSL and MA. They suggest that MSL consolidation is sleep-dependent and reflected by a differential increase of neural activity within the cortico-striatal system, while MA consolidation necessitates either a period of daytime or sleep and is associated with increased neuronal activity within the cortico-cerebellar system.

Introduction

Motor memory consolidation refers to the “off-line” process by which a memory trace initially labile becomes more robust and fixed. Accumulated evidence has shown that sleep contributes to this physiological process, but that its effect depends on the nature of the motor learning demands (see Diekelmann et al., 2009 for a review). For example, several researchers have demonstrated that the consolidation of a newly learned sequence of movements (motor sequence learning [MSL]) acquired through explicit mechanisms is sleep-dependent, as performance gains have been observed after nocturnal sleep, but not after the simple passage of time (Fischer et al., 2002, Walker et al., 2002, Morin et al., 2008, Doyon et al., 2009c). By contrast, the role of sleep in the consolidation of skills, in which subjects have to adapt to sensorimotor perturbations (motor adaptation [MA]), has been more controversial. While Huber and colleagues (2004) have reported that better performance on such task was only observed in subjects who slept following training, Doyon et al (2009) have recently demonstrated that similar performance gains could be seen after a night of sleep or an equivalent period of daytime.

Numerous studies have previously demonstrated that the striatum, cerebellum and motor-related cortical regions play a critical role into the acquisition of MSL and MA skill behaviors (Hikosaka et al., 1999, Doyon et al., 2002, Doyon and Benali, 2005b). Investigations of the brain regions mediating the consolidation process of these motor abilities, and how they relate to sleep, however, have revealed inconsistent findings. While sleep-dependent post-training improvement in performance on MSL has been associated with a reduction in brain activation in prefrontal, premotor and M1 areas, as well as increased activity in parietal regions (Fischer et al., 2005), a different pattern of results characterized by increases of activation in M1, medial prefrontal, hippocampus and cerebellum, along with a decrease of activity in parietal cortices have also been reported (Walker et al., 2005). Furthermore, the neural correlates associated with the consolidation of MA skill during sleep have never been fully studied. Using electroencephalography (EEG), it was suggested that slow wave activity localised in the parietal cortex contribute to the consolidation of a rotation adaptation task during sleep (Huber et al., 2004). Yet EEG recordings do not allow to measure sub-cortical activity changes in purportedly critical structures like the cerebellum.

The goal of this study was to determine, using functional magnetic resonance imaging (fMRI), the cerebral structures affected by the off-line consolidation of both MSL and MA skills, and how it relates to sleep versus passage of time. To do so, we compared activation maps between Night/sleep and Day/awake groups of young healthy subjects that were asked to execute motor tasks designed to measure one of these two forms of motor skills. The Night/sleep groups were thus trained and scanned in a post-training session after having reached asymptotic performance on a version of a 5-item finger sequence learning task (MSL) or an eight-target reaching task (MA) in the evening. They were then retested in the morning after a 12 hr delay encompassing a night of sleep in the laboratory. The Day/awake groups followed the same procedure, except that they were trained and scanned on either of these two tasks in the morning and retested 12 hours later in the evening (see Figure 1). Based on previous behavioural (Fischer et al., 2002, Walker et al., 2002) and imaging data (Doyon and Benali, 2005b), we hypothesised that MSL would elicit sleep-dependent changes in the cortico-striatal system, while MA would produce sleep-independent changes in the cortico-cerebellar network.

Results

Behavioral Data – Motor sequence learning. Performance on this task was measured using the execution time per sequence (TpS) for each block of trials. All subjects improved during the training session and reached asymptotic performance (for additional information regarding this initial session, see Supplemental information [SI] as only results collected while scanning are discussed below). To test for the amount of gains in performance following consolidation, a t-test comparing normalized data of the first block in the delayed retest session between the Night/sleep and Day/awake groups was used. This approach allowed us to minimise the possible influence of testing and retesting subjects at different times of day. As expected, the Night/sleep group at retest was significantly faster than the Day/awake group t(38)=12.4, p<.0001 (Figure 2A). Compared to its baseline performance in the evening, the Night/sleep group started off in the morning with a decreased TpS of 83ms (0.47 SD) in the delayed retest session, whereas the Day/awake group started off with a non-significant increase of 24ms TpS in the evening. In addition, to make sure this pattern of results was not driven by averaging across trials within the first block of the retest session (Rickard et al., 2008), the subjects' individual times to execute each sequence were analysed (see Figure 2B). The TpS were normalised on the last 5 blocks of the testing session, and the subject's performance on the first 5 sequences of the retest session was compared between the Night/sleep and Day/awake groups. Results of the t-test revealed again a significant difference between the Night/Sleep group (-.23 SD below their baseline) and the Day/awake group (.70 SD above their baseline) [t(22)=2.14, p=.04], hence demonstrating that subjects that slept experienced spontaneous gains in performance (consolidation) compared with the group that did not.

Behavioral Data – Motor adaptation. The main measure of performance on the MA task consisted of a ratio between the accuracy and the time taken to reach a target. Accuracy was calculated in pixels unit with the difference in surface (DS) between the subject's actual trajectory and the ideal one that had to be followed. Both the Night/sleep and Day/awake groups demonstrated learning across the training session and reached asymptotic performance (*see SI results*). The savings, a behavioural reflection of the consolidation process in motor adaptation learning (Krakauer et al., 2005, Krakauer and Shadmehr, 2006), were assessed by comparing the amount of execution that subjects required before reaching asymptotic performance in both test and delayed retest sessions. We found that for both

Night/sleep and Day/awake groups, there were significant savings from the immediate test to the delayed retest session, as evidenced by a significant interaction between cycles (i.e., the average performance on 8 targets) and sessions (immediate post-training test and retest) [$F_{(3,22)}$=4.30, p=.03] (Figure 2C), hence revealing a faster rate of relearning in the delayed retest session and an overall 16% increase in performance. Importantly, there was no Session X Group interaction, nor any difference between Groups [$F_{(1,22)}$<1.77, p>.20], suggesting a similar amount of savings for both the Night/sleep and Day/groups. Altogether, these results suggest that time alone was sufficient to elicit savings (consolidation) (Krakauer et al., 2005, Krakauer and Shadmehr, 2006).

Functional MRI Data: Motor sequence learning. Execution of the sequence at an asymptotic performance level during the immediate post-training test, for both Night/sleep and Day/awake groups, was associated with increased activity relative to baseline in bilateral primary motor cortex (M1), right sensorimotor cortex, cerebellum (Lobules IV, V on the left side, VIII bilaterally as well as VI and CrusII on the right), and ipsilateral putamen (at the junction of the caudate nucleus) (*see SI Table 1.1)*. To test for possible time of day effects, the immediate post-training test of the Day/awake and Night/sleep groups were compared (i.e., morning vs. evening), yet no region showed greater activity in either group. Sleep-dependent consolidation effects on the MSL task were then assessed by measuring the difference in activations between the delayed retest and immediate post-training test sessions in the Night/sleep group compared to the Day/awake group ($\text{Night/sleep}_{\text{delayed>immediate}}$ > $\text{Day/awake}_{\text{delayed>immediate}}$). As predicted, significant activations were found bilaterally in the basal ganglia (Figure 3A), and more specifically in the globus pallidus (Gp) and putamen ventrally. Other less extended activated regions at $P_{FDRcorr}$<0.05 included the left temporal pole, right superior temporal gyrus, left superior and middle frontal gyrus (see Table 1.1). Activity in the right putamen further showed that from the immediate to the delayed retest, activation in that region slightly increased for the Night/sleep group, but significantly decreased for the Day/awake group (Figure 3A). Note that a global conjunction analysis of the delayed > immediate test contrast revealed no region for which both Night/sleep and Day/awake groups had common increased activity, further suggesting that sleep had a differential effect on the off-line consolidation process. Finally, multiple regression analyses revealed significant correlations between the change in brain activity recorded in the cerebellum (Lobule VIII and Crus I) from the test to the retest session and the subject's gain in performance after sleep (*see SI Table 2.1*).

Functional MRI Data: Motor adaptation. Execution of the MA task during the immediate post-training test in both groups was associated with increased activity relative to baseline in cortical and subcortical regions including the M1, supplementary motor area (SMA), cerebellum, primary somatosensory cortex (S1), basal ganglia and thalamus bilaterally *(see SI Table 1.2)*. We also tested the possibility that time of day could influence the blood oxygenated level dependent (BOLD) signals during the first scanning session, but again the latter analysis did not reveal any significant effect, hence suggesting that the pattern of activation associated with early MA learning was not related to circadian effects. Furthermore, the Group X Session interaction revealed no significant brain activity (Table 1.2), suggesting that the consolidation process yielded similar activated maps in both groups, regardless of the type of delay (i.e. sleep or passage of time). Consolidation was therefore assessed through a global conjunction analysis of the two groups in the delayed > immediate contrast. This contrast revealed that activity in the right cerebellum (lobule VI) (X= 36, Y= -72, Z= -21), was significantly greater in the retest than in the test session ($svc_{10mm,}$ $P_{Fwe\text{-}corr}$=.04) (Figure 3B). Finally, multiple regression analyses revealed that this region of the cerebellum (Lobule VI), albeit slightly anterior (X = 36, Y = -60, Z = -21), correlated with the amount of savings found between the two sessions in the two groups (Figure 3B) *(see SI Table 2.2)*.

Discussion:

This study aimed at identifying the brain regions associated with the “off-line” consolidation process of two forms of motor skill learning: MSL and MA. As expected, the behavioural results yielded significant spontaneous gains in performance on an explicitly known MSL task that were observed following a night of sleep, but not after an equivalent diurnal period of time (Fischer et al., 2002, Walker et al., 2002, Morin et al., 2008, Doyon et al., 2009c). Importantly, such improvements were present as soon as participants began executing the sequence task in the retest session. This suggests that performance gains were not due to data averaging across trial blocks, hence masking a simple end product of continued learning within that session (Rickard et al., 2008), but rather that they reflect the expression of a real motor memory consolidation process.

By contrast, the amount of savings observed on the MA task was similar in both Night/sleep and Day/awake groups, thus implying that time alone is necessary, but sufficient, for consolidation of that memory trace to occur (Shadmehr and Brashers-Krug, 1997, Krakauer et al., 2005, Doyon et al., 2009c). The latter results do not corroborate those of Huber and colleagues (2004) who reported evidence of a sleep-dependent consolidation effect on a rotation adaptation task, but this apparent discrepancy may be explained by methodological differences in the two studies, as they differed in terms of their demands on upper-arm effectors and extent of kinematic adaptation needed.

Although the design of the present study does not allow us to exclude entirely the possibility of circadian influences on our pattern of results, most of the evidence to date suggests that such a confounding factor does not seem to play a major role in motor memory consolidation. Indeed, our own behavioural and imaging data did not yield any significant functional difference between the first evening and morning sessions. Gains in performance on motor sequence learning have also been demonstrated after diurnal sleep (Fischer et al., 2002) or following an afternoon nap of 90 minutes (Korman et al., 2007), hence suggesting further that the present results are probably not due to circadian effects, but rather to the sleep-dependent consolidation process of a motor memory trace.

Motor memory consolidation: Functional imaging data. The present study demonstrates that, as predicted, the effects of sleep and passage of time on motor memory consolidation of skills measuring MSL or MA are associated with distinct neuronal changes. For the MSL task, activity within the basal ganglia (and in the putamen, in particular) was significantly greater during the retest session in subjects who slept than in those who did not. Brain regions involved in the execution of the task after sleep included the striatum, as well as the cerebellum (lobules IV-V and VIII), bilateral primary motor cortex, the right sensory cortex and the supplementary motor area (SMA). Yet, only the striatal activity was strongly influenced by a night of sleep relative to daytime. Because scanning did not take place during sleep, it is not possible to state wether the striatum has an active role during sleep or appears as a consequence of it. Nevertheless, our results suggest that this structure does not only reflect the consolidation of a newly learned sequence of movements (Hikosaka et al., 1999, Doyon et al., 2002, Doyon et al., 2003, Doyon et al., 2009a), but that this physiological process is facilitated by sleep. Indeed, increases of activity within the striatum have previously been related to the acquisition of wrist movement sequences (Debaere et al., 2004, Puttemans et al., 2005, Remy et al., 2008) *per se*, as well as the learning of MSL, as opposed to the mere increase in speed of finger movements (Orban et al., 2010). Moreover, such increase in striatal activity has also been seen following motor memory consolidation when sleep (Walker et al., 2005), or a 24-hour delay including sleep is present after initial learning (Albouy et al., 2008). Altogether these findings suggest that sleep is critical for the striatum to assure its role following the consolidation of MSL.

Contrary to other researchers (Albouy et al., 2008), our results did not reveal any correlation between the amount of performance gains seen after sleep and the level of BOLD activity within the basal ganglia. Instead overnight gains were positively correlated with bilateral activity within the cerebellum (Lobule VIII A and B, Crus I). Although seemingly contradictory, the latter results are thought to reflect the inter-relationship existing between the subject's level of brain activation in those regions and the speed of execution of the motor sequence, rather than the process of consolidation *per se*. Indeed activity in Lobule VIII of the cerebellum has been related to the execution of discrete movements (Habas and Cabanis, 2008) and to the motoric implementation of a learned sequence, but not to the learning process as such (Orban et al., 2010).

In contrast to the sleep-dependent changes in the basal ganglia seen after MSL consolidation, sleep-independent functional changes in Lobule VI of the cerebellum were observed following consolidation of the newly-acquired adapted motor skill. Moreover, activity within this lobule was strongly correlated with the amount of savings that subjects exhibited in the retest session. These findings are consistent with our behavioural data, which demonstrated that both sleep and daytime produce better performance in the retest session. They also suggest that sleep does not bear any additional effects on the consolidation process of this form of motor learning. Such results further confirm the view that this region of the cerebellum is not only involved in the building and storing of an internal model necessary to execute the MA task efficiently (Shadmehr and Holcomb, 1997, Imamizu et al., 2000), but that it is also related to the consolidation process of this skill (Doyon and Benali, 2005b). Finally, although increased activity in the right parietal cortex was found during execution of the MA task before and after the 12-hour delay, no consolidation-related activity was observed in that region. This is again inconsistent with results from Huber et al (2004), but can be due to the differences in techniques used (fMRI *vs* EEG) and the physiological state (awaken during the retest session *vs* asleep) during which signals were recorded.

The fact that striatal activity related to MSL increased after sleep, but decreased after the passage of time, supports the notion that consolidation of a new motor sequence may rely on the covert reactivation, during sleep, of the brain regions involved in learning the motor skill in the first place (Maquet et al., 2000a). Based on this hypothesis, sleep would allow a “replay” of the neural representation for the sequence mediated by the striatum, thus enhancing the robustness of the initial motor memory trace. Such interpretation is consistent with rodents’ work, which showed that reactivation during post-training sleep can be observed in the ventral striatum after animals are trained on a reward-searching procedural task (Pennartz et al., 2004), as well as with previous positron emission tomography studies in humans, which have demonstrated that regional activity recorded during training on a probabilistic serial reaction time task is re-expressed in the post-training night (Maquet et al., 2000a, Peigneux et al., 2003).

Yet cerebral signs of memory reactivation are not limited to sleep, as they have also been observed during post-learning periods of wakefulness in both animals and humans. Reverse “replaying” of cell activity immediately after maze learning experience has been shown in rodents (Foster and Wilson, 2006), while reorganization of motor sequence related cerebral activity during awake post-training periods has also been demonstrated during an unrelated, attention cognitive task (oddball) in humans (Peigneux et al., 2006). The latter authors reported that the cortico-cerebellar and cortico-striatal systems known to be involved in the acquisition phase of motor skills interact early on during wakefulness after subjects have been trained on a MSL task. Although conjectural, a similar interplay between these two neuronal systems might explain the behavioural savings seen on the MA task after a 12-hour delay during daytime. As it is, our experimental design does not allow us to test this hypothesis directly, but it is possible that the cerebellar activation seen in the retest session corresponds to the end result of an interaction between these systems, a supposition that is consistent with Doyon’s model of cerebral plasticity associated with motor skill learning. (Doyon and Ungerleider 2002, Doyon and Benali, 2005b)

Finally, why are some motor learning processes dependent on sleep for consolidation to occur while others are not? One possible answer to that question relies on the difference in the acquisition mechanisms necessitated between our two motor tasks.

Indeed, MSL was based upon explicit mnemonic processes as the subject had prior declarative knowledge of the sequence, whereas learning during MA was implicit in nature. Indeed it has been suggested that an explicit strategy for MA would be counterproductive (Mazzoni and Krakauer, 2006). Such dissociation has previously revealed sleep effect differences in the consolidation of motor sequences (Robertson et al., 2004, Robertson and Cohen, 2006), and could thus explain the results reported above based upon our versions of the MSL and MA tasks. Furthermore, considering that “replay mechanisms” are critical for memory consolidation to take place, this suggests that time alone would permit implicit information acquired during MA practice to be replayed and consolidated, as it could be done in parallel to our everyday conscious activities. By contrast, replay of motor sequence representations acquired through explicit mechanisms could interfere with our thought processing during daytime, and thus requiring sleep for a reorganisation of the neural network involved in MSL. Alternatively, another possible answer to the sleep/no-sleep issue in motor memory consolidation comes from our own results, which suggest that such effects could be due to differences in neural networks supporting these mnemonic functions. Processes dependent on the striatum would rely on sleep, while others dependent upon the cerebellum would rely on daytime alone. Although probable, however, such a working hypothesis awaits further experimental investigation.

Method:

Subjects. Forty-eight young healthy subjects (mean age 23.0 years, 32 women) participated in the present study. They were divided into four groups: Motor sequence learning (MSL) Night/sleep (n=13; mean age: 23, 8 women), MSL Day/awake (n=11; mean age: 24, 6 women), Motor Adaptation (MA) Night/sleep (n=12; mean age: 22, 9 women) and MA Day/awake (n=12; mean age: 25, 9 women). Additional details on the specifications for the participants’ selection can be found in *SI Methods*. They gave their written informed consent to participate in the study. The project was approved by the “Regroupement Neuroimagerie/Québec” Ethics Committee at the Montreal Geriatric Institute.

Experimental Design: To compare the effects of sleep and passage of time on the consolidation of both motor sequence and motor adaptation tasks, and to identify the neural correlates mediating the consolidation process of each type of motor skill, between-subject (Night/sleep-Day/awake) and within-subject conditions (test and delayed retest) were implemented. In the Night/sleep group, subjects were first trained in the evening (9:00 p.m approximately) on one of the two motor tasks in a mock scanner and reached asymptotic performance. Following this training session, subjects were moved to the MRI room and scanned using a blocked design paradigm while executing the recently learned task. They then slept in the lab while PSG were recorded (data not reported here), and were scanned the following morning 12h after (9:00 a.m. approximately) in a retest session. In the Day/awake group, subjects were trained around 9:00 a.m. in the simulator and scanned after. They were then required to spend 12 hours in the lab under supervision (during which they could only read or watch T.V.) and were retested in a second scanning session around 9:00 p.m. (Figure 1A).

Behavioral paradigms: *Motor Sequence Learning Task.* A modified version of the finger tapping task (Karni et al., 1995) with a fixed number of sequences per block was used in order to control for the number of movements executed during the training and scanning sessions. The sequence 4-1-3-2-4 (1 being the index) was explicitly known to the subjects from the start, and was executed with the non-dominant hand using a MRI-compatible response box (custom made key pad) (Figure 1B). The training, immediate post-training (test) and delayed retest sessions consisted of 8 blocks of 20 sequences each. Once the training was completed, subjects entered the MRI room. All experimental blocks started with a 2.5s instruction where the word “Sequence” appeared in the middle of the screen, followed by a green square indicating that subjects could start producing the known sequence as fast and accurately as possible. After having completed 20 sequences, the color of the square changed to red to indicate the beginning of a 15s rest period. Time per sequence (TpS) and the number of correct sequences were recorded for each block.

Motor Adaptation task. A version of an eight-target tracking task (Doyon et al., 2009c) was used to measure motor adaptation (Figure 1B). In this task, subjects are required to manipulate a joystick with their dominant hand, in order to move a cursor positioned at the center of the screen to one of 8 targets (separated by 45 degrees) following an elliptical trajectory. Contrary to the MSL task, the dominant hand was chosen here in order to be able to compare our results to those from the motor adaptation literature, and because the results of the two tasks were never compared directly. The experimental task consisted of a “reversed mode”, where the relation between movements with the joystick and direction of the cursor were inverted by 180 degrees on each trial. The training was done in the MR simulator room and consisted of 10 blocks of 64 trials in order to make sure that all subjects had reached asymptotic performance. Once lying down in the scanner with the joystick apparatus on their stomach, subjects could then watch the projection of the instructions and targets to be reached displayed on the screen via the inverted mirrors. During the immediate post-training test and delayed retest sessions, subjects were required to complete 4 runs comprising 3 blocks of 16 trials each. Each block of trials was followed by a 32s “perceptual” condition where subjects were simply asked to observe (without making movements) the ideal elliptical trajectories that they needed to execute for reaching each target in the previous block of trials. Each trial began with a white circle (0.75cm in diameter) in the middle of the screen followed by the appearance of a small green-square cursor superimposed on top of the starting point. The target represented by a red square (1.5cm large) was displayed 10 cm away from the starting point, and an elliptical line (0.5cm in thickness; 2.5cm of radius) joining the starting point and the target were both displayed at the same time. Targets appeared randomly within the 8 targets locations, constituting one cycle. Subjects were instructed to reach the target as fast and accurately as possible within a time limit of 2900ms and to stay on the target for 100ms. If subjects reached the target on time, the color of the red square changed to green, whereas if subjects had not reached the target on time, the target disappeared and the trial was considered as an error. (Please see SI *Methods* for further information on the behavioural data analysis approaches.

MRI acquisition and analysis: Brain imaging data were obtained with a 3T scanner (Magnetom Trio Siemens AG, Germany), equipped with an 8-channel head coil. A high resolution anatomical T1-weighted scan was acquired for each subject (voxel size = 1 x 1 x 1 mm^3, TR = 23ms, TE = 2.98ms, FA = 90°□; FoV 256 x 240 mm^2; matrix 256 x 256; 176 slices). Functional T2*-weighted images were also acquired using a gradient echo-planar sequence sensitive BOLD signal (voxel size = 3.75 x 3.75 x 5 mm^3; TR = 2.5s for MSL (28 volumes) and 3.2s for MA tasks (28 volumes); TE = 30ms; FA = 90°; FOV = 240 x 240 mm^2, matrix size = 64 x 64; 28 slices). A different TR was used for scanning subjects in the two tasks in order to equate the number of volumes acquired in each block of trials. Data were and analyzed with SPM 2 software (http://www.fil.ion.ucl.ac.uk/spm/software). Preprocessing steps included the realignment, coregistration of functional and anatomical images, slice timing correction, spatial normalization into the MNI-152 stereotactic space, and smoothing using a Gaussian Kernel of 6mm full-width at half-maximum (FWHM). Consistent with the behavioural analyses, fMRI analyses for the MSL task comprised only the functional volumes obtained in the last 5 blocks of the immediate post-training test and the first 5 blocks of the retest. By contrast, all of the volumes were included in the analyses of the MA task.

Statistics were derived based upon the general linear model. First, an intra-individual analysis tested the effects of interest, using linear contrasts convolved with a standard canonical hemodynamic response function (HRF), generating statistical parametric maps. Movement parameters derived from realignment of the functional volumes were not included as it is not recommended when using a block design and manual responses (Johnstone et al., 2006). Linear contrasts estimated the main effects of either MSL or MA task, relative to its respective baseline, as well as the main effect of sleep ($\text{Night}_{\text{delayed retest}}$ >$\text{Night}_{\text{immediate post-training}}$) or passage of time ($\text{Day/awake}_{\text{delayed retest}}$ >$\text{Day/awake}_{\text{immediate post-training}}$). For both tasks, the baseline consisted of their respective rest period. The statistical images obtained at the individual level were then entered into a random-effects model. Participants were put in their respective groups (Night/sleep and Day/awake), and these were modelled as two distinct regressors of interest. For both tasks, commonalities between the Day/awake and Night/sleep groups during the immediate post-training test were assessed by a global conjunction analysis revealing activity levels that were jointly significant in the two groups ($\text{Night/sleep}_{\text{immediate}} \cap \text{Day/awake}_{\text{immediate}}$). We also assessed if there were common changes between the Night/sleep and Day/awake groups in both test and retest sessions using another conjunction analysis based on the results of the Delayed retest > Immediate post-training test contrast ($\text{Night/sleep}_{\text{delayed>immediate}} \cap \text{Day/awake}_{\text{delayed>immediate}}$). Similarly, for both tasks, we assessed Group X Session interactions ($\text{Night/sleep}_{\text{delayed>immediate}}$ > $\text{Day/awake}_{\text{delayed>immediate}}$) to assess any specific effect of sleep as opposed to the simple passage of time on motor memory consolidation of both motor tasks. To further assess the relationship between brain regions of the motor network and the behavioural reflection of consolidation, multiple regression analyses were carried out on the interaction and the conjunction contrast analyses for the MSL and MA tasks, respectively. The gain in TpS was used as the predictor for the MSL task, while the amount of savings served as the predictor for the MA task. All activation maps reported below are displayed at $p<0.001$ in order to better display the extent of the activity. Results that were significant at $p \leq .05$ after false discovery rate (FDR) correction (Genovese et al., 2002) for the whole brain volume are also reported in the tables. Because we formulated strong *a priori* hypotheses, we then used small volume correction (svc, radius = 10mm) for a structure of interest in which correction over the whole brain volume was too strict [cerebellum, 28, -74, -18 mm (Imamizu et al., 2003)]. In that case, we used family wise error (FWE) correction as it is known to better control for false positives than FDR (Nichols and Hayasaka, 2003).

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References

1. Diekelmann S, Wilhelm I, & Born J (2009) The whats and whens of sleep-dependent memory consolidation. *Sleep medicine reviews* 13(5):309-321.

2. Doyon J*, et al.* (2009) Contribution of night and day sleep vs. simple passage of time to the consolidation of motor sequence and visuomotor adaptation learning. *Experimental brain research. Experimentelle Hirnforschung* .

3. Fischer S, Hallschmid M, Elsner AL, & Born J (2002) Sleep forms memory for finger skills. *Proc Natl Acad Sci U S A* 99(18):11987-11991.

4. Walker MP, Brakefield T, Morgan A, Hobson JA, & Stickgold R (2002) Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron* 35(1):205-211.

5. Morin A*, et al.* (2008) Motor sequence learning increases sleep spindles and fast frequencies in post-training sleep. *Sleep* 31(8):1149-1156.

6. Hikosaka O*, et al.* (1999) Parallel neural networks for learning sequential procedures. *Trends in neurosciences* 22(10):464-471.

7. Doyon J*, et al.* (2002) Experience-dependent changes in cerebellar contributions to motor sequence learning. *Proc Natl Acad Sci U S A* 99(2):1017-1022.

8. Doyon J & Benali H (2005) Reorganization and plasticity in the adult brain during learning of motor skills. *Current opinion in neurobiology* 15(2):161-167.

9. Fischer S, Nitschke MF, Melchert UH, Erdmann C, & Born J (2005) *Motor Memory Consolidation in Sleep Shapes More Effective Neuronal Representations* (Journal of Neuroscience Vol 25(49) Dec 2005, 11248-11255).

10. Walker MP, Stickgold R, Alsop D, Gaab N, & Schlaug G (2005) Sleep-dependent motor memory plasticity in the human brain. *Neuroscience* 133(4):911-917.

11. Huber R, Ghilardi MF, Massimini M, & Tononi G (2004) Local sleep and learning. *Nature* 430(6995):78-81.

12. Rickard TC, Cai DJ, Rieth CA, Jones J, & Ard MC (2008) Sleep does not enhance motor sequence learning. *Journal of experimental psychology* 34(4):834-842.

13. Krakauer JW, Ghez C, & Ghilardi MF (2005) Adaptation to visuomotor transformations: consolidation, interference, and forgetting. *J Neurosci* 25(2):473-478.

14. Krakauer JW & Shadmehr R (2006) Consolidation of motor memory. *Trends in neurosciences* 29(1):58-64.

15. Shadmehr R & Brashers-Krug T (1997) Functional stages in the formation of human long-term motor memory. *J Neurosci* 17(1):409-419.

16. Korman M*, et al.* (2007) Daytime sleep condenses the time course of motor memory consolidation. *Nat Neurosci* 10(9):1206-1213.

17. Doyon J*, et al.* (2003) Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. *Neuropsychologia* 41(3):252-262.

18. Doyon J*, et al.* (2009) Contributions of the basal ganglia and functionally related brain structures to motor learning. *Behavioural brain research* 199(1):61-75.

19. Remy F, Wenderoth N, Lipkens K, & Swinnen SP (2008) Acquisition of a new bimanual coordination pattern modulates the cerebral activations elicited by an intrinsic pattern: an fMRI study. *Cortex* 44(5):482-493.

20. Debaere F, Wenderoth N, Sunaert S, Van Hecke P, & Swinnen SP (2004) Changes in brain activation during the acquisition of a new bimanual coodination task. *Neuropsychologia* 42(7):855-867.

21. Puttemans V, Wenderoth N, & Swinnen SP (2005) Changes in brain activation during the acquisition of a multifrequency bimanual coordination task: from the cognitive stage to advanced levels of automaticity. *J Neurosci* 25(17):4270-4278.

22. Orban P*, et al.* (2010) The multifaceted nature of the relationship between performance and brain activity in motor sequence learning. *Neuroimage* 49(1):694-702.

23. Albouy G*, et al.* (2008) Both the hippocampus and striatum are involved in consolidation of motor sequence memory. *Neuron* 58(2):261-272.

24. Habas C & Cabanis EA (2008) Neural correlates of simple unimanual discrete and continuous movements: a functional imaging study at 3 T. *Neuroradiology* 50(4):367-375.

25. Shadmehr R & Holcomb HH (1997) Neural correlates of motor memory consolidation. *Science (New York, N.Y* 277(5327):821-825.

26. Imamizu H*, et al.* (2000) Human cerebellar activity reflecting an acquired internal model of a new tool. *Nature* 403(6766):192-195.

27. Maquet P*, et al.* (2000) Experience-dependent changes in cerebral activation during human REM sleep. *Nature neuroscience* 3(8):831-836.

28. Pennartz CM*, et al.* (2004) The ventral striatum in off-line processing: ensemble reactivation during sleep and modulation by hippocampal ripples. *J Neurosci* 24(29):6446-6456.

29. Peigneux P*, et al.* (2003) Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. *Neuroimage* 20(1):125-134.

30. Foster DJ & Wilson MA (2006) Reverse replay of behavioural sequences in hippocampal place cells during the awake state. *Nature* 440(7084):680-683.

31. Peigneux P*, et al.* (2006) Offline persistence of memory-related cerebral activity during active wakefulness. *PLoS biology* 4(4):e100.

32. Della-Maggiore V & McIntosh AR (2005) Time course of changes in brain activity and functional connectivity associated with long-term adaptation to a rotational transformation. *Journal of neurophysiology* 93(4):2254-2262.

33. Doyon J & Benali H (2005) Reorganization and plasticity in the adult brain during learning of motor skills. *Curr Opin Neurobiol* 15(2):161-167.

34. Mazzoni P & Krakauer JW (2006) An implicit plan overrides an explicit strategy during visuomotor adaptation. *J Neurosci* 26(14):3642-3645.

35. Robertson EM, Pascual-Leone A, & Press DZ (2004) Awareness modifies the skill-learning benefits of sleep. *Curr Biol* 14(3):208-212.

36. Robertson EM & Cohen DA (2006) Understanding consolidation through the architecture of memories. *Neuroscientist* 12(3):261-271.

37. Karni A*, et al.* (1995) Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377(6545):155-158.

38. Johnstone T*, et al.* (2006) Motion correction and the use of motion covariates in multiple-subject fMRI analysis. *Human brain mapping* 27(10):779-788.

39. Genovese CR, Lazar NA, & Nichols T (2002) Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage* 15(4):870-878.

40. Imamizu H, Kuroda T, Miyauchi S, Yoshioka T, & Kawato M (2003) Modular organization of internal models of tools in the human cerebellum. *Proc Natl Acad Sci U S A* 100(9):5461-5466.

41. Nichols T & Hayasaka S (2003) Controlling the familywise error rate in functional neuroimaging: a comparative review. *Statistical methods in medical research* 12(5):419-446.

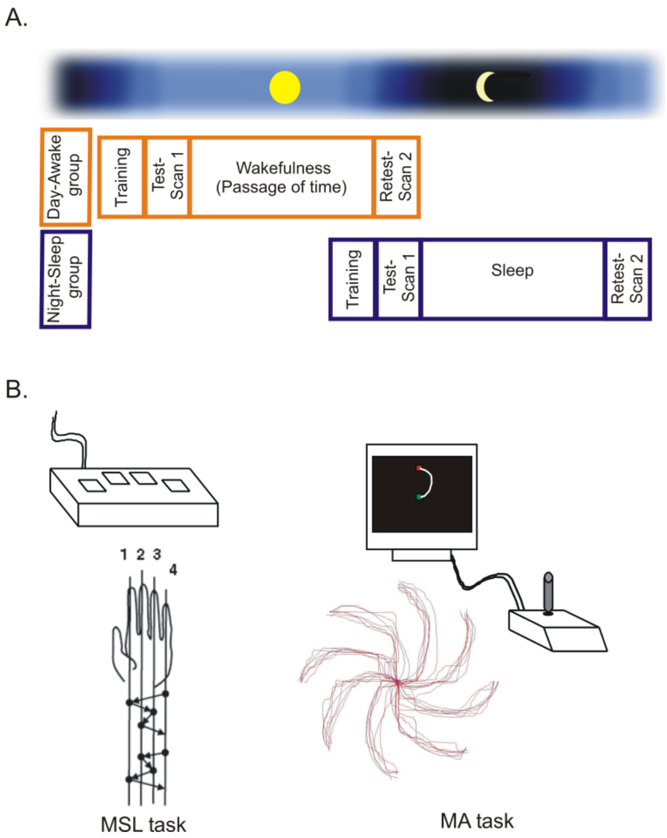

Figure 9 : Article 1 – Experimental design

Figure 1. In orange; the Day/awake group was tested and retested in the scanner while staying awake during the 12hr delay between-session. In blue; the Night/sleep group tested around 9 p.m and slept in the laboratory while polysomnographic measures were recorded.and was retested approximately two hours after waking up, around 9 a.m. B: Illustrations of the apparatus and order of finger presses used in the motor sequence learning (MSL) task, as well as the setup and performance of a representative block of trials in the motor adaptation (MA) task.

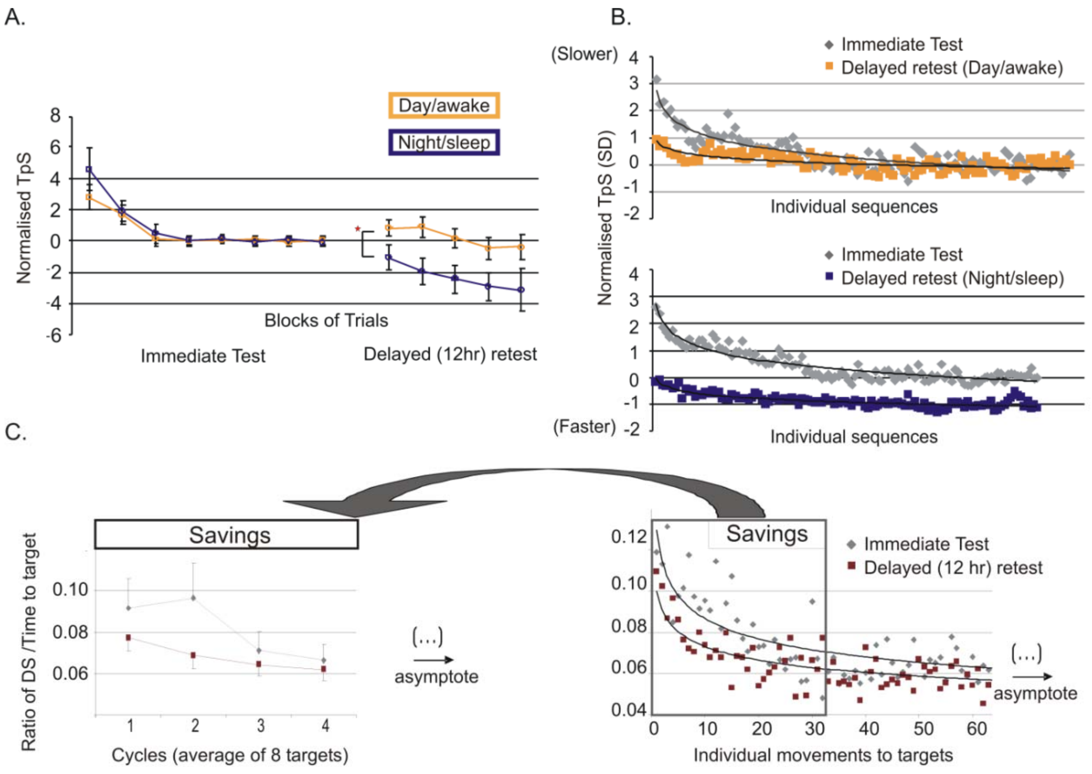

Figure 10 : Article 1 – Behavioral Results

Figure 2. A: Behavioural results of the MSL task. Performance of the two groups across blocks in both immediate and retest session are illustrated. B: This figure illustrates the groups' performance at the individual sequence level, with each curve representing a session. On top is the performance for the Day/awake group, while the one in the bottom is for the Night/sleep group. C: Behavioural results for the MA task. The Y axis represents the ratio of DS relative to the time taken to reach each target. Left graph: averaged subject's performance for each cycle. Right graph: performance on each individual movement to a single target. Each curve represents a session. Savings occurred within the first four cycles of the retest session. Yet there was no significant interaction or between-group differences (Night/sleep vs. Day/awake) with respect to the amount of savings, and thus the results of both groups were pooled together to look at test-retest differences (consolidation).

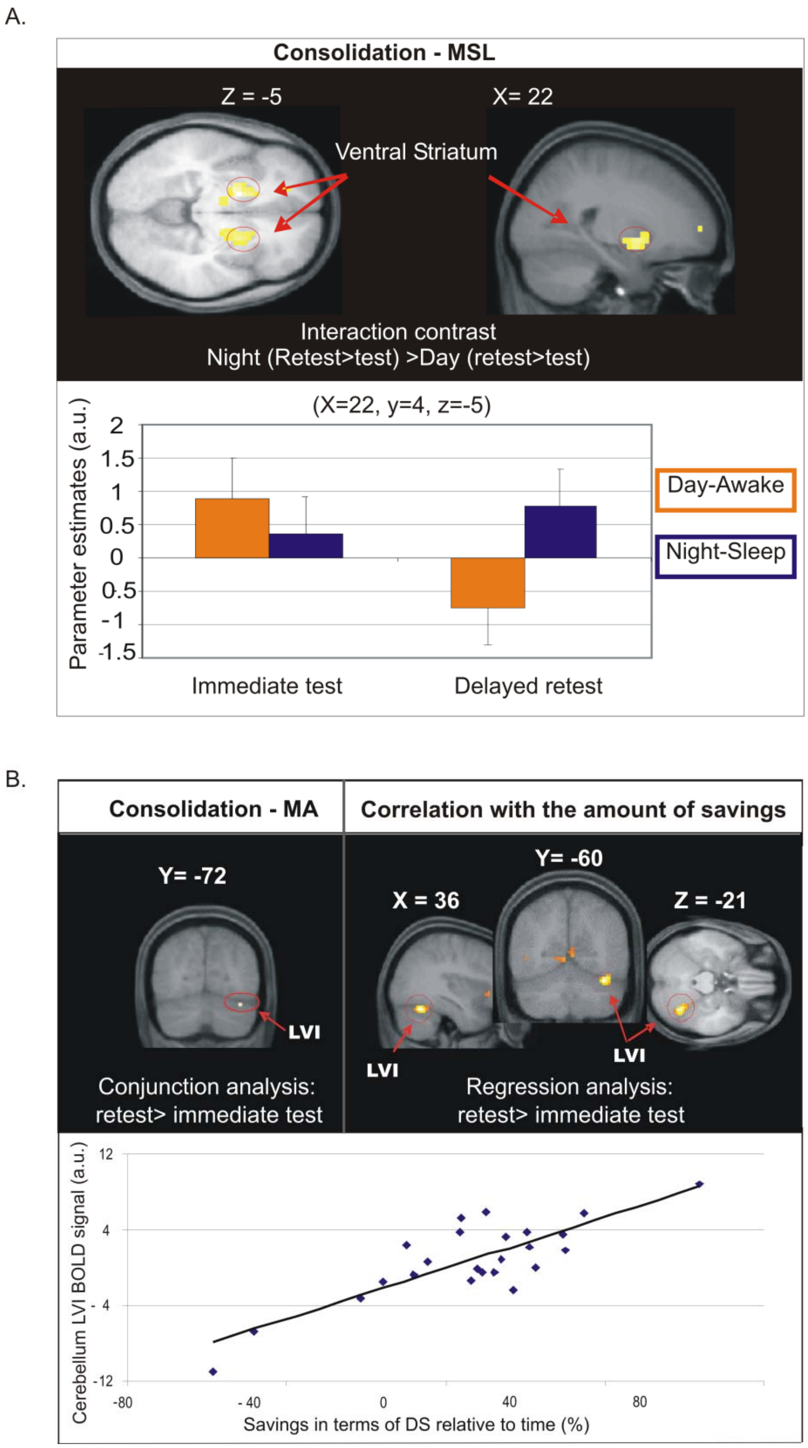

Figure 11 : Article 1- Functional imaging results

Figure 3. A: Functional data related to the MSL task. Top: Brain regions showing greater activity in the retest, compared to the immediate post-training test session for the Night/sleep group over the Day/awake group. The functional data are presented over an average of the anatomical scans (n=23) acquired in the whole group of subjects. Bottom: Bar graph of beta

values from the local maxima in the right putamen. B: Imaging results for the MA task shown on the averaged brain (n=24). Top left: Results of both Day/awake and Night/sleep groups combined, showing an increase of activity from the immediate post-training to the retest session in Lobule VI of the right cerebellum. Top right: Results of the correlation analysis between the increase in activity from the immediate post-training test to the retest session and the amount of saving observed in each subject. Bottom: Plot graph depicting a positive correlation between the amount of savings (X axis) and the strength of BOLD signal (Y axis) in Lobule VI of the right cerebellum (36, -60, -21).

Table 1. Article 1 - Effect of sleep

Brain regions showing greater BOLD activity in the Night/sleep group compared to the Day/awake group in the retest *vs.*test session.

Areas	x	y	z	$P_{FDR\text{-}corr}$	Z
1. MSLtask ($N_{(retest>test)}$ > $D_{(retest>test)}$)					
Ventral putamen L	-15	15	0	0.044	4.1
Ventral putamen R	23	4	-5	0.044	3.7
L	-15	4	-5	0.044	4.1
Temporal lobe (pole) L	-41	15	-20	0.044	4.0
(superior) R	45	0	15	0.044	3.8
Frontal lobe (superior) L	-23	53	0	0.044	3.8
R	26	56	0	0.049	3.5
Insula R	30	11	-15	0.049	3.5
2. MA task ($N_{(retest>test)}$ > $D_{(retest>test)}$)					
No significant responses	-	-	-		

2.1. Supplemental Information

Results

Training- *Motor sequence learning (MSL)***:** Subjects in both Night/sleep and Day/awake groups revealed a significant improvement in performance during the training phase, as measured by a decrease in execution time per sequence (TpS) across blocks ($F_{(7,22)}$=10.94, p<.0001). There was no significant difference between Groups ($F_{(1,22)}$=0.75, p=.40) nor any Group x Block interaction ($F_{(7,22)}$=0.41, p=.90), suggesting that Night/sleep and Day/awake groups did not differ with respect to their initial learning abilities of the MSL task. Subjects also reached asymptotic performance at the end of training (a condition known to be critical to enable consolidation [(Hauptmann and Karni, 2002)]), as there was no difference in their speed to execute the sequence across the last 3 blocks of training ($F_{(2,22)}$=0.05, p=.99). This was also true for the last 5 blocks of the immediate post-training test, which were used as baseline to measure the consolidation effect ($F_{(4,22)}$=0.06, p=.99). Accuracy measures, on the other hand, revealed no change across training in the two groups ($F_{(7,22)}$=0.91, p=.47). In the training session, performance started as high as 91% (standard deviation (SD) = 14%) and remained stable at the end of training 96 % (SD = 5%).

Motor adaptation (MA): Using the ratio between the distance in surface (DS) under the curve relative to the time to reach each target as a measure of performance on the MA task, both Night/sleep and Day/awake groups demonstrated similar learning capacities, as shown by a significant effect of blocks ($F_{(6,22)}$>20.33, p<.0001), but with no Group X Block interaction ($F_{(6,22)}$=2.13 p=.20) nor any Group differences ($F_{(1,22)}$=1.40, p=.25). Again, all subjects reached asymptotic performance at the end of the training period, as there was no difference between the last three blocks, $F_{(2,22)}$=0.23, p=.79.

Methods

Subjects: Participants for the MSL task had no experience playing a musical instrument and no training as a typist. Similarly, for the MA task, subjects who had extended practice in video games using joysticks were also excluded. All subjects were right handed, non-smokers and had no history of neurological or psychiatric disorders as well as no history of sleep disturbances or medication intake. Participants reported having a regular cycle of bedtime around 11:00 p.m. (+/- 1hour) and wake-up around 7:00 (+/- 1 hour) as they kept a sleep diary for the week prior to the study. Subjects with excessive depressive symptoms (score above 4) as measured by the short version of the Beck Depression Scale were excluded. All subjects participated to an adaptation night at the laboratory with polysomnographic (PSG) evaluations of their sleep. Subjects with an apnea-hypoapnea index >5 or a periodic limb movements index >5 were excluded. None of the subjects worked night shifts or were engaged in transmeridian trips 3 months prior to the study. During the whole experiment, subjects were asked to abstain from alcohol, nicotine and caffeine. They were also instructed to go to bed at 11:00 p.m. and wake-up at 7:00 a.m. for a week prior to the beginning of the imaging study and to abstain from taking day naps.

Behavioral data analysis-*MSL*: Two measures of interest were computed for each block: the mean time per sequences (TpS) and the accuracy, i.e. the number of correct sequences executed. A repeated-measure analysis of variance (ANOVA) on the TpS and accuracy per block; with blocks as a within-subject factor and Groups (Day or Night) as a between-subject factor, assessed the extent of learning during training. Although subjects reached asymptotic performance during training, they unexpectedly improved further during the first 3 experimental blocks of the immediate test session once in the scanner. Because we were specifically interested in comparing a stable state where asymptotic performance was reached during the test session with that of the delayed retest session, however, only the last 5 blocks of the former session and the first 5 blocks of the latter were included in the final analysis. Also, as there was great variability in the TpS between subjects, data were normalised according to the averaged TpS of the last five blocks of the immediate post-training test session for each subject individually. Hence, a t-test on the first block of the delayed retest was used to assess the difference in performance between the Night/sleep and the Day/awake groups after sleep or the passage of time, respectively. Furthermore, the performance of the first 5 sequences in the retest session was also analysed between the two groups, using an independent sample t-test.

MA task: The subject's trajectory during target reaching was represented by vectors [X_n, Y_n], and the difference in surface (DS) between the subject's vector and the ideal one was calculated in pixels unit (p) as a measure of accuracy. Time to reach a target was also computed. Because both variables showed a large amount of variation across blocks of trials, a ratio of DS (p) relative to Time to reach a target (s) was computed for each trial and averaged over cycles of eight targets each. This ratio was used as the dependent measure of interest and allowed to quantify global performance above any possible trade-offs. In order to measure the amount of savings (reflecting consolidation) that subjects exhibited in the retest session (Krakauer et al., 2005), we compared directly the amount of training that subjects required to reach asymptotic performance in both test and retest sessions using Tukey's tests of homogeneity on the number of cycles needed to attain the asymptote. While at first, subjects in the immediate test necessitated 10 cycles to reach a statistically stable level of performance, only four cycles were needed to get to an equivalent level of performance in the retest session. Consequently, only the group's performance from these first four cycles in the retest session was used in a second level of analyses. A mixed design repeated-measure ANOVA was then carried out to assess the difference in rate of learning between both test and retest sessions (cycles and sessions as within-subjects factors), as well as the possible difference between the Night/Sleep and Day/awake groups (between-subject factor).

Table 2 . SI Article 1- Test session
Brain regions revealing increased BOLD activity during the immediate post- training test in both Night/sleep and Day/awake groups.

Areas	x	y	z	$P_{FDR-corr}$	Z
1. Motor Sequence Learning task ($N_{test} \cap D_{test}$)					
Motor cortex R	41	-23	55	<0.001	5.4
	34	-15	65	<0.001	5.4
Motor cortex L	-30	-15	65	0.001	4.7
Somatosensory cortex R	38	-30	65	<0.001	5.8
Cerebellum (LIV-V) L	-4	-56	-5	<0.001	5.5
	-19	-49	-20	0.001	4.8
(LVIII) L	-4	-68	-30	0.001	4.8
	-19	-60	-50	0.020	3.6
R	30	-41	-45	0.050	3.3
(LVI) R	23	-56	-20	<0.001	5.1
(CRII) R	30	-83	-40	0.027	3.5
Ventral putamen L	-15	8	-5	0.027	3.5
2. Motor Adaptation learning task ($N_{test} \cap D_{test}$)					
Cerebellum (LIV-V) R	9	-57	-12	<0.001	8.5
(LVIII) R	21	-57	-45	<0.001	7.4
(LVIII) L	-27	-54	-48	<0.001	5.3
Motor cortex L	-39	-21	51	<0.001	7.4
Motor cortex R	54	0	33	<0.001	4.9
Sensory motor cortex R	33	-39	51	<0.001	5.3
Superior parietal R	16	-60	60	<0.001	6.5
Thalamus L	-15	-21	9	<0.001	6.0
R	12	-21	9	<0.001	3.6
Insula L	-45	-3	6	<0.001	5.2
R	33	12	9	<0.001	4.6
Temporal lobe R	45	-66	3	<0.001	5.6
Occipital L	-42	-72	9	<0.001	5.1
	-21	-90	9	<0.001	4.4
Putamen R	24	9	3	<0.001	4.6
Frontal lobe (middle) L	-33	33	27	<0.001	4.4
Supramarginal gyrus R	54	-30	33	<0.001	4.1

Table 3. SI Article 1 - Correlation with behavioral improvement
Brain regions showing increased BOLD activity in the Retest vs. Test sessions in both Night/sleep and Day/awake groups. (*) indicates that a FWE correction was used after small volume correction with a 10 mm sphere.

Areas	X	Y	Z	$P_{FDR-corr}$	Z
1. MSL Correlation with the gains in performance for the Night group (Nretest>test)					
Cerebellum (LVIII) L	-8	-64	-40	0.007*	3.45
R	15	-68	-45	0.008*	3.41
	23	-60	-55	0.018*	3.12
Crus I L	-38	-49	-40	0.006*	3.51
R	30	-56	-35	0.009*	3.35
2. MA- Correlation with the amount of savings for both Night and Day groups ($N_{retest>test} \cap D_{retest>test}$)					
Cerebellum (LVI) R	36	-60	-21	>0.001	5.88
Thalamus R	3	-24	0	0.031	4.20
Occipital lobe R	30	-84	18	0.039	4.09

3. Article II: “Off-line consolidation of motor sequence learning results in greater integration within the cortico-striatal functional network”

In preparation

Statement of Contribution:

Note that the data used for this article is the same as the one reported in the previous paper.

Julie Carrier, Abdallah Hadj Tahar, Avi Karni, Leslie G Ungerleider, Habib Benali and Julien Doyon participated in the elaboration of the design of the experiment.

Karen Debas participated in the data collection, did a literature review, elaborated the aim and hypothesis, performed all analyses with the innovative functional connectivity toolbox developed by HB’s group and wrote the manuscript.

Guillaume Marrelec and **Pierre Bellec** helped with technical and mathematical issues with the connectivity analyses.

Habib Benali’s group elaborated the functional connectivity platform.

Marc Barakat edited the manuscript.

Julien Doyon, as supervisor, participated in all the steps enumerated above.

Submitted: *Neuroimage*

Title: Off-line consolidation of motor sequence learning results in greater integration within the corstico-striatal functional network.

Running title: Connectivity and motor sequence consolidation

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Abstract

The consolidation of motor sequence learning is known to depend on sleep. Work in our laboratory and others have shown that the striatum is associated with this off-line consolidation process. In this study, we aimed to quantify the sleep-dependent dynamic changes occurring at the network level using a measure of functional integration. We directly compared changes in connectivity before and after sleep or the simple passage of daytime. As predicted, the results revealed greater integration within the cortico-striatal network after sleep, but not an equivalent daytime period. Importantly, a similar pattern of results was also observed using a data-driven approach; the increase in integration being specific to a cortico-striatal network, but not to other known functional networks. These findings reveal, for the first time, a new signature of motor sequence consolidation: a greater between-regions interaction within the cortico-striatal system.

Introduction

Contemporary theories of motor skill learning advocate that following encoding of a new motor ability, the memory undergoes “off-line” transformations allowing the initially labile trace to become somewhat fixed into the physical structure of the brain through a cascade of events occurring at both cellular and systems levels; a phase called “memory consolidation” (Dudai, 2004). A large number of studies have now convincingly demonstrated that sleep, during nighttime or daytime, plays a critical role in the off-line consolidation of some, but not all types of motor skills (see Diekelmann et al., 2009, Born and Wilhelm, 2012 for reviews). Indeed, sleep-dependent consolidation has particularly been observed following the acquisition of a new sequence of movements, as opposed to tasks requiring subjects to adapt to visuomotor changes in the environment (Doyon et al., 2009c, Albouy et al., 2012b). This mnemonic process has also been reported in conditions where motor sequences were acquired explicitly (Fischer et al., 2002, Walker et al., 2002) rather than when they are learned implicitly (Robertson et al., 2004), and more so for the allocentric (spatial) compared to the egocentric (motor) representation of a newly learned sequence of movements (Cohen et al., 2005, Witt et al., 2010, Albouy et al., 2013a).

Up to now, functional neuroimaging studies in the field have mainly attempted to target the specific brain regions mediating motor sequence learning (MSL) and consolidation. While the results have corroborated the contribution of both cortico-striatal and cortico-cerebellar systems in the acquisition of such skilled behaviours (e.g Doyon and Benali, 2005a, Floyer-Lea and Matthews, 2005, Doyon et al., 2009a), the off-line consolidation phase has been associated with increased activity in the striatum, and the putamen in particular (Debas et al., 2010), the hippocampus (Walker et al., 2005, Albouy et al., 2008), the cerebellum (Walker et al., 2005, Steele and Penhune, 2010) as well as other cortical regions including the primary motor (Steele and Penhune, 2010) and the medial prefrontal cortices (Walker et al., 2005). Interestingly, however, recent neuroimaging work has also begun to characterize the dynamic learning-dependent functional changes between cerebral regions through connectivity analyses, which are based upon correlations between time courses of brain areas (Friston et al., 1993b, Marrelec et al., 2008). Most of these studies have used hypothesis-driven, predefined motor networks or specific seed regions in order to identify the connectivity changes within or between networks during learning. Hence, MSL has been associated with greater connectivity within motor-related regions in the early learning phase of a new sequence of movements, followed by stabilization within the 2nd and 4th week of the acquisition process (Ma et al., 2010). Similarly, Sun and colleagues (2007) have found greater connectivity between the sensorimotor, premotor and the supplementary motor areas (SMA) within and between hemispheres during early learning of a bimanual motor sequence task. Yet although very informative, such an approach is limited by the fact that the pattern of changes in connectivity during learning can vary a great deal depending on the seed motor areas chosen within a given network. For example, Coynel et al. (2010) have reported decreases of integration within an associative cortico-striatal, but not within a sensorimotor cortico-striatal network, across 28 days of acquisition of an explicit sequence, hence demonstrating that the choice of motor regions within a network does have a critical effect on pattern of connectivity observed. In one study that used a data-driven approach to overcome this limit, Tamas Kincses and colleagues (2008) found that the acquisition of a motor sequence was associated with increased connectivity within a network comprising the premotor and posterior parietal cortices. Yet very few studies have investigated the change in functional connectivity related to the consolidation of a newly learned sequential motor skill (see Dayan and Cohen, 2011).

Task-related changes in connectivity between motor brain regions before and after sleep have previously been measured in order to gain insight into the brain network that could be involved in motor memory consolidation. For instance, a dynamic interplay between the hippocampus and the striatum during MSL training is known to predict overnight gains in performance (Albouy et al., 2008, Albouy et al., 2013b). While activity in the dorsal premotor cortex, posterior parietal cortex and pre-SMA were significantly correlated during REM sleep following sequence learning whereas no correlation was observed between these structures in a group that had not learned the motor sequence (Laureys et al., 2001). Despite such advances, however, it remains unclear whether such increase in connectivity possibly reflecting the consolidation process following sequence learning was strictly dependent on sleep*,* as no daytime control condition was used in either investigation. Furthermore, considering that the latter researchers analyzed connectivity changes through the use of specific seed regions based on *a priori* hypothesis, it is thus unknown whether the sleep-related changes in brain connectivity described above are specific to the motor regions mediating the learning process in the first place, or whether they can be observed in other brain networks.

The aim of the present study was thus to compare directly the changes in functional connectivity related to the consolidation process of a motor memory trace in two groups of young adults who participated in a test-retest paradigm, where motor sequence learning was measured before and after a 12 hr delay filled with either night sleep or the simple passage of daytime. Participants belonging to the Day/awake group were scanned in the morning and evening using functional magnetic resonance imaging (fMRI), while they executed a 5-element version of the finger sequence learning task (Karni et al., 1995), whereas those in the Night/sleep group were first tested on the same task in the evening, and then retested the following morning. Two different approaches in connectivity analysis were applied: First similar to studies summarized above, we used a hypothesis-driven method in which the brain activated regions forming the cortico-striatal network were selected based upon the results of a previous study that investigated the role of sleep versus the simple passage of time on the consolidation of MSL (Debas et al., 2010). Based on Doyon and colleagues' model, which predicts that the cortico-striatal system contributes to the consolidation process of a new sequence of movements (Doyon and Benali, 2005a, Doyon et al., 2009a) Doyon & Ungerleider, 2002), we hypothesized that subjects in the night group would show increased correlations between the learning-dependent motor regions (i.e., greater integration) when compared to subjects in the day group. Second, we used a data-driven approach that allowed functional network reorganization to be quantified without *a priori* assumptions. This method permitted us to measure, not only the change of integration within a cortico-striatal system before and after a night of sleep, but also, within other large-scale, functionally distinct networks extracted through an independent component analysis (ICA). It was predicted that the changes in integration after sleep, associated with off-line consolidation, would be spatially specific as it would only be observed within the cortico-striatal system, and not within the other extracted networks.

Results:

The present study uses a subset of the behavioral and fMRI data that we previously published where the results of a standard univariate approach were reported (see Debas et al., 2010, for more details).

Behavior

As previously reported in Debas et al. (2010), our behavioral results revealed significant test-retest gains in performance in participants who slept, as opposed to those who did not, further confirming the benefit of sleep in the off-line consolidation process of a newly acquired sequence of movements. Indeed, to test for the amount of gains in performance following sleep as opposed to daytime, a t-test was carried out to compare directly the results of the Night/sleep and Day/awake groups. The first two blocks of the retest session were used. These were normalized based upon the performance of the last five blocks of the testing session, where subjects had reached asymptotic performance. As expected, the results revealed a significant difference between the two groups ($t_{(1,22)} = 2.33$; $p=.03$) at retest, with the Night/sleep group performing faster than the Day/awake group.

Functional Connectivity

Network Identification

To calculate the levels of integration within the brain network associated with motor sequence consolidation, we first used a hypothesis-driven approach and identified a network comprising 7 regions of interest (ROI) which are known to be involved in the consolidation or learning of a new motor sequence **(see Table 1)**. Second, we used a data-driven approach through an ICA analysis as implemented in the Netbrainwork toolbox (http://sites.google.com/site/netbrainwork/). This toolbox allows the extraction of components statistically independent one from another. Networks were extracted for each individual, using the functional images derived from the test session while participants were performing the MSL task (i.e., active state). Networks at the group level were obtained using hierarchical clustering (see methods). ICA carried out on the entire test session, for both groups together, allowed the identification of seven, spatially independent, functional networks **(Figure 1)**. All were present in more than 72% of the participants, except for the executive network, which was present in only 54% of them, the latter being nevertheless reported here as it is a well-known functional network (Beckmann et al., 2005, Damoiseaux et al., 2006). Six of the networks (default-mode, motor, executive, fronto-parietal, visual lateral and visual medial) were spatially very similar to those previously reported in studies investigating resting-states (Beckmann et al., 2005), further supporting the relative stability of these functional networks regardless of the participant's state being either at rest or in action (Calhoun et al., 2008). A 7th large-scale network, called “*cortico-striatal”* was also identified. Multiple cortical areas were included in that network, notably the pre and post-central gyri as well as the superior and inferior parietal cortices. Yet, the strongest signal came from subcortical areas like the putamen and globus pallidus (**see Table 1 for more details**). The isolation of this cortico-striatal network was highly relevant as most of these brain regions have been associated with motor sequence learning or consolidation in multiple studies (see Doyon et al., 2009a for a review).

Functional integration

Large-scale integration is the term used to represent the idea that neural mechanisms select and coordinate distributed brain activity to produce a flow of adapted and unified cognitive movements (Varela et al., 2001). Network integration, as implemented in Netbrainwork, is a multivariate measure based on correlations between regions of interest (ROI) comprised in a given network (Marrelec et al., 2008). Changes in network integration relative to sleep or daytime were first measured within the hypothesis-driven, cortico-striatal network. The level of integration between the two groups (Day/awake and Night/sleep) in the first test session, i.e., morning vs. evening test groups, was not different ($t_{(22)}$, p=.87), hence suggesting that there were no significant effect of time of day on that measure at baseline. The relative change of integration following either sleep or the passage of daytime was then computed using the percent improvement between the test and retest sessions, and an independent t-test was used to compare the two groups. The results showed that connectivity within the hypothesis-driven network changed significantly depending on whether participants had slept or not: there was a decrease of 21% in integration level after the passage of time, as opposed to an increase of 7% following a night of sleep ($t_{(22)}$, p=.03) (Figure 2). The latter findings are consistent with our predictions and thus suggest a preserved level of synchrony in signals between regions of the hypothesis-driven cortico-striatal network following sleep-dependent motor memory consolidation.

Because the present study constitutes the first attempt to investigate dynamic changes in network integration after either sleep or the simple passage of time, we then assessed the *spatial specificity* between networks. This allowed us to verify whether the pattern of results described above within the cortico-striatal network is specific to the interaction between sleep/passage of time and the cortico-striatal network; or whether it were simply the result of a non-specific effect of sleep on brain regions at large (i.e., if all functional systems of the brain were generally more synchronous after sleep). We thus analysed the integration levels within each evoked large-scale connectivity network detected through ICA in the same way as described above. We calculated the relative changes of integration between the test and retest sessions in both groups, for each of our 7 large-scale networks which comprised between 15 to 32 ROIs (default-mode, fronto-parietal, motor, basal ganglia, executive, visual lateral, visual medial). Qualitatively we observe that sleep generally results in a greater level of integration as compared to daytime (see Figure 3A). Yet, independent t-tests comparing the relative change in integration of each network following either sleep vs the simple passage of daytime, revealed a difference that was only significant for the large-scale cortico-striatal network. Within that network, there was no significant difference in integration between our two groups in the test session ($t_{(22)}$, p=.11). Yet we found a decrease in integration of 7% with the passage of daytime as opposed to an increase of 10% following sleep ($t_{(22)}$=-3.1, p=.006). Only the motor network also showed a tendency for a greater integration after sleep as opposed to the passage of time ($t_{(22)}$=-1.7, p=.10), yet the difference was not significant. These results thus suggest that sleep profited specifically to the data-driven, large-scale cortico-striatal network, as opposed to other functional connectivity networks present during task execution. Unfortunately, however, there was no correlation between the changes in integration within this network and the observed overnight improvement in performance for the sleep group (r =.40, p=.23).

Discussion

As predicted, both hypothesis and data-driven methods revealed that off-line motor sequence consolidation is associated with a greater level of integration within the cortico-striatal system. Importantly, the two approaches confirm that the increased integration within this task-related network is sleep-dependent, as no such change in integration was observed after a similar period of daytime. Furthermore, our findings show that this within-network dynamic found following motor sequence consolidation is specific to the cortico-striatal system since none of the other networks identified through the data-driven analysis demonstrated significant sleep-related changes in integration. Altogether, our findings are suggestive of a new mechanism associated with the off-line sleep-dependent motor memory consolidation process, that is, a greater synchrony of activity between regions forming the cortico-striatal network.

The present increase in interactions between regions of the cortico-striatal network, as shown with the hypothesis-driven network analysis are in line with previous studies that have demonstrated greater strength in regional brain connectivity during the initial learning phase of MSL task (Sun et al., 2007, Coynel et al., 2010, Ma et al., 2010). For example, our findings are in accord with those from Ma and colleagues (2010) who have reported that the connectivity between the basal ganglia and M1 is strengthened across 2 and 4 weeks of learning of a sequence of movements. They also corroborate the results from Sun et al. (2007) who have shown that activity in the parietal cortex becomes significantly more correlated with other motor-related during early vs. late MSL, as well as the results from Peigneux and collaborators (2003) who have demonstrated that probabilistic sequence learning increases connectivity between the striatum and cuneus. Such a hypothesis-driven approach does not exclude the possibility that other structures like the cerebellum (Steele and Penhune, 2010) and the hippocampus (Albouy et al., 2008, Albouy et al., 2013a) may also contribute to the consolidation process of sequential types of skills. Yet our findings suggest that the brain regions constituting the cortico-striatal network, namely the primary motor cortex, SMA, superior parietal cortex, putamen, globus pallidus and thalamus, are not only involved in the acquisition of a new motor sequence; but also that the increased coherence in activity between these brain structures following sleep can be a good marker (and thus a possible mechanism) of the sleep-dependent off-line consolidation process of MSL.

As a complementary approach, we also used spatial independent component analysis (sICA) at the group level during task execution in order to test the specificity of the sleep-dependent increase in cortico-striatal integration. Remarkably, this method allowed the isolation of a large-scale cortico-striatal system (see Table 1) in which more than half of the automatically detected seeds have previously been directly associated to motor learning or motor memory consolidation (Hikosaka et al., 1999, Doyon et al., 2002, Doyon et al., 2003, Fischer et al., 2005, Walker et al., 2005, Albouy et al., 2008, Dayan and Cohen, 2011). This data-driven approach did not only recruit multiple motor cortical regions, but also a large number of sub-cortical structures like the putamen, globus pallidus and the hippocampus. Interestingly, similar to the hypothesis-driven analysis, this network demonstrated a significant increase in integration after sleep, while simple passage of daytime provoked decreased within-system integration. Such pattern of findings is in accord with previous work in our laboratory, which demonstrated that the putamen and globus pallidus are related to motor sequence consolidation (Debas et al., 2010). They are also consistent with an increasing number of studies, which are suggesting that the hippocampus is implicated in this process, as it appears to be responsible for consolidating contextual information and high order associations in motor sequence learning (Schendan et al., 2003, Albouy et al., 2008, Gheysen et al., 2010, Rose et al., 2011, Albouy et al., 2013a). More importantly, however, only this large-scale cortico-striatal network revealed a sleep-dependent increase in integration, as no other functional network presented a significant change in integration associated with sleep or daytime, hence further confirming the specificity of the association between sleep-dependent consolidation and the cortico-striatal system. Altogether our results support the hypothesis elaborated in Doyon's model, endorsing a role for the cortico-striatal system in the consolidation of motor sequences.

Systemic sleep-dependent consolidation

The present study is the first, to our knowledge, to look at changes in connectivity related to motor memory consolidation at the systemic level following sleep. Qualitatively, we found that almost all functional networks identified through the data-driven approach revealed higher connectivity following sleep, compared to after the simple passage of daytime (Figure 3). This observation supports the idea that sleep provokes a diffuse increase in integration within networks at the whole brain level (Boly et al., 2012). In addition to this global increased interaction, however, the specificity of this effect on the cortico-striatal system also suggests that consolidation during sleep provides an active protection, by facilitating the cohesion of the functional system mediating the type of learning that previously occurred during daytime.

The physiological mechanism responsible for this increase in integration within the cortico-striatal system after sleep remains unknown. Furthermore, the present study does not admittedly allow to address this issue directly as group network analyses were only measured before and after, but not during sleep. Yet because such a change in integration was only found after a night of sleep, but not following a similar amount of time in a wake state, at least two hypothetical physiological processes could account for the sleep-dependent consolidation mechanism reported here. First, it is possible that the cortico-striatal system is the actual network that shows reactivation during sleep. Indeed, findings reveal that following training, the memory trace is maintained during post-learning rest periods, as demonstrated by increased connectivity in regions that were previously recruited for learning (Albert et al., 2009b, Lewis et al., 2009a, Stevens et al., 2010, Vahdat et al., 2011). In addition the brain regions initially involved during training are also reactivated during post-learning sleep period (see Rauchs et al., 2005 for a review). In several electrophysiological studies in rodents, recordings of place cells in the hippocampus have revealed that the firing pattern of these cells is repeated in the same sequence during sleep (e.g Wilson and McNaughton, 1994), and importantly, post-learning sleep reactivation has also been reported in the striatum (Pennartz et al., 2004, Lansink et al., 2009). Finally, additional support for the “reactivation hypothesis” have come from human neuroimaging studies using positron by emission tomography (PET), which showed that regional activity recorded during training on a probabilistic serial reaction time task is re-expressed in the post-training night (Maquet et al., 2000b, Peigneux et al., 2003), notably within the cuneus and striatum (Peigneux et al., 2003). Thus it could be hypothesized that sleep-dependent reactivation of the cortico-striatal system favors optimal interaction between the regions of that specific network, as one executes the task the following morning. This would be in opposition to other unrelated functional networks, which would not benefit from the same sleep-dependent replay. A second possible mechanisms which could explain our pattern of results is based on the work by Boly et al (2012) who have shown that during non-rapid eye movement (NREM) sleep, each functional network detected via ICA is characterized by greater within- as compared to between- system connectivity. Interestingly, the increased functional clustering of brain activity, suggesting an independent operation of networks during NREM sleep, might actually support the specificity of changes in integration that we found for a system which was involved in prior daytime learning. The latter hypothesis is also supported by the fact that different sleep characteristics of NREM sleep in particular (see Diekelmann and Born, 2010), notably spindles (Fogel and Smith, 2011, Fogel et al., 2012), have been linked with memory consolidation and have been shown to correlate with activity in the putamen, in association with motor sequence consolidation (Barakat et al., 2012b).

Conclusion

The results of the present study suggest a new mechanism with which motor sequence consolidation is processed in the brain, i.e., through an increased level of integration within the cortico-striatal network. Furthermore, these results were reproducible using both a hypothesis- and a data-driven approach. The latter approach has proved to be an efficient way to isolate a task-specific network which demonstrated a pattern of connectivity change that was not observed in any other functional network. These findings support the strong specificity of the effect of sleep, which allows associating with confidence the changes in the pattern of connectivity to the off-line consolidation process of motor sequence learning.

Methods:

Subjects: 24 young healthy subjects aged between 19 and 30 years old (13 women) participated in the present study. Participants had no experience playing a musical instrument, nor had received previous training for speed typing. Participants reported having a regular cycle of bedtime starting around 11:00 p.m. (+/- 1hour) and waking-up around 7:00 (+/- 1 hour), and this was confirmed through a sleep diary for a full week prior to the study.

Subjects with depressive symptoms (score above 4) as measured by the short version of the Beck Depression Scale were excluded. All subjects participated in an adaptation night at our laboratory, during which polysomnographic (PSG) measures of their sleep were recorded (data reported in Barakat et al., 2010, Barakat et al., 2012a). Subjects with an apnea-hypoapnea index >5 or a periodic limb movements index >5 were excluded. None of the subjects worked night shifts or were engaged in trans-meridian trips 3 months prior to the study. During the entire period of the experiment, subjects were asked to abstain from alcohol. They were non-smokers and remained caffeine free prior to scanning sessions. They were also right handed, had no history of neurological or psychiatric disorders as well as no history of sleep disturbances or medication intake. They gave their written informed consent to participate in the study. The project was approved by the “Regroupement Neuroimagerie/Québec” Ethics Review Committee at the “Centre de recherche de l’Institut universitaire de gériatrie de Montréal, Université de Montréal”.

Experimental Design: To study the effect of sleep on the integration of cerebral networks, we used a between (Night/sleep-Day/awake) and within-subject (test and retest sessions) design (see Debas et al., 2010 for more details). Briefly, the Night/sleep group (n=13, 7 female) was first trained in the evening (9:00 p.m approximately) on the motor sequence learning task, in which subjects were asked to practice an explicitly known 5-item sequence (4-1-3-2-4) of finger movements of the left, non-dominant hand (Karni et al., 1995) until they reached asymptotic performance. This training session took place in a mock scanner to simulate the MRI environment. Participants then moved to the MRI room and were scanned (i.e., test session) in a blocked paradigm while executing the newly learned motor sequence using a MRI-compatible response box. Following this first scanning session, subjects were required to sleep in the laboratory and polysomnographic (PSG) data were recorded (see Barakat et al., 2010 for a report of the EEG results). They were then rescanned the following morning 12 hours later (9:00 a.m. approximately) in a retest session. The training, test and retest sessions consisted of 8 blocks during which subjects had to practice 20 sequences each (i.e., 100 finger movements). All experimental blocks started off with a 2.5s instruction period where the word “Sequence” appeared in the middle of the screen seen through mirrors inserted in the head coil, followed by a green square indicating that subjects could start producing the known sequence as fast and accurately as possible. After having completed 20 sequences, the color of the square on the screen changed to red, indicating the beginning of a 15s rest period. The Day/awake group (n=11, 6 female) followed a similar procedure, except that the first training and scanning sessions took place in the morning (around 9:00 a.m.) whereas the retest session was completed 12hr later in the evening (around 9:00 p.m.). Time per sequence and the number of correct sequences were recorded for each block in the two groups of subjects.

MRI acquisition: Brain imaging data were acquired with a 3T scanner (Magnetom Trio Siemens AG, Germany) equipped with an 8-channel head coil. A high resolution anatomical T1-weighted scan was obtained for each subject (voxel size = 1 x 1 x 1 mm^3, TR = 23ms, TE = 2.98ms, FA = 9°; matrix 256 x 256; 176 slices). Functional T2*-weighted images were also acquired using a standard gradient echo-planar sequence sensitive to the BOLD signal (voxel size = 3.75 x 3.75 x 5 mm^3; TR = 2.5s; TE = 30ms; FA = 90°; FOV = 240 x 240 mm^2, matrix size = 64 x 64; 28 slices). Pre-processing steps using Statistical Parametric Mapping (SPM5) (http://www.fil.ion.ucl.ac.uk/spm/software) included realignment and smoothing using a Gaussian Kernel of 6mm full-width at half-maximum (FWHM).

Behavioral analyses: Time to execute a sequence was averaged for each of the 8 blocks of the test and delayed retest sessions. Data for both sessions were normalised based upon the results of the last 5 blocks of the test session, a period in which subjects had reached asymptotic performance. We then used the first two block of the delayed retest session to directly compare the behavioral performance between the two groups, using an independent t-test.

Connectivity Analyses

Hypothesis-driven functional network identification

The hypothesis-driven cortico-striatal network was built based on regions known to contribute to consolidation using the coordinates corresponding to the local peaks of activity during task execution and found in our standard main effects analyses (Debas et al., 2010). Two ROI's in the precentral cortex were chosen, as the latter region has been suggested to be important for motor sequence consolidation (Robertson et al., 2005, Steele and Penhune, 2010). Similarly, the putamen, globus pallidus, SMA and the superior parietal cortex were also chosen for their role in motor sequence learning or consolidation (Doyon et al., 2002, Grafton and Ivry, 1995, Fischer et al., 2005, Debas et al., 2010). Finally, because we aimed to study levels of information exchange within the cortico-striatal network via levels of integration, the thalamus, which constitutes a primordial relay station (Alexander and Crutcher, 1990) was also added to the network. ROI's sizes were 7 and 4 voxels in the cortex and in sub-cortical areas respectively.

Data-driven functional network identification

For this approach, we used the NetBrainWork toolbox (http://sites.google.com/site/netbrainwork/) comprising NEDICA, a mathematical method used for detecting group functional large-scale networks in fMRI data using spatial independent component analysis (sICA) (Perlbarg et al., 2008). The default number of spatial components (i.e., 40) was calculated using the infomax ICA algorithm. After registration into the MNI standardized space, a hierarchical clustering of the individual spatial components allowed to group components. This clustering algorithm was based on the maximization of two indices: the degree of representativity (i.e., the proportion of subjects represented in the group component) and the degree of unicity (i.e., the proportion of subjects contributing to only one component in the group component).

A first analysis using fMRI images of the test session from both Night/sleep and Day/awake groups gave rise to the group components. The two indices (representativity and unicity) as well as guidelines (Kelly et al., 2010) were used to visually screen the group components in order to exclude maps comprising regions distributed around ventricules, sinus or blood vessels. The remaining 7 group components are reported in the “Results” section and are referred to as the functional networks of interest.

Region extraction

The data-driven large scale networks were built using an automatic approach. ROIs were formed automatically around the peak signal of each of the seven large-scale networks. ROIs were built using a region-growing algorithm as implemented in the toolbox. At each step of this algorithm, the voxel included in the ROI corresponded to the local highest t-score. Although it is possible to choose the size of the ROI wanted, only one ROI size can be selected for a given large-scale network .Thus ROI’s of 7 voxels, with a minimal distance between 2 ROIs of 30mm, was established for networks that had a majority of cortical areas; whereas ROIs of 4 voxels extension, and a minimal distance of 25mm between 2 ROIs, was chosen for the network presenting primarily sub-cortical areas.

Integration analysis

Quantification of the level of network integration implemented in NetBrainWork was previously defined by Tononi et al. (1994) and has successfully been applied to fMRI results in Benali’s group (Marrelec et al., 2008). Integration is mainly based on the correlation between the temporal courses of the regions of interest of a given network, as the amplitude of the signal of the ROI was normalised. This metric allows to quantify the global level of statistical dependence within the network. It summarizes the within system organisation using all correlation coefficients that can be computed with the ROIs comprised in the given network into a single number, which can then be statistically compared between session and between groups. The underlying assumption is that the larger the correlation coefficient, the greater the integration and information exchange within the system. The absolute measure of integration being dependent upon the number of ROIs selected within a given network, no statistical comparison was done between networks. Integration was thus computed here for each session and each subject. Independent t-test between the Day/awake group and the

Night/sleep group's test session assessed whether there were any time of day effects. Then, relative changes of integration between the test and retest sessions were calculated for the two groups. Independent t-tests were first used to assess the difference in connectivity changes associated with sleep vs. the simple passage of daytime for the hypothesis-driven cortico-striatal network. Similarly, a measure of integration was computed for each subject and each session for the 7 identified data-driven networks. Seven independent t-tests were then performed to test the effect of sleep vs. daytime. Finally, we used Pearson's correlation analyses to assess the possible relationship between the subject's behavioural improvements in performance from test to retest with changes in the levels of integration.

References

Albert NB, Robertson EM, Miall RC (2009) The resting human brain and motor learning. Current biology : CB 19:1023-1027.

Albouy G, Fogel S, Pottiez H, Nguyen VA, Ray L, Lungu O, Carrier J, Robertson E, Doyon J (2013a) Daytime sleep enhances consolidation of the spatial but not motoric representation of motor sequence memory. PLoS One 8:e52805.

Albouy G, Sterpenich V, Balteau E, Vandewalle G, Desseilles M, Dang-Vu T, Darsaud A, Ruby P, Luppi PH, Degueldre C, Peigneux P, Luxen A, Maquet P (2008) Both the hippocampus and striatum are involved in consolidation of motor sequence memory. Neuron 58:261-272.

Albouy G, Sterpenich V, Vandewalle G, Darsaud A, Gais S, Rauchs G, Desseilles M, Boly M, Dang-Vu T, Balteau E, Degueldre C, Phillips C, Luxen A, Maquet P (2013b) Interaction between Hippocampal and Striatal Systems Predicts Subsequent Consolidation of Motor Sequence Memory. PLoS One 8:e59490.

Albouy G, Vandewalle G, Sterpenich V, Rauchs G, Desseilles M, Balteau E, Degueldre C, Phillips C, Luxen A, Maquet P (2012) Sleep Stabilizes Visuomotor Adaptation Memory: a functional Magnetic Resonance Imaging Study. J Sleep Res.

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends in neurosciences 13:266-271.

Barakat M, Carrier J, Debas K, Lungu O, Fogel S, Vandewalle G, Hoge RD, Bellec P, Karni A, Ungerleider LG, Benali H, Doyon J (2012) Sleep spindles predict neural and behavioral changes in motor sequence consolidation. Human brain mapping.

Barakat M, Doyon J, Debas K, Vandewalle G, Morin A, Poirier G, Martin N, Lafortune M, Karni A, Ungerleider LG, Benali H, Carrier J (2010) Fast and slow spindle involvement in the consolidation of a new motor sequence. Behavioural brain research.

Beckmann CF, DeLuca M, Devlin JT, Smith SM (2005) Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc Lond B Biol Sci 360:1001-1013.

Boly M, Perlbarg V, Marrelec G, Schabus M, Laureys S, Doyon J, Pelegrini-Issac M, Maquet P, Benali H (2012) Hierarchical clustering of brain activity during human nonrapid eye movement sleep. Proceedings of the National Academy of Sciences of the United States of America 109:5856-5861.

Born J, Wilhelm I (2012) System consolidation of memory during sleep. Psychol Res 76:192-203.

Calhoun VD, Kiehl KA, Pearlson GD (2008) Modulation of temporally coherent brain networks estimated using ICA at rest and during cognitive tasks. Human brain mapping 29:828-838.

Cohen DA, Pascual-Leone A, Press DZ, Robertson EM (2005) Off-line learning of motor skill memory: a double dissociation of goal and movement. Proceedings of the National Academy of Sciences of the United States of America 102:18237-18241.

Coynel D, Marrelec G, Perlbarg V, Pelegrini-Issac M, Van de Moortele PF, Ugurbil K, Doyon J, Benali H, Lehericy S (2010) Dynamics of motor-related functional integration during motor sequence learning. Neuroimage 49:759-766.

Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF (2006) Consistent resting-state networks across healthy subjects. Proceedings of the National Academy of Sciences of the United States of America 103:13848-13853.

Dayan E, Cohen LG (2011) Neuroplasticity subserving motor skill learning. Neuron 72:443-454.

Debas K, Carrier J, Orban P, Barakat M, Lungu O, Vandewalle G, Hadj Tahar A, Bellec P, Karni A, Ungerleider LG, Benali H, Doyon J (2010) Brain plasticity related to the consolidation of motor sequence learning and motor adaptation. Proc Natl Acad Sci U S A 107:17839-17844.

Diekelmann S, Born J (2010) The memory function of sleep. Nature reviews Neuroscience 11:114-126.

Diekelmann S, Wilhelm I, Born J (2009) The whats and whens of sleep-dependent memory consolidation. Sleep medicine reviews 13:309-321.

Doyon J, Bellec P, Amsel R, Penhune V, Monchi O, Carrier J, Lehericy S, Benali H (2009a) Contributions of the basal ganglia and functionally related brain structures to motor learning. Behavioural brain research 199:61-75.

Doyon J, Benali H (2005) Reorganization and plasticity in the adult brain during learning of motor skills. Curr Opin Neurobiol 15:161-167.

Doyon J, Korman M, Morin A, Dostie V, Tahar AH, Benali H, Karni A, Ungerleider LG, Carrier J (2009b) Contribution of night and day sleep vs. simple passage of time to the consolidation of motor sequence and visuomotor adaptation learning. Experimental brain research Experimentelle Hirnforschung.

Doyon J, Penhune V, Ungerleider LG, Doyon J, Penhune V, Ungerleider LG (2003) Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. Neuropsychologia 41:252-262.

Doyon J, Song AW, Karni A, Lalonde F, Adams MM, Ungerleider LG (2002) Experience-dependent changes in cerebellar contributions to motor sequence learning. Proc Natl Acad Sci U S A 99:1017-1022.

Dudai Y (2004) The neurobiology of consolidations, or, how stable is the engram? Annual review of psychology 55:51-86.

Fischer S, Hallschmid M, Elsner AL, Born J (2002) Sleep forms memory for finger skills. Proc Natl Acad Sci U S A 99:11987-11991.

Fischer S, Nitschke MF, Melchert UH, Erdmann C, Born J (2005) Motor Memory Consolidation in Sleep Shapes More Effective Neuronal Representations: Journal of Neuroscience Vol 25(49) Dec 2005, 11248-11255.

Floyer-Lea A, Matthews PM (2005) Distinguishable Brain Activation Networks for Short- and Long-Term Motor Skill Learning: Journal of Neurophysiology Vol 94(1) Jul 2005, 512-518.

Fogel S, Martin N, Lafortune M, Barakat M, Debas K, Laventure S, Latreille V, Gagnon JF, Doyon J, Carrier J (2012) NREM Sleep Oscillations and Brain Plasticity in Aging. Front Neurol 3:176.

Fogel SM, Smith CT (2011) The function of the sleep spindle: a physiological index of intelligence and a mechanism for sleep-dependent memory consolidation. Neuroscience and biobehavioral reviews 35:1154-1165.

Friston KJ, Frith CD, Liddle PF, Frackowiak RS (1993) Functional connectivity: the principal-component analysis of large (PET) data sets. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 13:5-14.

Gheysen F, Van Opstal F, Roggeman C, Van Waelvelde H, Fias W (2010) Hippocampal contribution to early and later stages of implicit motor sequence learning. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale 202:795-807.

Hikosaka O, Nakahara H, Rand MK, Sakai K, Lu X, Nakamura K, Miyachi S, Doya K (1999) Parallel neural networks for learning sequential procedures. Trends in neurosciences 22:464-471.

Karni A, Meyer G, Jezzard P, Adams MM, Turner R, Ungerleider LG (1995) Functional MRI evidence for adult motor cortex plasticity during motor skill learning. Nature 377:155-158.

Kelly RE, Jr., Alexopoulos GS, Wang Z, Gunning FM, Murphy CF, Morimoto SS, Kanellopoulos D, Jia Z, Lim KO, Hoptman MJ (2010) Visual inspection of independent components: defining a procedure for artifact removal from fMRI data. J Neurosci Methods 189:233-245.

Lansink CS, Goltstein PM, Lankelma JV, McNaughton BL, Pennartz CM (2009) Hippocampus leads ventral striatum in replay of place-reward information. PLoS biology 7:e1000173.

Laureys S, Peigneux P, Phillips C, Fuchs S, Degueldre C, Aerts J, Del Fiore G, Petiau C, Luxen A, van der Linden M, Cleeremans A, Smith C, Maquet P (2001) Experience-dependent changes in cerebral functional connectivity during human rapid eye movement sleep. Neuroscience 105:521-525.

Lewis CM, Baldassarre A, Committeri G, Romani GL, Corbetta M (2009) Learning sculpts the spontaneous activity of the resting human brain. Proc Natl Acad Sci U S A 106:17558-17563.

Ma L, Wang B, Narayana S, Hazeltine E, Chen X, Robin DA, Fox PT, Xiong J (2010) Changes in regional activity are accompanied with changes in inter-regional connectivity during 4 weeks motor learning. Brain research 1318:64-76.

Maquet P, Laureys S, Peigneux P, Fuchs S, Petiau C, Phillips C, Aerts J, Del Fiore G, Degueldre C, Meulemans T, Luxen A, Franck G, Van Der Linden M, Smith C, Cleeremans A (2000) Experience-dependent changes in cerebral activation during human REM sleep. Nature neuroscience 3:831-836.

Marrelec G, Bellec P, Krainik A, Duffau H, Pelegrini-Issac M, Lehericy S, Benali H, Doyon J (2008) Regions, systems, and the brain: hierarchical measures of functional integration in fMRI. Med Image Anal 12:484-496.

Peigneux P, Laureys S, Fuchs S, Destrebecqz A, Collette F, Delbeuck X, Phillips C, Aerts J, Del Fiore G, Degueldre C, Luxen A, Cleeremans A, Maquet P (2003) Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. Neuroimage 20:125-134.

Pennartz CM, Lee E, Verheul J, Lipa P, Barnes CA, McNaughton BL (2004) The ventral striatum in off-line processing: ensemble reactivation during sleep and modulation by hippocampal ripples. J Neurosci 24:6446-6456.

Rauchs G, Desgranges B, Foret J, Eustache F (2005) The relationships between memory systems and sleep stages. Journal of sleep research 14:123-140.

Robertson EM, Pascual-Leone A, Press DZ (2004) Awareness modifies the skill-learning benefits of sleep. Curr Biol 14:208-212.

Robertson EM, Press DZ, Pascual-Leone A (2005) Off-line learning and the primary motor cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:6372-6378.

Rose M, Haider H, Salari N, Buchel C (2011) Functional dissociation of hippocampal mechanism during implicit learning based on the domain of associations. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:13739-13745.

Schendan HE, Searl MM, Melrose RJ, Stern CE (2003) An FMRI study of the role of the medial temporal lobe in implicit and explicit sequence learning. Neuron 37:1013-1025.

Steele CJ, Penhune VB (2010) Specific increases within global decreases: a functional magnetic resonance imaging investigation of five days of motor sequence learning. J Neurosci 30:8332-8341.

Stevens WD, Buckner RL, Schacter DL (2010) Correlated low-frequency BOLD fluctuations in the resting human brain are modulated by recent experience in category-preferential visual regions. Cereb Cortex 20:1997-2006.

Sun FT, Miller LM, Rao AA, D'Esposito M (2007) Functional connectivity of cortical networks involved in bimanual motor sequence learning. Cerebral cortex 17:1227-1234.

Vahdat S, Darainy M, Milner TE, Ostry DJ (2011) Functionally specific changes in resting-state sensorimotor networks after motor learning. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:16907-16915.

Varela F, Lachaux JP, Rodriguez E, Martinerie J (2001) The brainweb: phase synchronization and large-scale integration. Nature reviews Neuroscience 2:229-239.

Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R (2002) Practice with sleep makes perfect: sleep-dependent motor skill learning. Neuron 35:205-211.

Walker MP, Stickgold R, Alsop D, Gaab N, Schlaug G (2005) Sleep-dependent motor memory plasticity in the human brain. Neuroscience 133:911-917.

Wilson MA, McNaughton BL (1994) Reactivation of hippocampal ensemble memories during sleep. Science (New York, NY 265:676-679.

Witt K, Margraf N, Bieber C, Born J, Deuschl G (2010) Sleep consolidates the effector-independent representation of a motor skill. Neuroscience 171:227-234.

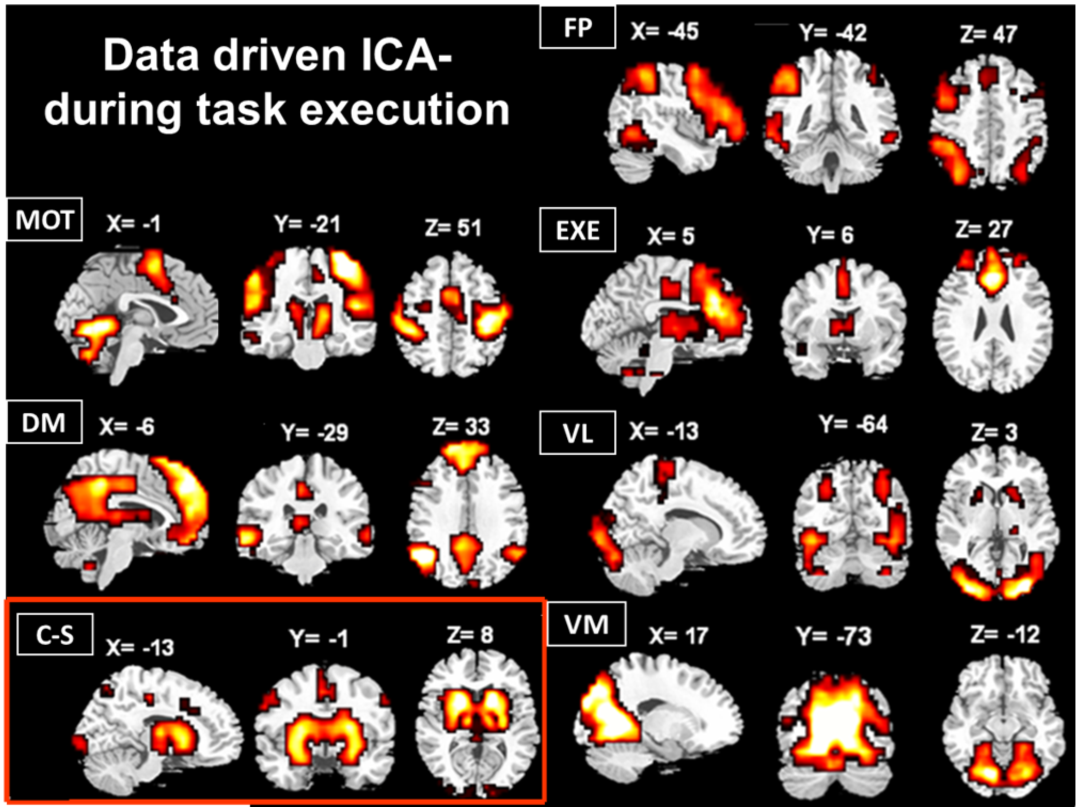


Figure 12 : Article 2 – Functional Large Scale Networks

Figure 1: large scale networks as isolated via ICA during the first testing session of both Sleep and Wake groups. Visual display was set for a best representativity of the regions part of the networks and the most similar coordinates as presented in Beckmann et la., 2005 were used to facilitate visual comparison. MOT=motor; DM=default-mode; C-S=cortico-striatal; FP=fronto-parietal; EXE=executive; VL=visual lateral; VM=visual medial

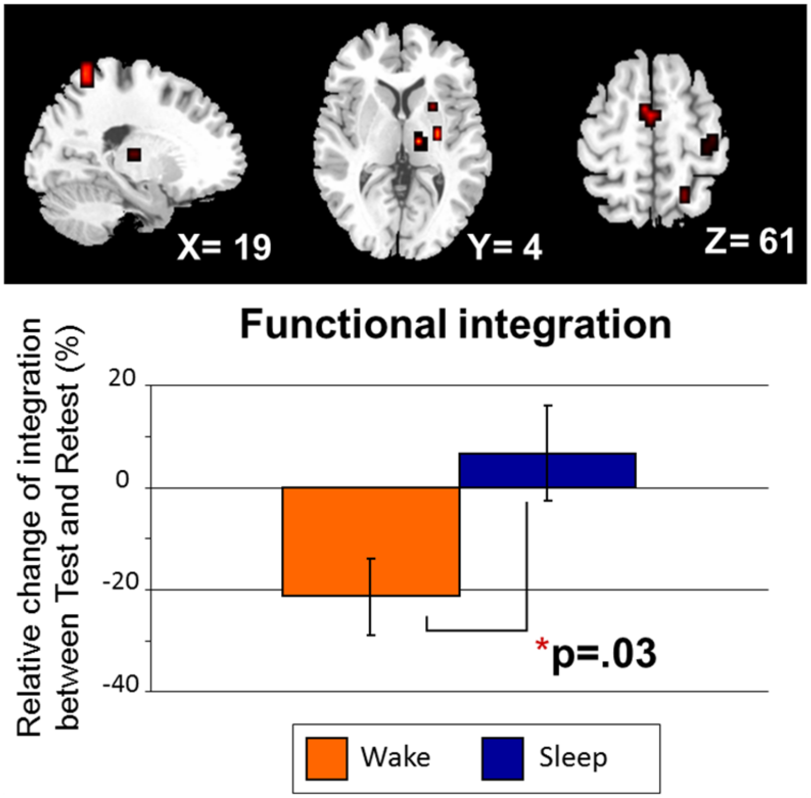
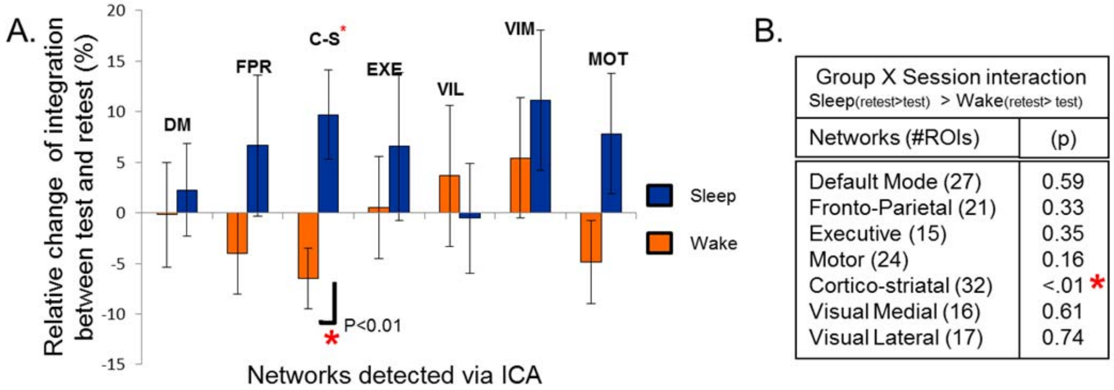


Figure 13 : Article 2- Change of integration – hypothesis driven network

Figure 2: relative change of integration for the hypothesis-driven network for which most of the regions are represented above, see Table 1 for details of the regions included. Results show a differential effect of sleep on the level of interaction amongst regions of the network. The group who remained awake during daytime presented a 21% decrease in the levels of integration, in opposition with the sleep group who presented 7% increases of integration from test to retest.



Group X Session interaction Sleep(retest>test) > Wake(retest> test)	
Networks (#ROIs)	(p)
Default Mode (27)	0.59
Fronto-Parietal (21)	0.33
Executive (15)	0.35
Motor (24)	0.16
Cortico-striatal (32)	<.01 *
Visual Medial (16)	0.61
Visual Lateral (17)	0.74

Figure 14 : Article 2- Change of integration – data-driven networks

Figure 3: A. Relative change of integration from test to retest for all data-driven networks. Sleep had a significant beneficial effect on within network interaction, when compared to daytime, only for the cortico-striatal network. B. on the right side, all resulting (p) values of the measure of interaction are presented. The interaction tested if levels of integration were higher in the retest vs. test session for the sleep group vs. the wake group.

Table 4. Article 2 - cortico-striatal networks

Table 1: regions selected for the corticostriatal (C-S) networks and the corresponding Talairach coodinates. SMA=supplementary motor area; sup = superior; mid=middle; Ant= anterior; Inf= inferior.

A. Hypothesis-driven C-S network

ROIs	X	Y	Z
precentral	39	-22	51
precentral	31	-12	63
Putamen	26	7	5
SMA	-1	-4	53
Parietal sup	22	-54	63
Globus pallidus	30	-13	-1
Thalamus	16	-18	6

B. Data-driven C-S network

ROIs	X	Y	Z
CB Crus II	35	-80	-30
CB Crus II	10	-79	-28
Vermis	2	-60	-33
Putamen	-19	6	7
Globus Pallidus	16	6	1
Globus pallidus	30	-13	-1
Amygdala	-26	-2	-13
Amygdala	24	-1	-17
Thalamus	-10	-15	7
hippocampus	-29	-18	-4
Caudate	19	1	18
Post-central	-50	-10	36
Precentral	-57	4	18
Precentral	60	10	21
Cingulum Ant	-4	18	27
Cingulum Mid	-9	-25	43
Cingulum Mid	-3	-2	42
Parietal Inf	-47	-33	49
Parietal Sup	-10	-67	54
Cuneus	-1	-76	37
Frontal Mid	-34	49	13
Insula	-27	-22	11
Insula	-39	11	2
fusiform	28	-73	-9
lingual	-22	-85	-8
Calcarine	-8	-97	-2
Occipital Inf	-42	-86	1
Occipital Inf	-48	-63	-9
Calcarine	18	-97	5

Chapter III: General Discussion

Motor learning is characterised behaviorally by a fast, within session learning phase, followed by a slower learning phase, during which improvement takes place across multiple learning sessions. To date, the behavioural determinants and neural substrates mediating these two acquisition phases have been well documented. Yet, the brain physiological mechanisms responsible for the consolidation process known to occur off-line between these two phases have remained poorly understood until recently. Indeed, new evidence now supports the notion that the latter process depends on sleep when it involves motor sequence learning, but not when it implicates motor adaptation (Doyon et al., 2009c) hence suggesting that different mechanisms do mediate their respective consolidation processes. Interestingly, Doyon et al., (2002, 2005)' model has proposed that the acquisition and long term maintenance of these two types of motor skill rely on different brain networks. Motor sequence learning is now known to rely more importantly on the cotrico-striatal system, whereas motor adaptation learning is believed to depend on the cortico-cerebellar system. Yet, are these systems critical for the consolidation of these two types of motor skilled behaviors? What is the role of sleep in these motor learning processes? Is sleep necessary to trigger the cerebral changes mediating the consolidation process or is the simple passage of time sufficient? Over and above the sleep-dependent or –independent influence of specific brain regions in consolidation, what is the brain dynamic associated with the consolidation process at the level of networks? Importantly, how specific at the brain systems level are the changes in connectivity associated with consolidation? To answer these questions, we set out to decipher the brain regions associated with motor skill consolidation, as well as clarify the involvement of sleep in motor memory consolidation. We did so by characterizing both the changes in cerebral activity and in levels of network integration occurring after a night of sleep or the simple passage of daytime, for both motor sequence learning and motor adaptation.

4. Summary of the results

The aim of the first article was to better characterize the process of motor consolidation at the regional level, using two types of learning, one for which the occurrence of consolidation depends on sleep while the other does not. Behaviorally, we replicated the findings suggesting a benefit of sleep solely for motor sequence learning (MSL). Indeed, for MSL, consolidation was reflected as a gain in performance that was absent when participants remained awake for the same period of time. Importantly, motor adaptation learning was not differentially affected by sleep or daytime, such that both types of delay resulted in a faster relearning rate when retested. Using fMRI, we found that the putamen was the most active motor region following sleep as opposed to following daytime in participants who learned a motor sequence. By contrast, for motor adaptation, in line with the behavioral results, there was no differential effect of sleep, rather a slight increase in activity was observed in the cerebellum (lobule VI) following both sleep and daytime. Furthermore, the strength of activation of that region was correlated with the amount of savings. These results suggest a dissociation in the mechanisms by which motor skills are consolidated. For MSL, the consolidation process is sleep-dependent and is associated with the putamen, whereas for MA, the consolidation process is independent from sleep and seems to rely on the cerebellum.

We know from studies measuring inter-regional connectivity, that motor regions do not act in isolation to subserve motor learning. Thus for the second article, we aimed to quantify changes in connectivity, i.e. changes in brain region interaction, that were associated with the sleep-dependent consolidation process of MSL. The results revealed that as one consolidates, the regions of the network involved in the task operate in a more synchronous and integrated fashion. Indeed, we found greater integration within the cortico-striatal network following sleep, but a decrease after the simple passage of daytime. Being it the first study assessing changes of integration at the system's level following a night of sleep, we also verified that these increases of integration were not simply an unspecific effect of sleep, but rather a consequence of the consolidation process. Using a data-driven approach, we were then able to show that the results were spatially specific to a cortico-striatal network, such that no other known functional network demonstrated this differential pattern of connectivity change between sleep and daytime.

In the next sections, I will first address the main limitations of these two studies, before moving on to a general discussion on the sleep-dependent and independent types of consolidation processes that occur with motor sequence learning and motor adaptation. Finally, I will discuss the possible mechanisms that could underlie the sleep-dependent consolidation process of a newly learned sequence of movements.

4.1. Limits

1. "Associated with" does not mean "responsible for"

The first main limitation addressed here is simply the extent of the interpretation that can be drawn from our results. Given the fact that for both articles we tested and retested participants before and after sleep, the changes objectified can only be seen as associated to the consolidation process. Indeed, the differences in activity or connectivity could simply be a reflection of consolidation, being the state of the mnesic trace at the time of retesting. Changes in cerebral activity from testing to retesting are taken as indicators of offline memory processing and are an indirect measure of consolidation. Yet, the chances are that as long as the off-line period itself is not fully understood and studied, the exact processes specifically underlying consolidation will remain uncertain.

2. Influence of time of day

The second major limitation is based on the idea that each human being has a circadian rhythm, such that according to a 24 hour cycle, endogenous changes occur at the biological, physiological and behavioral level. According to these changes, comparing the same behavior in the morning vs. the evening includes some unknown endogenous changes which might have interacted with the off-line, between-session process. This is a confound of time of day (Carrier and Monk, 2000, Cajochen et al., 2004) which is a concern when interpreting results based on designs like the one we used in the present thesis.

One way to overcome time of day influences is to test and retest participants at the same time of day. Yet, to do so while testing the effect of sleep, one has to use a sleep deprivation paradigm, which also has its limits. Indeed, other types of biological changes occur after sleep deprivation obliging retesting to take place two days after the initial training session for fatigue effects to dissipate; testing and retesting 3 days apart leaving more time for other types of confounds to take place (e.g., more interference). Furthermore, although we know that consolidation takes place during the first post-learning sleep period, it is unclear whether the changes it triggers would last or even be the same after 3 days. Fischer et al., (2005) who used this paradigm found some changes in brain activity 3 days following training. Yet because of the laps of time between testing sessions, it is also delicate to associate any change to the consolidation process itself. Particularly in the field of functional connectivity for which the brain dynamics of functional systems are less understood, it is unclear whether we would have found sleep-dependent changes of integration (study 2) as we do not know how long this effect lasts above the first post-sleep day. In summary, I believe that although testing and retesting at different time of day has its limits, until we can measure efficiently the off-line period itself through simultaneous EEG-fMRI studies for example, I would like to propose that the latter approach constitutes an efficient way to study the effect of sleep on consolidation.

In addition, I argue that time of day, if playing a role in the off-line consolidation process, plays only a minor role as compared to the active role of sleep itself. Three main points justify the latter statement: first, as we have shown in our two studies and as others who have used the same paradigm have also shown (Walker et al., 2002, Walker et al., 2003) there are no significant differences in performance between a first learning session taking place in the morning vs.evening. This was also true in our studies, both at the behavioral and imaging level, as well as for our measure of integration. Namely differences between the first morning and evening sessions were not significant. Secondly, to overcome the influence of time of day, some have used diurnal sleep (Fischer et al., 2002) or nap paradigms (Korman et al., 2007, Nishida and Walker, 2007, Albouy et al., 2013b). In both cases, diurnal sleep also resulted in gains in performance, albeit sometimes to a lesser extent than when subjects were retested after a full night of sleep. Finally, as reviewed in the introduction, studies in humans and animals have shown reactivation of the same regions that were involved in initial learning during the following sleep period, including following a sequence learning task (Maquet et al., 2000a, Peigneux et al., 2003) further supporting the hypothesis that sleep plays an active role in the consolidation process. Thus I believe that sleep, as oppose to changes attributed to time of day, is necessary for consolidation of sequential learning to occur as suggested by our results.

5. The Role of the Striatum and Cerebellum in Memory Consolidation

The implication of the striatum in the early and later phases of motor sequence learning has been extensively described in the last two decades (Doyon and Benali, 2005b, Doyon et al., 2009a). Its role, however, particularly in the mnemonic processes allowing for skill learning, is still uncertain. Some have suggested that the basal ganglia contributes through reinforcement signals mediated by dopaminergic neurons (Doya, 1999, 2000); and through the process of chunking of different units together, hence resulting in a better performance of the sequence (Graybiel, 1998, 2005). In support of this viewpoint, a recent review has reinforced the role of the sensorimotor striatum in the chunking process, and suggested that the associative striatum would be responsible for the selection of responses and evaluation of outcome or reward (Penhune and Steele, 2012). In addition to such processes that allow performance to improve during the early acquisition phase, our results further suggest that the putamen in particular is strongly associated with the off-line memory consolidation process that follows this initial learning stage. The putamen is not only involved once consolidation has taken place, (Debas et al., 2010); we also know that its activity is correlated with the amount of spindles recorded during nighttime, a sleep characteristic that is associated with gains in performance observed following sleep (Barakat et al., 2012b). Additional support for its role in motor sequence consolidation also comes from a primate study. At the cellular level, blocking the striatal-dopaminergic receptor (D2) with the use of raclopride (D2-antagonist) in primates, affected specifically the transition from the fast learning phase to an automatized execution of a sequence, defined as a more consistent performance with the presence of chunked movements. Under raclopride, monkeys were able to learn a new sequence in the same way during an On vs. OFF drug period at the beginning of learning. They demonstrated in both cases a decrease in performance fluctuation as well as an increase in chunking efficacy in the first training session. Yet, across the multiple days of training, monkeys under raclopride required two to four times as many trials during the ON vs. OFF period to reach optimal performance. These results thus suggest that even when initial learning of a new sequence is accomplished, the consolidation is largely affected when deprived of a normal level of striatal-D2 receptors and thus support the idea that this structure plays a critical role in the consolidation of motor sequences (Levesque et al., 2007). Other animal studies have also found the striatum to be associated with consolidation during sleep specifically, yet the learning was not specific to motor sequences and this topic will be briefly discussed in section 9.4. Finally, our results, together with previous findings reporting the implication of the striatum to the MSL consolidation process (Peigneux et al., 2003, Walker et al., 2005, Albouy et al., 2008), support Doyon's predictions based on his model of motor learning (Doyon and Benali, 2005b).

Interestingly, Doyon's model also predicts a different role for the cerebellum, as it suggests this structure to be involved specifically in both the early learning phase and long-term maintenance of a visuomotor adaptation task rather than the learning of sequences, a hypothesis that is also supported by our findings (study 1). Again though, the nature of its functional contribution remains conjectural. Contrary to the striatum, the role of the cerebellum has been associated with supervised learning based on processes related to sensorimotor integration (Doya, 1999). It has been suggested that this structure is a site of development and storage of an internal model of motor action into space (Imamizu et al., 2000, Imamizu et al., 2003, Penhune and Steele, 2012). In both MSL and MA one needs to learn and adjust motor output based on errors. However only in MA one has to adapt the new motor output based on an adjustment from the visual feedback. Thus, although the role of the cerebellum is recognized in early MSL, results from study 1 suggest it is not a key structure for the consolidation process of this task, but rather for one that requires constant online adjustment of the motor output. Learned sequences have triggered activity in the cerebellum, mostly in Lobules VI and dentate nuclei (Doyon et al., 2002). Additional increased activity has also been observed in lobule VIII, an area associated with a high frequency of finger movement, but not necessarily related to the sequence learning itself (Orban et al., 2010). In fact, in a study designed to dissociate learning from strict motoric performance of MSL, Orban and colleagues (2010) have found that most of the cerebellum (Lobules IV-V, bilateral VIII and ipsilateral VI) were associated with performance, a pattern of findings consistent with Seidler et al. (2002) who reported that this structure was mostly associated with performance rather than learning. By contrast, the contralateral lobule VI was associated with motor sequence learning *per se*, suggesting that part of this system contributes to the early phase of motor sequence learning. Accordingly, they posited that this region might play a role in the temporal restructuring (i.e. chunking) of a sequence. Yet, they further suggested that, although this region might be necessary for acquiring these sequential temporal properties, the cerebellum might not necessarily be a critical node of the brain for the storage of automatized motor sequences. The latter statement is in agreement with our findings as we found no difference in activity in that structure in association with the consolidation of motor sequences. Instead, we found ipsilateral lobule VI of the cerebellum to be more active for participants who show greater savings of the learned motor adaptation. This is in agreement with other motor adaptation studies reporting activity in the cerebellum (Shadmehr and Holcomb, 1997, Krakauer et al., 2004, Izawa et al., 2012), and altogether, suggest a role of the cerebellum in the memory process of visuomotor adaptation learning.

Beyond the importance of these two structures in memory consolidation, results from Study 2 also suggest that brain regions do not act in isolation, as their strength of interaction is associated with the memory consolidation. To better understand the latter process, the next section (6.) will first address the role played by sleep, while the subsequent one (7.) will address the dynamic reorganization associated with sleep and presumably, the consolidation process.

6. What are the pre-requisite for sleep-dependent consolidation?

Our results support the idea that not all motor memories require sleep to undergo consolidation. To better understand the possible factors that will dictate if sleep is required for consolidation we can either put emphasis on the importance of the structures involved in the sleep-dependent memory, or on the nature of the memory process itself. The first approach will only be briefly addressed as it is mostly founded on cellular mechanisms, such as cell replay or synaptic homeostasis; while the second approach will be discussed more thoroughly.

6.1. Targeting specific brain regions as a way to understand sleep-dependent memory consolidation

We often focus on some particular memory processes in order to find the brain regions subserving memory consolidation. Here, the possibility that some specific brain regions are more predisposed to be associated with sleep-dependent memory processes is explored. More precisely, we could consider that some brain regions are more prone to be reactivated during sleep. For example, we found that motor adaptation consolidation was associated with the cerebellum, whereas sleep-dependent consolidation of motor sequence learning was associated with the striatum. Would it be possible that some biological characteristics of the striatum favor its reactivation during sleep compared to the cerebellum? Answering this question is beyond the aim of the thesis, but that is worth rising. In the literature, the hippocampus is one region that is most often cited as being reactivated during post-learning sleep, yet it does project directly to the ventral striatum (see Buhry et al., 2011). In rats, significant reactivation of the ventral striatum during slow wave sleep was observed following a reward-searching T-maze task. Furthermore, this physiological effect was stronger for about 20% of their units which were modulated by high frequency activity (sharp/wave ripples) in the hippocampus (Pennartz et al., 2004, Pennartz et al., 2009). Thus these results further support the strong functional linkage between the striatum and hippocampus. This was also true when rodents learned both spatial and emotional information, as the learned experience is replayed in the hippocampus jointly with the ventral striatum during sleep (Lansink et al., 2009). Yet again, there were more cell pairs in which hippocampal cells fired before the striatal reward-related neuron. Thus, although these results suggest that memory consolidation depends not only on hippocampal-dependent sleep activity, but also on replay activity in the striatal system, there seems to be an important contribution from the interaction between the two structures.

These results suggest a role of the striatum in learning and sleep-dependent memory processes. Yet, much less seems to be published about reactivations in the cerebellum, a structure absent from a recent review on reactivation and replay of memories by Buhry et al., (2011). It should be noted that the hippocampus, being a structure historically important in memory, was the focus of studies together with closely linked structures such as the striatum and other subcortical regions. Thus, if little studies actually targeted the cerebellum when studying memory replay, there are little chances to find results about that structure. Nevertheless, a recent study found a role of the cerebellum during sleep. During the post-learning sleep period, cue sounds that were previously learned were presented to participants during SWS as a manner to reactivate the memory. Brain activity during cue presentation was associated with better memory retention and included medial temporal lobe, thalamus and cerebellum (van Dongen et al., 2012). The authors suggest that the cerebellum played a significant role considering the nature of the object location task which required subjects to associate an object with a particular location as well as the movement of the joystick to that particular location. Thus they argue that activity of the cerebellum could represent the reactivation of the specific motor patterns.

In sum, much more studies have reported reactivations within the striatum as opposed to the cerebellum; yet, this is possibly biased by the focus of most memory studies on the medial temporal lobe structures and their close link with the striatum.

6.2. Different motor memory processes, different needs for consolidation

One of the major concerns with regards to the necessity of sleep in consolidating memories is whether it plays a passive or an active role. A passive role would suggest that sleep aids memory simply because it is a period in which there is no interference with our everyday endeavor (Mednick et al., 2011). Yet sleep itself is an active process, and the associated brain activity, although different from wake state, could also be interfering. Based on our results and what is known in the literature, I argue that sleep participates actively to the consolidation of explicit form of sequence learning and is a privileged moment of brain state to favor mnemonic processes. An important consideration is that using the same protocol for two different types of learning, we find that consolidation mechanism differs regarding (1) their dependence to sleep and (2) the neuronal systems involved. Thus, most probably, some particular aspects of motor learning, which are prominent in explicit MSL, need sleep to consolidate; while other characteristics of motor learning more prominent in MA don't. Actually some authors are in favor of a segregation of the different types of memory into multiple components in order to better understand memory consolidation (Robertson and Cohen, 2006). Recent theories in the field of declarative memories, favor an important role of sleep in consolidation selectivity, i.e. retaining the relevant vs. irrelevant information (Fischer and Born, 2009, Fischer et al., 2011, Wilhelm et al., 2011, Oudiette et al., 2013); these studies modulated the intention to remember and anticipation of future reward. However emotions and reward did not play a significant role in our studies and can hardly explain why the motor sequence task, but not the motor adaptation task required sleep for consolidation to occur. Thus in an attempt to decipher the most favorable conditions for consolidation to take place, the next sections will briefly overview the motor consolidation processes that are sleep-dependent and those that are not.

6.3. Benefit of daytime for consolidation

It is acknowledged that although sleep aids memory, some processing still occurs during post-learning wakefulness. Most of the evidence up to now (described below), comes from motor adaptation studies, yet other types of learning are also suggested to process off line, such as motor sequences that are learned implicitly, or more specifically the egocentric (motor/effector dependent) aspect of a sequence.

6.3.1. Motor adaptation learning

In the present thesis, we have seen that daytime was sufficient for the consolidation of motor adaptation learning to occur and that this process seemed to depend on the cerebellum (lobule VI). Others had also observed behavioral gains (Doyon et al., 2009c) or savings following daytime delays (Brashers-Krug et al., 1996, Shadmehr and Holcomb, 1997, Krakauer et al., 2005) and irrespective of whether participants slept or were sleep deprived (Albouy et al., 2012). Also, the cerebellum was found in other studies to be associated with this type of learning (Shadmehr and Holcomb, 1997, Krakauer et al., 2004, Izawa et al., 2012). Yet learning a motor adaptation task is quite complex and entails multiple facets. One has to analyze the visual feedback, adjust the motor output while adapting to the imposed visual rotation and finaly reach the target appropriately. With a complex task comes multiple ways to measure performance which give rise to conflicting results while measuring the effect of sleep. Indeed, not all laboratories measure the different facets of the task in the same way and one group has found a beneficial role of sleep for the consolidation of MA (Huber et al., 2004).

In an attempt to unravel these conflicting results, one group cleverly examined 7 different parameters of speed and accuracy (Albouy et al., 2012). The latter authors suggested a protective effect of sleep by means of stabilization of the memory trace, which did not occur if sleep deprivation followed the learning period. They measured both the possibility of having savings and/or gains in performance 2 days after the first learning period. In agreement with our results of study 1, they found savings in both the sleep and sleep deprived group, indicating no specific effect of sleep on that measure. Yet, they found that the sleep deprived group started off the retest session with a decreased performance while the sleep group's performance was maintained from test to retest. Only the measure of directional error, the amount of deviation from an ideal curve, did not improve differently between the two groups. These results thus suggest a protective effect of sleep on some particular components of the task, yet no cerebral changes were observed in association with this stabilization. Furthermore, it is unclear whether this stabilization process would be strictly dependent on sleep or could also happen during daytime following learning. Indeed, the brain state being different during daytime then during the wake state of a sleep deprivation night, it is hard to clearly associate the stabilization of the task with sleep itself. Importantly however, the study showed that not all measures of performance behaved the same with sleep or sleep deprivation, suggesting that the different learning components of the task might take different paths to consolidation.

As discussed in the introduction and in the second article, activity during the post-learning rest periods was shown to be modulated by the previous learning experience (Albert et al., 2009b, Lewis et al., 2009b, Vahdat et al., 2011). Although it is still unclear what these modulations mean, it should be kept in mind that if learning influences post-learning resting state, the functional dynamic observed during resting state is also known to influence subsequent learning (Fox et al., 2007). Thus spontaneous “resting activity” represents a good opportunity to explore what could possibly underlie off-line consolidation processes. As a reminder, the work of Albert et al (2009) and Vahdat et al (2011) demonstrated dynamic changes reflected by increased connectivity in the off-line period following a visuomotor adaptation task. Although conjectural, these changes could advocate in favor of at least some sleep-independent mnemonic processes following motor adaptation learning.

6.3.2. Motor sequence learning

Implicit motor sequences

Apart from motor adaptation, other types of motor learning have been shown to go through a sleep-independent consolidation process. Implicit MSL is one of them (Robertson et al., 2004). The latter authors behaviorally compared the off-line process of explicit MSL vs. implicit MSL and found that only the consolidation of implicit MSL was time-dependent, as opposed to sleep-dependent for explicit MSL. These results thus suggested that awareness of the task was an important factor influencing the off-line consolidation process. Similar conclusions were drawn with the use of a probabilistic sequence, for which learning does not reach awareness, such that improvements in performance were not depend on sleep (Sun et al., 2007). Yet one study using an implicit oculomotor SRT found no gains in performance over the day, while the following night of sleep did trigger improved performance (Albouy et al., 2008). In sum, although questions remain, it seems clear that motor sequence learning does not always undergo consolidation with sleep and that awareness of the task influences the path to consolidation. In accord with this hypothesis, the MA task is one that is learned implicitly and does not seem to require sleep for consolidation. The hypothesis puts emphasis on the nature of the memory, being explicit or implicit as a way to determine whether sleep is required for consolidation.

No studies have examined the effect of sleep on explicit vs. implicit MSL or MA using fMRI, yet, theories of motor sequence learning (Ashe et al., 2006) argue that subcortical structures interact with different cortical areas depending of awareness. Implicit sequence learning would rely on the primary motor cortex, reflecting element-to-element association within the sequence. With practice, the sequence would then be supported through interactions between the medial motor areas such as SMA and pre-SMA, to eventually reach the prefrontal cortex when the representation of the sequence becomes explicit and rehearsal becomes possible. In all cases, subcortical structures are thought to interact with cortical areas with no modulation from awareness. Integrating this theory to the differential effect of sleep on explicit-implicit memory made above, it could be suggested that subcortical areas are involved in consolidation of MSL whether the process depends on sleep or not. These structures would act as pillar of two parallel networks, responsible for the explicit and implicit sequential mnemonic processes. In our study, it could be that sleep allowed consolidation because of the explicit nature of the task and consequently, the striatum was recruited more importantly for participants who slept. Accordingly, it can be considered that the striatum would still be involved following daytime if a sequence was learned implicitly, because we know that daytime is sufficient to trigger consolidation for this type of mnemonic process.

Egocentric component of MSL

Above an explicit-implicit MSL distinction it is also argued that implicit MSL can be segregated into two components such that only one of them would benefit from sleep, while the other could improve with wakefulness (Cohen et al., 2005). One component is about the specific finger movement and is called egocentric or effector-dependent, while the second component is about the goal of the movement and is called allocentric or effector-independent. They hypothesize that the consolidation of the egocentric component is sleep-independent and relies on what Hikosaka et al., described in 2002 to apply to the motor sequence.

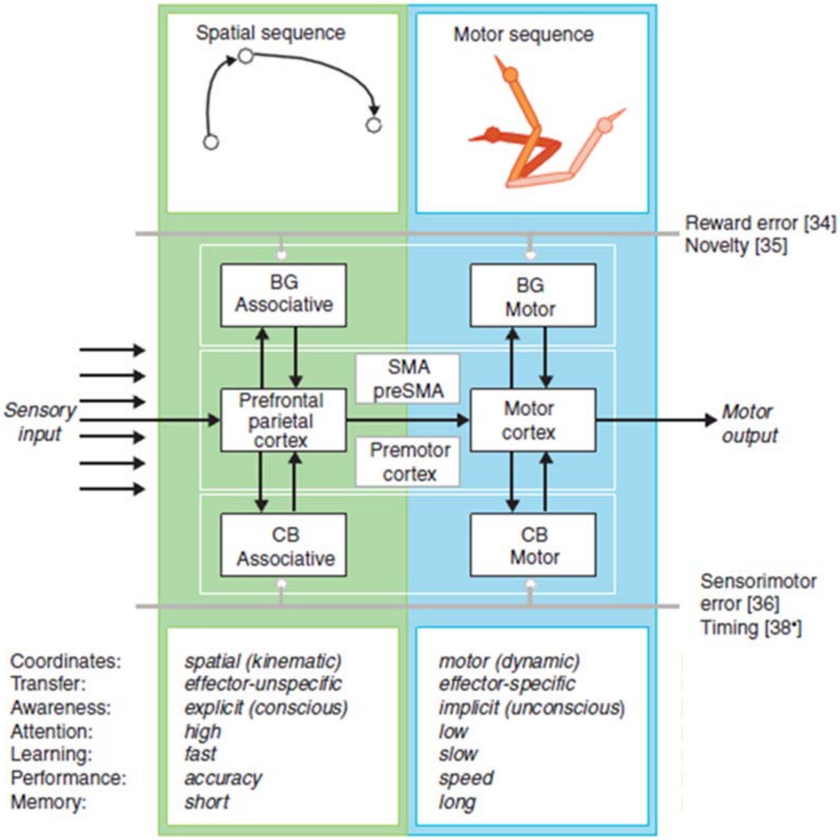


Figure 15: Spatial and motor sequences – adapted from Hikosaka et al. (2002)

Namely, that the egocentric component of the sequence would rely primarily on M1 and SMA, while the parietal and frontal cortices would support goal-based learning (Grafton et al., 1998, Hikosaka et al., 1999, Robertson and Cohen, 2006). Cohen et al., (2005) found that indeed, only the allocentric component of the sequence required sleep to undergo consolidation, as opposed to the egocentric component. Yet no study has yet studied the neural correlates of such dissociation. Interestingly this hypothesis is not at odds with the theory of Ashe et al., 2006 stated earlier, as the egocentric component of the sequence seems to rely on implicit more than explicit demands as compared to the allocentric component of the sequence.

Although one might think that our results are at odds with this allo/ego distinction as our articles focus on the motor system involved in the sleep-dependent consolidation of MSL, I believe it is not. First, both the MSL and MA tasks we used encompass both allocentric and egocentric components, as they were not designed to dissociate these processes. Second, we used explicit sequence learning and the task did not require any adjustment of the visual input to reach button presses as the hand of the participants remained on the response box as they laid in the scanner. Thus I believe our sequence task had more of an allocentric, than egocentric component and the results are further discussed in section 6.4.2. As for motor adaptation learning, even if it is not a sequential task, I also questioned myself as to the allocentric/egocentric nature of the task and to the possibility that this allocentric/egocentric hypothesis could explain our dissociative effect of sleep on our two motor learning tasks. At a first glimpse, our results did not seem compatible with the idea that allocentric motor skills require sleep to consolidate, because MA learning consolidated over the day and the spatial aspect of the task is important. Furthermore, executing the task also requires monitoring and spatial working memory capacities (Seidler et al., 2012). Yet, it should be kept in mind that even if constant monitoring is needed, the learning does mostly occur implicitly (Mazzoni and Krakauer, 2006). Furthermore, although the spatial aspect of the task is important the task remains a reaching task and consequently a highly effector-specific task. What allows optimal performance in such a task is an analysis based on the subjective perception of the participant in order to adapt to the transformation and reach the target, in an egocentric space; as opposed to an analysis based on a target in the allocentric space, independent from the viewpoint of the participant. Although speculative, it could be suggested that the paradigm that we used for motor adaptation learning was more egocentric in nature and for this reason, did not require sleep for consolidation. If one considers that the task is equally allocentric and egocentric, then at least our results are not necessarily in contradiction with the allocentric/egocentric distinction.

In sum, on one hand it seems that motor adaptation learning, which takes place mostly implicitly, and implicit motor sequence learning, particularly the egocentric component of the sequence, bear consolidation processes that are generally independent from sleep. While on the other hand, explicitly learned motor sequences or possibly the allocentric component of a sequence, seem to require sleep to undergo consolidation. Furthermore, if we use the model cited above (Hikosaka et al., 2002), the latter process would notably recruit the prefrontal and parietal areas in addition to a motor network.

6.4. Benefit of sleep for consolidation

6.4.1. Motor adaptation

Although our study and other’s also suggest that off-line processing of motor adaptation learning takes place during daytime, as described earlier, one study found an importance of sleep. Huber et al., 2004 found sleep-dependent improvement in performance, which was associated with an increase in slow wave activity in the right, ipsilateral, parietal lobe. This result paired with the ones from Albouy et al., 2012 who found a protective effect of sleep on some behavioral measures suggests that some components of motor adaptation learning would benefit from sleep to result in improved or maintained performance. In addition the latter study did not report any change in activity associated with sleep, thus the exact motor aspects that might require sleep for consolidation are still unclear.

6.4.2. Explicit Motor sequence learning

We found explicit MSL to consolidate specifically with a night of sleep, as no consolidation took place for a group that stayed awake during daytime. Others have also shown a sleep dependent consolidation process, as reflected by gains in performance, for an explicit motor sequence task (Robertson et al., 2004), after a period of sleep during nighttime (Walker et al., 2002, Walker et al., 2003, Fischer et al., 2005, Walker et al., 2005, Doyon et al., 2009c) or daytime (Fischer et al., 2002) and even following a nap (Korman et al., 2007, Nishida and Walker, 2007, Albouy et al., 2013a).

As discussed earlier, the allocentric (effector-independent) aspect of a sequence might be the particular learning component that requires sleep for consolidation, and for the expression of improved performance (Cohen et al., 2005). According to that hypothesis, the allocentric map of the sequence, irrespective of the actual fingers involved, is the aspect that requires sleep to undergo off-line consolidation. Support for this idea comes from findings suggesting that the generalization of a learned skill, tested by means of intermanual transfer, is helped by sleep. The authors found that only the allocentric coordinate frame of a learned sequence is better consolidated during night time than during daytime; yet transfer of the motor coordinates to the other hand was not affected by neither daytime or nighttime (Witt et al., 2010). If sleep does contribute more importantly to the consolidation of the allocentric aspect of motor sequences, our results would suggest the implication of the striatum in the allocentric component of sequence learning. As discussed earlier, this does not imply that the striatum would not play a role in the egocentric aspect as well, only that probably, the explicit nature of the task used in our study, favored a sleep-dependent consolidation process, which in turn required the implication of the striatum.

6.5. Summary - current hypothesis

Although our studies did not aim at understanding which exact memory process undergoes sleep-dependent consolidation, our results support a dissociation in the role of sleep between the consolidation of an explicit vs. implicit motor memory. Namely, consolidation of explicit motor learning was sleep dependent and associated with the striatum, as opposed to the consolidation of implicit visuomotor adaptation learning associated with the cerebellum. Yet it has to be kept in mind that an explicit-implicit categorization is quite large and remains a poor criterion when trying to distinguish different memory types (Henke, 2010). Thus I also introduced another hypothesis that mostly stands for sequence learning for the moment, which is that the consolidation of an allocentric, effector-independent facet of learning is sleep-dependent, as opposed to the egocentric aspect of sequence learning.

Ideas in favor of an explicit-implicit dissociation of consolidation were brought up in study 1. As opposed to most studies using an explicitly learned sequence, those who used implicit learning with the use of different variants of SRT did not find specific effect of sleep in the maintenance or improvement of performance (Robertson et al., 2004, Song et al., 2007, Nemeth et al., 2010) but see (Albouy et al., 2008). Some authors have given broad explanations to this explicit-implicit dissociative effect of sleep, suggesting that sleep has a greater impact on cognitive functions connected to the frontal lobe, but less on the subcortical structures; while awareness of sequential structure could be related primarily to the frontal lobe (Janacsek and Nemeth, 2012). Saying so, however, they claim that implicit learning would rely on subcortical structures and would not necessarily benefit from sleep. Our data do not support this idea as we found the implication of the striatum specifically in a group that showed consolidation of an explicit sequence. Thus, as mentioned earlier, the striatum is most probably involved in both implicit and explicit sequence learning consolidation. Furthermore our results also suggest that the striatum interacts with a whole system, including the hippocampus, in order to favor optimal consolidation.

The interaction between explicit and implicit memory system has also been studied and findings suggest that sleep can modulate the interaction between memory systems (Born and Wagner, 2004, see also Robertson, 2012). Indeed, participants demonstrate gains in explicit knowledge when implicit learning is followed by sleep. Furthermore, improved reaction times is observed following sleep in subjects whom did not gain explicit knowledge, indicating enhanced procedural memory at the expense of declarative information (Wagner et al., 2004). Interestingly it was also demonstrated that children exhibit greater explicit knowledge from implicit learning, in parallel with a much greater amount of slow wave sleep compared to adults, suggesting a role of sleep, most probably SWS, in the gain of insight into hidden structures (Born and Wilhelm, 2012, Wilhelm et al., 2012). Marshall & Born took into account some of the findings from the declarative memory domain and suggested that when both explicit and implicit information are in competition, explicit information preferentially undergoes sleep-dependent consolidation (Marshall and Born, 2007). They suggest that explicitly learned material is supported by prefrontal-hippocampal circuitry during encoding, which is what enables access of a memory to sleep-dependent consolidation. Thus, although they seem to put emphasis on the structures involved in the process as a way to dictate the implication of sleep in consolidation, they also specify that these structures would subserve specifically explicitly learned material. It could further be hypothesized, based on our results, that when the explicit material is sequential motor information, a prefrontal-hippocampal circuitry would also recruit the striatum to optimize memory consolidation. Support for this possibility comes from a recent review actually suggesting that “a hippocampal-striatal replay mechanism should not be excluded as a mechanism for motor skill consolidation” (Spencer et al., 2013). Furthermore, interaction between the hippocampus and the putamen during initial training of a motor sequence has also been found to predict overnight gains in performance (Albouy et al., 2013b).

The group of Cohen et al (2005) nicely disentangled implicit sequence learning into allocentric and egocentric components. Yet, no task is learned in a purely implicit or explicit fashion (Robertson et al., 2004), thus it should be noted that the amount of explicit learning developed for each of these components is uncertain. Indeed, the spatial map developed in allocentric learning might require more monitoring then the egocentric aspect, which by definition is more procedural in nature and so for which implicitness remains the most efficient way to perform. The model by Hikosaka actually suggests that the prefrontal cortex is involved more importantly in the goal of the movement (see Figure 15) and I remind that the regions of the PFC are also more involved in explicit learning (Ashe et al., 2006). Could it be the case that allocentric learning is consolidated during sleep because the learning is more explicit then implicit in nature, whereas egocentric learning could consolidate over the day because it is more implicit then explicit in nature?

In future avenues, it would be interesting to firstly explore the underlying neural correlates of an allocentric and egocentric components of motor sequence learning. This would firstly allow verifying which regions are involved in the sleep-dependent facet of sequence learning and whether the striatum is part of that system. Secondly, it has recently been suggested that the default-mode (DM) remains more active during an implicit memory task as compared to a comparable explicit memory task in which the default mode was significantly deactivated (Yang et al., 2010). Thus activity of the DM could be used as a “marker” of implicitness in both allocentric and egocentric tasks. If activity of the DM is greater in the egocentric component of learning, then it might mean that sleep-dependent consolidation relies on the level of implicitness of the task, whereas if activity of the DM is not significantly different in the two conditions then sleep-dependent consolidation would rather rely on the allocentric facet of motor learning.

7. Possible mechanisms associated with sleep-dependent consolidation

Results from study 2 revealed that motor sequence consolidation was reflected as a higher level of interaction between the regions of a cortico-striatal system. Importantly, no other functional networks presented this differential effect of sleep on within-system integration. Although quantitative comparison of between-networks integration was not possible because of methodological concerns, it seems that after sleep, all network demonstrate higher within-network levels of integrations as compared to following time spent awake. What happens during sleep, which could lead to higher levels of integration is uncertain but was briefly discussed in study 2. Sleep is hypothesized to be a privileged moment for consolidation to occur, most probably because it allows for different levels of engagement and disengagement between functional memory networks (Massimini et al., 2005, Robertson, 2009, Boly et al., 2012). Although we did not record brain activity during sleep, and so presumably during the consolidation process per se, the last section aims at discussing the possible mechanisms that could be responsible for consolidation, in light of our results suggesting greater synchrony between the regions of the system involved following consolidation.

At the systemic level, Massimini et al., (2005) showed that brain stimulation, using transcranial magnetic stimulation (TMS), provoked waves of signal to the connected cortical areas during quiet wakefulness. Yet, during NREM sleep, the initial response of the stimulation was stronger, but faded rapidly. Importantly, it did not expand beyond the site of stimulation in the way it did in the wake state. The authors interpreted these results by associating sleep to a state of breakdown in cortical effective connectivity. Yet more recent findings suggest a more complex hierarchical dynamic change during sleep state (Boly et al., 2012). As discussed in study 2, their results suggest that the whole brain demonstrates greater overall interaction, yet is characterized by greater within- as opposed to between-system integration in each and every functional networks. These results could thus suggest that there is disengagement between memory systems during sleep. This pattern of connectivity change might allow the memory systems to operate independently (Robertson, 2009, 2012). In our precise case, it might be that the explicit/allocentric aspects of the MSL task were processed independently from the implicit/egocentric aspect of the task. This independent processing might subsequently favor an optimized interaction between the memory systems the following morning, when executing the task. If we push it further, since the large scale cortico-striatal network was the only one showing greater within-system integration following consolidation, it could possibly represent the re-engagement between the different networks associated with the different facets of sequence learning. Similarly, it could also be hypothesized that during wake state, the different memory system did not benefit from this disengagement to favor independent processing, most possibly because of the constant input from the external environment which favors interaction amongst memory system or simply because the brain is in a conscious state. Consequently, as we observe in our study 2 (Figure 14 of the Thesis), system integration within large scale functional networks following a day spent awake is sub-optimal at the whole brain level. It should be noted that up to now, however, there is no scientific evidence to support the idea that because memory systems, or network, are allowed to function more independently during nighttime, they will interact more efficiently the following morning.

8. Perspective

We aimed to better understand the neural correlates and the dynamic functional reorganisation of systems that are associated with the consolidation of motor skills, particularly of motor sequence learning, which is known to depend on sleep.

Numerous questions about the sleep-dependent consolidation process of motor sequences could be better answered if the post-learning sleep period *itself* was studied, using combined fMRI-EEG recordings. This method would allow to directly associate the sleep characteristics at the microarchitectural level, with the changes in brain activity at the systemic level. Of our interest, we could verify whether the striatum is part of a network that is reactivated during post-learning sleep. Furthermore, the association between the different sleep characteristics and brain reactivation could be explored. It would also be interesting to measure within-system integration at different time points during sleep as a way to describe the evolution of a brain state that “is consolidating” a specific type of learning with one that is not. Finally relating causally these neurophysiological phenomena, as well as brain activity reactivations, to post-sleep improvements in behavioral performance would also be important in order to validate that all these processes are reflections of the consolidation process.

It would be equally important to study sleep-independent consolidation processes in order to understand what allows consolidation during daytime, for example with the use of an implicit/motoric sequence. We could thus verify whether a cortico-striatal system can also be isolated with the use of ICA and whether the effect that we observed following sleep-dependent consolidation can be generalized to sequence consolidation at large (i.e. independent from sleep). It should be explored whether an increased interaction is present within a specific cortico-striatal network, following daytime, in association with implicit sequence learning. If this is so, it would support the idea that integration within a network could be a marker of sequence consolidation.

Finally, in the long term it would be crucial to further understand whether what is seen at the systemic level can actually be explained by what is observed at the cellular level. As reviewed in the introduction, post-learning sleep studies with the use of EEG has allowed to target multiple sleep characteristics associated with the consolidation process. With MSL in particular, most recent studies have focused on a role of NREM sleep, with emphasis on slow oscillations, spindles and sharp/wave ripples (Rauchs et al., 2005, Diekelmann and Born, 2010, Fogel and Smith, 2011). Interestingly, when exploring these sleep characteristics at the cellular level, we also found notions of synchrony and correlations between neurons or brain regions. Thus it would be interesting to verify whether these neurophysiological phenomena are linked in any ways to system integration, also defined as a higher level of temporal correlation between brain regions. For example, slow oscillations originate in the neocortex with a peak frequency of 0.8 Hz and are thought to synchronize neuronal activity into down-states of widespread hyperpolarization and neuronal silence, followed by up-state depolarization (see Diekelmann and Born, 2010). Is this descent to the down-state, characterized with neuronal synchrony, an optimal period for information exchange between regions? And is this type of synchrony somehow associated with the within-system higher interaction that we observed following sleep? Massimini (2005) reported that the more prior activation during wake state, the more transitions between up-state and down-states are observed during sleep. An avenue of research could be to decipher any association between these transitions and the nighttime engagement-disengagement of the different functional brain systems (Robertson, 2009) hypothesized to underlie memory consolidation.

9. Implication

Finally, through behavioral, as well as different brain imaging analysis, we characterized the effect of sleep versus the simple passage of time, on the consolidation of two different types of motor skills. Although numerous questions remain, these studies have expanded our knowledge base of the distinct neural networks mediating sleep-dependent vs. independent consolidation of motor skills. We also provided new insights as to the dynamic organization of brain networks following sleep-dependent consolidation, with the use of innovative data-driven methods. Clinically these findings could have repercussion for the understanding of the changes in learning abilities in the elderly in whom sleep is known to deteriorate, as well as for patients recovering from damage to the motor system.

References

Agostino R, Sanes JN, Hallett M (1996) Motor skill learning in Parkinson's disease. Journal of the neurological sciences 139:218-226.

Albert NB, Robertson EM, Mehta P, Miall RC (2009a) Resting state networks and memory consolidation. Commun Integr Biol 2:530-532.

Albert NB, Robertson EM, Miall RC (2009b) The resting human brain and motor learning. Current biology : CB 19:1023-1027.

Albouy G, Fogel S, Pottiez H, Nguyen VA, Ray L, Lungu O, Carrier J, Robertson E, Doyon J (2013a) Daytime sleep enhances consolidation of the spatial but not motoric representation of motor sequence memory. PLoS One 8:e52805.

Albouy G, Sterpenich V, Balteau E, Vandewalle G, Desseilles M, Dang-Vu T, Darsaud A, Ruby P, Luppi PH, Degueldre C, Peigneux P, Luxen A, Maquet P (2008) Both the hippocampus and striatum are involved in consolidation of motor sequence memory. Neuron 58:261-272.

Albouy G, Sterpenich V, Vandewalle G, Darsaud A, Gais S, Rauchs G, Desseilles M, Boly M, Dang-Vu T, Balteau E, Degueldre C, Phillips C, Luxen A, Maquet P (2012) Neural correlates of performance variability during motor sequence acquisition. Neuroimage 60:324-331.

Albouy G, Sterpenich V, Vandewalle G, Darsaud A, Gais S, Rauchs G, Desseilles M, Boly M, Dang-Vu T, Balteau E, Degueldre C, Phillips C, Luxen A, Maquet P (2013b) Interaction between Hippocampal and Striatal Systems Predicts Subsequent Consolidation of Motor Sequence Memory. PLoS One 8:e59490.

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends in neurosciences 13:266-271.

Ashe J, Lungu OV, Basford AT, Lu X (2006) Cortical control of motor sequences. Current opinion in neurobiology 16:213-221.

Barakat M, Carrier J, Debas K, Lungu O, Fogel S, Vandewalle G, Hoge RD, Bellec P, Karni A, Ungerleider LG, Benali H, Doyon J (2012) Sleep spindles predict neural and behavioral changes in motor sequence consolidation. Human brain mapping.

Barakat M, Doyon J, Debas K, Vandewalle G, Morin A, Poirier G, Martin N, Lafortune M, Karni A, Ungerleider LG, Benali H, Carrier J (2010) Fast and slow spindle involvement in the consolidation of a new motor sequence. Behavioural brain research.

Beckmann CF, DeLuca M, Devlin JT, Smith SM (2005) Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc Lond B Biol Sci 360:1001-1013.

Benecke R, Rothwell JC, Dick JP, Day BL, Marsden CD (1987) Disturbance of sequential movements in patients with Parkinson's disease. Brain 110 (Pt 2):361-379.

Boly M, Perlbarg V, Marrelec G, Schabus M, Laureys S, Doyon J, Pelegrini-Issac M, Maquet P, Benali H (2012) Hierarchical clustering of brain activity during human nonrapid eye movement sleep. Proceedings of the National Academy of Sciences of the United States of America 109:5856-5861.

Boly M, Phillips C, Tshibanda L, Vanhaudenhuyse A, Schabus M, Dang-Vu TT, Moonen G, Hustinx R, Maquet P, Laureys S (2008) Intrinsic brain activity in altered states of consciousness: how conscious is the default mode of brain function? Ann N Y Acad Sci 1129:119-129.

Born J, Wagner U (2004) Awareness in memory: being explicit about the role of sleep. Trends in cognitive sciences 8:242-244.

Born J, Wilhelm I (2012) System consolidation of memory during sleep. Psychol Res 76:192-203.

Brashers-Krug T, Shadmehr R, Bizzi E (1996) Consolidation in human motor memory. Nature 382:252-255.

Buhry L, Azizi AH, Cheng S (2011) Reactivation, replay, and preplay: how it might all fit together. Neural Plast 2011:203462.

Caithness G, Osu R, Bays P, Chase H, Klassen J, Kawato M, Wolpert DM, Flanagan JR (2004) Failure to consolidate the consolidation theory of learning for sensorimotor adaptation tasks. J Neurosci 24:8662-8671.

Cajochen C, Knoblauch V, Wirz-Justice A, Krauchi K, Graw P, Wallach D (2004) Circadian modulation of sequence learning under high and low sleep pressure conditions. Behavioural brain research 151:167-176.

Calhoun VD, Kiehl KA, Pearlson GD (2008) Modulation of temporally coherent brain networks estimated using ICA at rest and during cognitive tasks. Human brain mapping 29:828-838.

Carrier J, Monk TH (2000) Circadian rhythms of performance: new trends. Chronobiol Int 17:719-732.

Censor N, Sagi D, Cohen LG (2012) Common mechanisms of human perceptual and motor learning. Nature reviews Neuroscience 13:658-664.

Cohen DA, Pascual-Leone A, Press DZ, Robertson EM (2005) Off-line learning of motor skill memory: a double dissociation of goal and movement. Proceedings of the National Academy of Sciences of the United States of America 102:18237-18241.

Cohen NJ, Squire LR (1980) Preserved learning and retention of pattern-analyzing skill in amnesia: dissociation of knowing how and knowing that. Science (New York, NY 210:207-210.

Coynel D, Marrelec G, Perlbarg V, Pelegrini-Issac M, Van de Moortele PF, Ugurbil K, Doyon J, Benali H, Lehericy S (2010) Dynamics of motor-related functional integration during motor sequence learning. Neuroimage 49:759-766.

Damoiseaux JS, Beckmann CF, Arigita EJ, Barkhof F, Scheltens P, Stam CJ, Smith SM, Rombouts SA (2008) Reduced resting-state brain activity in the "default network" in normal aging. Cerebral cortex 18:1856-1864.

Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF (2006) Consistent resting-state networks across healthy subjects. Proceedings of the National Academy of Sciences of the United States of America 103:13848-13853.

Dang-Vu TT, Schabus M, Desseilles M, Albouy G, Boly M, Darsaud A, Gais S, Rauchs G, Sterpenich V, Vandewalle G, Carrier J, Moonen G, Balteau E, Degueldre C, Luxen A, Phillips C, Maquet P (2008) Spontaneous neural activity during human slow wave sleep. Proceedings of the National Academy of Sciences of the United States of America 105:15160-15165.

Dayan E, Cohen LG (2011) Neuroplasticity subserving motor skill learning. Neuron 72:443-454.

Debaere F, Wenderoth N, Sunaert S, Van Hecke P, Swinnen SP (2004) Changes in brain activation during the acquisition of a new bimanual coodination task. Neuropsychologia 42:855-867.

Debas K, Carrier J, Orban P, Barakat M, Lungu O, Vandewalle G, Hadj Tahar A, Bellec P, Karni A, Ungerleider LG, Benali H, Doyon J (2010) Brain plasticity related to the consolidation of motor sequence learning and motor adaptation. Proc Natl Acad Sci U S A 107:17839-17844.

Della-Maggiore V, McIntosh AR (2005) Time course of changes in brain activity and functional connectivity associated with long-term adaptation to a rotational transformation. Journal of neurophysiology 93:2254-2262.

Diekelmann S, Born J (2010) The memory function of sleep. Nature reviews Neuroscience 11:114-126.

Diekelmann S, Wilhelm I, Born J (2009) The whats and whens of sleep-dependent memory consolidation. Sleep medicine reviews 13:309-321.

Doya K (1999) What are the computations of the cerebellum, the basal ganglia and the cerebral cortex? Neural Netw 12:961-974.

Doya K (2000) Complementary roles of basal ganglia and cerebellum in learning and motor control. Current opinion in neurobiology 10:732-739.

Doyon J, Bellec P, Amsel R, Penhune V, Monchi O, Carrier J, Lehericy S, Benali H (2009a) Contributions of the basal ganglia and functionally related brain structures to motor learning. Behavioural brain research 199:61-75.

Doyon J, Benali H (2005) Reorganization and plasticity in the adult brain during learning of motor skills. Current opinion in neurobiology 15:161-167.

Doyon J, Korman M, Morin A, Dostie V, Hadj Tahar A, Benali H, Karni A, Ungerleider LG, Carrier J (2009b) Contribution of night and day sleep vs. simple passage of time to the consolidation of motor sequence and visuomotor adaptation learning. Exp Brain Res 195:15-26.

Doyon J, Korman M, Morin A, Dostie V, Tahar AH, Benali H, Karni A, Ungerleider LG, Carrier J (2009c) Contribution of night and day sleep vs. simple passage of time to the consolidation of motor sequence and visuomotor adaptation learning. Experimental brain research Experimentelle Hirnforschung.

Doyon J, Penhune V, Ungerleider LG, Doyon J, Penhune V, Ungerleider LG (2003) Distinct contribution of the cortico-striatal and cortico-cerebellar systems to motor skill learning. Neuropsychologia 41:252-262.

Doyon J, Song AW, Karni A, Lalonde F, Adams MM, Ungerleider LG (2002) Experience-dependent changes in cerebellar contributions to motor sequence learning. Proc Natl Acad Sci U S A 99:1017-1022.

Dudai Y (2002) Molecular bases of long-term memories: a question of persistence. Current opinion in neurobiology 12:211-216.

Dudai Y (2004) The neurobiology of consolidations, or, how stable is the engram? Annual review of psychology 55:51-86.

Eichenbaum H, Cohen NJ (2001) From conditioning to conscious recollection : memory systems of the brain. Oxford ; New York: Oxford University Press.

Filippini N, MacIntosh BJ, Hough MG, Goodwin GM, Frisoni GB, Smith SM, Matthews PM, Beckmann CF, Mackay CE (2009) Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. Proc Natl Acad Sci U S A 106:7209-7214.

Fischer S, Born J (2009) Anticipated reward enhances offline learning during sleep. Journal of experimental psychology Learning, memory, and cognition 35:1586-1593.

Fischer S, Diekelmann S, Born J (2011) Sleep's role in the processing of unwanted memories. Journal of sleep research 20:267-274.

Fischer S, Hallschmid M, Elsner AL, Born J (2002) Sleep forms memory for finger skills. Proc Natl Acad Sci U S A 99:11987-11991.

Fischer S, Nitschke MF, Melchert UH, Erdmann C, Born J (2005) Motor Memory Consolidation in Sleep Shapes More Effective Neuronal Representations: Journal of Neuroscience Vol 25(49) Dec 2005, 11248-11255.

Floyer-Lea A, Matthews PM (2005) Distinguishable Brain Activation Networks for Short- and Long-Term Motor Skill Learning: Journal of Neurophysiology Vol 94(1) Jul 2005, 512-518.

Fogel SM, Smith CT (2011) The function of the sleep spindle: a physiological index of intelligence and a mechanism for sleep-dependent memory consolidation. Neuroscience and biobehavioral reviews 35:1154-1165.

Foster DJ, Wilson MA (2006) Reverse replay of behavioural sequences in hippocampal place cells during the awake state. Nature 440:680-683.

Fox MD, Raichle ME (2007) Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nature reviews Neuroscience 8:700-711.

Fox MD, Snyder AZ, Vincent JL, Raichle ME (2007) Intrinsic fluctuations within cortical systems account for intertrial variability in human behavior. Neuron 56:171-184.

Friston KJ (2011) Functional and effective connectivity: a review. Brain Connect 1:13-36.

Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ (1997) Psychophysiological and modulatory interactions in neuroimaging. NeuroImage 6:218-229.

Friston KJ, Frith CD, Frackowiak RS (1993) Principal component analysis learning algorithms: a neurobiological analysis. Proceedings 254:47-54.

Gabrieli JD, Stebbins GT, Singh J, Willingham DB, Goetz CG (1997) Intact mirror-tracing and impaired rotary-pursuit skill learning in patients with Huntington's disease: evidence for dissociable memory systems in skill learning. Neuropsychology 11:272-281.

Genovese CR, Lazar NA, Nichols T (2002) Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage 15:870-878.

Gheysen F, Van Opstal F, Roggeman C, Van Waelvelde H, Fias W (2010) Hippocampal contribution to early and later stages of implicit motor sequence learning. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale 202:795-807.

Ghilardi M, Ghez C, Dhawan V, Moeller J, Mentis M, Nakamura T, Antonini A, Eidelberg D (2000) Patterns of regional brain activation associated with different forms of motor learning. Brain research 871:127-145.

Grafton ST, Hazeltine E, Ivry RB (1998) Abstract and effector-specific representations of motor sequences identified with PET. The Journal of neuroscience : the official journal of the Society for Neuroscience 18:9420-9428.

Grafton ST, Mazziotta JC, Presty S, Friston KJ, Frackowiak RS, Phelps ME (1992) Functional anatomy of human procedural learning determined with regional cerebral blood flow and PET. J Neurosci 12:2542-2548.

Graybiel AM (1998) The basal ganglia and chunking of action repertoires. Neurobiology of learning and memory 70:119-136.

Graybiel AM (2005) The basal ganglia: learning new tricks and loving it. Current opinion in neurobiology 15:638-644.

Habas C, Cabanis EA (2008) Neural correlates of simple unimanual discrete and continuous movements: a functional imaging study at 3 T. Neuroradiology 50:367-375.

Hauptmann B, Karni A (2002) From primed to learn: the saturation of repetition priming and the induction of long-term memory. Brain research 13:313-322.

Henke K (2010) A model for memory systems based on processing modes rather than consciousness. Nature reviews Neuroscience 11:523-532.

Hikosaka O, Nakahara H, Rand MK, Sakai K, Lu X, Nakamura K, Miyachi S, Doya K (1999) Parallel neural networks for learning sequential procedures. Trends in neurosciences 22:464-471.

Hikosaka O, Nakamura K, Sakai K, Nakahara H (2002) Central mechanisms of motor skill learning. Current opinion in neurobiology 12:217-222.

Horovitz SG, Fukunaga M, de Zwart JA, van Gelderen P, Fulton SC, Balkin TJ, Duyn JH (2008) Low frequency BOLD fluctuations during resting wakefulness and light sleep: a simultaneous EEG-fMRI study. Human brain mapping 29:671-682.

Huber R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and learning. Nature 430:78-81.

Imamizu H, Kuroda T, Miyauchi S, Yoshioka T, Kawato M (2003) Modular organization of internal models of tools in the human cerebellum. Proc Natl Acad Sci U S A 100:5461-5466.

Imamizu H, Miyauchi S, Tamada T, Sasaki Y, Takino R, Putz B, Yoshioka T, Kawato M (2000) Human cerebellar activity reflecting an acquired internal model of a new tool. Nature 403:192-195.

Izawa J, Criscimagna-Hemminger SE, Shadmehr R (2012) Cerebellar contributions to reach adaptation and learning sensory consequences of action. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:4230-4239.

Janacsek K, Nemeth D (2012) Predicting the future: from implicit learning to consolidation. Int J Psychophysiol 83:213-221.

Jenkins IH, Brooks DJ, Nixon PD, Frackowiak RS, Passingham RE (1994) Motor sequence learning: a study with positron emission tomography. The Journal of neuroscience : the official journal of the Society for Neuroscience 14:3775-3790.

John ER (1967) Mechanisms of memory. New York: Academic Press.

Johnstone T, Ores Walsh KS, Greischar LL, Alexander AL, Fox AS, Davidson RJ, Oakes TR (2006) Motion correction and the use of motion covariates in multiple-subject fMRI analysis. Human brain mapping 27:779-788.

Jueptner M, Frith CD, Brooks DJ, Frackowiak RS, Passingham RE (1997) Anatomy of motor learning. II. Subcortical structures and learning by trial and error. Journal of neurophysiology 77:1325-1337.

Kandel ER, Squire LR (2000) Neuroscience: breaking down scientific barriers to the study of brain and mind. Science (New York, NY 290:1113-1120.

Karni A (1995) When practice makes perfect. Lancet 345:395.

Karni A (1996) The acquisition of perceptual and motor skills: a memory system in the adult human cortex. Brain research 5:39-48.

Karni A, Bertini G (1997) Learning perceptual skills: behavioral probes into adult cortical plasticity. Current opinion in neurobiology 7:530-535.

Karni A, Meyer G, Jezzard P, Adams MM, Turner R, Ungerleider LG (1995) Functional MRI evidence for adult motor cortex plasticity during motor skill learning. Nature 377:155-158.

Karni A, Meyer G, Rey-Hipolito C, Jezzard P, Adams MM, Turner R, Ungerleider LG (1998) The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. Proc Natl Acad Sci U S A 95:861-868.

Karni A, Sagi D (1993) The time course of learning a visual skill. Nature 365:250-252.

Kitazawa S, Kimura T, Yin PB (1998) Cerebellar complex spikes encode both destinations and errors in arm movements. Nature 392:494-497.

Knopman D, Nissen MJ (1991) Procedural learning is impaired in Huntington's disease: evidence from the serial reaction time task. Neuropsychologia 29:245-254.

Korman M, Doyon J, Doljansky J, Carrier J, Dagan Y, Karni A (2007) Daytime sleep condenses the time course of motor memory consolidation. Nature neuroscience 10:1206-1213.

Krakauer JW, Ghez C, Ghilardi MF (2005) Adaptation to visuomotor transformations: consolidation, interference, and forgetting. J Neurosci 25:473-478.

Krakauer JW, Ghilardi MF, Mentis M, Barnes A, Veytsman M, Eidelberg D, Ghez C (2004) Differential cortical and subcortical activations in learning rotations and gains for reaching: a PET study. Journal of neurophysiology 91:924-933.

Krakauer JW, Pine ZM, Ghilardi MF, Ghez C (2000) Learning of visuomotor transformations for vectorial planning of reaching trajectories. J Neurosci 20:8916-8924.

Krakauer JW, Shadmehr R (2006) Consolidation of motor memory. Trends in neurosciences 29:58-64.

Kuriyama K, Stickgold R, Walker MP (2004) Sleep-dependent learning and motor-skill complexity. Learning & memory (Cold Spring Harbor, NY 11:705-713.

Lansink CS, Goltstein PM, Lankelma JV, McNaughton BL, Pennartz CM (2009) Hippocampus leads ventral striatum in replay of place-reward information. PLoS biology 7:e1000173.

Lee AK, Wilson MA (2002) Memory of sequential experience in the hippocampus during slow wave sleep. Neuron 36:1183-1194.

Lee L, Harrison LM, Mechelli A (2003) The Functional Brain Connectivity Workshop: report and commentary. Network 14:R1-15.

Lehericy S, Benali H, Van de Moortele PF, Pelegrini-Issac M, Waechter T, Ugurbil K, Doyon J, Lehericy S, Benali H, Van de Moortele P-F, Pelegrini-Issac M, Waechter T, Ugurbil K, Doyon J (2005) Distinct basal ganglia territories are engaged in early and advanced motor sequence learning. Proceedings of the National Academy of Sciences of the United States of America 102:12566-12571.

Lehericy S, Ducros M, Krainik A, Francois C, Van de Moortele PF, Ugurbil K, Kim DS (2004) 3-D diffusion tensor axonal tracking shows distinct SMA and pre-SMA projections to the human striatum. Cerebral cortex 14:1302-1309.

Levesque M, Bedard MA, Courtemanche R, Tremblay PL, Scherzer P, Blanchet PJ (2007) Raclopride-induced motor consolidation impairment in primates: role of the dopamine type-2 receptor in movement chunking into integrated sequences. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale 182:499-508.

Lewis CM, Baldassarre A, Committeri G, Romani GL, Corbetta M (2009) Learning sculpts the spontaneous activity of the resting human brain. Proceedings of the National Academy of Sciences of the United States of America 106:17558-17563.

Ma L, Wang B, Narayana S, Hazeltine E, Chen X, Robin DA, Fox PT, Xiong J (2010) Changes in regional activity are accompanied with changes in inter-regional connectivity during 4 weeks motor learning. Brain research 1318:64-76.

Maquet P (2001) The role of sleep in learning and memory. Science (New York, NY 294:1048-1052.

Maquet P, Laureys S, Peigneux P, Fuchs S, Petiau C, Phillips C, Aerts J, Del Fiore G, Degueldre C, Meulemans T, Luxen A, Franck G, Van Der Linden M, Smith C, Cleeremans A (2000) Experience-dependent changes in cerebral activation during human REM sleep. Nature neuroscience 3:831-836.

Marin MF, Pilgrim K, Lupien SJ (2010) Modulatory effects of stress on reactivated emotional memories. Psychoneuroendocrinology 35:1388-1396.

Marshall L, Born J (2007) The contribution of sleep to hippocampus-dependent memory consolidation. Trends in cognitive sciences 11:442-450.

Martin TA, Keating JG, Goodkin HP, Bastian AJ, Thach WT (1996) Throwing while looking through prisms. I. Focal olivocerebellar lesions impair adaptation. Brain : a journal of neurology 119 (Pt 4):1183-1198.

Martone M, Butters N, Payne M, Becker JT, Sax DS (1984) Dissociations between skill learning and verbal recognition in amnesia and dementia. Archives of neurology 41:965-970.

Massimini M, Ferrarelli F, Huber R, Esser SK, Singh H, Tononi G (2005) Breakdown of cortical effective connectivity during sleep. Science (New York, NY 309:2228-2232.

Mazzoni P, Krakauer JW (2006) An implicit plan overrides an explicit strategy during visuomotor adaptation. J Neurosci 26:3642-3645.

McClelland JL, McNaughton BL, O'Reilly RC (1995) Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. Psychological review 102:419-457.

McKeown MJ, Makeig S, Brown GG, Jung TP, Kindermann SS, Bell AJ, Sejnowski TJ (1998) Analysis of fMRI data by blind separation into independent spatial components. Human brain mapping 6:160-188.

Mednick SC, Cai DJ, Shuman T, Anagnostaras S, Wixted JT (2011) An opportunistic theory of cellular and systems consolidation. Trends in neurosciences 34:504-514.

Miall RC, Christensen LO, Cain O, Stanley J (2007) Disruption of state estimation in the human lateral cerebellum. PLoS biology 5:e316.

Miall RC, Robertson EM (2006) Functional imaging: is the resting brain resting? Current biology : CB 16:R998-1000.

Middleton FA, Strick PL (2000) Basal ganglia and cerebellar loops: motor and cognitive circuits. Brain Res Brain Res Rev 31:236-250.

Morin A, Doyon J, Dostie V, Barakat M, Hadj Tahar A, Korman M, Benali H, Karni A, Ungerleider LG, Carrier J (2008) Motor sequence learning increases sleep spindles and fast frequencies in post-training sleep. Sleep 31:1149-1156.

Nader K (2003) Memory traces unbound. Trends in neurosciences 26:65-72.

Nader K, Schafe GE, LeDoux JE (2000) The labile nature of consolidation theory. Nature reviews 1:216-219.

Nemeth D, Janacsek K, Londe Z, Ullman MT, Howard DV, Howard JH, Jr. (2010) Sleep has no critical role in implicit motor sequence learning in young and old adults. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale 201:351-358.

Nezafat R, Shadmehr R, Holcomb HH (2001) Long-term adaptation to dynamics of reaching movements: a PET study. Experimental brain research Experimentelle Hirnforschung 140:66-76.

Nichols T, Hayasaka S (2003) Controlling the familywise error rate in functional neuroimaging: a comparative review. Statistical methods in medical research 12:419-446.

Nishida M, Walker MP (2007) Daytime naps, motor memory consolidation and regionally specific sleep spindles. PLoS One 2:e341.

Orban P, Peigneux P, Lungu O, Albouy G, Breton E, Laberenne F, Benali H, Maquet P, Doyon J (2010) The multifaceted nature of the relationship between performance and brain activity in motor sequence learning. Neuroimage 49:694-702.

Oudiette D, Antony JW, Creery JD, Paller KA (2013) The Role of Memory Reactivation during Wakefulness and Sleep in Determining Which Memories Endure. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:6672-6678.

Peigneux P, Laureys S, Delbeuck X, Maquet P (2001) Sleeping brain, learning brain. The role of sleep for memory systems. Neuroreport 12:A111-124.

Peigneux P, Laureys S, Fuchs S, Destrebecqz A, Collette F, Delbeuck X, Phillips C, Aerts J, Del Fiore G, Degueldre C, Luxen A, Cleeremans A, Maquet P (2003) Learned material content and acquisition level modulate cerebral reactivation during posttraining rapid-eye-movements sleep. Neuroimage 20:125-134.

Peigneux P, Orban P, Balteau E, Degueldre C, Luxen A, Laureys S, Maquet P (2006) Offline persistence of memory-related cerebral activity during active wakefulness. PLoS biology 4:e100.

Penhune VB, Doyon J (2002) Dynamic cortical and subcortical networks in learning and delayed recall of timed motor sequences. J Neurosci 22:1397-1406.

Penhune VB, Steele CJ (2012) Parallel contributions of cerebellar, striatal and M1 mechanisms to motor sequence learning. Behavioural brain research 226:579-591.

Pennartz CM, Berke JD, Graybiel AM, Ito R, Lansink CS, van der Meer M, Redish AD, Smith KS, Voorn P (2009) Corticostriatal Interactions during Learning, Memory Processing, and Decision Making. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:12831-12838.

Pennartz CM, Lee E, Verheul J, Lipa P, Barnes CA, McNaughton BL (2004) The ventral striatum in off-line processing: ensemble reactivation during sleep and modulation by hippocampal ripples. J Neurosci 24:6446-6456.

Perlbarg V, Bellec P, Anton JL, Pelegrini-Issac M, Doyon J, Benali H (2007) CORSICA: correction of structured noise in fMRI by automatic identification of ICA components. Magnetic resonance imaging 25:35-46.

Press DZ, Casement MD, Pascual-Leone A, Robertson EM (2005) The time course of off-line motor sequence learning. Brain research 25:375-378.

Puttemans V, Wenderoth N, Swinnen SP (2005) Changes in brain activation during the acquisition of a multifrequency bimanual coordination task: from the cognitive stage to advanced levels of automaticity. J Neurosci 25:4270-4278.

Rauchs G, Desgranges B, Foret J, Eustache F (2005) The relationships between memory systems and sleep stages. J Sleep Res 14:123-140.

Remy F, Wenderoth N, Lipkens K, Swinnen SP (2008) Acquisition of a new bimanual coordination pattern modulates the cerebral activations elicited by an intrinsic pattern: an fMRI study. Cortex 44:482-493.

Rickard TC, Cai DJ, Rieth CA, Jones J, Ard MC (2008) Sleep does not enhance motor sequence learning. Journal of experimental psychology 34:834-842.

Robertson EM (2004) Skill learning: putting procedural consolidation in context. Current biology : CB 14:R1061-1063.

Robertson EM (2009) From creation to consolidation: a novel framework for memory processing. PLoS biology 7:e19.

Robertson EM (2012) New insights in human memory interference and consolidation. Current biology : CB 22:R66-71.

Robertson EM, Cohen DA (2006) Understanding consolidation through the architecture of memories. Neuroscientist 12:261-271.

Robertson EM, Pascual-Leone A, Press DZ (2004) Awareness modifies the skill-learning benefits of sleep. Curr Biol 14:208-212.

Rose M, Haider H, Salari N, Buchel C (2011) Functional dissociation of hippocampal mechanism during implicit learning based on the domain of associations. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:13739-13745.

Schendan HE, Searl MM, Melrose RJ, Stern CE (2003) An FMRI study of the role of the medial temporal lobe in implicit and explicit sequence learning. Neuron 37:1013-1025.

Scoville WB, Milner B (1957) Loss of recent memory after bilateral hippocampal lesions. Journal of neurology, neurosurgery, and psychiatry 20:11-21.

Seidler RD, Bo J, Anguera JA (2012) Neurocognitive contributions to motor skill learning: the role of working memory. J Mot Behav 44:445-453.

Shadmehr R, Brashers-Krug T (1997) Functional stages in the formation of human long-term motor memory. J Neurosci 17:409-419.

Shadmehr R, Holcomb HH (1997) Neural correlates of motor memory consolidation. Science (New York, NY 277:821-825.

Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, Filippini N, Watkins KE, Toro R, Laird AR, Beckmann CF (2009) Correspondence of the brain's functional architecture during activation and rest. Proc Natl Acad Sci U S A 106:13040-13045.

Song S, Howard JH, Jr., Howard DV (2007) Sleep does not benefit probabilistic motor sequence learning. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:12475-12483.

Spencer J, Patel M, Mehta N, Simmons HC, 3rd, Bennett T, Bailey JK, Moses A (2013) Special consideration regarding the assessment and management of patients being treated with mandibular advancement oral appliance therapy for snoring and obstructive sleep apnea. Cranio 31:10-13.

Squire LR, Knowlton B, Musen G (1993) The structure and organization of memory. Annual review of psychology 44:453-495.

Squire LR, Zola SM (1996) Structure and function of declarative and nondeclarative memory systems. Proc Natl Acad Sci U S A 93:13515-13522.

Steele CJ, Penhune VB (2010) Specific increases within global decreases: a functional magnetic resonance imaging investigation of five days of motor sequence learning. J Neurosci 30:8332-8341.

Stickgold R, Walker MP (2013) Sleep-dependent memory triage: evolving generalization through selective processing. Nature neuroscience 16:139-145.

Sun FT, Miller LM, Rao AA, D'Esposito M (2007) Functional connectivity of cortical networks involved in bimanual motor sequence learning. Cerebral cortex 17:1227-1234.

Toni I, Rowe J, Stephan KE, Passingham RE (2002) Changes of cortico-striatal effective connectivity during visuomotor learning. Cerebral cortex 12:1040-1047.

Tononi G, Cirelli C (2003) Sleep and synaptic homeostasis: a hypothesis. Brain research bulletin 62:143-150.

Ungerleider LG (1995) Functional brain imaging studies of cortical mechanisms for memory. Science (New York, NY 270:769-775.

Ungerleider LG, Doyon J, Karni A (2002) Imaging brain plasticity during motor skill learning. Neurobiology of Learning and Memory 78:553-564.

Vahdat S, Darainy M, Milner TE, Ostry DJ (2011) Functionally specific changes in resting-state sensorimotor networks after motor learning. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:16907-16915.

van Dongen EV, Takashima A, Barth M, Zapp J, Schad LR, Paller KA, Fernandez G (2012) Memory stabilization with targeted reactivation during human slow-wave sleep.

Proceedings of the National Academy of Sciences of the United States of America 109:10575-10580.

Wagner U, Gais S, Haider H, Verleger R, Born J (2004) Sleep inspires insight. Nature 427:352-355.

Walker MP, Brakefield T, Hobson JA, Stickgold R (2003) Dissociable stages of human memory consolidation and reconsolidation: Nature Vol 425(6958) Oct 2003, 616-620.

Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R (2002) Practice with sleep makes perfect: sleep-dependent motor skill learning. Neuron 35:205-211.

Walker MP, Stickgold R, Alsop D, Gaab N, Schlaug G (2005) Sleep-dependent motor memory plasticity in the human brain. Neuroscience 133:911-917.

Wilhelm I, Diekelmann S, Molzow I, Ayoub A, Molle M, Born J (2011) Sleep selectively enhances memory expected to be of future relevance. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:1563-1569.

Wilhelm I, Metzkow-Meszaros M, Knapp S, Born J (2012) Sleep-dependent consolidation of procedural motor memories in children and adults: the pre-sleep level of performance matters. Dev Sci 15:506-515.

Winocur G, Moscovitch M (2011) Memory transformation and systems consolidation. Journal of the International Neuropsychological Society : JINS 17:766-780.

Witt K, Margraf N, Bieber C, Born J, Deuschl G (2010) Sleep consolidates the effector-independent representation of a motor skill. Neuroscience 171:227-234.

Wu T, Wang L, Chen Y, Zhao C, Li K, Chan P (2009) Changes of functional connectivity of the motor network in the resting state in Parkinson's disease. Neuroscience letters 460:6-10.

Yang J, Weng X, Zang Y, Xu M, Xu X (2010) Sustained activity within the default mode network during an implicit memory task. Cortex; a journal devoted to the study of the nervous system and behavior 46:354-366.