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THE NEURAL CONTROL OF RHYTHMIC MOTOR SEQUENCES

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To my family …

ABSTRACT

Sequence learning is one of the most important functions of the motor system since almost every advanced motor skill consists of many component movements that need to be executed in sequential order. For successful performance both, the correct serial order of movements and adequate timing are of importance. Learning a new sequence does not necessarily require conscious awareness: it can occur implicitly. This thesis investigates both the behavioral (**Study I**) and neural (**Study II, Study III)** basis of timing control and implicit sequence learning. Additionally, we study if brief periods of motor sequence learning can induce short-term plasticity in the functional connectivity between brain regions (**Study IV**).

In **Study I**, we use the process dissociation procedure to show that distinct implicit and explicit systems exist for temporal sequence learning: Whereas implicit learning is gradual and gives rise to knowledge that is inaccessible to conscious control, the explicit system is fast and results in representations that can be consciously accessed. In **Study II**, we used fMRI to investigate the influence of the training and pacing modality on the neural control of rhythmic sequence performance. We showed that the dorsal auditory pathway was activated during the performance of both visual and auditory rhythms, suggesting that over-learned rhythms, even those that were both trained and paced in visual modality, are transformed into auditory-motor representations. In **Study III**, we used PET to investigate if individual differences in implicit and explicit sequence learning are related to dopamine receptor densities in the functional sub-regions of the striatum. We found that densities in the limbic striatum were specifically related to implicit but not explicit learning, supporting the idea that implicit and explicit sequence learning depend on partly distinct neural circuitry. In **Study IV**, we used TMS to investigate if motor sequence training can induce training-dependent transient

changes in the functional connection between posterior parietal cortex (PPC) and primary motor cortex (M1). We could show that brief periods of motor sequence training did induce plasticity-like effects in the PPC-M1 connection, suggesting that motor training has a very powerful modulatory effect on brain connectivity.

In sum, our results offer both behavioral and anatomical support for the fact that implicit and explicit learning seem to rely on at least partially different neural circuits. They also show that that the influence of stimulus modality on the neural activity and functional connectivity is rather small, at least when the sequences are over-learned. Finally, they suggest that already short periods of motor sequence training can induce short-term plasticity-like effects in the connectivity of different motor regions.

LIST OF PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their roman numerals (Study I-IV):

I. **Karabanov, A.**, Ullén, F. (2008). Implicit and explicit learning of temporal sequences studied with the process dissociation procedure. *Journal of Neurophysiology*, 100: 733-739.

II. **Karabanov, A.**, Blom, Ö., Forsman, Ullén, F. (2009). The dorsal auditory pathway is involved in the performance of visual and auditory rhythms. *NeuroImage,* 44(2): 480-488.

III. **Karabanov, A.**, Çervenka, S., de Manzano, Ö., Forssberg, H., Farde, L., Ullén, F. (2010). Dopamine D2 receptor density in the limbic striatum is related to implicit but not explicit movement sequence learning. *Proceedings of the National Academy of Sciences of the United States of America.* 107(16): 7574-7549.

IV. **Karabanov, A.**, Joutsen, A., Poston, B., Hallett M. Changes in the posterior parietal cortex – primary motor cortex pathway induced by sensorimotor training. *Manuscript*

ADDITIONAL PUBLICATIONS

I. Ullén, F., Forsman, L., Blom, Ö., **Karabanov, A**., Madison, G. (2008). Intelligence and variability in a simple timing task share neural substrates in the prefrontal white matter. *The Journal of Neuroscience.* 28(16): 4232-4238.

II. Madison, G., Forsman, L., Blom, Ö., **Karabanov, A**., Ullén, F. (2009). Correlations between intelligence and components of serial timing variability. *Intelligence.* 37: 68-75.

III. De Manzano, Ö., Çervenka, S., **Karabanov, A**., Farde, L., Ullén, F. (2010). Thinking outside a less intact box: thalamic dopamine D2 receptor densities are negatively related to psychometric creativity in healthy individuals. *Public Library of Science One*. 5(5): e 10670

IV. Forsman, L., Blom, Ö., **Karabanov, A.**, Madison, G., Ullén, F. Differences in regional brain volume related to the Extroversion-Introversion dimension – a voxel based morphometry study. *Manuscript.*

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LIST OF ABBREVIATIONS

BOLD	Blood Oxygenated Level Dependent
BP	Binding Potential
CS	Conditioning Stimulus
D2BP	D2 Binding Potential
DA	Dopamine
DLPFC	Dorsolateral Prefrontal Cortex
fMRI	Functional Magnetic Resonance Imaging
GLM	General Linear Model
ISR	Immediate Serial Recall
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
MR	Magnetic Resonance
MRE	Mean Relative Error
MRI	Magnetic Resonance Imaging
PDP	Process Dissociation Procedure
PET	Positron Emission Tomography
PMC	Premotor Cortex
PMd	Dorsal Premotor Cortex
PMv	Ventral Premotor Cortex
PPC	Posterior Parietal Cortex
RE	Relative Error
RMT	Resting Motor Threshold
ROI	Region Of Interest
RSI	Response to Stimulus Interval
RT	Reaction Time
S1	Primary Somatosensory Cortex
SMA	Supplementary Motor Area
SMG	Supramarginal Gyrus
SN	Substantia Nigra
SRTT	Serial Reaction Time Task
STG	Superior Temporal Gyrus
TAC	Time Activity Curve
TMS	Transcranial Magnetic Stimulation
TPJ	Temporo-Parietal Junction
TS	Test Stimulus
VTA	Ventral Tegmental Area

FOREWORD

When I started to work on my thesis work in fall 2006 my project had the title *Neural control of rhythmic motor sequences - normal physiology and functional plasticity in professional musicians* and contained three larger projects: One investigating the role of the superior temporal gyrus (STG) during rhythmic sequence production and two projects investigating functional and anatomical reorganizations in professional musicians. Now, four years later, one of these projects, the one on the role of the STG, is realized and part of this thesis. One of the other projects, investigating anatomical and functional changes in musicians using TMS, was also realized, but unfortunately by another group and before I even had the time to start the project (Rosenkranz et al., 2007). The third project was supposed to investigate functional plasticity in professional musicians using fMRI, and I have to confess it got lost on the way. Even though brain plasticity in professional musicians is truly a fascinating subject, I was led astray, investigating normal physiology during sequence learning. I followed the intriguing questions that arose along the way of my first experiment. After investigating modality differences in rhythmic sequence learning, I entered the wide realm of implicit learning by wondering if rhythmic sequences also could be learned implicitly. Fascinated by the differences (and similarities) between implicit and explicit sequence learning and with the methodology for measuring these different learning types, I expanded my interest in the unconscious learning of sequences by investigating whether dopaminergic function in different parts of the striatum is differentially correlated to implicit and explicit learning. Finally, on the other side of the Atlantic and equipped with a new technique, I returned to my initial interest in rhythmic sequences and plasticity. However, instead of long term plastic changes induced by motor sequence learning, as seen in musicians, I grew interested in short term plasticity induced by just a couple of minutes of motor training. So now, at the end of four years

of research, the thesis you are holding in your hand is called: *Neural control of rhythmic motor sequences*; a title that describes the essence of my work rather well, even if it may not pay full acknowledgement to the implicit/explicit aspect of some of my studies.

1. INTRODUCTION

1.1 DEFINING MOTOR LEARNING

Humans are born with a very limited repertoire of movements and throughout their lifetime they never stop to learn new motor patterns or to shape their performance to meet ever-changing environmental conditions. The ability to learn and modify motor skills therefore seems to be one of the most crucial functions of the nervous system. As evident from the wide range of motor skills that can be acquired, motor leaming is a very complex phenomenon, occurring on different time-scales with many influencing factors and it can be studied from many different aspects. Therefore it is important to carefully define the different concepts used when talking about motor learning. I would like to start with a very general definition: Motor learning refers to "a change in motor performance with practice" (Hallett, 2003). Often the term 'procedural' learning is used interchangeably and relates motor learning into a larger theoretical framework of human learning. Procedural learning classically refers to learning that cannot be transmitted verbally and is usually contrasted with 'declarative learning', which refers to learning facts and information that can be communicated verbally (Squire, 1986). However, motor learning can also be broken down into at least two subgroups: *motor skill learning* and *motor adaptation* (Sanes et al., 1990; Doyon et al., 2003; Hallett, 2003; Doyon and Benali, 2005) To understand the difference between motor skill learning and motor adaptation, it is probably easiest to refer to the concept of operating characteristics. As Hallett (2003) describes: '*An operating characteristic is a descriptor of a set of movements that relate different movement variables to each other. It describes the current state of capability of the system'*. A good example for an operating characteristic is Fitt's law (Fitts, 1954) relating movement speed and

movement accuracy. A movement from A to B can be made with different speeds, however as the speed increases the accuracy drops. *Motor adaptation* can be classified as a change in motor performance without a change in the operating characteristic; e.g., adaptation learning means to react at a different movement speed but with the predictable decrease in accuracy. A good example for motor adaptation is manipulating objects while looking through a magnifying glass: it will require learning a new gain between visual distance and movement amplitude. *Motor skill learning* on the other hand can be defined as a change in motor performance with a change in the operating characteristic, e.g., learning a faster movement without increasing inaccuracy. By this violation of Fitt's law a new operating characteristic emerges, which often would be recognized as a new skill. All the work contained in this thesis will focus on the acquisition of new motor skills, so in the following I will discuss both physiological and behavioral aspects of motor skill learning in greater detail.

1.2 STAGES OF MOTOR LEARNING

Learning new motor skills usually takes some time and can be separated in different phases of learning. Most research differentiates two or sometimes three different learning stages. Since I believe that three stages describe motor learning more accurately, I will present a three-stage model put forth by Halsband (Halsband and Freund, 1993). Even though the terminology is somewhat different from earlier models (Posner, 1967), all models essentially agree on the main characteristics of each learning phase. During the *initial stage,* movements are still slow and the integration of sensory information is central to executing the new motor skill. The precision and speed of the newly learned movement varies greatly from trial to trial. In the *intermediate stage,* sensory-motor links become more stable and sensory feedback becomes less important, movements become faster, smoother and are executed with smaller variance between

single trials. Finally during the *consolidation stage,* movements are automated, smooth and very fast. Executing the movements does not require overt attention anymore and the variance between trials is small. Besides the behavioral differences, brain activity during the different learning stages can vary greatly. I will discuss these differences in chapter 1.6 in more detail.

1.3 SEQUENTIAL MOTOR SKILLS

Most motor skills are complex and consist of several multi-joint movements that have to be connected in a sequential fashion. Only if the movements are executed in the right sequential order, can strumming on the piano turn into Bach and uncoordinated movements into dance. So learning a new motor skill often means learning a new motor sequence too. Studying motor sequence learning is interesting, not only because it can give us insights into the motor system and the mechanisms underlying the acquisition of new motor skills but also because it can serve as a proxy to understand the physiology of the control of sequences in general: sequencing is not only required of the motor system but plays a pivotal role in almost any cognitive task.

It is important to realize that movement sequences have two dimensions: The *ordinal structure* of a sequence describes the serial order of the individual movements whereas the *temporal structure* of the sequence describes the series of temporal intervals between the onsets of the movements. Taking a simple melody on the piano as an example, the ordinal structure is the serial order of key presses, one for each note, whereas the temporal structure describes the temporal intervals that are determined by the durations of the notes of the melody. In many cases, only if both the serial order of the movements and the timing are executed correctly, movement sequences become functional. Similar to the 'where' and 'what' stream of the visual system, there is evidence that the brain represents order and timing of motor sequences independently

and that also the neural systems controlling these two sequence dimensions are, at least partially, independent. Also the differences between ordinal and temporal sequences will be discussed in greater detail in chapter 1.6.

1.4 IMPLICIT AND EXPLICIT LEARNING

Learning a new sequence, both motor and non-motor, does not necessarily require conscious awareness and can occur in an incidental manner. Most people will have experienced incidental learning; For example, after having punched in a phone number or pin code often enough, the fingers know where to move even if we were not trying to remember the sequence and might not be able to verbally recall it. Learning with conscious awareness is usually called '*explicit*' whereas unconscious learning is classified as '*implicit*'.

The term implicit learning was coined by Reber (Reber, 1967) who could show that participants were able to learn rules of an artificial grammar by studying a series of letter strings. Participants could classify new letter strings with an above-chance accuracy as grammatical or ungrammatical but had little ability to describe the underlying grammar rules. Reber described his results as a *'peculiar combination of highly efficient behavior with complex stimuli and almost complete lack of verbalization knowledge'*. The differentiation between conscious and unconscious learning has created a lot of interest in the scientific community throughout the last 20 years, and many other paradigms beside Reber's grammar learning have emerged. Maybe the most widely used paradigm for studying sequence learning is the serial reaction time (SRT) task. The SRT task 'has spawned a small industry' during the last years (Shanks, 2005) with much controversy about both whether sequence learning in this task is consciously accessible and whether sequence learning is due to the motor or the sensory aspect of the task. A whole introduction could be written solely on these

issues. While introducing several behavioral paradigms that I used to measure both implicit and explicit learning, I will try to touch on the question of awareness in the SRT task but will leave the sensory/motor discussion largely untouched. I would like to refer the interested reader to several fascinating papers discussing the perceptual component of the SRT task (Robertson et al., 2001; Robertson, 2007).

1.4.1 The serial reaction time task

In the SRT task, a target appears on a computer display, and participants are instructed to press the response key assigned to that stimulus as fast as possible. However, the order of target presentation is not random, rather it follows, without the participant's knowledge, a predictable, or partially predictable, sequence of locations. During the task, reaction times decrease progressively over the course of training and increase as soon as the underlying sequence shifts to a random sequence. These decreases in reaction time can be seen even if participants report little or no awareness of the underlying sequence (Nissen, 1987; Willingham et al., 1989; Willingham et al., 2002). Awareness is classically tested at the end of the SRT task by verbal assessment: only if the participants report to be unaware of the underlying sequence is their knowledge thought to be truly implicit. However, verbal assessments are a good example for one of the notorious methodological difficulties that differentiating implicit and explicit knowledge faces (for a discussion see: Shanks, 1994; Stadler, 1997). Awareness tests might not be sensitive enough to pick up all conscious knowledge that the participant is in possession of. Verbal questions can fail to detect partial explicit knowledge simply because the participants might choose to withhold conscious knowledge with a very low confidence, or they could probe for knowledge that is not necessary to perform the task (knowledge of rules when knowledge of instances is sufficient). As a result, several researchers have suggested that tests of explicit knowledge administered after

the SRT task should involve forced-choice tasks, such as recognition or generation (Shanks, 1994; Jimenez, 1999). Traditionally, recognition and generation tasks are seen as an expression of explicit learning. The fact that they correlate very well with learning measured by decreased reaction time in the SRT task (thought to measure implicit learning) has led some researchers to conclude that learning is essentially explicit in nature, and that there is very little or no truly implicit learning (Shanks and Johnstone, 1999). However, the idea that forced-choice tasks exclusively measure explicit learning whereas decreased reaction time in the SRT task exclusively measures implicit learning is rooted in the dubious assumption that tasks can be 'process pure'. The assumption of 'process purity' is a second major concern for awareness tests. A generation task, for example, which is classically aimed at assessing explicit knowledge, might be influenced by implicit knowledge to an unknown degree. This poses a serious dilemma for implicit learning research since it shows that unconscious learning also most easily contaminates the tests most sensitive to awareness.

1.4.2 The process dissociation procedure

The Process Dissociation Procedure (PDP) is an approach that tries to avoid assumptions about process purity in forced choice tests. The basic idea (Jacoby, 1991) is to design two tasks: One in which both explicit and implicit learning help performance and a second task where only explicit knowledge increases performance whereas implicit learning acts as interference. Jacoby applied the original version of the PDP to a stem completion-task used to study verbal implicit memory. In this experiment, participants had to complete word stems in two different conditions: In the *Inclusion* condition where participants were instructed to try to use words from a previously studied list when trying to complete the word stems and in the *Exclusion* condition where participants were asked to exclude the studied words when trying to

complete word stems. The idea underlying these tasks is that whereas both implicit and explicit knowledge aid performance on the *Inclusion* task, implicit knowledge should interfere with performance on the *Exclusion* task, making it impossible to the truly implicit learner to suppress the previously studied words during *Exclusion*. The PDP has been adapted to study implicit motor sequence learning by several researchers (Buchner et al., 1997; Buchner et al., 1998) but most notably by Destrebecqz and Cleeremans (Destrebecqz and Cleeremans, 2001). They added two free generation tasks to the SRT task. In the *Inclusion* task, participants were instructed to generate sequences that were similar to the sequence in the SRT task, whereas in the *Exclusion* condition participants were instructed to generate sequences that were not similar to the sequence presented during the SRT task. Destrebeqcz and Cleeremans could show that, if the response-to–stimulus interval (RSI) during the SRT task was 0 ms, the likelihoods of producing similar sequences during the *Exclusion* and the *Inclusion* condition were both above chance level and did not significantly differ from each other. This suggests that the knowledge that was obtained by the participants was inaccessible to intentional control and therefore truly implicit.

1.4.3 Immediate serial recall

The immediate serial recall (ISR) paradigm is often used to study purely explicit memory and working memory and faces somewhat less methodological concerns than the paradigms testing implicit memory. Learning is measured by the error rate during reproduction. In the ISR paradigm, an entire sequence of stimuli is first presented to the participant who afterwards has to recall the complete sequence in the correct order. When using ISR for movement sequence learning, each stimulus typically represents a particular movement. During recall, the participant has to produce the entire movement sequence from memory. The ISR paradigm is more suited than the SRT task to study

temporal sequence learning since the whole target rhythm can be presented before reproduction. The temporal structure of the SRT task on the other hand, is influenced by the participant's reaction time.

1.5 NEURAL CORRELATES OF IMPLICIT AND EXPLICIT LEARNING

The implicit/explicit distinction is closely related to the non-declarative/declarative distinction which arose out of work in amnesiacs like H.M. who, after surgical removal of regions in both medial temporal lobes, was unable to form new conscious memories (declarative) but could still acquire new memories of which he was not consciously aware (procedural) (Eichenbaum, 2002). These early lesion studies clearly suggested at least partially different neural correlates underlying conscious and unconscious memory systems. Since the advent of neuroimaging techniques, researchers do not need to solely rely on lesion studies to investigate the neural underpinnings of cognitive functions. As a result, a more differentiated picture of similarities and differences in brain activation during implicit and explicit motor sequence learning arose. Whereas all of the brain regions important in motor skill learning have been implicated to some extent in both implicit and explicit sequence learning (Rauch et al., 1995; Berns et al., 1997; Hazeltine et al., 1997; Rauch et al., 1997; Honda et al., 1998; Peigneux et al., 2000; Willingham et al., 2002; Schendan et al., 2003; Aizenstein et al., 2004; Thomas et al., 2004), there seems to be some differences in brain activity. The basal ganglia are more consistently activated in studies of implicit sequence learning and a functional magnetic resonance study (fMRI) using the PDP specifically related basal ganglia activity to the implicit component of the learning (Destrebecqz et al., 2005). The prefrontal cortex, in contrast, appears to play specific roles for explicit sequence learning (Jenkins et al., 1994; Berns et al., 1997; Destrebecqz et al., 2005). This picture is further supported by clinical findings. Impaired implicit learning with small or no

defects in explicit learning is found in Parkinson patients (Pascual-Leone et al., 1993; Dominey et al., 1997; Siegert et al., 2006), meanwhile selectively impaired explicit sequence learning has been reported in Korsakoff (Nissen and Bullemer, 1987) and Alzheimer patients (Knopman and Nissen, 1987).

1.6 ANATOMY OF MOTOR SKILL LEARNING

During the last decades we have learned a lot about the neural substrates of sequence learning. A large number of studies, using both electrophysiology and different imaging techniques, have helped to unveil its neuronal structures. Although the explosion of imaging studies has surely helped to understand they mechanisms underlying sequence learning a detailed picture of the functional properties of many of the involved areas is just evolving. Several factors discussed above, contribute to the difficulties of determining the functional properties of different structures. First, sequence learning can be separated in different stages: The initial, intermediate and advanced stage. When looking at the neural mechanism underlying motor sequence learning it is important to study the involvement of brain structures during the different stages of learning separately. Second, sequences can be learned in an implicit and in an explicit fashion. As mentioned before, the implicit and the explicit systems often interact, and it is very difficult to clearly disentangle them. Third, different brain structures might focus on either the ordinal or temporal aspects of motor sequence production. In the following I will discuss cortical and sub-cortical structures important in motor sequence learning, with a special focus on their differential roles for the three mentioned aspects. For an overview of the cortical structures see Figure 1. The chapter will be concluded discussing dopamine, an important neurotransmitter involved in sequential movement acquisition.

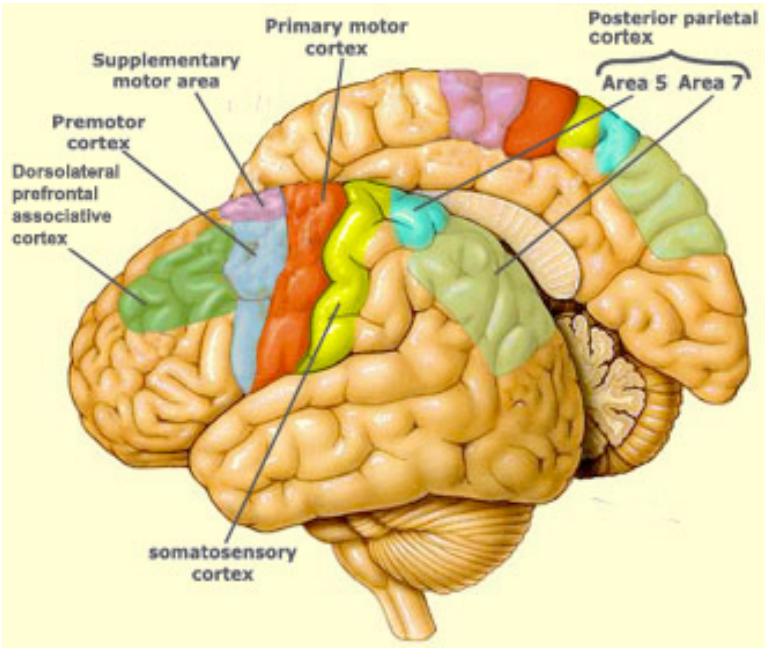

Figure 1: Cortical areas involved in the control of motor sequence production. (Image taken from www.thebrain.mcgill.ca; ↄ - 'copyleft' allows publication for non-commercial purposes)

1.6.1 Cortical control of motor sequences

Prefrontal cortex

There is evidence that the prefrontal cortex, and most notably the dorsolateral prefrontal cortex (DLPFC), is involved in the initial stages of explicit learning of motor sequences (Jueptner et al., 1997; Sakai et al., 1998; Lewis and Miall, 2003). Generally it seems that the more 'cognitively controlled' the movement sequence is (i.e., if conscious attention is paid to the sequence execution), the more reliable the activation of the DLPFC (for review see: Lewis and Miall, 2003). What is less clear is which of the DLPFC putative functions are being engaged, since sequence learning requires working memory, as well as executive control. However, electrophysiological studies suggest that the involvement of the DLPFC is not only due to working memory requirements but that cells in that region fire selectively depending on the abstract temporal representation of the temporal order in a sequence (Hasegawa et al., 2004; Ninokura et al., 2004; Ryou and Wilson, 2004). The notion that the DLPFC is involved in the temporal ordering of a sequence also is supported by older clinical observations of patients with prefrontal lesions (Milner, 1971; Petrides and Milner, 1982).

Posterior parietal cortex

The posterior parietal cortex (PPC) receives cortical afferents from visual, auditory and somatosensory areas and projects mainly to frontal premotor areas (Kolb, 2003). Its anatomical connections and its location between the occipital and the sensorimotor cortex allow the posterior parietal cortex to integrate motor and sensory stimuli. It plays an important role in planned movement execution by acting as sensorimotor interface and by locating both one's own body and external objects in space. Even though most studies have focused on visual-motor integration and specifically visually guided

reaching and grasping tasks (Andersen, 1987; Buneo and Andersen, 2006; Iacoboni, 2006; Rushworth and Taylor, 2006) there is evidence that also auditory-motor integration activates the PPC (Lewis et al., 2004; Karabanov et al., 2009). Several imaging studies suggest that the PPC is acivated to a higher degree after the initial stage of motor learning (Shadmehr and Holcomb, 1997; Honda et al., 1998). It should also be noted that an increase in parietal activation in the PPC after the initial learning stage could only be found if the learning tasks were explicit and not implicit (Hazeltine et al., 1997; Honda et al., 1998; Eliassen et al., 2001).

Superior temporal gyrus

Even though the superior temporal gyrus (STG) is primarily an auditory area and cannot be formally regarded as belonging to motor network activated during motor sequence acquisition it is included in this summary since it is commonly activated during the acquisition and performance of *rhythmic* sequences (Bengtsson et al., 2004; Lewis et al., 2004; Bengtsson et al., 2005; Chen et al., 2006). For ordinal sequences it is usually not activated. It has been suggested that the activation of the STG reflects the auditory memory of the temporal sequence (Hickok et al., 2003; Bengtsson et al., 2004, 2005). However, since almost all studies in rhythmic sequence production have only worked with auditory stimuli it is not entirely clear if the activation of the STG is specific to rhythms trained in the auditory modality only or if they are an intrinsic feature of the temporal sequence network.

Pre-supplementary motor area

Based on anatomical and physiological evidence in animals, the medial premotor area has been divided into two distinct areas: the supplementary motor area (SMA, sometimes referred to as the SMA-proper) and the pre-supplementary motor area (pre-

SMA). In comparison to the SMA the pre-SMA receives most of its input from frontal and parietal association areas, as well as from the associative striatum (Akkal et al., 2007) and does not project directly to the primary motor areas. It also does not show a somatotopic organization as observed in SMA (Kurata, 1992) and the primary motor cortex (Picard and Strick, 1996; Lehericy et al., 2004). Primate studies have shown that both cells in the pre-SMA and in the SMA show strong sensitivity to the rank-order of movement elements within a sequence (Shima and Tanji, 2000). The pre-SMA, however, is especially activated during the initial stage of motor learning (Nakamura et al., 1998); after the initial stage of learning is over, the pre-SMA appears only to be activated during the first movement of the sequence or when changing to a new sequence (Nakamura et al., 1998; Kennerley et al., 2004). This suggests that the pre-SMA may be particularly important for higher-order hierarchical control of sequences, e.g. the chunking of sequence elements and providing a temporal template for the production of sequence segments (Ashe et al., 2006). In a study investigating differences in neural activity during the acquisition of ordinal and temporal sequences Bengtsson et al. (2004) found the pre-SMA more active during the performance of temporal sequences than ordinal sequences.

Supplementary motor area

The SMA receives pre-central and post-central afferents from primary motor cortex as well as afferents from the caudal premotor areas and primary and secondary motor areas (Lehericy et al., 2004) and from the sensorimotor striatum (Akkal et al., 2007) and sends direct corticospinal efferents to primary motor cortex. Despite the differences in anatomical connectivity between SMA and pre-SMA their functional similarity is considerable. As mentioned above, also neurons in the SMA are rank order sensitive and seem to play an important role in ‘chunking’ a long motor sequence into smaller

elements. However, in comparison to the pre-SMA, SMA activation increases with practice, and can also be observed if the sequence is learned implicitly (Grafton et al., 1995; Hazeltine et al., 1997). Due to its direct connections to the primary cortex, Ashe et al. (2006) proposed that: '*It seems likely that the pre-SMA is concerned primarily with aspects of temporal control, and that the SMA integrates this temporal coding with the required motor output'*. This conclusion is supported by electrophysiological findings showing that activity in the SMA neurons is phase-locked to the onset of a movement whereas activity in pre-SMA neurons often precedes the movement onset (Rizzolatti et al., 1990; Picard and Strick, 1996). The idea that the SMA is involved in the temporal performance of sequential movements is supported by clinical observations in patients with SMA lesions. Halsband et al. (2006) reported strong disturbances in the performance of rhythmic movement sequences in patients with SMA lesions. Also the SMA seems to be more active during the acquisition of temporal than of ordinal sequences.

Premotor Cortex

The lateral premotor cortex (PMC) receives its input mainly from the parietal cortex and the cerebellum and has both direct corticospinal projections and afferents to the primary motor cortex (Dum and Strick, 2002; Chouinard and Paus, 2006). Based on anatomical connections the lateral premotor cortex can be divided in the ventral premotor region (PMv) and the dorsal premotor region (PMd). Whereas the PMv receives its main input from Brodmann area 7, the PMd receives afferents from Brodmann area 5 (Chouinard and Paus, 2006). Functional imaging studies have shown both these regions to be activated during the earlier stages of sequential motor skill learning tasks (Jenkins et al., 1994; Bengtsson et al., 2004; Garraux et al., 2005; Pope et al., 2005). Evidence from a wide range of physiological and functional studies suggests

that PMd and PMv have functional differences: Whereas the PMd is critical for implementing associations between sensory cues and a particular motor response (Kurata and Wise, 1988; Kurata and Hoffman, 1994; Chouinard et al., 2005), the PMv contributes to the control of hand movements during reaching and grasping (Rizzolatti et al., 1988; Hepp-Reymond et al., 1994; Ehrsson et al., 2001). The PMv also contains the so-called 'mirror neurons' that discharge both when a subject performs a specific action and when another individual is observed performing the same action (Rizzolatti et al., 1996; Rizzolatti et al., 2002; Binkofski et al., 2004; Ferrari et al., 2005). PMv and PMd are both active in implicit and explicit sequence learning (Keele et al., 2003). However, activity in the PMd seems to be higher during the acquisition of ordinal as compared to temporal sequences (Bengtsson et al., 2004).

Primary motor cortex

The somatotopically organized primary motor cortex contains many of the large corticospinal neurons and it receives its input mainly from the premotor areas as well as from primary and secondary somatosensory areas and from posterior regions (BA 5) (Murray and Coulter, 1981). Because the primary motor cortex is so intimately involved in the generation of movements it is difficult to entangle this function from involvement specific to sequence learning. It has however been suggested that M1 plays a crucial role in early implicit memory of motor sequences (Honda et al., 1998; Ashe et al., 2006) and it has been shown that M1 plays a crucial role in early motor memory consolidation even if sequences were not purely implicitly learned (Muellbacher et al., 2002; Robertson et al., 2005).

1.6.2 Sub-cortical control of motor sequences

Besides the cortical motor areas also two subcortical areas have been identified in being crucial for the acquisition and retention of motor skills: the cerebellum and the basal ganglia. Each structure forms a distinct cortico-subcortical circuit and a wide range of clinical, neurophysiological and functional studies supports the role in both circuits during motor skill acquisition and retention (for review see (Doyon et al., 2003).

The Basal Ganglia

The basal ganglia are a group of nuclei at the base of the forebrain and consist of the striatum, the palladium, the substantia nigra and the subthalamic nucleus. The striatum (consisting of the putamen and the caudate nucleus) is the largest part of the basal ganglia. The striatum can be divided in sub-compartments depending on the cortical connections it receives. The ventral striatum, also called limbic striatum, has close connections to the limbic structures, whereas the dorsal caudate and the precommisural putamen, together called the associative striatum, have strong connections to the premotor areas as well as to the dorsolateral prefrontal cortex. The sensorimotor striatum finally, comprised of the postcomissural part of the dorsal putamen, has strong connections to the SMA and primary motor areas (Haber and McFarland, 1999; Cervenka et al., 2008). The striatal complex as well as a diagram of the three different striatal loops can be seen in Figure 2. The striatum is strongly activated during sequence learning (for review of imaging studies see Doyon et al., 2003) with increasing activity over the course of learning. This has led several researchers to speculate that the striatum might play an important role in the storage of sequences (for reviews see Doyon et al., 2003; Halsband, 2006; Halsband and Lange, 2006). Recent studies have shown that activity in the striatum shifts from the associative region to the sensorimotor striatum during sequence learning (Lehericy et al., 2004) suggesting that

motor sequences might be stored in the sensorimotor part of the striatum. It is noteworthy that the striatum is not only involved during the learning of explicit sequences but that striatal activity is the most consistently activated structure in studies investigating implicit sequence learning (Grafton et al., 1995; Doyon et al., 1996; Hazeltine et al., 1997) and more recent studies even have related the amount of implicit knowledge obtained with striatal activity (Destrebecqz et al., 2005).

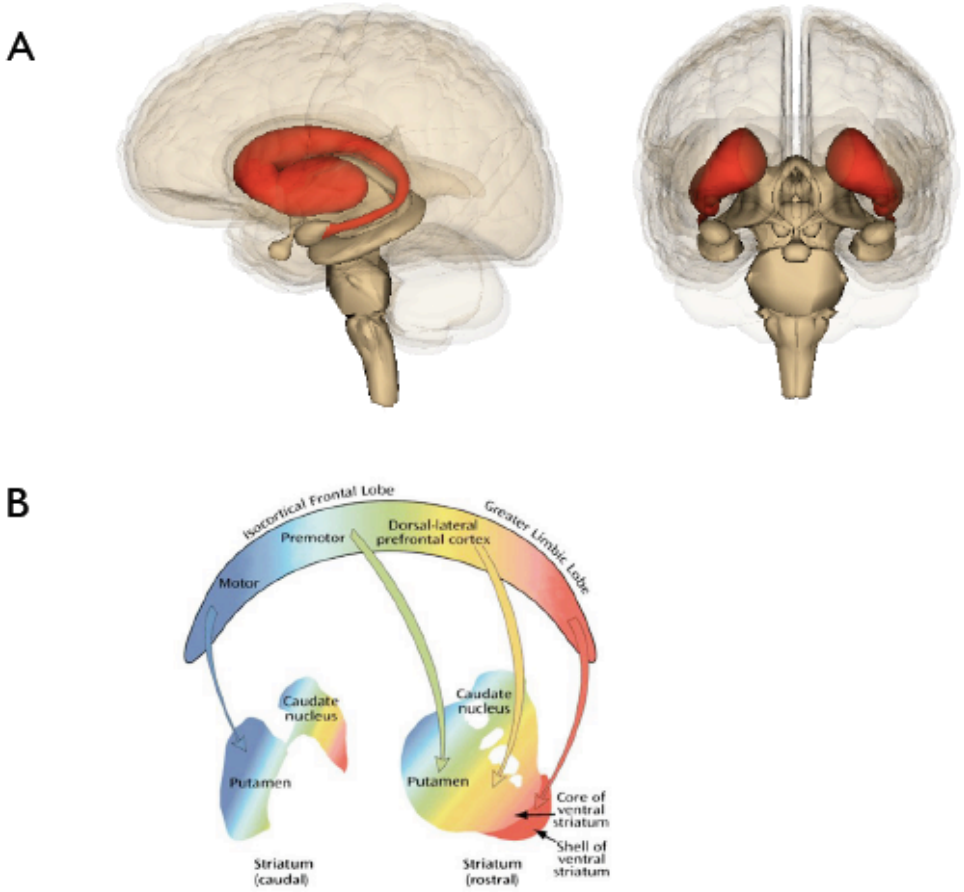

Figure 2: Schematic representation of the location of the basal ganglia (modified from a download from Wikimedia Commons. No known restrictions on publication). Figure 2B shows a schematic figure of the anatomical connectivity in striatum (originally published in: Haber and McFarland, 1999. Used with permission from Suzanne Haber).

The Cerebellum

The cerebellum plays an important role in the control of voluntary movements as well as in balance and the muscle tone. It influences the motor system by evaluating disparities between the intended movement and the actual outcome by adjusting motor areas both in the cortex and in the brain stem while the movement is in progress (Ghez, 2000). After cerebellar injury, both animals and humans show slow and uncoordinated movements and tend to stagger and sway while walking. It is clear that a structure of such primal importance in the control of movement also is important for the learning of sequential motor skills. Indeed, many imaging studies have reported strong and consistent cerebellar activation in the early stages of motor learning (Jenkins et al., 1994; Doyon and Benali, 2005). In contrast to the striatum, however, the activation of the cerebellum decreases during the course of learning (Penhune and Doyon, 2005). Once the movement sequences are encoded and store into memory the adjusting and evaluating role of the cerebellum is not longer required to the same extent.

1.7 SEQUENCE LEARNING AND THE DOPAMINERGIC SYSTEM

Dopamine (DA) is a neurotransmitter that is produced mainly in the substantia nigra (SN) of basal ganglia and the ventral tegmental area (VTA). It has been implicated in a broad array of cognitive functions such as voluntary movement, motivation and reinforcement learning. The dopamine system interacts with other parts of the brain via four major pathways: The mesolimbic and the mesocortical pathway both originate in the VTA and project to the amygdala/hippocampus and to the frontal/pre-frontal areas, respectively. These pathways play an important role in reinforcement learning, motivation and reward. The tuberoinfundibular pathway transmits dopamine from the hypothalamus to the pituitary gland and is primarily involved in hormone regulation.

Finally, the nigrostriatal pathway connects the SN to the striatum, and is implicated in voluntary movements, motor skill and habit learning. Loss of dopaminergic neurons in this pathway can lead to Parkinson's disease. This pathway seems to also play an important role during motor sequence learning. Impairment of motor sequence learning is seen after down regulation of nigrostriatal DA (Matsumoto et al., 1999); conversely, sequence learning is facilitated by dopamine agonists (Kumari et al., 1997). Several PET studies have also showed correlations between dopamine release and sequence learning in the striatum (Badgaiyan et al., 2007; Garraux et al., 2007; Badgaiyan et al., 2008).

1.8 REORGANIZATION AND PLASTICITY DURING MOTOR LEARNING

Learning new motor skills does not only result in functional cerebral adaptations as discussed in the chapter above. Often, the functional changes seen during the acquisition of motor skills are correlated with long-term micro and macro anatomical changes. In a series of animal studies, it could be shown that rigorous motor training results in an increased number of synapses per neuron in both the motor cortex and the cerebellum of rats (Black et al., 1990; Kleim et al., 1996). Also in humans it has been shown that even relatively short periods of motor training can cause changes in the motor system. Pascual-Leone et al., (Pascual-Leone et al., 1995) could show that after a 5-day period of piano practicing the cortical representations of the long finger flexor and extensor muscles enlarged, However, if those short term changes are functional or anatomical is not clear. Motor practice over years can also induce long-lasting changes in cortical excitability; Nordstrom and Butler (Nordstrom and Butler, 2002) found that interhemispheric inhibition is altered in professional musicians. If the motor training is more extensive, and continues over the course of several years as in the case of

musicians, even macro anatomical changes can be observed in the human brain, leading to volumetric changes in the gray matter and to higher fractional anisotropy in the white matter of the human brain. These macro anatomical changes have been shown in the corpus callosum, motor cortex, cerebellum and planum temporale of professional musicians (Gaser and Schlaug, 2003a, b; Hyde et al., 2009). These studies show that the brain is constantly reshaped in response to skill learning and the sensory stimulation connected to learning new skills.

2. AIMS

The research presented in this thesis focuses on different aspects of human movement sequence learning. It brings together insights from behavioral studies, functional and receptor neuroimaging as well as electrophysiological techniques to investigate the acquisition of rhythmic motor sequences and the behavioral and neural underpinnings of unconscious sequence learning. The ultimate goal of the studies presented is to contribute to unravelling the mechanisms of sequential skill acquisition in healthy individuals.

The specific objectives were to investigate:

- If implicit learning of rhythmic sequences can be demonstrated using the process dissociation procedure (PDP). **(Study 1)**
- If some of the brain areas commonly seen activated by rhythm reproduction are specific to training or pacing modality. **(Study 2)**
- If individual differences in implicit and explicit sequence learning are related to dopamine receptor densities in the striatum. **(Study 3)**
- If brief periods of sequence training can induce short-term plasticity-like effects in the functional connection between the posterior parietal cortex and the primary motor cortex. **(Study 4)**

3. METHODS

This thesis combines different imaging techniques, electrophysiology and behavioral measurements to investigate the neural underpinnings of rhythmic and ordinal sequence learning. In this section we will summarize the different behavioral tasks, the basic concepts for data analysis as well as the imaging and electrophysiological techniques used in the different studies. First, the behavioral tasks and the basic concepts for the analysis of the behavioral data will be described. This will be kept rather brief since many of the theoretical considerations underlying the behavioral tasks have already been described in the introduction. Second, we will give a somewhat more thorough description of the different techniques used in this thesis. More detailed descriptions of all methods can be found in the method sections of each study.

3.1 BEHAVIORAL MEASUREMENTS

The behavioral data measured in all experiments consisted of the identity and/or of the onset time of all key presses (study I-IV) and self reported questionnaires (study I and study III). Statistical analysis was performed in Statistica (StatSoft), Matlab (MathWorks) and Excel (Microsoft). Experiments I-III were implemented in E-prime ((Psychological Software Tools, Inc); experiment IV was implemented in Stim2 (NeuroScan).

3.1.1 Immediate serial recall

An immediate serial recall task (ISR) was used in both study I and in study II. In study I, the ISR task was used to test implicit learning of temporal sequences. We used the same paradigm in study II to explicitly train participants on different rhythmic sequences. The ISR task in study I was comprised of 50 repetitions of an 8-interval

sequence. Stimuli were presented either visually, or audio-visually depending on experimental group. Performance in study I was analyzed by calculating the mean relative error (MRE, Eq.1) for each repetition of the full sequence.

In study II the ISR task was repeated until participants could reproduce the complete 8-interval sequence correctly six times in a row. Performance for each interval was evaluated independently by calculating the relative error (RE, Eq.2). This calculation was done on-line during sequence training. An interval was considered correct if the RE did not exceed 0.3. Stimuli in study II were auditory or visual depending on experimental group.

$$\text{(Eq.1)}\quad MRE = \frac{\sum_{i=1}^{n} \left| \frac{p_i - s_i}{s_i} \right|}{n}$$

$$\text{(Eq.2)}\quad RE = \left| \frac{p_i - s_i}{s_i} \right|$$

Equation 1 and **2**: RE is the absolute relative error and MRE is the mean relative error; p_i is the duration of the produced interval; s_i is the duration of the stimulus interval. n is the number of intervals in the sequence.

3.1.2 Free recall

In study II recollection of the sequences previously practiced during an ISR task was tested using a free recall paradigm. During free recall participants had to recall the trained sequences from memory. The free recall was performed in the fMRI scanner approximately 24 h after the ISR task. Each recall session started with four beats of a pacing metronome, presented either visually or auditorily, to ensure that all participants followed the same beat. After the metronome subjects had 32.5 seconds to repetitively

produce the sequence by free recall. During the free recall phase the performance was measured by calculating the MRE for each repetition of the full rhythmical sequence.

3.1.3 Process dissociation procedure

We used the PDP (for background on the PDP, see Introduction) to study learning of temporal sequences in study I and to study learning of ordinal sequences in study III. Performance during Inclusion and Exclusion tasks was measured by calculating similarity scores between the produced temporal (study I) and ordinal (study III) sequences and their respective target sequences. Similarity scores were determined by calculating the number of correct triplets. This was done by dividing the number of generated three-element chunks that were part of the training sequence by the total number of triplets produced during the generation task. The triplet analysis was done to assess knowledge of fragments of the sequence. From the performance in the Inclusion and Exclusion tasks it was possible to derive estimates of implicit and explicit learning (Destrebecqz and Cleeremans, 2001): An assumption of the PDP is that both implicit and explicit knowledge act additively to increase the similarity scores in the Inclusion task. In the Exclusion task however, the similarity scores should decrease with higher explicit knowledge (meaning that the participant successfully suppresses the earlier sequence) whereas implicit knowledge should lead to high similarity scores since it acts as interference in this task. These assumptions can be formulized as follows:

(Eq. 3) Inclusion = I + E + Baseline, and
Exclusion = I - E + Baseline

These formulas can then be transformed into:

(Eq. 4) E = Inclusion-Exclusion

I = (Inclusion +Exclusion) / Baseline

Equation 3 and **4**: Inclusion and Exclusion are similarity scores in the corresponding generation tasks; Baseline is the expected similarity score during random performance; I and E are implicit and explicit learning scores, respectively.

Note that good performers have a low similarity score in Exclusion, since the task is to avoid producing the target sequence. Baseline is defined as the expected similarity score during random performance with a uniform distribution, i.e. equal probability for all responses. For ordinal sequences, as in study III, the expected similarity score during random performance is easy to calculate since the response variable, i.e. which one of the four possible keys the subject pressed, is discrete. For the temporal sequences in study I however, the response variable, i.e. the duration of the produced temporal interval, is continuous. Generally, the understanding of how temporal sequences are represented is still limited, and it is therefore very difficult to determine what a random performance would look like. As a result we could only calculate an explicit learning score for study I. In study I, both generation tasks ended after the participants had reproduced an 8-interval sequence 10 times. In Study III the tasks ended after a 12-item sequence had been reproduced 8 times.

3.1.4 Serial reaction time task

A serial reaction time task was used prior to the PDP in study III. Discussion of the SRTT will be kept to a minimum here since we have discussed this test at length in the

Introduction. The SRTT consisted of 15 training blocks, with each block containing 8 repetitions of the 12-item ordinal target sequence. All stimuli were presented visually. During the SRTT reaction time (RT) was used to measure performance. RT was defined as the time difference between stimulus onset and response time.

3.1.5 Synchronized tapping

Synchronized finger tapping was used for explicit rhythmic sequence training in study IV. Participants had to synchronize their tapping to continuously presented visual or auditory stimuli depending on experimental group. Participants trained to synchronize their tapping to the 8-element long sequence for 10 minutes. Performance was measured by how many key presses were on target. A tap was considered to be correct if it was made within one third of the interstimulus interval preceding or succeeding the pacing stimulus. The number of on-target intervals for each rhythm repetition was used to quantify learning.

3.1.6 Rhythmic and ordinal structures and stimuli modality

All rhythms were metrical (Essens and Povel, 1985) rhythmic structures commonly found in western classical and popular music and all the rhythmic intervals had durations between 375 and 1500 ms. All ordinal sequences were sequences of key presses on a normal PC keyboard or on a response pad. All auditory stimuli were presented via headphones and the sound level was adjusted to the preference of the participant. All auditory stimuli were natural sounds downloaded from a database of licensed sounds such as a bongo beat or a clapping sound. All visual stimuli were presented either on a computer screen in front of the participant or on a small screen in the scanner room.

3.1.7 Questionnaires

In studies I and III participants had to answer multiple-choice questionnaires on the perceived regularities of the stimuli after completing either the SRTT or the ISR task. After completing the tasks, the questionnaires were displayed on the screen and the participants had to choose which of the three following alternatives best characterized the pattern of stimuli: 1, “the pattern was always predictable”; 2, “the pattern was sometimes predictable”; and 3, “the pattern was always random.”

3.2 IMAGING TECHNIQUES

3.2.1 Functional magnetic resonance imaging (fMRI)

Functional MRI was recorded for study II and for the additional material presented in section 4.5. Anatomical MRI images were also collected for study III and IV. Functional MRI data for study II as well as anatomical MRI data for study III were obtained using a GE Signa Horizon Echospeed 1.5T scanner at the MR-Center/Stockholm and an 8-channel head-coil. Anatomical MRIs for study IV were obtained by Radiology at the National Institutes of Health. fMRI is one of the most commonly used methods to investigate neural activity in the human brain (Ogawa et al., 1992). It is completely non-invasive and has a good spatial (1-10mm) resolution. fMRI detects regional changes in the cerebral blood flow (rCBF) and thereby indirectly measures neural activation. If larger neuron assemblies are activated in the brain their metabolic demands change resulting in an increase in blood supply to this region. The increase in blood flow however exceeds the oxygen utilization of the neurons. The gain in oxygenated blood, the so-called blood-oxygenated-level-dependent (BOLD) effect, is used to measure neural activity. Oxygenated haemoglobin is not magnetic

(diamagnetic) whereas deoxygenated haemoglobin is paramagnetic and thereby disturbs the magnetic field. BOLD effects are measured using rapid volumetric acquisition, whereby one whole slice image is acquired for every radio-frequency excitation pulse. Because of the rapid acquisition of images, fMRI is rather noisy and highly susceptible to artefacts. To account for these problems, all images were preprocessed before statistical analysis. Preprocessing consisted of realignment to correct for head movements, spatial normalization to a standard brain, and spatial smoothing to reduce noise. For statistical analysis, the data was modelled as a block design (each experimental condition presented continuously for approximately 32 seconds) and a standard general linear model (GLM) as implemented in SMP5. Also the anatomical MR images for study III were realigned, co-registered with the PET data, and normalized using SPM2.

3.2.2 Positron emission tomography (PET)

In study III we used positron emission tomography (PET), a technique that offers the unique possibility to visualize and measure densities of neuroreceptors and transporter proteins *in vivo*. For imaging receptors and proteins, a ligand that selectively binds to the target is coupled with a positron-emitting radionuclide. Radionuclides are isotopes with a relatively short half-life. In our study, carbon-11 [11C] was used. During PET measurements, the radioligand is injected into the bloodstream and quickly passes the blood-brain-barrier where it binds to its target molecules, emitting positrons. These positrons travel in the tissue for a very short distance (≈ 1.6mm for [11C]) until they collide with an electron. This encounter annihilates both electron and positron but also emits two 511 keV γ-particles (photons) at almost an 180˚ angle relative to each other. The photons are registered by the PET detectors and allow the system to estimate where the collision took place. Following data acquisition, a series of images is

reconstructed showing the distribution of radioactivity over time. For study III, PET measurements were acquired using an ECAT Exact HR system (CTI Siemens) run in 3D mode. Radioligand distribution was quantified by calculating the binding potential (BP) representing the product of receptor density (Bmax), apparent affinity (1/Kd), and the free fraction of free and non-specific bound ligand (f2) (Mintun et al., 1984). D2 receptor density was measured using two different radioligands: [11C]raclopride for striatal regions and [11C]FLB 457 for extra-striatal regions. Due to the moderate binding affinity of [C11]raclopride for dopamine receptors, it can only be used in the dopamine receptor dense regions of the striatum. High affinity ligands such as [11C]FLB 457 can only be used in extrastriatal regions, since binding-equilibrium within the striatum would not be reached during the measurement time. For each subject, the PET data was co-registered to an MRI image. For determination of regional ligand binding, regions of interest (ROIs) were manually delineated on each individual MR using the Human Brain Atlas software. For the striatal region, a detailed sub-regional analysis was performed, identifying ROIs for the whole striatum as well as separately for the limbic, associative and sensorimotor subregions (Mawlawi et al., 2001; Martinez et al., 2003). Figure 3 shows the ROIs for the striatal subregions. For [11C]FLB 457, a wide range of extrastriatal regions was identified (for details see(Cervenka et al., 2006). The ROIs were transferred to a series of PET images to generate decay-corrected time activity curves (TACs) and BP values were calculated using the Simplified Reference Tissue Model (SRTM) with the cerebellum as a region of reference ((for details see: Lammertsma and Hume, 1996). For all regions, TACs for left and right sides were spatially averaged to improve ROI statistics.

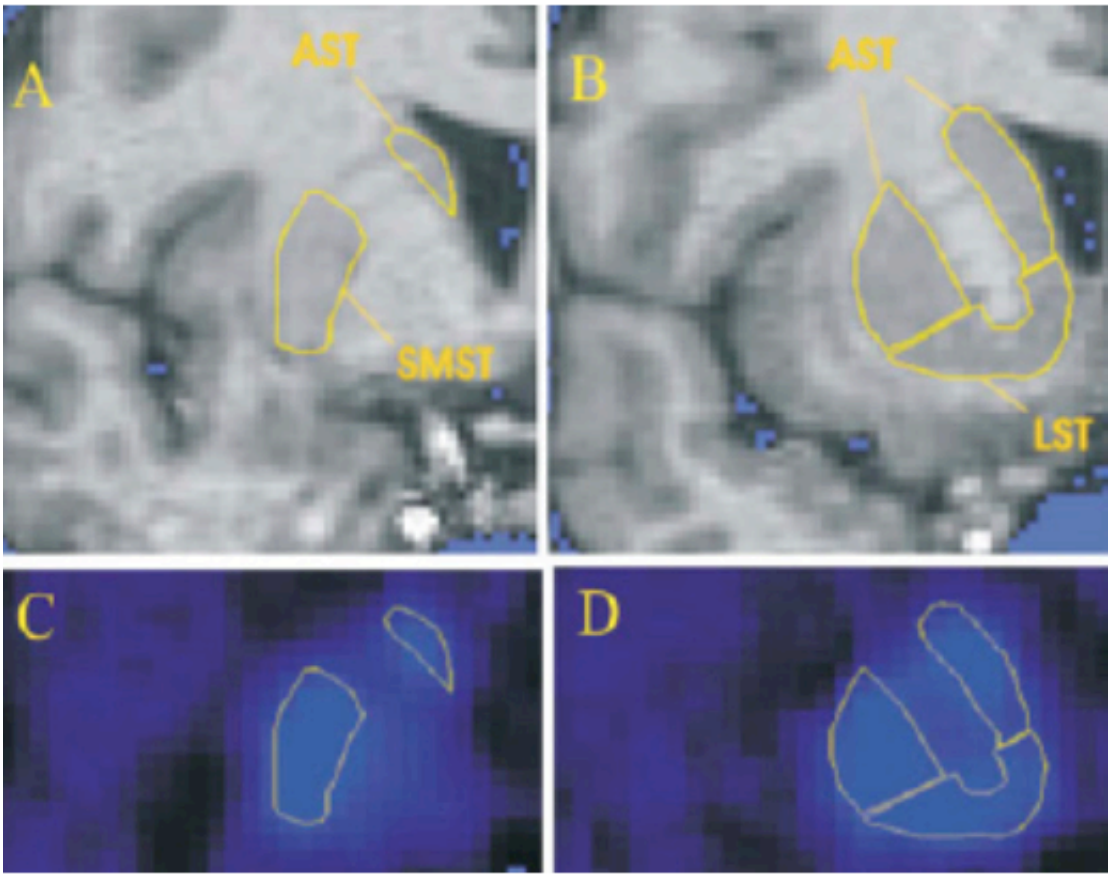

Figure 3: Functional regions of interest (ROIs) in the striatum. LST, limbic striatum; AST, associative striatum; SMST, sensorimotor striatum.

3.2.3 Transcranial magnetic stimulation (TMS)

In study IV we used TMS to investigate functional connectivity between different motor areas. TMS is a non-invasive method for stimulating the human brain. Stimulation is produced by an electric current passing through a magnetic coil that generates a brief, high-intensity magnetic field. Figure 4 shows the coils used in study IV and their placement. If a single stimulation pulse is given to the brain, the underlying tissue gets depolarized and discharges action potentials. If administered over M1, it excites neurons contributing to the pyramidal tract and produces a brief, relatively synchronous muscle response, the motor-evoked potential (MEP), which can be recorded by electromyography. This is the basis for observing changes in the

excitability of the motor cortex and how it is modulated by projections from other cortical areas. Paired-pulse paradigms as used in study IV provide the opportunity to probe inputs to M1 from other areas in the human cortex. The modulation of M1 excitability is tested by giving a conditioning stimulus (CS) prior to the test stimulus (TS) over M1. In study IV we paired a CS over the PPC with a TS over M1. MEPs were recorded from the first dorsal interosseous (FDI) muscle. The TS was given over the 'hotspot' for that muscle, i.e., the area of the motor cortex where the MEP response to stimulation was greatest. During the experiment, the intensity of the stimulus was set individually, so that the TS alone evoked an MEP of approximately 1 mV. The location for the CS over the PPC was determined individually with the help of a neuronavigation tool (BrainSight) and the intensity was set to 90% of the resting motor threshold (RMT) for each participant individually. The RMT is defined as the lowest intensity at which stimulation over the hotspot elicits a reliable response of at least 50 μV. A paired-pulse session contained both TS alone stimulations as well as TS-CS pairings at 2, 4 and 6 ms. Statistical analysis of the MEPs amplitudes were done in Excel and Statistica. For details see the Methods section of study IV.

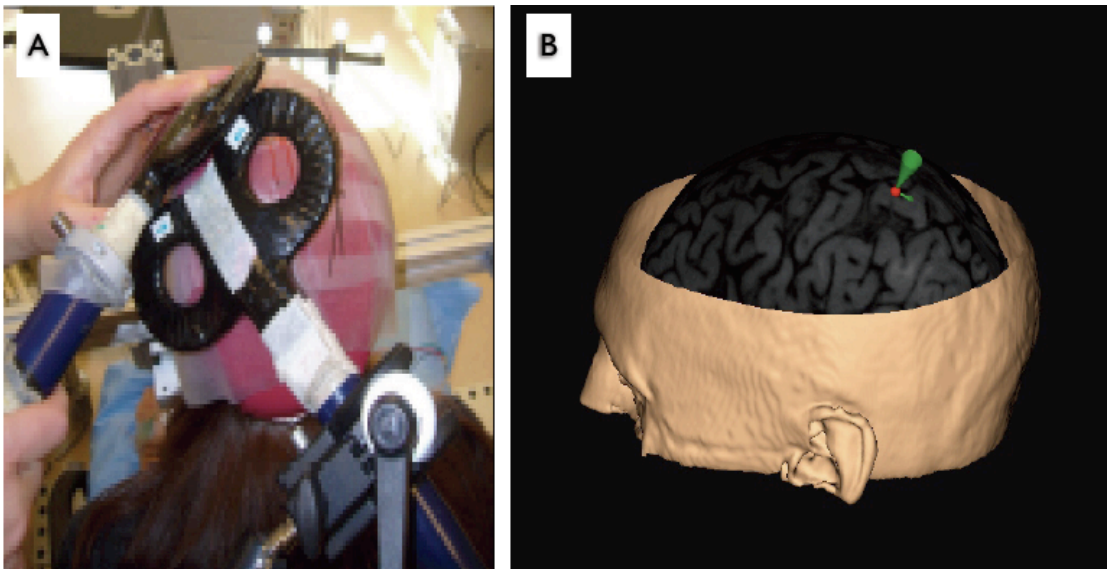

Figure 4: Coil coordinates and the coil placement for the TMS experiments. (*A*) Displays the placement of both coils on the head. (*B*) Shows the stimulation coordinates averaged over all participants.

3.3 PARTICIPANTS

A total of 93 healthy volunteers (56 female, 37 male) participated in the four different experiments. An additional 18 participants (7 female, 11 male) participated in the study presented as additional material. Age ranged from 18 to 65 years. Participants were recruited via advertisement or they were part of a database of healthy volunteers from the National Institute of Neurological Disorders and Stroke. All participants were paid for participation and all experimental procedures were undertaken with the understanding and written consent of each participant. Age distribution, number of participants and inclusion criteria were different between studies. No participant took part in multiple experiments. Table 1 summarizes the number of participants in each study as well as age ranges, inclusion criteria, and the institution that granted ethical approval.

Study	N	Excluded	Female	Male	Age (Ø ±SD)	Inclusion Criteria	Ethics
I	43	3	28	15	28±10.1	**Neurologically Healthy** (self report) **Under 40** years of age **No musical training** > 4 years **No hearing impairment** (self report)	Ethical Committee KI Dnr. 2007/83–/32
II	16	4	9	7	29±6.3	**Neurologically Healthy** (self report) **Right-handed** (Edinbourgh Inventory) **Under 40** years of age **No musical training** > 4 years **No hearing impairment** (self report)	Ethical Committee KI Dnr 2007/ 83–/32
III	15	0	8	7	56±8.0	**Neurologically Healthy** (Exam) **Nonsmokers** (self report)	Ethics and Radiation Safety committees KI Dnr. 2007/704–31/4, 02–431
IV	19	1	11	8	32±8.3	**Neurologically healthy** (Exam) **Under 50** years of age **Right-handed** (self report) **No musical training** > 4 years **No hearing impairment**	Institutional Review Board NINDS Protocol: 09-N-0146

Add. Material	18	2	7	11	27±6.2	**Neurologically Healthy** (self report) **Right-handed** (Edinburgh Inventory) **Under 40** years of age **No musical training** > 4 years **No hearing impairment** (self report)	Ethical Committee KI Dnr 2007/83--/32

Table 1 shows the distribution of participants in the different experiment.

4. RESULTS AND SHORT DISCUSSION

4.1 STUDY I

Implicit and explicit learning of temporal sequences studied with the process dissociation procedure

In this study we investigated whether temporal sequences can be learned implicitly using a process dissociation procedure (PDP). Participants performed repeated ISR trials of sequential stimuli with a random ordinal structure and fixed temporal structure. Explicit knowledge was evaluated through verbal questions and the process dissociation prodecure. Participants were divided into two groups (n =20): in Group 1 (‘ordinal’/implicit group) stimulus presentation was visual, and the participants were instructed to repeat the ordinal structure; in Group 2 (‘ordinal+temporal’/explicit group) stimulus presentation was audio-visual, and the participants were instructed to repeat temporal and ordinal structure. During the SRTT the ‘ordinal’ group was able to significantely improve their performance of the rhythm. Whereas their error rate at the beginning of the SRTT was higher than that of the ‘ordinal+temporal’ group, there was no significant difference in error rate in the last trials of the SRTT (Figure 5A). Interestingly, the ordinal+temporal group had consistently low error rates but did not significantly improve their performance throughout the SRTT. In the PDP, the ‘ordinal’ group did not show a significant difference between Inclusion and Exclusion task. The ‘ordinal+temporal’ group did display a significant difference between Inclusion and Exclusion tasks despite the lack of improvement during the SRTT (Figure 5B). These results suggest that the learning showed in the SRTT by the ordinal group is largely implicit, whereas the ‘ordinal+temporal’ was able to establish an explicit representation of the sequence even though they did not improve their performance during the SRTT. We found a negative correlation between the degree of improvement during serial

recall and explicit knowledge measured by the PDP for both the 'ordinal' group and the 'ordinal+temporal' group (Figure 5C and 5D). This relation was independent of the final level of performance during the serial recall. Taken together, these data suggest that distinct implicit and explicit learning systems might exist for temporal sequences. Whereas the implicit system is gradual and inaccessible to conscious control, the explicit system is fast and results in representations that can be used to control performance in inclusion and exclusion tasks.

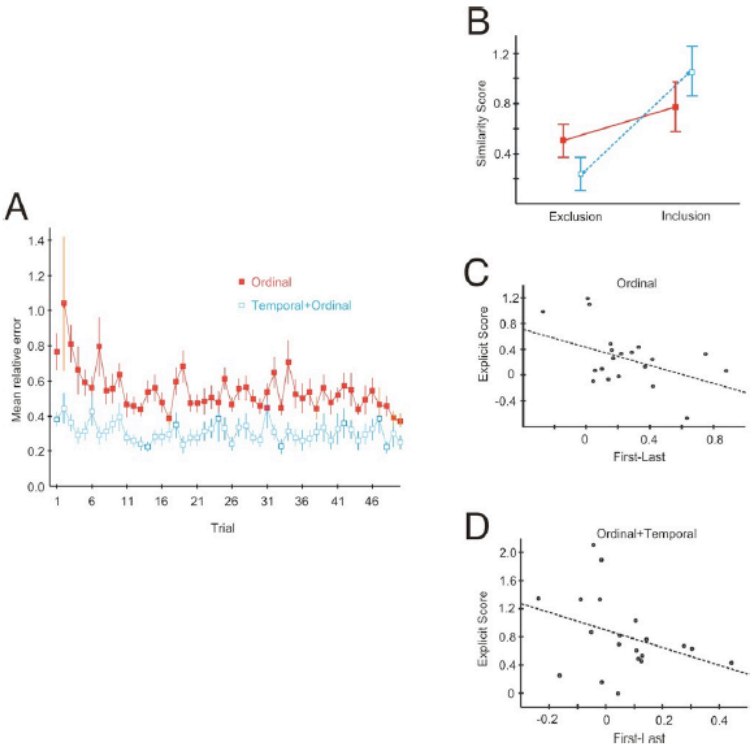

Figure 5: Performance in the serial recall task, for the Ordinal and the Temporal+Ordinal groups. A: Mean relative error of each reproduction across all participants is shown as a function of trial number. Error bars represent SE. B: Mean relative error in the generation tasks for the Ordinal and Temporal + Ordinal groups. Bars show 95% CIs. C: Correlation between improvement of performance during serial recall and explicit generation task performance for the Ordinal group. D: The same correlation for the Temporal+Ordinal group.

4.2 STUDY II

The dorsal auditory pathway is involved in the performance of both visual and auditory rhythms

We used functional magnetic resonance imaging to investigate the effects of two factors on the neural control of temporal sequence performance: the modality (visual/auditory) in which the rhythms were trained approximately one day prior to the scan, and the modality (visual/auditory) of the pacing stimuli preceding self-paced rhythm performance. Data from the self-paced performance phase of 12 participants were analyzed. We only found a significant main effect for the visual metronome modality in the left angular gyrus due to a deactivation of this region after auditory pacing (Figure 6A) No significant differences could be detected for the training modality. The conjunction of all conditions revealed a set of brain areas that included dorsal auditory pathway areas (left temporo-parietal junction area and ventral premotor cortex), as well as the dorsal premotor cortex, the supplementary and presupplementary premotor areas, the cerebellum and the basal ganglia. All these areas were active during rhythm production independent of the training and pacing modality (Figure 6B). Behaviorally, there were no significant differences between the performance of the sequences learned by a visual or auditory stimulus. We conclude that the regions seen in the conjucntion analysis are involved in controlling performance of well-learned rhythms, regardless of the modality. This suggests that after extensive short-term training, both visual and auditory trained rhythms are transformed into auditory-motor representations. The deactivation of the angular cortex following auditory pacing may represent cross-modal auditory-visual inhibition.

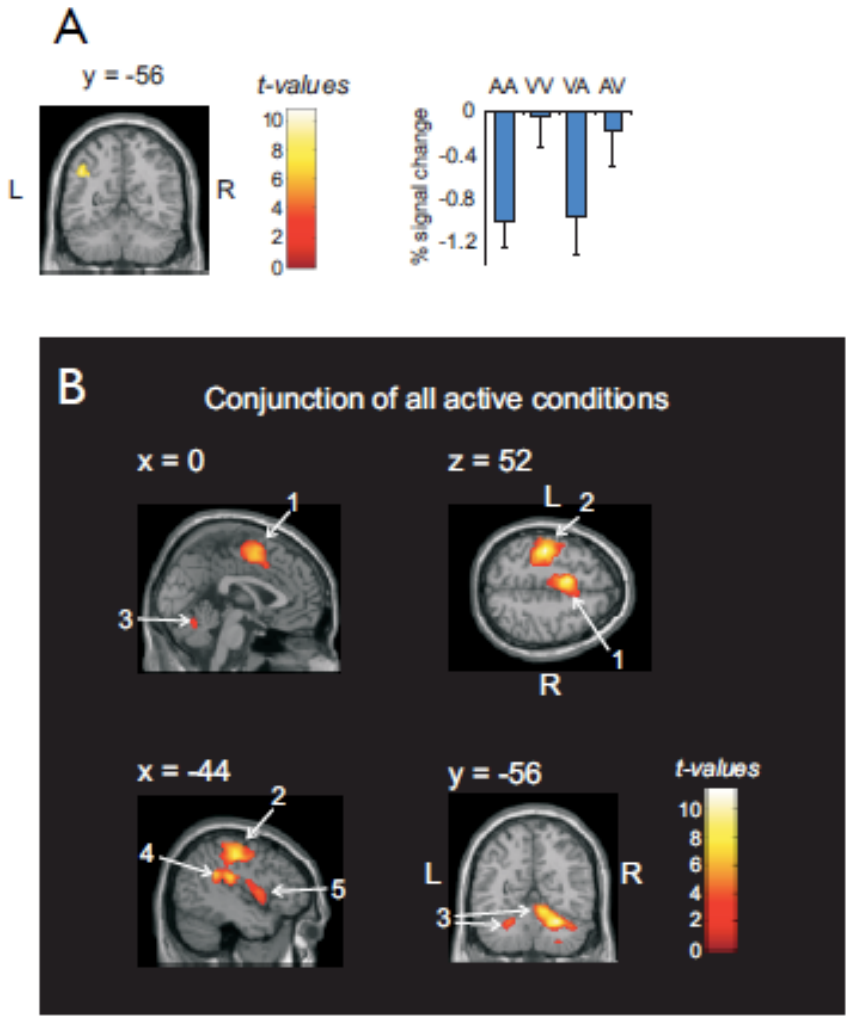

Figure 6: 6A shows activation of the left angular gyrus in the main effect contrast for the visual metronome. The color scale represents t values. R and L denote the right and left sides, respectively. The bar diagram shows mean the percent signal change in BOLD signal for each condition. Error bars show standard error of the mean. Conditions are abbreviated as follows: AA, auditory training, auditory metronome; VV, visual training, visual metronome; VA, visual training, auditory metronome; AV, auditory training, visual metronome. 6B shows activated brain regions in a conjunction analysis across all conditions. Active regions were found in SMA, preSMA and CMA(1); in a large cluster that included left primary sensorimotor and lateral premotor areas, and extended caudally to the intraparietal sulcus (2); bilaterally in the cerebellar hemispheres (3); in cortical regions around the temporo-parietal junction (4); and in the insula (5).

4.3 STUDY III

Dopamine receptor density in the limbic striatum is related to implicit but not to explicit movement sequence learning

In this study we used PET to investigate whether individual differences in implicit and explicit motor sequence learning are related to dopamine D2 receptor densities in functional subregions of the striatum. Sequence learning was assessed using the serial reaction time task, and measures of implicit and explicit knowledge were estimated using a process dissociation procedure. Correlation analyses were performed between PDP measures and D2 binding potential (D2BP). In the three striatal subregions (sensorimotor, associative, limbic striatum) a differential pattem of correlations with implicit and explicit learning was found: In the limbic subregion, D2BP was specifically related to implicit but not explicit learning (Figure 7A). The associative and sensorimotor striatum showed negative correlations with both implicit and explicit learning (Figure 7B and 7C). In these regions the correlations between D2BP and implicit and explicit learning were not significantly different. These findings suggest that individual differences in striatal DA function underlie differences in sequence learning ability and support the idea that implicit and explicit sequence learning depend on partly distinct neural circuitry.

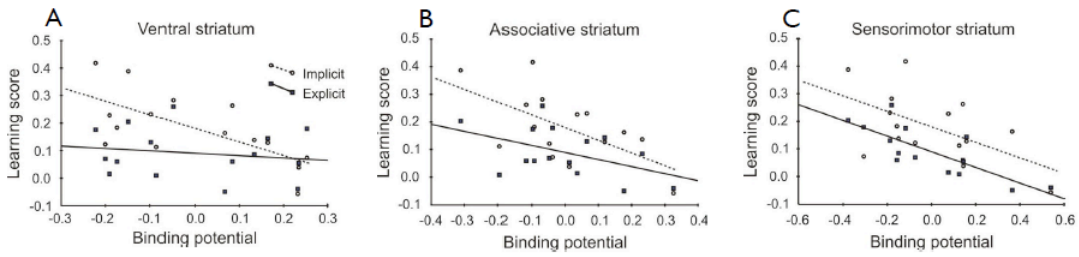

Figure 7: Explicit and implicit learning as a function of D2 receptor binding potential. Data are shown for the ventral (A), associative (B), and sensorimotor (C) subregion of the striatum. Correlation statistics are controlled for age and ROI volume.

4.4 STUDY IV

Changes in the posterior parietal cortex – primary motor cortex pathway induced by sensorimotor training

In this study we used paired pulse TMS to investigate if the functional connection between the PPC and M1 can be modulated by short periods of rhythmic sequence learning. Additionally, we were interested to see if visual learners (n=9) would show higher modulation than auditory learners (n=9). To assess functional connectivity, TMS was done before and at three time points after the rhythmic motor training had been concluded. We found that prior to motor training, stimulation of the PPC facilitated the motor-evoked potentials evoked by a test stimulus over the ipsilateral M1 if the inter-stimulus interval between both pulses was 2 ms. Directly after the motor training session, the facilitation observed at rest was completely absent and the PPC stimulation had no effect on M1 at any inter-stimulus interval (Figure 8). This training-induced modulation of the PPC-M1 connection was transient, since no significant changes compared to rest could be observed at testing sessions 30 minutes and 60 minutes after training was concluded. We were not able to find statistically significant differences between the visual and the auditory learners, even though that might be due to the relatively small subject number in each group. Figure 9 shows that there is a trend for a differential modulation of the PPC-M1 connection 30 minutes after training has been concluded. Taken together these results show, that even short periods of motor training can influence connectivity between different brain regions, and that both visual and auditory motor integration influence the PPC-M1 pathway.

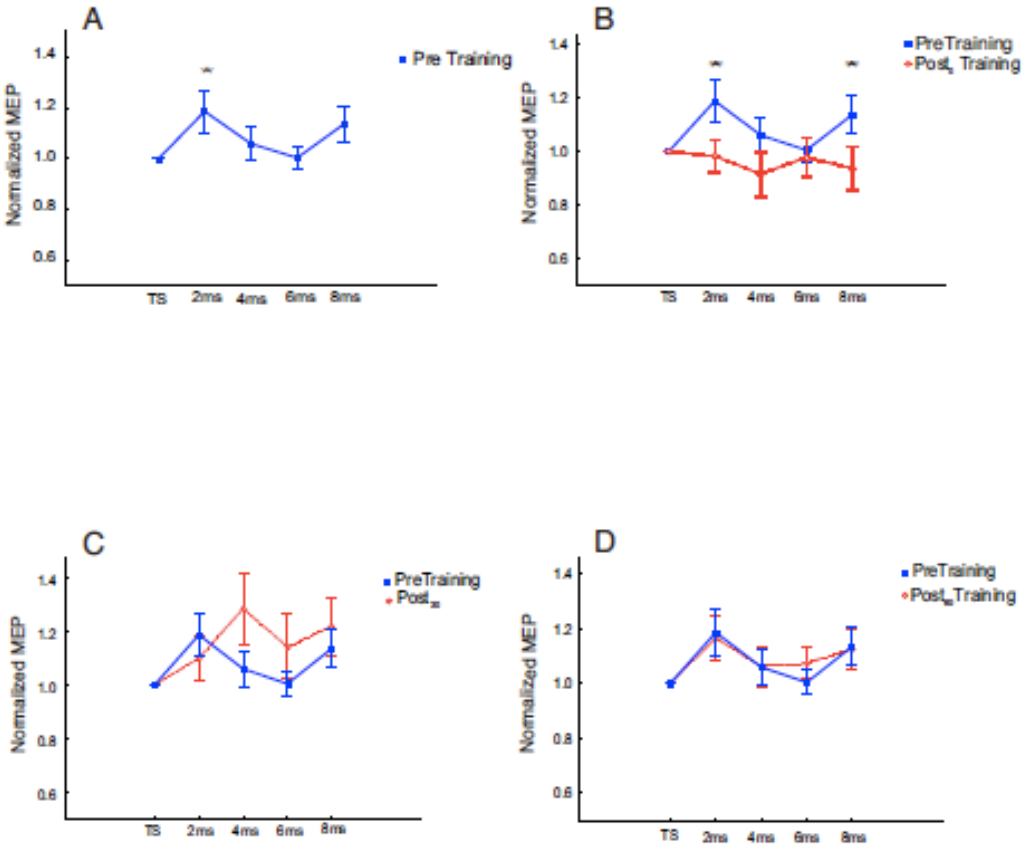

Figure 8: TMS results (mean ± SE) for the test stimulus alone as well as for all four paired-pulse ISIs (2, 4, 6, 8 ms) before and after training across all participants. All results are normalized to the test stimulus (TS). (*A*) Normalized MEP data before the sequence tapping. The asterisk indicates significant differences to the TS. (*B*) Normalized MEPs directly after the tapping session. The pre-training results are displayed for comparison. Asterisks indicate significant differences between pre and $post_0$. (*C*) Normalized MEPs 30 minutes after the tapping session, the pre-training results are displayed for comparison. (*D*) Normalized MEPs 60 minutes after the tapping session. The pre-training results are displayed for comparison.

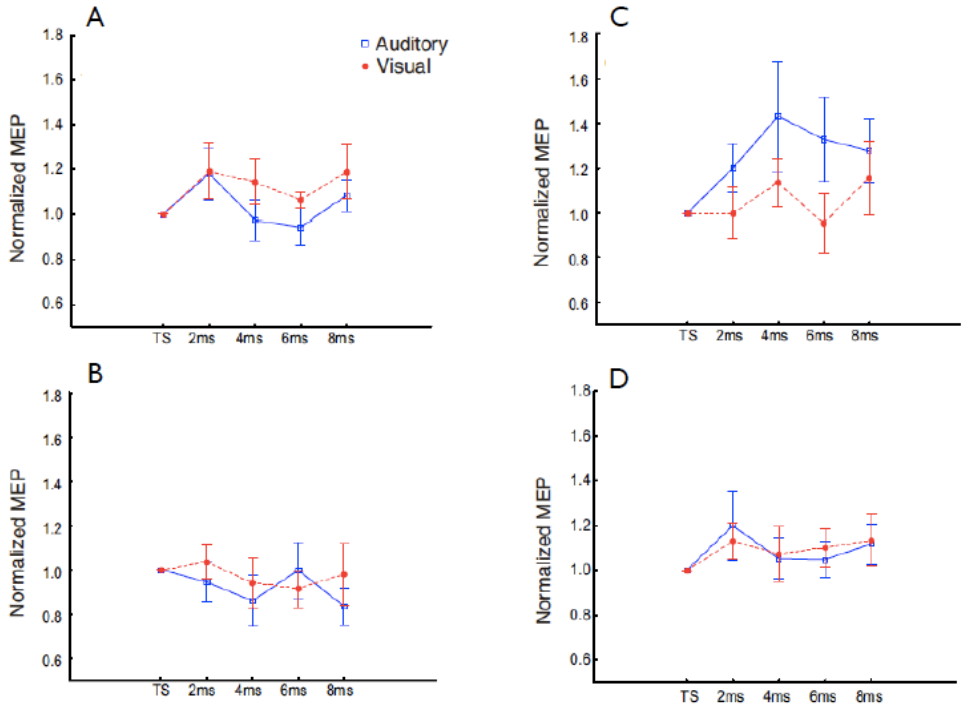

Figure 9: TMS results (mean ± SE) for the visual and the auditory learners separately. TS and ISI are displayed on the x-axis and MEP amplitude (%) is shown on the y-axis. (*A*) Shows the normalized MEP data before the sequence tapping. (*B*) Depicts the normalized MEPs directly after the tapping session. (*C*) Depicts the normalized MEPs 30 minutes after the tapping session. (*D*) Shows the normalized MEPs 60 minutes after the tapping session.

4.5 ADDITIONAL MATERIAL

Hearing, seeing, feeling rhythms - modality differences in reproduction and early phase learning of rhythmic sequences

These preliminary results are a direct continuation of study II. They further explore the effects of modality differences on brain activity during encoding. fMRI was used to investigate if non-auditorily presented rhythms activate auditory regions already during early learning phases or if a transformation to auditory representations takes place later, e.g. during the consolidation phase. Here we report preliminary results collected from 16 subjects. Participants learned rhythmic sequences in blocks. Each block contained

10 consecutive presentations and reproductions of a 10-interval rhythm. Presentation modality was fixed within a block (either visual, auditory or tactile) but pseudo-randomly distributed in-between blocks. Note that only activity during sequence production is reported. No activations were found specifically for the visual and auditory modality, thus further confirming our main hypothesis that visual and auditory rhythms are processed by an overlapping set of brain regions already during reproduction and early stages of learning. In contrast, several brain regions were active in the main effect contrast for the tactile modality (Figure 10A). Tactile specific activations were seen bilaterally in the superior posterior and medial temporal area extending into the parietal operculus, in the putamen exceeding into the claustrum and insula, as well as in the supramarginal gyrus (SMG), hippocampus, thalamus and the primary sensory cortex (S1). A conjunction analysis of rhythm production across all modalities revealed a network very similar to the results in study II (Figure 10B), with the exception of higher frontal activity most probably due to the increased working memory demands during early sequence learning.

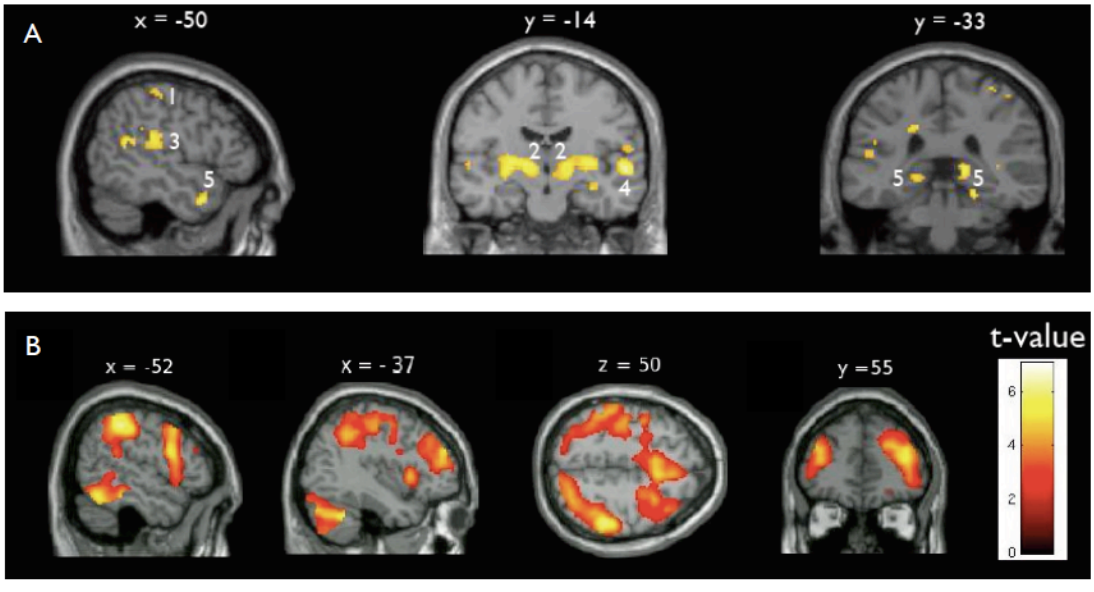

Figure 10: (A) Activations for the main effect for reproduction of rhythms presented in tactile modality. Active regions in this graph are: (1) primary sensory cortex, (2) putamen and insula, (3) supramarginal gyrus, (4) superior temporal gyrus and (5) hippocampus. Activity maps of brain regions with significantly increased BOLD contrast signals are shown projected onto axial and coronal sections. (B) Activated brain regions in a conjunction analysis for the rhythm reproduction across all modalities. Activity maps of brain regions with significantly increased BOLD contrast signals are shown projected onto sagital, axial and coronal sections. In both graphs the color scale represents the t-values. All activations are FDR corrected at $p < 0.05$.

5. GENERAL DISCUSSION

The general aim of this thesis was to study the behavioral and neural mechanisms underlying motor sequence learning in healthy humans. We have focused on four major aspects: 1) the peculiarities of temporal sequence learning, 2) the differences between implicit and explicit sequence learning, 3) the modality differences in sequence learning and 4) learning induced plasticity. A broad range of methods was applied to answer those research questions. In the following, I will discuss the results of this thesis aspect by aspect.

5.1 TEMPORAL SEQUENCE LEARNING

In **Study I**, **II** and **IV** the stimulus material consisted of temporal sequences. Whereas the findings of **Study IV** might be somewhat more applicable to sequences in general, **Study I** and **II** focus on aspects unique to temporal sequence learning. **Study I** showed that not only ordinal but also temporal sequences can be learned implicitly. Earlier studies (Salidis, 2001; Ullen and Bengtsson, 2003) did not use the PDP so they are open to criticisms about process purity. By directly comparing explicit and implicit temporal sequence learning using the PDP, **Study I** added evidence in favour of implicit temporal sequence learning. **Study II** showed that the activity in the temporo-parietal junction (TPJ), which is part of the dorsal auditory stream (Hickok et al., 2003) and commonly activated during the production of auditory rhythms, is not dependent on the modality in which the sequences were learned. Our findings that the TPJ is activated independent of sequence modality can be related to existing studies in two ways: First, by showing that the TPJ is part of the 'intrinsic rhythm network' and is not only activated by a certain stimulus modality. We are able to add support to earlier studies finding that only temporal and not ordinal sequences activate auditory brain areas (Bengtsson et al., 2004). Our findings suggest that the higher auditory areas found

active by Bengtsson et al. are part of a general temporal sequence network and do not merely process modality-specific aspects of the rhythmic stimuli. Second, by showing that auditory areas are activated even after visual training we were able to add neurobiological evidence to the behavioral observation that the auditory modality is dominant for processing temporal information and representation of temporal sequences (Fendrich and Corballis, 2001; Repp and Penel, 2002). Taken together these two studies suggest that temporal sequences also allow implicit and explicit learning and that the dorsal auditory pathway is part of the "intrinsic" rhythmic sequence production network.

5.2 IMPLICIT AND EXPLICIT LEARNING

Study I and **III** investigated differences between implicit and explicit sequence learning. As already mentioned we could show in **Study I** that temporal sequences can be learned both implicitly and explicitly. Furthermore we showed that there was a negative correlation between the degree of improvement during serial recall and a measure of explicit knowledge in the generation task, meaning that participants showing an improvement over the ISR trials had worse explicit scores than participants who did not. This seeming paradox is explained by the fact that especially explicit learners seemed to instantly get the rhythmic structure leading them to perform on a stable level from the first trials of the experiment. That fast learning in the ISR was paired with high explicit scores, whereas slow gradual improvement during the ISR was paired with low explicit scores. This suggests that distinct implicit and explicit systems may exist for learning of temporal sequences: implicit learning is gradual and gives rise to knowledge that is inaccessible to conscious control while the explicit system is fast and results in representations that can be used to control performance in inclusion and exclusion tasks. In **Study III** we focused on ordinal sequences and

investigated whether functional subregions of the striatum are differently associated with measures of implicit and explicit sequence knowledge. The main finding of this study was that indeed implicit and explicit sequential learning correlate differently with dopamine D2BP in functional subregions of the striatum. Specifically, D2BP in the limbic striatum showed a significant correlation only with implicit learning. These findings tie in with earlier studies (Badgaiyan et al., 2007, 2008) that did not separate striatal subregions and hence could not find differences between learning type and striatal involvement. The specific correlation between D2BP in the limbic striatum and implicit learning also fits the view that implicit learning systems in general tend to involve phylogenetically old parts of the nervous system (Reber et al., 1991) since the limbic subregion is phylogenetically the oldest subregion of the striatum, present even in primitive vertebrates such as the lamprey. In summary, **Study I** and **Study III** offer both behavioral and anatomical support that implicit and explicit learning seem to rely on partially different neural circuits. They are also in line with the evolutionary perspective on implicit learning offered by Reber. **Study III** offers anatomical evidence showing that implicit learning relies on phylogenetically older structures of the striatum. The behavioral data from **Study I** is also in line with this idea. It is conceivable that the slow, gradual improvement as seen for implicit learners in **Study** I could also be displayed by more primitive vertebrates, whereas the fast, conscious representations formed by the explicit system cannot.

5.3 MODALITY DIFFERENCES

Study II and **IV** as well as the additional material described in section 4.5 look at modality differences in temporal motor sequence learning. As mentioned in section 5.1, **Study II** showed that the dorsal auditory stream including the TPJ is activated independent of the modality of the pacing and training stimulus. This was to our

knowledge the first study directly comparing rhythms trained in visual and auditory modalities. Our fMRI data suggests that well learned visual temporal sequences get 'transformed' into auditory representations and are stored that way. Collier and Logen (Collier and Logan, 2000) as well as Guttman et al. (Guttman et al., 2005) offered behavioral evidence for the transformation of visually presented sequences into auditory representations. Our results are able to add physiological evidence to their behavioral observations. Recently, Grahn (Grahn, 2010) could confirm that the TPJ was active independently of stimulus modality not only during rhythmic sequence performance but also even during rhythmic sequence perception. Since **Study II** focused on well-learned sequences, we conducted additional experiments (see section 4.5) to investigate if the TPJ is already activated during the early learning of rhythmic sequences. We found that tactile, visual and auditory sequence production activated areas in the TPJ already during the very early learning stage. Interestingly, these data also suggests that whereas visual and auditory sequences activate the exact same brain network during reproduction, tactile sequences involve additional modality specific areas even during reproduction. This might imply that whereas visual rhythms are processed entirely as auditory-motor representations, there is additional modality-specific circuitry for handling rhythms in tactile modality. Also in **study IV** we were comparing auditory and visual rhythms. We investigated if auditory or visual learning of temporal sequences has a differential influence on the PPC-M1 connection. No significant difference could be seen in the modulation of this pathway. Taken together these data show that the influence of stimulus modality on the neural activity and functional connectivity is rather small. These findings further support that temporal sequences are stored in a modality independent network that includes the dorsal auditory stream. However, it has to be noted that there might be modality differences

that we were not able to detect and that other parietal-M1 connections might show stronger modality dependent modulation than the PPC-M1 pathway tested by study IV.

5.4 TRAINING INDUCED PLASTICITY

In **Study IV** we also investigated if short periods of motor training could modulate the functional connectivity between the PPC and M1. We choose the PPC due to its important role in sensory-motor integration. Earlier studies reported facilitation from PPC to M1 *during* reaching and grasping movements (Koch et al., 2009; Koch et al., 2010). We show that already short periods of motor sequence training influenced the connectivity between M1 and the PPC. The observed effect was transient with peak facilitation found directly after the training was concluded. Already 60 minutes after motor training, the M1-PPC connectivity had returned to baseline. These results fit nicely into a whole row of TMS experiments investigating the involvement of the PPC in different aspects of motor function (Koch et al., 2009; Koch et al., 2010; Ziluk et al., 2010) and demonstrate the powerful effect that even short periods of motor training can have on functional brain connectivity.

6. FUTURE PERSPECTIVES

Investigating the behavioral and neurobiological underpinnings of motor sequence learning may not only allow us to better understand one of the most important functions of the motor system, but it may also serve as a window to further unravel the physiology of many other skills requiring sequential representation in memory. Ultimately, a better understanding of the neurobiology of motor sequence learning may also help with the development of new treatments and therapies for different motor disorders. In the last two decades the interest in investigating different aspects of motor sequence learning has been immense, and thanks to physiological studies as well as to the advent of neuroimaging, a lot is known about the brain regions involved in motor sequence learning. Even though much is known about the neural circuits involved in sequence learning, the full mechanisms that govern motor control, from molecules over neuronal circuits to human behavior, are far from being understood. Taking the methods and results of this thesis into consideration the field of human motor sequence learning could benefit from the following developments: First, with the rise of different imaging techniques purely behavioral paradigms have become somewhat outdated. However, well-designed behavioral paradigms can offer a lot of insight about the detailed factors that influence sequence learning and should keep their place amongst research tools. It also is of crucial importance for imaging experiments to carefully evaluate the behavioral paradigm used during scanning. This will help not only to draw important conclusions about the connection between individual performance and brain activity, but it will also help to obtain more consistent scanning data across studies and groups. Second, future imaging studies should focus less on whole brain studies reporting single active brain regions but should rather decide on a ‘micro’ or ‘macro’ approach. In an ideal micro approach, a very specific sub-region of the motor system is studied to answer a clearly defined a priori hypothesis about the function or the

mechanisms of this region. The 'macro' approach, on the other hand, looks at the whole brain but focuses on brain connectivity instead of isolated regions. Third, the field will profit from the application of methods such as diffusion tensor imaging or morphometic measures that go beyond the "classical" fMRI experiments, as well as from using a multi-technique approach, including combinations of physiology, functional, anatomical and molecular imaging and behavioral research. By investigating specific questions in motor sequence learning research from all those angles, we will be better able to understand the complete mechanisms governing motor learning. Finally, the inclusion of genetic information will be an important factor to further understand the bases of individual differences in motor learning as well as the molecular basis of these processes.

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