ABSTRACT

Title of Document:	**LEARNING PROCESSES UNDERLYING IMPLICIT MOTOR SEQUENCE ACQUISITION IN CHILDREN AND ADULTS.**
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Children and adults are able to learn a motor sequence quickly, usually over a course of one learning session consisting of 4-8 learning blocks. This initial acquisition is referred to as fast learning. However, little is known about the learning processes underlying the fast acquisition of motor sequences. Therefore, the overarching objective of this dissertation was to examine the underlying processes that drive rapid motor sequence learning in children and adults. In a series of studies, children and adults performed a modified serial reaction time (SRT) task, a primary window into understanding implicit motor sequence learning. Study I demonstrated that fast learning of implicit motor sequences in six- and 10-year-old children was comparable to adults, while the performance (i.e., reaction time, RT) during learning was reflected by two age-related processes. Learning in six-year-old children dominantly relied on an offline process where RT improved after a short rest, while

offline enhancement as well as online progressive improvement in RT reflected sequence learning in 10-year-old children and adults. In studies II, III, and IV, we demonstrated that the online and offline processes were neither by-products of task pacing constraints nor illusory effects of fatigue or reactive inhibition. Instead, these two age-related processes were more likely to be functional mechanisms underlying implicit motor sequence learning, which could be modulated by the involvement of procedural and declarative memory. In addition, study III characterized the developmental landscape of 5- to 14-year-old children and found that the developmental changes of online and offline learning were primarily present in early childhood. As fast learning is known to enable generalization (or transfer) of sequences learning, we expected, given the findings in studies I through IV, age-related differences in the generalization of implicit motor sequence learning. The results in study V, interestingly, demonstrated that the generalization of implicit motor sequence learning was better in children than in adults. However, in study VI, when greater procedural memory was required in the SRT task, learning in adults largely depended on offline learning; and, the age-related differences in learning generalization vanished, suggesting that offline learning may facilitate the generalization of implicit motor sequence learning. Taken together, results from these studies found two age-related learning processes (i.e., online and offline learning) that drive the fast implicit sequence acquisition and demonstrated that the age-related online and offline learning may lead to children a superior ability in the generalization of motor sequence learning. These results extend our understanding of

the age-related development of implicit motor sequence learning and provide

potential insights into the question of why childhood is an optimal period for learning.

LEARNING PROCESSES UNDERLYING IMPLICIT MOTOR SEQUENCE
ACQUISITION IN CHILDREN AND ADULTS.

By

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Dedication

To my loving wife, Xia Li
Your love, support, understanding, and inspiration keep me going every day.

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Chapter 1: **Introduction**

Overall Purpose

Childhood is a critical period for the development of motor skills. The experiences we have and the skills we learn during this period can greatly affect the motor skills of our later life. For example, the acquisition of fundamental motor patterns before the age of seven years affects the ability to perform the complex motor behaviors in the adolescent and adult years (Clark & Metcalfe, 2002; Watanabe, Savion-Lemieux, & Penhune, 2007). Learning that takes place at early ages also affects brain functions in our later life (Elbert, Pantev, Wienbruch, Rockstroh, & Taub, 1995; Schlaug, Jancke, Huang, Staiger, & Steinmetz, 1995). Given the importance of childhood in learning, therefore, there is a need for a clearer understanding of motor skill learning in childhood and how it might develop with age.

One of the most important aspects of learning motor as well as cognitive skills involves sequence learning. Specifically, an imperative feature of playing musical instruments, spoken language, and motor skills is that they emerge from a sequence of movements produced in a specific order. For example, in speaking, words are pronounced in a specific order, reading requires the eyes to move sequentially from word to word, and playing the piano involves multiply ordered finger movements. Thus, learning motor sequences is a fundamental and crucial ability in our daily life. A growing literature demonstrates that motor sequence learning involves memory systems of procedural and declarative learning (Brown & Robertson, 2007a; Curran

& Keele, 1993; Destrebecqz & Cleeremans, 2001; Reber & Squire, 1994; Robertson, 2007; Willingham & Goedert-Eschmann, 1999; Willingham, Nissen, & Bullemer, 1989). However, unlike the clear developmental picture of declarative learning that becomes better as age increases until the twenties (Craik & Bialystok, 2006), the development of motor sequence learning that requires greater procedural memory is equivocal (Hodel, Markant, Van Den Heuvell, Cirilli-Raether, & Thomas, 2014; Janacsek, Fiser, & Nemeth, 2012b; Meulemans, Van der Linden, & Perruchet, 1998; Nemeth, Janacsek, & Fiser, 2013a; Thomas et al., 2004; Vinter & Perruchet, 2000).

Research has shown that both adults and children as young as six years of age can learn motor sequences quickly, usually over a course of one learning session (Meulemans, Van der Linden, & Perruchet, 1998; Reber, 1996), and referred to as fast learning (Censor, Sagi, & Cohen, 2012; Dayan & Cohen, 2011; Honda et al., 1998; Karni et al., 1998a; Walker, Brakefield, Morgan, Hobson, & Stickgold, 2002). Since the memory of sequences encoded during fast learning is usually fragile, slow learning, involving memory consolidation that follows fast learning, allows the newly acquired memory to stabilize and become resistant to interference (Brown & Robertson, 2007a; Nettersheim, Hallschmid, Born, & Diekelmann, 2015; Robertson, Pascual-Leone, & Press, 2004; Robertson, Press, & Pascual-Leone, 2005). Surprisingly, memory consolidation in children is superior (Wilhelm et al., 2013) and occurs earlier (Adi‑Japha, Badir, Dorfberger, & Karni, 2014) compared to that in adults, allowing the newly acquired memory to quickly become resistant to interference from a competing motor skill in children (Adi‑Japha, et al., 2014; Dorfberger, Adi-Japha, & Karni, 2007). To date, the mechanism underlying the

superiority of children's motor sequence learning is unclear. Given that slow learning builds on the memory of sequences encoded during fast learning, a clear understanding of age-related differences in fast learning is essential. Therefore, the primary purpose of this dissertation is to programmatically investigate the underlying processes that drive fast motor sequence learning in children and adults.

Most often, motor sequences are learned implicitly where performer is not aware of there is a sequence to the movements. In this dissertation, the serial reaction time (SRT) task (and its modified version) is employed as it has been used as the primary paradigm to study implicit motor sequence learning (Nissen & Bullemer, 1987). In the SRT task, six (or four) visual stimuli appear one at a time following a specific order. Children and adults are required to respond to these stimuli as quickly and accurately as possible without being instructed about the presentation of the stimulus sequence. There are 6 to 8 learning blocks with mandatory rest of 3 minutes between each block. Reaction time (RT) is used to access learning and its underlying processes. Specifically, mean RT of each learning block is used as a marker of implicit sequence learning (Cleeremans, Destrebecqz, & Boyer, 1998; Clegg, DiGirolamo, & Keele, 1998; Cohen, Ivry, & Keele, 1990; Jimenez, Mendez, & Cleeremans, 1996; Robertson, 2007). The changes in RT within and between learning blocks are used to assess underlying learning processes. Specifically, the progressive improvement in RT within each block is an indicator of the online process and the RT enhancement after a short rest reflected the offline process.

Specific Aims (SA) and Backgrounds

There are six specific aims (SA) by which we programmatically investigated the development of learning processes underlying motor sequence acquisition. SA1 demonstrates whether the acquisition of an implicit motor sequence during fast learning is driven by different processes (i.e., online and offline processes) in children and adults. SAs 2 to 4 aim to investigate the mechanisms underlying these two different processes. Specifically, these SAs examine whether online and offline processes are active learning mechanisms, artifacts of fatigue or reactive inhibition, or by-products of task pacing. Given the common notion that fast learning facilitates the generalization of motor sequence learning (Censor, 2013; Perez et al., 2007), the distinct fast learning processes in children and adults raises the possibility that there are age-related differences in generalizing sequence learning. Therefore, SAs 5 and 6 demonstrate whether the generalization of motor sequence learning is age-related and whether the age-related differences are, in part, explained by online and offline learning.

<u>SA1 (Study I; Chapter 3): To determine if the acquisition of implicit motor sequences during fast learning is driven by age-related learning processes.</u>

Many have suggested that sequence learning in adults is driven by trial-by-trial online learning where an iterative mental computation is performed (Bornstein & Daw, 2012, 2013; Cleeremans & McClelland, 1991; Verstynen et al., 2012). Although online learning allows performance to improve quickly within a single learning session (Censor, et al., 2012; Dayan & Cohen, 2011), this iterative process may impose demanding mental computation requirements for children. In addition to

online learning, implicit acquisition of motor sequences may arise from offline learning that facilitates performance after rest without physical practice of the sequence (Censor, et al., 2012; Robertson, Pascual-Leone, & Miall, 2004).

Hypothesis 1.1: Sequence learning in adults dominantly relies on an online process where RT progressively improves within each learning block.

Hypothesis 1.2: Sequence learning in children primarily relies on an offline process where RT improves following short rest.

Results in study I demonstrate that fast sequence learning in children and adults may be driven by different learning processes. In particular, learning in six-year-old children dominantly relied on an offline process where RT improves after a short rest, while offline enhancement and online progressive improvement in RT concurrently resulted in sequence learning in 10-year-olds and adults. However, the mechanisms underlying the online and offline processes are unclear. A dominant explanation for these two processes is that they result from the emergence of fatigue or reactive inhibition (Brawn, Fenn, Nusbaum, & Margoliash, 2010; Rickard, Cai, Rieth, Jones, & Ard, 2008a; Rieth, Cai, McDevitt, & Mednick, 2010). For example, fatigue or reactive inhibition that is elicited by motivation and attentional factors accumulates while an individual is practicing the task and thus prevents progressive improvements in RT (Ammons, 1947; Bourne & Archer, 1956; Denny, Frisbey, & Weaver, 1955). The effect of fatigue or reactive inhibition dissipates following a rest, which results in the recovery of RT and consequently leads to offline RT improvements (Brawn, et al., 2010; Rickard, et al., 2008a; Rieth, et al., 2010). The fatigue or reactive inhibition explanation appears to be more critical in developmental

studies as fatigue or reactive inhibition is more likely to accumulate in children compared to adults when they perform the same task.

In addition, the age-related differences in online and offline processes may also be by-products of the task pacing conditions. In study I, children and adults performed the task under the same inter-stimulus-interval (ISI). The same ISI was relatively shorter in children than adults. Thus, the ISI employed may have prevented children from learning the sequence online as online learning requires iterative mental computations that need an adequate amount of time between stimuli that the fixed ISI might not have provided. In addition, a shorter ISI in children made the task relatively faster, which may induce greater fatigue or reactive inhibition accumulation that slowed down the online RT.

SAs 2 and 3 are built to investigate whether the age-related online and offline processes are by-products of task pacing or artifacts of fatigue or reactive inhibition. Since performing the SRT task under a fixed ISI condition could lead to the children performing under a relatively shorter ISI, we conducted study II (Chapter 4) in adults only to examine the effects of ISI on offline and online processes. In this study, adults performed the modified SRT task under different ISI conditions (i.e., short vs long ISI) and the online and offline processes were examined. In addition, study III (Chapter 5) was conducted where children and adults performed a self-paced SRT task. In the self-paced SRT task, the length of the ISI was determined by participants' own response speeds, which, therefore, eliminated the confounding effect of task pacing in study I. Furthermore, in study III, two hypotheses of fatigue or reactive inhibition were tested.

SA2 (Study II; Chapter 4): To determine if task pacing influences online and offline processes in adults.

Hypothesis 2.1: Despite the ISI, sequence learning in adults mainly arises from the online process where RT progressively improves within learning blocks.

SA3 (Study III; Chapter 5): To characterize the age-related differences in online and offline processes when children and adults perform a self-paced SRT task and determine if these two processes are artifacts of fatigue or reactive inhibition.

Hypothesis 3.1: Sequence learning in children and adults arise from age-related online and offline processes in the self-paced SRT task.

Hypothesis 3.2: The age-related online and offline processes are not illusory effects of fatigue or reactive inhibition.

Results of studies II and III suggest that the age-related online and offline processes are neither by-products of task pacing nor artifacts of fatigue or reactive inhibition. An alternative to the effects of task pacing and fatigue or reactive inhibition is that the age-related online and offline processes are active learning mechanisms. Since children are less likely to acquire declarative knowledge of sequences compared to adults (Meulemans, et al., 1998) and the offline process dominates their learning, while learning in adults largely relies on the online process and yields greater declarative knowledge (study I), we proposed that procedural and declarative memory may serve as the substrates for online and offline processes that occur during fast learning in the SRT task. That is to say, modulating the involvement of procedural and declarative memory would bias the online and offline processes. To

test this hypothesis, we asked adults to perform the SRT task where they learned either a fixed or probabilistic sequence and with or without preliminary knowledge of the presence of a sequence. The sequence and preliminary knowledge were manipulated to emphasize either procedural (probabilistic sequence; no preliminary knowledge) or declarative (fixed sequence; with preliminary knowledge) memory.

<u>SA4 (Study IV; Chapter 6): To determine if online and offline processes during fast motor sequence learning are active learning mechanisms that are linked to procedural and declarative memory.</u>

Hypothesis 4.1: Sequence learning arises from greater offline improvement in RT when a probabilistic sequence, compared to a fixed sequence, is learned.

Hypothesis 4.2: Sequence learning arises from greater online improvement in RT when the preliminary knowledge of the sequence is given before learning starts.

Results in study IV demonstrate that probabilistic sequence learning arises from a greater offline process, while fixed sequence learning is reflected by a greater online process, suggesting that online and offline processes can be mediated by the declarative and procedural memory that are required to learn motor sequences. Taken together, studies I to IV demonstrate age-related online and offline processes that underlie the acquisition of motor sequences during fast learning. The online and offline processes were neither by-products of task pacing constraints nor illusory effects of fatigue or reactive inhibition. Instead, these two age-related processes were more likely to be functional mechanisms underlying implicit motor sequence learning, which appear to be tied to procedural and declarative memory.

The distinct fast learning processes in children and adults raises the possibility of the age-related differences in the generalization of motor sequence learning (i.e., generalizing the learning of a sequence in one context to another novel context), as generalization of sequence learning is facilitated by fast learning where an abstract representation of the task develops (Censor, 2013; Perez, et al., 2007). To date, little is known whether learning a sequence is generalizable to another novel sequence that shares the same underlying structure with the learned sequence. In addition, a paucity of studies has investigated age-related differences in the generalization of implicit motor sequence learning. Therefore, SA5 addresses these two questions.

SA5 (Study V; Chapter 7, Experiment 1): To determine if the generalization of implicit motor sequence learning is age-related.

In study V, children and adults performed the same SRT task as that employed in study I. A generalization block was added at the end of the SRT task to examine whether adult and children were able to generalize the learning of one sequence to a novel sequence that shared the same underlying structure with the learned sequence. The underlying structure was referred to as the first-order transitional probabilistic structure given that learning a sequence is indeed learning its probabilistic structure (Bornstein & Daw, 2013; Visser, Raijmakers, & Molenaar, 2007). To date, little is known whether the generalization of motor sequence learning is facilitated by online or offline learning, making it challenging to hypothesize whether children or adults would have greater generalization of sequence learning. Thus, we aimed to explore the age-related differences in the generalization of implicit motor sequence learning.

Hypothesis 5.1: Children and adults are able to generalize implicit learning of one motor sequence to another with the same probabilistic structure. However, there may be age-related differences to the extent of sequence learning generalization.

Results in study V demonstrate that the generalization of implicit motor sequence learning was inferior in adults compared to children, raising the possibility that offline learning rather than online learning may facilitate sequence learning generalization. The question here is whether the generalization of motor sequence learning is age-related when learning in both children and adults largely relies on offline learning. To modulate the learning process toward offline learning in adults, in study VI (experiment 2 in Chapter 7), we asked children and adults to learn a probabilistic sequence that had been shown to strengthen offline learning in adults (Study IV, Chapter 6).

SA6 (Study VI; Chapter 7, Experiment 2) To determine if age-related differences in the generalization of implicit motor sequence learning are, in part, explained by online and offline learning.

Hypothesis 6.1: Generalization of probabilistic sequence learning is comparable between children and adults. That is to say, there are no age-related differences in generalizing probabilistic motor sequence learning.

Organization of the Dissertation Proposal

The dissertation consists of eight chapters. Following chapter 1, the second chapter is a review of the relevant literature. Chapters 3 through 7 describe study I to VI and are written in a journal manuscript format. Specifically, Chapter 3 reports study I (SA1) that investigates whether the acquisition of implicit motor sequences

during fast learning is driven by age-related learning processes. This chapter was submitted for publication and is currently under review. Chapter 4 details study II (SA2) that examines the effect of task pacing on online and offline processes underlying implicit motor sequence learning in adults. This chapter was submitted for publication and is currently under revision. For publication purpose, this chapter does not include results of online and offline learning processes. Results of online and offline learning processes are detailed in Appendix 1. Chapter 5 describes study III (SA3) that characterizes the age-related differences in online and offline processes when children and adults performed a self-paced SRT task and demonstrates whether these two processes are artifacts of fatigue or reactive inhibition. Chapter 6 reports study IV (SA4) that investigates whether online and offline processes during fast motor sequence learning are active learning mechanisms that are linked to procedural and declarative memory. This chapter was recently published in *Frontier in Human Neuroscience*. Chapter 7 details studies V and VI (SA5 and SA6) that explore age-related differences in the generalization of implicit motor sequence learning and investigates whether the age-related differences in the generalization of implicit motor sequence learning are, in part, explained by online and offline learning. Last, chapter 8 provides a general discussion of the experimental findings, implications, and direction of future research.

Chapter 2: Review of Literature

Overall Structure

Sequence learning is a widely used term in learning a variety of tasks: artificial intelligence (i.e., planning and reasoning), machine learning, decision making, time series prediction in finance and economics, natural language processing, handwriting recognition, adaptive control, and motor/cognitive skill learning. While each of these task domains has a unique perspective on understanding sequence learning, the common concept in all areas is the acquisition of ordered sequences underlying the task being performed. In this dissertation, sequence learning and its development focus on those sequences rooted in the motor domain.

This chapter reviews the extant literature about motor sequence learning in both adults and children. Six sections are included. Following this introductory section, the second section focuses on implicit and explicit memory/learning and their interactive relationship. Section 3 discusses paradigms that have been used to investigate motor sequence learning, including the serial reaction time (SRT) task whose modified version was employed in this proposed dissertation. Section 4 provides an extensive discussion on existing findings for sequence learning with the SRT task. Based on the discussion of the adult literature through chapters 1 to 4, Section 5 describes the age-related differences in motor sequence learning, particularly between children and adults. Last, Section 6 highlights knowledge gaps in the extant literature and how this dissertation addresses these gaps.

Explicit and Implicit Memory and Learning

Dissociable Forms of Memory

The dissociable memory system has been well demonstrated by numerous studies of amnesic patients and animal models. Theoretical and experimental findings in the literature have been thoroughly reviewed in several papers (Milner, Squire, & Kandel, 1998; Squire, 1992, 2004; Squire, Knowlton, & Musen, 1993; Squire & Wixted, 2011; Squire & Zola, 1996). Here, we only summarize the major concepts associated with these different forms of memory.

Learning depends on multiple forms of memory. One important form of memory refers to declarative memory which provides the basis for acquiring, retaining, and retrieving information of experienced events (episodic memory) and facts (semantic memory) (Tulving, 1985). Declarative memory presents the capacity to verbally articulate knowledge that is learned or memorized, thus it is often measured by recall and recognition tests in the laboratory (Purves et al., 2013). In contrast, non-declarative knowledge is typically acquired without intention and awareness and this type of memory is expressed through performance rather than recollection of facts and events. Non-declarative memory is responsible for conditioning, priming, and skill learning. Furthermore, skill learning, including perceptual-motor, perceptual, and cognitive learning, depends on one particular type of non-declarative memory, which is known as procedural memory. The declarative and non-declarative memory is interchangeably used with explicit and implicit memory in the literature.

Learning is closely related to memory since it involves three phases of the memory process: encoding, storing, and retrieving. Thus the dissociation between implicit and explicit memory leads to two different types of learning - implicit and explicit learning. In the following sub-section 'Explicit and Implicit Learning', we summarize findings from studies investigating skill learning that are related to the research purposes of this dissertation.

Explicit and Implicit Learning

The first evidence confirming that the learning system is not unitary but dissociable comes from a seminal study conducted by Brenda Milner and colleagues (as cited in Purves, et al., 2013; Squire, 1992, 2004; Squire & Wixted, 2011). In this study, amnesic patient H.M. (Squire, 2009) practiced a mirror-tracing task for three days. H.M. showed learning of this motor task, observed by persistent improvement with a comparable level as normal participants, even though he failed to report that he had extensive practice on this task, suggesting that learning to perform the mirror-tracing task is intact in amnesia patients. Since the deficit of declarative/explicit memory system in amnesia patients did not affect the ability to learn such a motor skill, the learning relied on the procedural/implicit memory system and thus is considered to be implicit learning. The preserved implicit learning ability in amnesia patients has been further confirmed in cognitive (e.g., Knowlton, Mangels, & Squire, 1996) , perceptual (e.g., Cohen & Squire, 1980), and motor skill learning (e.g., Nissen & Bullemer, 1987).

Given the common finding that explicit and implicit learning are dissociable in amnesia, it is important to know whether implicit learning can occur when the

explicit/declarative learning system is intact. Many tasks have been designed to investigate implicit learning in typically developed participants, including dynamic system control (Berry & Broadbent, 1984), hidden covariation detection (Lewicki, 1986; Lewicki, Czyzewska, & Hoffman, 1987), second language learning (Michas & Berry, 1994), weather prediction (Knowlton, et al., 1996), sequence learning (Nissen & Bullemer, 1987), and artificial grammar learning (Reber, 1967). In all these studies, participants are instructed to perform a task without knowing the specific rule underlying the task. For example, in the seminal study of artificial grammar learning (AGL), Reber (1967) instructed participants to memorize letter strings generated by an artificial grammar machine. After this learning phase, novel strings were presented and participants were asked to classify them as grammatical or non-grammatical. Although participants could not verbally describe the rule embedded in the artificial grammar machine, their performance to classify strings was better than chance. This result in AGL and similar results in other paradigms lead to the conclusion that typically developed participants can implicitly learn a task.

However, implicit learning inevitably interacts with explicit learning in typically developed participants. For example, it was found that participants always exhibited at least partial declarative knowledge of the artificial grammar (see, Frensch & Rünger, 2003, for a review). Evidence of the co-existence of explicit and implicit learning also comes from an fMRI study (Poldrack et al., 2001). In this study, activities of the medial temporal lobe that are related to explicit learning and of the basal ganglia that are responsible for implicit learning depend on whether the task requires explicit or implicit memory. Furthermore, it was found that the activities in

these two brain areas are negatively correlated, suggesting that implicit and explicit memory compete with each other during learning.

In sum, two types of learning, implicit and explicit learning, play a vital role in the acquisition of cognitive and cognitive-motor skills. Since motor skills normally emerge from a sequence of movements that follow a specific order, it is fundamental to learn the sequential order before a good performance can be achieved. Importantly, a motor sequence can also be learned explicitly and implicitly. In the next section, the extant literature of explicit and implicit motor sequence learning is discussed.

Motor Sequence Learning: Paradigms, and Frameworks

Explicit and Implicit Sequence Learning

Explicit sequence learning is associated with awareness of what should be learned or what has been learned. In contrast, implicit learning takes place without such awareness (Berry, 1997; Cleeremans, et al., 1998; Frensch & Rünger, 2003). For example, children learn to pronounce sequential words both explicitly and implicitly. Specific instructions are sometimes received so that children learn to pronounce several syllables in an order to speak a word, while most times, they learn to speak by intuitively echoing sounds and repeating words that adults say with little understanding of the words.

Paradigm: The Finger Tapping Task/Finger-to-thumb Tapping Task

Explicit sequence learning has been widely studied through the finger-tapping task (also known as the finger-to-thumb tapping task) (Karni et al., 1995; Walker, Brakefield, Hobson, & Stickgold, 2003; Walker, et al., 2002). In the finger-tapping

task, a finger sequence is given to participants before they perform a task in which they follow the specific order to tap fingers as fast as they can. Thus, explicit learning plays a vital role in the early phase of learning this task (Doyon & Benali, 2005; Fitts & Posner, 1967), while procedural memory is involved in the later learning phase (Hikosaka et al., 1999; Nakahara, Doya, & Hikosaka, 2001; Walker, et al., 2003). Given the vital role of procedural learning in the later phase, this task, in addition to the motor adaptation paradigm, has been considered as the most common paradigm designed to study motor learning (Censor, et al., 2012).

Paradigm: The Serial Reaction Time Task

The serial reaction time (SRT) task was originally designed to assess implicit learning (Nissen & Bullemer, 1987), while recent studies considered this task as a motor task and used it to study motor sequence learning (e.g., Doyon, et al., 2001, 2003; Penhune, et al., 2009; Willingham, 1998, 1999). In the SRT task, participants respond to stimuli by pressing corresponding keys as quickly and accurately as possible without being informed that the stimuli follow a specific order. Traditionally, the sequential stimuli are formed by 10 repetitions of an 8-12 element stimulus sequence (i.e. fixed-repeated sequence) (Nissen & Bullemer, 1987; Reed & Johnson, 1994a; Stadler & Frensch, 1998). Learning can take place without any prior knowledge of the stimulus sequence as revealed by a faster mean reaction time (RT)/response time to this sequence compared to the mean RT/response time to a novel sequence (Cleeremans, et al., 1998; Clegg, et al., 1998; Cohen, et al., 1990; Jimenez, et al., 1996; Robertson, 2007). However, implicit learning in the SRT task inevitably accompanies the acquisition of explicit/declarative knowledge of

sequences, indicated by the capability of participants to verbally recall/recognize, at least, a part of the sequence after performing the SRT task (Cleeremans, et al., 1998; Clegg, et al., 1998; Destrebecqz & Cleeremans, 2001; Jimenez, et al., 1996; Remillard & Clark, 2001; Thomas & Nelson, 2001; Weiermann & Meier, 2012a). This result is not surprising. The co-existence and competition between explicit (or declarative) and implicit (or procedural) learning are natural hallmarks of the interactive learning mechanisms in our brain (Brown & Robertson, 2007a, 2007b; Keisler & Shadmehr, 2010b; Poldrack, et al., 2001; Sun, Slusarz, & Terry, 2005).

This dissertation will employ the modified SRT task in which the feet take the place of fingers in moving to targets around the body (please see section 'effectors to perform the SRT task').

Stages of Motor Sequence Learning

Fitts and Posner (1967) proposed that there are three stages in the acquisition of motor skills. During the first stage, referred to as the cognitive stage, an individual understands what the task to perform is and develops the required movement patterns. Performance is refined with practice through the second stage, the associative stage. Finally during the autonomous stage, performing the task is automatic and requires minimum cognitive resources.

Fitts and Posner's framework of motor learning has been supported and further elaborated by recent studies in motor sequence learning. In a review paper, Doyon and Benali (2005) proposed a five-stage model of motor skill learning: 1) (Fast learning) Fast learning develops within a single session when an individual practices a new motor task, in which considerable improvements in the performance

take place and a short memory of this task is formed (Honda, et al., 1998; Karni et al., 1998b; Karni & Sagi, 1993; Walker, et al., 2002); 2) (Slow learning) When practice continues, performance improves but at a slow rate; 3) (Consolidation) After practice stops, learning does not terminate. Rather, memory formed in the first stage is stabilized so that it is resistant to interference from a new task that needs to be acquired (Brashers-Krug, Shadmehr, & Bizzi, 1996; Cohen & Robertson, 2011; Shadmehr & Brashers-Krug, 1997), or performance is enhanced without additional practice through offline learning (Karni & Sagi, 1993; Robertson, Pascual-Leone, & Press, 2004; Robertson, et al., 2005; Walker, et al., 2003; Walker, et al., 2002); 4) (Automatic stage) performance in this stage requires few cognitive resources; and, 5) (Retention stage) The long-term memory is formed and thus the performance level is maintained after a long period in the absence of further practice (Abe et al., 2011; Karni & Sagi, 1993; Savion-Lemieux & Penhune, 2005). Besides these five stages, it has been found recently that memory can be degraded, refined, and modified during additional practice or sleep. This stage is known as the re-consolidation stage (Censor, Dimyan, & Cohen, 2010; Walker, et al., 2003).

It is important to note that these learning stages are observed in the finger tapping task and other procedural learning tasks (see, Censor, et al., 2012; Doyon & Benali, 2005, for a review), while it is unknown if these stages are common in learning the SRT task. However, the extant studies have shown fast learning and offline learning when an individual performs the SRT task. This dissertation focuses on the fast learning stage (i.e., the first training session) and investigate if offline learning takes place in this early stage.

Research Issues in the Serial Reaction Time Task in Adults

Since the seminal study using the SRT task in 1987 (Nissen & Bullemer), many variations of this task have been used, including variations in the effector used to perform the SRT task, the sequence structure, measurement to assess learning, and dual-task versus single-task design and so forth. Some of these variations were found to affect sequence learning, while others were not. In the following sub-sections, the extant literature pertaining to these variations is briefly reviewed. In addition, existing findings of learning models, consolidation, and interference of motor sequence learning is then discussed.

Effectors to Perform the SRT Task

While the classic SRT typically requires bi-manual finger tapping, sequence learning has been investigated using a variety of movements, including uni-manual finger tapping (Andresen & Marsolek, 2012), target tracing (Lang, Gapenne, Aubert, & Ferrel-Chapus, 2013; Panzer et al., 2009; Park & Shea, 2005), oculomotor task (Marcus, Karatekin, & Markiewicz, 2006), and arm reaching (Moisello et al., 2009). These SRT tasks are performed with participants seated. Much of our daily motor sequence skills, however, require us to perform actions while standing, which involves postural control. The postural control introduced in standing vs. sitting offers a challenge that may be a rate limiter to performance and perhaps to learning (Thelen, 1989; Thelen, Ulrich, & Jensen, 1989). Therefore, this dissertation extends the SRT task to a whole body foot stepping task.

Sequence Structure in the SRT task

In the seminar study of the SRT task (Nissen & Bullemer, 1987), the 10-item sequence, D-B-C-A-C-B-D-C-B-A, was used and each learning block comprised 10 repetitions of this sequence. Nissen and Bullemer failed to observe learning when a secondary task (i.e., tone-counting task) was performed concurrently. It was later found that the impaired learning may not result from the secondary task. Instead, it was likely that the sequence structure affected the acquisition of the sequences (Cohen, et al., 1990). Cohen and colleagues referred to the sequence that Nissen and Bullemer used as an ambiguous sequence. In an ambiguous sequence, all locations are followed by more than one other location. In contrast, a hybrid sequence contains some trials that are followed by only one other location. Additionally, in a unique sequence (also known as the first-order-conditional sequence), all trials are followed by only one other location. Cohen, et al., (1990) observed learning of these three types of sequences when the SRT task was performed alone, while participants cannot learn an ambiguous sequence under the distraction of a secondary task.

While learning was observed in the unique, hybrid, and ambiguous sequences, it was found that such learning may simply result from the acquisition of the relative frequencies of locations rather than the sequence itself (Perruchet, Gallego, & Savy, 1990). In addition, Reed and Johnson (1994a) argued that other properties other than the sequence itself may be acquired, such as transition frequency and reversal frequency. To circumvent this issue, a second-order-conditional (SOC) sequence was proposed, in which each trial was exclusively determined by the preceding two trials. The SOC sequence perhaps is the most common sequence used in the literature and

most importantly, converging evidence demonstrates that the SOC sequence can be learned in the SRT task (Curran, 1997b; DeCoster & O'Mally, 2011; Reed & Johnson, 1994a).

As put forth previously, an interactive relationship between implicit and explicit learning has been observed in many studies using the deterministic sequences introduced above (Cleeremans, et al., 1998; Clegg, et al., 1998; Destrebecqz & Cleeremans, 2001; Jimenez, et al., 1996; Thomas & Nelson, 2001; Weiermann & Meier, 2012a). Explicit learning emerges in the implicit sequence learning task (i.e., the SRT task) because the simple sequence is likely to be detected and therefore explicit to the learner. Many other types of sequences have been used to minimize the effect of explicit learning in the SRT task. These manipulations include an alternative serial reaction time (ASRT) task (Howard et al., 2004), intermixing two fixed sequences (Schvaneveldt & Gomez, 1998), incorporating a fixed sequence with some random trials (Savion-Lemieux, Bailey, & Penhune, 2009), fixed sequences with statistical transitions on some trials (Stadler, 1992), a sequence in which each position can be following by any other positions with an equal likelihood (Reber & Squire, 1994), and probabilistic sequences produced by an artificial grammar or a Markov chain (Deroost & Soetens, 2006; Deroost, Zeeuws, & Soetens, 2006; Jimenez, et al., 1996).

However, no sequence can completely prevent explicit learning. In some studies that compared sequence learning between amnesic participants and their typically developed peers, it has been found that the control groups exhibited greater learning than amnesic patients (Nissen & Bullemer, 1987; Reber & Squire, 1994).

These results imply that explicit learning, at least partially, contributes to sequence learning in the SRT task. In addition, although complex probabilistic sequences can reduce the acquisition of explicit knowledge (Cleeremans & Jiménez, 1998), sequences even embedded with a third-order probabilistic transition can be explicitly acquired after extensive practice (Remillard & Clark, 2001).

In this dissertation, the fixed and probabilistic sequences generated by a first-order Markov process are used. It is infeasible to eliminate or isolate either of implicit or explicit learning from the SRT task. Nonetheless, manipulating the sequence structure can modulate the involvement of implicit and explicit memory required by the SRT task.

Measurement Used to Assess Sequence Learning

Reaction time (RT) has been used to measure performance in the SRT task since it reflects the amount of time spent processing information and indeed indicates the dynamics in the brain during cognitive learning (Donders, 1969; Laming, 1968; Luce, 1991; Sternberg, 1969a). Based on the performance change indicated by RT, sequence learning can be assessed in the SRT task. In the original SRT study (Nissen & Bullemer, 1987), some participants practiced a structured sequence while others were exposed to a random sequence. The mean RT difference between these two groups indicated learning of the structure sequence. Recently, a more common method has been used. Participants practiced the same structured sequence several times and without notice, a novel sequence is presented instead of the practiced sequence (Cohen, et al., 1990; Willingham, et al., 1989). Two measurements can be used to infer sequence learning: 1) the progressively improved mean RT of each

learning block when participants practice the same sequence; and, 2) a slower mean RT when the novel sequence is presented. However, the progressively improved RT could be caused by general motor improvements, such as increased familiarities with the task and improve stimulus-response mapping (Robertson, 2007).

While the mean RT provides overall information in the performance of each block, little of the dynamic changes that take place within each block are revealed. Recent studies have suggested that the performance changes within each block play a crucial role in understanding sequence learning (Brawn, et al., 2010; Nemeth, Janacsek, Király, et al., 2013; Rickard, Cai, Rieth, Jones, & Ard, 2008b; Rieth, et al., 2010), thus there is a need to examine the progressive changes that occur within each learning block.

In addition to these three measurements, the difference of RT between the high and low frequency trials has been used in the SRT task with probabilistic sequences or in the ASRT task (Howard, Howard, Japikse, et al., 2004; Janacsek, Fiser, & Nemeth, 2012b). This method allows us to assess learning not only after several learning blocks, but also within each learning block where RT may progressively improve.

In this dissertation, the RT difference between the novel and practiced sequences are used to assess sequence learning when the SOC sequence is used. The difference in RT between the high and low frequency trials will be used when a probabilistic sequence is used in the SRT task. In addition, the progressive change in RT within each learning block will be examined.

The Effect of Attentional Load on Motor Sequence Learning

In addition to manipulating sequence structure, the presence of a secondary task appears to reduce the acquisition of explicit knowledge. While Nissen and Bullemer (1987) found that a secondary task completely impairs implicit learning, the results were not replicated by subsequence studies (Cleeremans & McClelland, 1991; Cohen, et al., 1990; Frensch, Buchner, & Lin, 1994; Frensch, Lin, & Buchner, 1998; Frensch, Wenke, & Runger, 1999; Keele & Jennings, 1992; Schmidtke & Heuer, 1997; Schvaneveldt & Gomez, 1998; Shanks & Channon, 2002; Shanks, Rowland, & Ranger, 2005). In contrast, results from these studies suggest that a secondary task attenuates, but not completely impairs implicit learning. Although several hypotheses, including suppression of behavioral expression (Frensch, et al., 1998), disruption of short-term memory (Frensch & Miner, 1994), and disruption of temporal organization (Stadler, 1995) have been proposed as the mechanism underlying the detrimental effect of the secondary task on sequence learning, converging evidence demonstrates that sequence learning is reduced due to the attentional load imposed by the secondary task (Cohen, et al., 1990; Keele & Jennings, 1992; Nissen & Bullemer, 1987; Shanks & Channon, 2002; Shanks, et al., 2005; Willingham, Greenberg, & Thomas, 1997). Furthermore, it has been reported that this attentional load does not affect the learning of a probabilistic sequence (Jimenez, et al., 1996) where explicit learning is reduced. Therefore, the attentional load caused by a secondary task appears to diminish the effects of explicit learning in the SRT task.

Response-Stimulus Interval and Inter-Stimulus Interval Effect

As an alternative explanation for the detrimental effects of a dual task on sequence learning, the effect of response-stimulus interval (RSI) have been proposed. In the SRT task, the RSI is known as the delay from a response to the subsequence stimulus. Frensch and Miner (1994) suggested that a tone between stimuli lengthens the RSI and indeed prevents adjacent stimuli to be presented in short-term memory, which affects sequence learning under a secondary task. On the other hand, Stadler (1993) has found that inserting random pauses between adjacent stimuli impaired sequence learning and in a subsequent study (Stadler, 1995) suggested that a tone-counting task, similar to a random pause, imposes variability into the RSI and thus disorganizes the temporal organization of the sequential stimuli. Interestingly, these two views were not supported by other researchers, leading to numerous subsequent studies that investigated the effect of RSI on motor sequence learning.

After the studies of Frensch and Miner (1994) and Stadler (1993, 1995), the effect of the response-stimulus interval (RSI) has been widely investigated. To examine the RSI effect, three types of manipulation on RSI have been employed.

The first type of RSI is the constant RSI. A constant RSI (e.g., 200-500ms) has been used in the first SRT task (Nissen & Bullemer, 1987) and most subsequent studies (Cohen, et al., 1990; Curran & Keele, 1993; Frensch, et al., 1999; Heuer & Schmidtke, 1996; Willingham, et al., 1989). In these studies, learning was observed by slower RT when random sequential stimuli were presented. The converging evidence from these studies suggests that sequence learning in adults appears to be robust to the variations of the RSI length. Willingham, Greenberg, and Thomas

(1997) extended this conclusion to longer RSI, such as 2000ms. It was found that manipulation of RSI influences the behavior expression but not learning. Other evidence comes from Destrebecqz and Cleeremans (2003). Their finding confirmed that sequence learning measured by RT does not rely on the RSI, even when the RSI is set to 0ms. In addition, Destrebecqz and Cleeremans have observed that long RSI promotes acquisition of explicit sequence knowledge, while this statement has been challenged by numerous recent studies (Norman, Price, & Duff, 2006; Norman, Price, Duff, & Mentzoni, 2007; Rünger, 2012; Shanks, et al., 2005; Shanks, Wilkinson, & Channon, 2003; Wilkinson & Shanks, 2004).

The second type of manipulation on RSI is to mix RSIs with different lengths, which originates from the hypothesis that variations in RSI would impair the temporal organization of sequences (Stadler, 1993, 1995). Evidence supporting this hypothesis was provided by one study (Miyawaki, 2006), however, most other studies suggested that variations of RSI do not impair sequence learning although they could suppress the behavior expression of learning (Shin & Ivry, 2002; Willingham, et al., 1997). Furthermore, it has been found that learning was the same regardless of the type of RSI (random mixed RSI, constant RSI, and fixed mixed RSI) (Shin, 2008).

The constant RSI and mixed RSI control the interval between each response and its subsequent stimulus. However, the total task duration for each sequence varied among individuals. Recent research, especially brain image studies, circumvented this issue by using the third type of manipulation - fixed interval stimulus interval (ISI) in which the interval between stimuli is fixed (Bischoff-Grethe, Goedert, Willingham, & Grafton, 2004; Hodel, Markant, Van Den Heuvell, Cirilli-Raether, & Thomas, 2014;

Savion-Lemieux, et al., 2009; Thomas et al., 2004). In these studies, learning was observed although the ISI, instead of RSI, was controlled.

In this dissertation, to avoid the possibility that the expression of learning is influenced by the mixed RSI, only the fixed RSI and ISI will be used.

Motor-based or Perceptual-based Sequence Learning

Information processing comprises three different stages: stimulus identification, response selection, and response execution. Learning on each of these stages can contribute to the improvement in RT performance (Sternberg, 1969a, 1969b). Given that reduced RT in the SRT task may result from one or more of these stages, stimulus-based, response-based, and stimulus-response-based hypotheses have been proposed as the mechanisms underlying sequence learning. Evidence of stimulus-based learning come from perceptual sequence learning that does not involve motor actions (Fiser & Aslin, 2002; Fiser, Berkes, Orban, & Lengyel, 2010) and observational sequence learning studies (Heyes & Foster, 2002; Song, Howard, & Howard, 2008; Vinter & Perruchet, 2002). The stimulus-based hypothesis is also supported by studies that investigated transfer of learning between response effectors (Cohen, et al., 1990; Deroost, et al., 2006; Verwey & Wright, 2004).

On the other hand, there is also evidence suggesting that sequence learning is motor-based. It has been suggested that participants who did not acquire explicit knowledge of sequences failed to transfer learning between different response configurations (Willingham, 1999), suggesting that sequence learning is not effector-independent for all participants. In a subsequence study, Willingham and colleagues (2000) manipulated the stimulus-response mapping. Specifically, participants were

asked to tap the one on the right of this stimulus when a stimulus was on. After training sessions, participant switched back to the normal stimulus-response mapping. Results showed that participants exhibited learning when the response sequence did not change, while the learning effect was not observed when the stimulus sequence was changed. Other evidences supporting motor-based sequence learning comes from brain image studies (Bischoff-Grethe, et al., 2004).

It is most likely that sequence acquisition is not exclusively attributed to either stimulus- or response-based learning (Fendrich, Healy, & Bourne, 1991; Kirsch & Hoffmann, 2012; Verwey & Wright, 2004). In Verwey and Wright's study (2004), half of participants performed the SRT task with three fingers on one hand, while other participants used three fingers on two hands. The hand configuration was switched after participants learned a sequence. RT to the practiced sequence was faster on the hand configuration used during learning than the switched hand configuration, while the RT to the practiced sequence using the non-trained hand configuration was faster than the RT to a new sequence using the same non-trained hand configuration. These results provide support that both stimulus- and motor-based learning benefit the acquisition of motor sequences. Furthermore, it has been found recently that whether sequence learning is stimulus-based or response-based also depends on the sequence structure (Andresen & Marsolek, 2012) or the context of stimulus presentation (Kirsch & Hoffmann, 2012) .

Information Acquired in the SRT Task and Its Generalization

Learning a sequence has been considered to be the acquisition of the underlying structure or abstraction rule (Cleeremans & McClelland, 1991; Reber,

1967; Restle, 1976), while others have argued that acquiring segments or chunks is sufficient to learning a sequence (Perruchet & Pacton, 2006). In the literature, the notion of a chunking mechanism has been widely accepted (Gobet et al., 2001; Jimenez, 2008; Koch & Hoffmann, 2000; Sakai, Kitaguchi, & Hikosaka, 2003; Servanschreiber & Anderson, 1990). However, a chunking mechanism is contradicted by the consolidation of procedural memory discussed in previous sections. Particularly, there is no time available for consolidation since chunks are performed continuously in a sequence, so that each chunk would interfere with each other. In addition, a chunking mechanism fails to explain why a random sequence cannot be learned (DeCoster & O'Mally, 2011; Nissen & Bullemer, 1987). At last but not least, learning chunks makes it harder for the generalization of sequence learning. For example, a 10-trial sequence has 120 three-trial chunks and thus effective generalization to other 10-trial sequences requires learning of all 120 chunks.

Like the learning of chunks, learning the fixed sequence itself is hard to be generalized to other sequences since each fixed sequence is unique. In contrast, statistical learning, rooted in language science (Saffran, 2003; Saffran, Aslin, & Newport, 1996; Saffran, Johnson, Aslin, & Newport, 1999), allows the probabilistic transitions between trials to be acquired (Hunt & Aslin, 2001). Indeed, acquisition of probabilistic transitions among all trials means the acquisition of the underlying structure of the sequence. Evidence has shown that adult participants are able to learn the probabilistic structure that is used to produce the sequences (Visser, et al., 2007) and the acquired probabilistic structure may have a positive effect on generalization (Tenenbaum, Kemp, Griffiths, & Goodman, 2011). In recent years, such structural

learning has been found to advance the learning of different tasks with the same structure in motor learning studies (Braun, Aertsen, Wolpert, & Mehring, 2009; Braun, Mehring, & Wolpert, 2010; Braun, Waldert, Aertsen, Wolpert, & Mehring, 2010). In addition, structural learning has been extended to sequential decision making (Acuna & Schrater, 2010), while little is known if sequence learning in the SRT task can be generalized.

The knowledge gap in sequence learning generalization is due to the sequences (i.e., the SOC sequence) used in previous studies. Each sequence is unique, making it impossible to create a different sequence with the same structure. In this dissertation, the generalization effect between adults and children is investigated. Like the probabilistic sequence, the underlying probabilistic structure of SOC sequences will be computed and used to produce generalization sequences. If sequence generalization is observed, it is predicted that the transitional probability underlying the sequence is more likely to be acquired. In contrast, if the unique fixed sequence is acquired instead, there will be no generalization effect.

Error-based Learning: Model-based vs Model-Free

Learning takes place when the actual outcome differs from the desired outcome, resulting in a prediction error. This is known as error-based learning (Schultz & Dickinson, 2000). There are two types of prediction errors, sensory- and reward-prediction errors, corresponding to two learning strategies, model-based and model-free learning (Haith & Krakauer, 2012). There is ample evidence for error-based learning (i.e., model-based and model-free learning) in motor learning studies, including motor adaptation (Bhushan & Shadmehr, 1999; Burge, Ernst, & Banks,

2008; Donchin, Francis, & Shadmehr, 2003; Izawa, Rane, Donchin, & Shadmehr, 2008; Izawa & Shadmehr, 2011; Shadmehr & Mussa-Ivaldi, 1994; Thoroughman & Shadmehr, 2000; van Beers, 2012), oculomotor learning (Wong & Shelhamer, 2011), and sequence learning (Cleeremans & Dienes, 2008; Cleeremans & McClelland, 1991; Cleeremans, Servan-Schreiber, & McClelland, 1989; Verstynen, et al., 2012).

In sequence learning, participants anticipate the next outcome (Schvaneveldt & Gomez, 1998; Willingham, et al., 1989), therefore the error is formed as the mismatch between the predicted and actual outcomes (Dale, Duran, & Morehead, 2012; Marcus, et al., 2006), computed using reward or state information (Fermin, Yoshida, Ito, Yoshimoto, & Doya, 2010; Glascher, Daw, Dayan, & O'Doherty, 2010). Further evidence for the model-free and model-based learning in sequence acquisition comes from brain image studies. It has been suggested that model-based learning is related to the cerebellum (Miall, Weir, Wolpert, & Stein, 1993; Wolpert, Miall, & Kawato, 1998) and the prefrontal lobe (Daw, Niv, & Dayan, 2005) while model-free learning is related to the basal ganglia (Daw, et al., 2005; Doya, 1999, 2000; Nakahara, et al., 2001). Meanwhile, these brain areas, including the cerebellum, prefrontal cortex, and the basal ganglia, are responsive to motor sequence learning (Ashe, Lungu, Basford, & Lu, 2006; Doyon, Penhune, & Ungerleider, 2003; Willingham, Salidis, & Gabrieli, 2002), suggesting that both model-based and model-free learning contribute to the acquisition of motor sequences. Furthermore, these two learning strategies do not only act simultaneously, but also optimally act together with their dominances shifting based upon the learning phase and available feedback information (Daw, et al., 2005; Fermin, et al., 2010; Glascher, et al., 2010).

It is important to note that error-based learning typically requires an iterative update of the parameters of the learning system (Elman, 1990; Thoroughman & Shadmehr, 2000). This trial-by-trial error-based process is also known as online learning, reflected by a continuous decrease in RT while the SRT task is performing (Verstynen, et al., 2012).

Consolidation in the SRT Task

As put forth previously in the section 'stages of motor sequence learning,' consolidation is a critical period in sequence learning, during which fragile memory of one newly learned skill can be stabilized to be resistant to interference from other memories of competing skills, or performance can be enhanced without additional practice after its first acquisition (i.e., offline learning) (Censor, et al., 2012; Robertson, Pascual-Leone, & Miall, 2004). Memory stabilization plays an important role in motor adaption, while offline learning is more common in motor sequence learning. There is considerable evidence supporting that conclusion that offline learning of explicit knowledge is sleep-dependent (Brawn, et al., 2010; Fischer, Hallschmid, Elsner, & Born, 2002; Robertson, Pascual-Leone, & Press, 2004; Walker, et al., 2002; Wilhelm, Metzkow-Meszaros, Knapp, & Born, 2012; Wilhelm, et al., 2013). Offline learning of implicit knowledge, however, does not rely on sleep and it takes place with a 4- or 12-hour delay after the initial learning in the SRT task (Press, Casement, Pascual-Leone, & Robertson, 2005; Robertson, Pascual-Leone, & Press, 2004). Thus, one can infer that offline gain that occurs without sleep indicates implicit learning.

Recent studies have begun to suggest that offline learning cannot be observed until after 12 hours elapsed because explicit memory may suppress implicit learning. As aforementioned in previous sections, explicit knowledge can be acquired in the SRT task (Cleeremans, et al., 1998; Clegg, et al., 1998; Destrebecqz & Cleeremans, 2001; Jimenez, et al., 1996; Remillard & Clark, 2001; Thomas & Nelson, 2001; Weiermann & Meier, 2012a). In addition, Destrebecqz and colleagues (2005) found that the prefrontal cortex that supports explicit learning can inhibit the activities of the striatum that is related to implicit learning. When the explicit knowledge was disturbed by a secondary declarative memory task, offline learning took place with a delay of four hours after its initial acquisition (Brown & Robertson, 2007a). Such an interference of the declarative knowledge has been found to positively affect implicit learning in several studies (Keisler & Shadmehr, 2010b; Nemeth, Janacsek, Polner, & Kovacs, 2012). On the other hand, implicit learning can also inhibit the development of declarative knowledge (Brown & Robertson, 2007a, 2007b). This reciprocal relationship between implicit and explicit learning has been found to depend on the dorsal lateral prefrontal cortex (DLPFC) (Cohen & Robertson, 2011; Diekelmann, Buchel, Born, & Rasch, 2011) Thus, disruption of the DLPFC with transcranial magnetic stimulation (TMS) prevents the interference between two different memory tasks (i.e., the implicit and explicit memory task) (Cohen & Robertson, 2011).

Recently, offline enhancement in performance has been observed after a 5-30-minutes rest. (Albouy et al., 2006a; Hotermans, Peigneux, de Noordhout, Moonen, & Maquet, 2008; Hotermans, Peigneux, Maertens, Moonen, & Maquet, 2006). Such offline improvement is known as an offline boost. The relationship between an offline

boost and offline learning that occurs hours later remains unclear (Nettersheim, et al., 2015). It is necessary to point out that the offline learning and offline boost observed in the extant literature has been widely considered to occur only after the initial acquisition of sequences (i.e., following fast learning). However, it is unclear whether offline learning drives the acquisition of sequences in the fast learning stage. This dissertation investigates whether offline learning drives the fast acquisition of sequences within the first learning session in the SRT task (see the sections 'the development of motor sequence learning in the SRT task' and 'knowledge gaps').

Interference in the SRT Task

Besides the interference between the implicit and explicit memory discussed in the preceding section, interference also exists between the learning of two different tasks that requires the same type of memory. Learning task B with a short delay after the acquisition of task A impairs the memory of task A (Brashers-Krug, et al., 1996) and surprisingly, limited practice trials on task B (e.g., 20 trials) are sufficient to cause this interference (Criscimagna-Hemminger & Shadmehr, 2008). Evidence of interference in sequence learning is lacking but one study has demonstrated that learning a new sequence interfered with the learning of a pre-learned sequence despite when the new sequence was learned (Goedert & Willingham, 2002), while sufficient practice on the pre-learned sequence can prevent the interference (Ghilardi, Moisello, Silvestri, Ghez, & Krakauer, 2009). However, in both studies, extensive practice on the new sequence is required to produce the interference effect, while it is unknown whether a short practice of the new sequence, such as one learning block

used in the SRT task, can result in the interference effect. This question will be addressed in this dissertation.

The Development of Motor Sequence Learning in the SRT Task

Sequence learning can occur at any age, for example, learning to ski during childhood, learning a musical instrument in middle age, and learning how to speak a second language at age 60. However, since this dissertation investigates the development of motor sequence learning between children and adults, the following discussion will focus on the extant literature of sequence learning in children, but not in the aging population.

Compared to the adult literature (i.e., section 2 to section 4 in this chapter), there is a paucity of studies that investigated motor sequence learning in the SRT task in children. While the results from early studies on the age effect in sequence learning in the SRT task remain equivocal, converging evidence from recent research has demonstrated that the development of motor sequence learning is age-related (Adi‑Japha, et al., 2014; Dorfberger, et al., 2007; Janacsek, Fiser, & Nemeth, 2012a; Meulemans, et al., 1998; Nemeth, Janacsek, & Fiser, 2013b; Savion-Lemieux, et al., 2009; Thomas, et al., 2004; Thomas & Nelson, 2001; Weiermann & Meier, 2012b; Wilhelm, et al., 2013).

An early hypothesis suggested that implicit learning was age-independent. Supporting evidence for this hypothesis came from an implicit drawing task (Vinter & Perruchet, 2000, 2002), an oculomotor task (Karatekin, Marcus, & White, 2007), and a hidden covariance detection task. The same age-independent result also were found in the SRT task (Meulemans, et al., 1998; Weiermann & Meier, 2012a),

suggesting that motor sequence learning in the SRT task does not depend on age. However, subsequent studies have shown that adults exhibited greater learning than children (Savion-Lemieux, et al., 2009; Thomas, et al., 2004; Thomas & Nelson, 2001). Hodel, et al., (2014) suggesting that these differences may result from the variations in the SRT task used in previous studies. For example, Thomas and Nelson (2001), Thomas, et al., (2004) and Savion-Lemieus, et al., (2009) used fixed inter-stimulus-intervals (ISI) that could lead to a relative shorter ISI in children compared to adults, while others (Meulemans, et al., 1998; Weiermann & Meier, 2012a) used fixed response-stimulus-intervals (RSI) in children and adults, which produced self-paced ISIs in each individual participant. In their experiment, Hodel and colleagues (2014) found that children exhibited greater learning when they performed the self-paced SRT task compared to the SRT task with fixed ISIs, a result that could explain the age effect on sequence learning in the study of Thomas, et al., (2004).

Although age-independent motor sequence learning has been demonstrated in the literature, an increasing amount of evidence suggests that the ability to learn motor sequences develops with age. First, although children and adults exhibited a comparable level of learning as revealed by RT, children have been found to acquire less explicit knowledge of the sequences (Meulemans, et al., 1998; Weiermann & Meier, 2012a). Given that adults exhibited better explicit learning, superior implicit learning in children is required to achieve the same level of learning in adults. Recent studies have confirmed this hypothesis, demonstrating the superiority of children in acquiring probabilistic sequences that require greater implicit learning (Janacsek, et al., 2012b; Nemeth, Janacsek, & Fiser, 2013a). Second, given greater implicit

learning and an immature prefrontal cortex (Lenroot & Giedd, 2006) in children that may reduce the interference between implicit and declarative memory, memory consolidation (see the section 'consolidation in the SRT task') is likely to occur faster in children compared to adults. It has been found that an interval of 15 minutes is sufficient for children to stabilize (at least part of) the memory of a newly acquired skill, while it takes more than 2 hours for memory to be stable in adults (Adi‑Japha, et al., 2014). Indeed, the faster memory stabilization process allows the newly acquired memory become resistant to the interference from a competing motor skill in children (Adi‑Japha, et al., 2014; Dorfberger, et al., 2007). Last, children outperform the adults' capacity in offline learning. It has been found that children exhibited greater performance in explicit sequence knowledge with sleep after they performed the SRT task, suggesting greater offline gains were produced during the sleep period (Wilhelm, et al., 2013).

The literature discussed in this section provides evidence of the age-related development of motor sequence learning in the SRT task, however, there are several knowledge gaps that need to be investigated and answered to better understand the development of motor sequence learning. These knowledge gaps will be discussed in the next section and will be addressed in this dissertation.

Knowledge Gaps

<u>Knowledge Gap 1: Can sequence learning take place in the foot stepping SRT task?</u>

Learning a motor sequence in the SRT task has been observed in a finger tapping, eye moving, or arm reaching task, while it is unclear if such learning can be

extended to an SRT task that requires postural control, such as a foot stepping task that better approximates daily activities. The majority of studies in the dissertation (except study IV in chapter 6) extended the classic finger-pressing SRT task to a foot stepping SRT task.

Knowledge Gap 2: Is sequence learning in foot stepping SRT task age-related?

Children and adults exhibit a comparable level of learning in the classic SRT task that involves finger tapping. Since the postural control introduced in the foot stepping SRT task offers a challenge that may be a rate limiter to performance and perhaps to learning (Thelen, 1989; Thelen, et al., 1989) in children, it is unclear whether learning of the foot stepping sequence is age-related. The majority of studies in the dissertation (except study IV in chapter 6) extended the classic finger-pressing SRT task to a foot stepping SRT task.

Knowledge Gap 3: Is the learning process underlying fast sequence acquisition age-related?

It is well documented that adults use the online trial-by-trial learning to acquire motor sequences, while it remains unclear if children use the same online learning strategy. In addition, limited acquisition of declarative sequence knowledge in children may induce faster offline learning, which has not been examined to date. Study I addressed this knowledge gap.

Knowledge Gap 4: Are the age-related processes are active learning mechanisms that connect with procedural and declarative memory, artifacts of fatigue/reactive inhibition, or by-products of task constraints?

A dominant explanation considers the online and offline changes in RT as performance-based. That is to say, rather than being a learning-based mechanism, the online and offline changes in RT may result from the emergence of fatigue or reactive inhibition (Brawn, et al., 2010; Rickard, et al., 2008a). Although previous studies that claimed the effect of fatigue or reactive inhibition focused on explicit sequence learning, this effect can also be postulated as a dominant source of online and offline processes in implicit sequence learning (Rieth, et al., 2010). In addition, the age-related differences may also be a by-product of task constraints (i.e., task pacing conditions). Alternatively, the online and offline processes may connect with declarative and procedural memory systems as children are less likely to acquire declarative knowledge of sequences compared to adults (Meulemans, et al., 1998). However, to date, it remains unclear about the mechanisms underlying the online and offline processes. Studies II to IV addressed this knowledge gap.

Knowledge Gap 5: Are there age effects on the generalization of motor sequence learning?

Fast learning seems to facilitate the generalization of sequence learning, which raises the possibility of age-related differences in the generalization. However, to our knowledge, the age-effect on the generalization of motor sequence learning has not been investigated. Studies V and VI addressed this knowledge gap.

Chapter 3: [*](Study I) Children and adults both learn motor sequences quickly, but do so differently

Abstract

Both children and adults can learn motor sequences in one learning session, yet little is known about potential age-related processes that underlie this sequence acquisition. Here, we examined the learning processes of a foot-stepping serial reaction time task in 6- and 10-year-old children and adults. We observed that a motor sequence was acquired through online learning, as revealed by progressively improved reaction time (RT) while the task is performed in a continuous manner, and offline learning, as revealed by improved RT after 3-minute rests. Remarkably, offline and online learning occurred differently between children and adults: Learning in 6-year-olds relied primarily on offline learning while learning in 10-year-olds and adults relied on both processes equally. We subsequently found that offline learning after a rest was related to the performance variability before the rest, suggesting that the offline learning may originate from an exploration process as the sequence is learned. Our results suggest that motor sequence learning during a single learning session is driven by multiple age-related processes.

Introduction

Throughout our day, we effortlessly produce sequences of actions from getting out of bed in the morning, tying our shoes, to pouring a cup of coffee and drinking it. While these motor sequences comprise much of what we do in our activities of daily living, their acquisition is not altogether well understood. For

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example, using the serial reaction time (SRT) task (Nissen & Bullemer, 1987; Robertson, 2007) in the laboratory, adults (Cleeremans, et al., 1998; Seger, 1994; Stadler & Frensch, 1998) and children as young as six years of age (Meulemans, et al., 1998; Thomas & Nelson, 2001; Weiermann & Meier, 2012b) learn motor sequences to a comparable level (Meulemans, et al., 1998). This sequence acquisition develops quickly (within a single learning session), as revealed by considerable improvements within that session as well as a reversal of improvement if the sequence changes. However, little is known about the age-related processes underlying the sequence acquisition that takes place so quickly over the course of one learning session. The purpose of this study, therefore, was to examine whether the same or different processes drive motor sequence learning in children and adults.

Many have suggested that learning motor sequences in adults is driven by an incremental so-called "online" process through iterative computations (Bornstein & Daw, 2012, 2013; Cleeremans & McClelland, 1991; Verstynen, et al., 2012). Online learning allows performance to improve quickly within a single learning session (i.e., over a short continuous bout of practice) (Censor, et al., 2012; Dayan & Cohen, 2011). Yet, it is not clear whether children also learn a motor sequence in an 'online' fashion. Since online learning involves using the previous trial's information to update performance on the next trial, this iterative process may impose demanding computational requirements for children. As children are able to learn motor sequences at a comparable level to adults (Karatekin, et al., 2007; Meulemans, et al., 1998), we speculate that in addition to online learning, another process may contribute to the children's acquisition of motor sequences.

We hypothesize that offline learning may be one possible process that children employ. Offline learning is usually referred to as a process that leads to the improvement in performance without practice (Robertson, Pascual-Leone, & Miall, 2004). Specifically in a sequence learning task, performance is further strengthened, by an early offline boost (Hotermans, et al., 2006; Schmitz et al., 2009; Zhang et al., 2011) 5-30 minutes after the acquisition of motor sequences or via a later occurring (hours later) memory consolidation (Albouy et al., 2006b; Brown & Robertson, 2007a; Robertson, Pascual-Leone, & Press, 2004). Thus, offline learning is typically considered to appear only after the motor sequence is learned. To date, it is unknown whether offline learning may drive the acquisition of motor sequences over an initial single learning session.

An additional aspect of learning that underlies sequence acquisition is the variability in performance. Recently, it has been reported that variability promotes the learning of motor sequences (Verstynen, et al., 2012). Specifically, variability reflects the exploration of the context in which the task is performed so as to discover unknown knowledge about the context (e.g., stimulus sequence). Given that children usually exhibit greater variability/exploration in performance compared to adults (Goldfield, Kay, & Warren, 1993; Piek, 2002b; Thelen, Ulrich, & Wolff, 1991), we examined whether variability of RT is correlated with motor sequence learning; either for online or offline learning.

Here, we employed the SRT task to examine the concurrent effect of online and offline learning, as well as their relationship to exploration, to acquire motor sequences in adults and children. The SRT task consisted of 6 learning blocks, in

which 6- and 10-year-old children and adults responded to a 100-trial sequence of visual stimuli that followed either a fixed order A (sequence A in blocks 1-4 and 6) or a fixed order B (sequence B in block 5) (Robertson, 2007). We measured online learning as the change in performance (i.e., reaction time) within each learning block. After each block, participants took a three-minute break. The change in performance right before and after each rest was computed to infer offline learning. We found that the fixed motor sequence A was acquired through both online and offline learning. Indeed the offline learning we observed may be an exploration process as offline learning after a rest was positively correlated to the variability in reaction time preceding the rest. Significantly, our results reveal that offline and online learning processes differ with age. Adults and 10-year-olds acquired the sequence through online and offline learning equally, while 6-year-olds' learning was dominated by offline learning.

Materials and Methods

Ethics statement

This study was approved by the Institutional Review Board at the University of Maryland, College Park and it was performed in accordance with the approved guidelines. Signed written informed consent forms from the adult participants/parents and assent forms from child participants were received prior to their participation. Each participant received a $15 monetary compensation upon the completion of the experiment. Child participants also received a small toy prize for completing the Movement Assessment Battery for Children 2 (MABC2) (Henderson, Sugden, & Barnett, 2007).

Participants

Ten 6-year-old children (6.65 ± 0.83 years, male = 6) and thirteen 10-year-old children (10.5 ± 0.68 years, male = 5) were recruited for this study. Prior to the experiment, children were screened by the MABC2 to determine if movement difficulties existed. One 10-year-old male child was excluded because he scored below the 5th percentile on the MABC2. The remaining 22 children scored above the 25th percentile and so were included in this study. Ten young non-musician adults (20.47 ± 0.9 years, male = 5) were recruited from the University of Maryland, College Park. For one adult participant, data from the last block (i.e., block 6) were excluded from the data analysis due to unexpected equipment problems when he performed the task. Prior to participation, adults completed a neurological health questionnaire. No participants reported neurological health issues. In addition, participants were screened for their experience with the video game, Dance Dance Revolution (DDR) since the SRT task we employed was similar to the DDR video game. All participants had little DDR experience (i.e., equal or less than 2 hour experience) and no participants had played the DDR game more than twice in the past year.

Experimental task

Participants stood on a home position (two 18cm x 11cm felt mats) and performed a whack-a-mole type game with sequential foot stepping. Six stepping targets (six 12cm x 12cm felt mats) were positioned to the front, back, and side of the home position (see Figure 3.1). The distances between the targets and home position were marked at 60% of the largest step that the participants were able to accomplish

in each direction. This step length was chosen for comfortableness that required appropriate but not a maximum reach for each step. Six visual cues (i.e., six holes) were presented on the monitor positioned in front of the participants. These visual cues were spatially compatible with the targets on the floor. One mole successively popped up from one of the six holes to represent the sequence order (see Figure 3.1). A laptop computer with a customized Labview (National Instruments, Austin, TX, USA) program controlled the sequence. A Vicon motion capture system (Oxford Metrics, Oxford, UK) recorded the real-time three-dimensional positions of reflective markers attached to the participants' toe, heel, and the 5th metatarsal on both feet with a sampling frequency of 200Hz.
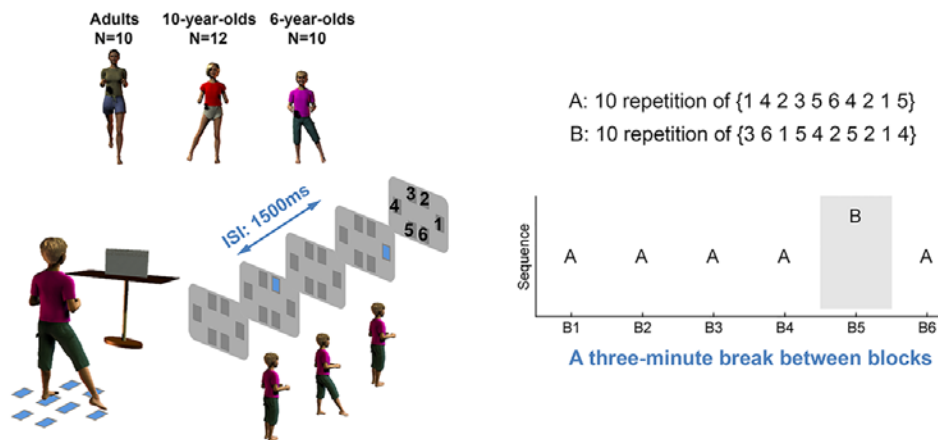
Figure 3.1: Experimental setup and protocol.
Ten 6-year-old children, thirteen 10-year-old children (twelve were included for data analyses), and 10 adults performed a foot stepping SRT task. Participants responded to each visual stimulus as quickly and accurately as possible by stepping to the spatially-matched target on the floor. The stepping performance was measured by reaction time, defined as the elapsed time from stimulus onset and foot movement onset. In blocks 1-4, the visual stimuli followed sequence A (10 repetitions of 1423564215). In block 5, the stimulus followed sequence B (ten repetitions of 3615425214). Each number was associated with one spatially located square, but the numbers were not displayed to participants. Both sequences consist of 100 trials and each stimulus appeared 1500ms after the preceding stimulus. After each learning block, participants had a 3-minute rest. Participants were not told that the stimuli followed a sequence until they completed all 6 blocks. This figure was drawn by Yue Du.

Procedures

Participants stood on the home position before starting each experimental block (Figure 3.1). They were instructed to step to the appropriate target on the floor as quickly and accurately as possible when the mole appeared in the corresponding location on the screen. After each step, they were required to step back to the home position for the next stimulus, appearing 1500ms after the previous stimulus. An accurate hit on the target mat or home position was encouraged, but not strictly required because, during the continuous stepping movement, participants, especially children, would shift their positions slightly. However, stepping in the right direction was required. Before the experimental trial blocks began, participants practiced with a random sequence of 36 trials to become familiar with the task. Then, they performed six blocks of 100 foot stepping movements. For blocks 1-4, the stimuli followed sequence A (ten repetition of the sequence 1423564215). Each number was associated with one spatially located square, as shown in Fig 1, but the numbers were not displayed to participants nor described to them as such. A novel sequence (sequence B), ten repetitions of the sequence 3615425214, was provided for block 5 followed by block 6 when participants again performed sequence A. After each block, participants took a short break lasting about three minutes. Until the completion of all 6 blocks, participants were not informed that the visual stimuli followed any order. Upon completion of all six blocks, participants were asked whether they noticed there was a sequence to the presentation of the visual stimuli. Subsequently, participants were given 4 different 10-element long sequences and were asked to choose the one they thought had appeared in their task (i.e., recognition task I). Participants were

then asked to complete a recognition task II that consisted of 4 trials. In each trial, they were given four sequence segments and were asked to choose the ones they thought had appeared in the task. Finally, participants were asked to recall and write down the sequence. These were done to investigate whether participants had declarative knowledge of the sequence they had been practicing.

Data analysis

A customized MATLAB^{TM} (MathWorks, Naticks, MA, USA) script was used to derive the reaction time (RT), a measure of the temporal difference between the stimulus onset and the movement initiation. The time series of the three-dimension trajectory of markers on the toes, heels, and the fifth metatarsals were filtered by an eighth-order Butterworth filter with a cutoff frequency of 10Hz. We marked the movement onset as the first sample when the foot reached 10% of the maximum height of movement. Steps were considered an error and discarded if one of the following two conditions occurred: 1) stepping to a wrong target; or, 2) stepping to the correct target but from other targets and not from the home position as required. A trial's RT also was excluded if its absolute magnitude was out of the range from (μ – 2.5×δ) to (μ + 2.5×δ), where μ and δ are the mean and standard deviation of the raw RT for each block (Ratcliff, 1993). The exclusion criterion reduced the effect of extreme outliers on variability calculation (see below). We chose this specific range rather than other smaller ones to preserve as much of the raw RT data in our data analysis as possible. These criteria resulted in excluding 1.98% ± 0.42% (mean ± standard error) of the RTs in adults, 2.28% ± 0.29% in 10-year-olds, and 2.75% ± 0.36% of the RTs in 6-year-olds in each block.

The mean RT for each block (BMRT) was employed to assess the summative performance throughout the entire task. The magnitude of learning was measured by the BMRT difference between blocks 4 and the novel block 5 (Robertson, 2007). We chose the mean rather than median of RT as: 1) the sample median may provide a biased estimation of RT (Miller, 1988); and 2) after excluding outliers, the mean RT represented the performance as effectively as the median of raw RT, which was revealed by a significantly high correlation ($r = 0.98$, $p<0.00001$ for all three age groups).

Exploration during learning is usually equivalent to variability. To examine the RT variability, we used a linear regression to remove extraneous changes in RT caused by time and location effects from the original RT observations. The variability of these extraneous RT changes would result from the fluctuations with time and between stimulus locations. The residual of the linear regression, referred to as refined RT, was of interest and its variability was computed to reflect exploration in learning. Particularly in the regression, we included the first-, second-, and third-order effects of time, location, and their interactions as explanatory factors.

To assess offline and online learning, RMRT – the mean magnitude of RT for each repetition of 10-trial responses (the sequence was 10-elements long) was used. According to the definition of offline learning, the magnitude of offline gain was computed as the discrepancy between the last RMRT in one block and the first RMRT in the succeeding block. The 10 RMRTs within each block were fitted using a linear regression whose slope characterized the online learning rate. The magnitude of online learning was then computed by multiplying the online learning rate by 10 (i.e.,

10 RMRT used to fit the linear regression). We chose the linear regression on the 10 RMRTs rather than the original 100 individual RT within each block, because our preliminary analysis suggested that the 100 individual RTs were substantially affected by stimulus locations and its interaction with time. This effect could greatly influence the slope estimation in the linear regression analysis. However, grouping sets of 10-trial responses together eliminated the location effect and provided a robust estimation.

To determine the RT changes during offline and online periods when participants were learning the motor sequence through blocks 1 to 4, we averaged offline and online RT changes before block 5 in which a novel sequence B was introduced. We used average rather than total RT change since there were four blocks (i.e., blocks 1 to 4) where online learning could take place and only three breaks (i.e., between blocks 1 and 2, blocks 2 and 3, and blocks 3 and 4) where offline learning could occur before a novel sequence B was given. When comparing the percentage of offline (i.e., offline/(offline + online)) and online learning (i.e., online/(offline + online)), negative magnitudes of offline or online learning were treated as zero as they did not contribute to the improvement in RT.

A controversy regarding offline learning is whether it is a byproduct of the reactive inhibition or fatigue effect (Brawn, et al., 2010; Rickard, et al., 2008a) as fatigue may deteriorate performance before a rest, which leads to larger offline learning. In order to examine the likelihood of a fatigue effect, we computed the mean RT for the first 50 trials (i.e., the first half) and second 50 trials (i.e., the second half) in each block to examine whether learning was inferior in the second 50 trials

(Nemeth, Janacsek, Kiraly, et al., 2013). As well, we computed online learning for both the first and last trials in each block to examine whether there were greater RT declines at the end of each block. Finally, corrected offline learning was calculated by removing the RT decrement within the preceding block from the total offline gain.

To determine the amount of declarative knowledge of sequence A, we counted the number of participants who chose the correct sequence in the recognition task I. We computed the recognition score in the recognition task II as the number of trials (normalized by 4) that participants correctly chose the sequence segments. To calculate the recall score, we counted the number of correct 2-, 3-, and 4-element chunks in the sequence participants wrote down. These numbers were normalized by the total correct 2-, 3-, and 4-element chunks in sequence A.

Data analysis

We employed a two-way mixed effect ANOVA to examine the effects of learning block and age on BMRT and variability. In these analyses, the age group was treated as a between-subject factor (6yrs, 10yrs, and adults) and block (blocks 1 to 6) was considered as a within-subject factor. Contrast analyses were used to assess the magnitude of learning as the performance changes from block 4 to 5. Separate one-way ANOVAs were used to examine the age effect on the (corrected) offline/online changes in RT. For all ANOVA tests, post hoc tests employed a Tukey-Kramer correction. The correlation between the average offline/online learning and the average variability of RT 'noise' across the first 4 blocks (i.e., learning on sequence A) was examined using a partial correlation analysis with the age effect controlled.

Non-parametric ANOVAs (i.e., Kruskal-Wallis test) were used to examine the age effect on recognition and recall scores.

A three-way mixed effect ANOVA was employed to examine the effects of learning block, age, and phase of block (i.e., the first and last 50 trials) on mean RT with block and phase of block as within-subject factors. A two-way mixed effect ANOVA was used to investigate the effect of block and block phase on online learning in six-year-olds because only six-year-olds exhibited increased RT within blocks (see results). In this analysis, both block and block phase were treated as within-block factors. For all mixed effect ANOVA, the co-variance matrix was determined by the Akaike's Information Criterion (AIC). All effects were tested at a significance level p = 0.05.

Results

Children and adults learn the motor sequence at a comparable level

Performance was measured by the mean reaction time of 100 steps in each block (BMRT) and the magnitude of learning was assessed by the BMRT difference between sequence A in block 4 and the novel sequence B in block 5(Robertson, 2007) (see Methods). Our analyses revealed (repeated measures 3 groups × 6 blocks ANOVA) significant effects for learning blocks ($F_{5,29} = 16.01$, $p<0.0001$) and age ($F_{2,29} = 22.18$, $p<0.0001$) but no interaction on the BMRT (Figure 3.2A). Post hoc analyses with a Tukey-Kramer correction found that 10-year-olds had the same BMRT as adults (p=0.062). Both groups were faster than the 6-year-olds (p<0.0005), whereas all three groups decreased their BMRT (i.e., faster reaction time) from block

1 to 4 (p<0.0001) and the BMRT became longer when sequence B was introduced in block 5 (p<0.0001). These results indicate that all groups, regardless of age, learned sequence A. No differences were found between blocks 1 and 5, indicating that the improvement in RT from block 1 to 4 was due to sequence learning, and not motor improvements. The same BMRT before (block 4) and after (block 6) sequence B suggests that all age groups preserved the learning of sequence A even after practicing sequence B. Most importantly, contrast analyses found that the increase in BMRT from block 4 to 5 did not differ among the age groups (all p > 0.47), indicating that the magnitude of learning was consistent for 6-year-olds, 10-year-olds, and young adults (Figure 3.2B).
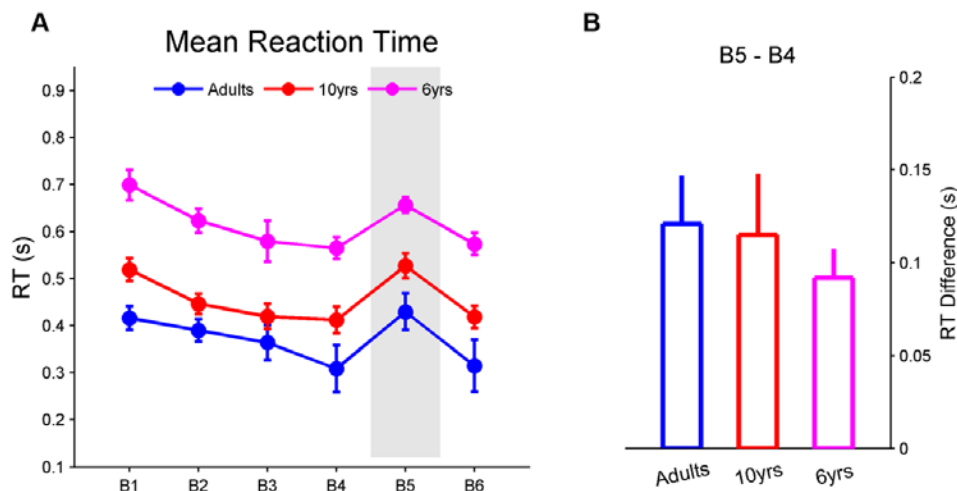
Figure 3.2. No age effect on sequence learning as measured by the mean RT.
(A) The mean reaction time for each block (BMRT). The gray area represents the block in which the stimuli follows a novel sequence. The BMRT depended on learning blocks and age. Adults and 10-year-olds were faster than the 6-year-olds, while there was a trend that 10-year-olds had the same BMRT as adults. All three groups learned sequence A, as revealed by decreased BMRT from block 1 to 4 and increased BMRT from block 4 to 5. Such learning did not result from motor improvements as the BMRTs in blocks 1 and 5 were the same; (B) The difference in BMRT between blocks 4 (primary sequence A) and 5 (novel sequence B), representing the absolute magnitude of learning. All three groups learned sequence A to a comparable level. Error bars represent standard errors of the mean performance within each block. RT = reaction time.

Online and offline learning: Two age-related processes drive motor

sequence learning

Visually, it is clear that the three groups demonstrated different RT patterns (Figure 3.3A). The adults' RTs gradually decreased within each block, while there were offline boosts in RT in 6-year-olds. Ten-year-olds' RTs exhibited a mixed pattern similar, in part, to the adults and the 6-year-olds.

Figure 3.3B displayed the total amount of offline (between blocks) and online (within blocks) learning that took place when participants learned sequence A through blocks 1 to 4 (see Methods). Both offline (one-way ANOVA, $F_{2, 29} = 20.07$, p < 0.0001) and online (one-way ANOVA, $F_{2, 29} = 7.97$, p < 0.005) changes in RT were affected by age. Specifically, 6-year-olds produced greater offline RT changes than 10-year-olds *(p<0.0001)* and adults (p < 0.0001). In contrast, 10-year-olds *(p<0.01)* and adults (p < 0.005) exhibited greater online RT changes than 6-year-olds whose RT deteriorated 'online' *(less than zero; p $< 0.0005)$.

The deteriorated online learning in 6-year-olds is possibly due to the small online RT improvements in some blocks that were cancelled out by large online RT decrements in other blocks when we averaged the RT changes across blocks 1 to 4. Thus, we decomposed these changes in RT into improvements and declines that took place online or offline (Figure 3.3C). There was a significant effect of age on offline (one-way ANOVA, $F_{2, 29} = 18.35$, p < 0.0001) but not online (one-way ANOVA, $F_{2, 29} = 1.16$, p $= 0.33$) improvements in RT. Specifically, 6-year-olds produced a greater offline improvement than 10-year-olds (p<0.0001) and adults (p < 0.0001). There was not a significant effect of age on offline declines in RT (but $F_{2, 29} = 2.72$, p $= 0.08$),

while the online declines in RT were affected by age ($F_{2, 29}$ = 13.32, p < 0.0001). Six-year-olds exhibited greater declines in RT compared to 10-year-olds (p < 0.005) and adults (p < 0.0001).

We further compared the percentage of offline and online learning as a proportion of the improvement of RT as shown in Fig 3C. The contribution percentage of offline learning was affected by age (one-way ANOVA, $F_{2, 29}$ = 6.96, p < 0.005) (Figure 3.3D). Specifically, offline learning contributed more to sequence learning in 6-year-olds than 10-year-olds (p < 0.05) and adults (p < 0.005). Indeed, the offline learning contributed more than 50% ($mean_{offline}$ = 87.64%) to motor sequence learning in 6-year-olds (one-tailed t-test, p<0.0005), while offline and online improvements contributed equally in 10-year-olds and ($mean_{offline}$ = 55.44%) and in adults ($mean_{offline}$ = 41.65%). The percentage of online learning equals one minus the percentage of offline learning, thus the result of online learning is identical to that of offline learning reported above.

RT variability is correlated with offline learning

To quantify the variability associated with learning, we removed extraneous changes in RT caused by time and location effects through a linear regression (see Methods). The refined RT exemplar (i.e., RT after removing extraneous time and location effects) for one individual in each age group is displayed in Fig 4. It was found that the variability of the refined RT depended on the interaction between age group and block (repeated measures 3 group × 6 blocks ANOVA, $F_{10, 29}$ = 2.8, p < 0.05, Figure 3.4). Tukey-Kramer corrected post hoc analyses revealed that six-year-olds exhibited larger variability in block 2 than 10-year-olds (p < 0.05) and adults (p

< 0.005) and the difference between six-year-olds and adults in block 1 approached

significance (p=0.059). There were no differences in variability among groups from

block 3 to 6. Unlike the mean magnitude of RT, there were no differences in RT

variability between blocks 4 and 5 in all age groups.
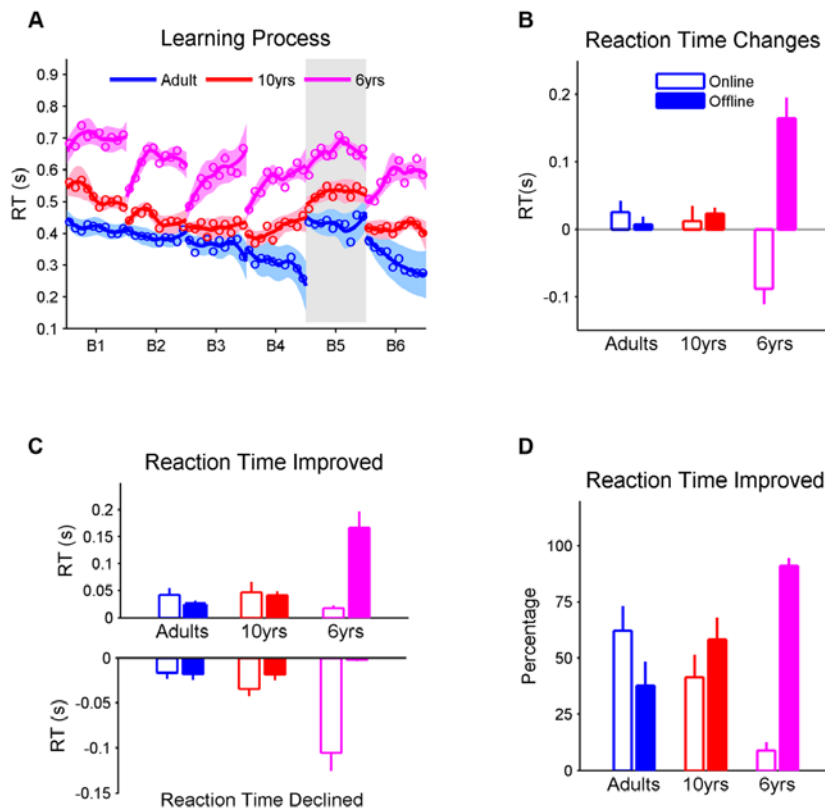
Figure 3.3: Offline and online learning differed between children and adults.
(**A**) Changes in RT within and between blocks. The solid line represents the trend as RT progressively changes, estimated by a local weighted regression. Shaded areas are the standard errors of the trend. Circles represent RMRTs (i.e. the mean RT of one repetition of stimulus sequence). The adults' RTs progressively decreased within each block, while there were offline boosts in RT in 6-year-olds; (**B**) Average online and offline RT changes when learning sequence A from blocks 1 to 4. Note that positive values imply decreases in RT (i.e., RT becomes faster). Both offline and online changes in RT relied on age. Specifically, the offline change was greater in 6-year-olds than 10-year-olds and adults. The online change was greater in 10-year-olds and adults than 6-year-olds whose RT deteriorated 'online'; (**C**) Offline but not online improvements in RT was affected by age. Six-year-olds produced a greater offline improvement than 10-year-olds and adults. Online but not offline declines in RT were affected by age. Six-year-olds exhibited greater declines in RT compared to 10-year-olds and adults; and, (**D**) The percentage of RT improvement arising from offline and online learning. Offline learning mediated sequence learning differently at different ages. Six-year-olds relied more on offline learning than 10-year-olds and adults. Specifically, 87.64% (for 6-year-olds), 55.44% (for 10-year-olds) and 41.65% (for adults) of improved RT is driven by offline learning. The percentage of online learning equals 1 minus the percentage of offline learning. Error bars represent standard errors.
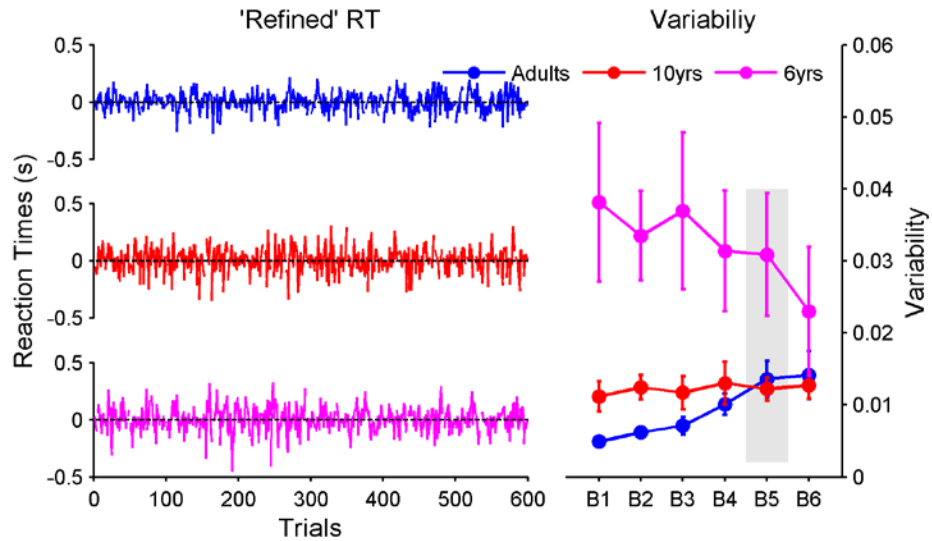
Figure 3.4: RT variability between age groups.
(left) An individual example of the refined RT in each age group (i.e., RT after the time and location effects were removed). (right) Six-year-olds showed greater variability in RT 'noise' at early phase of the task, while the variability reduced to the same level as 10-year-olds and adults as learning progressed. Error bars represent standard errors.

A partial correlation analysis between the mean online RT change and the mean variability across the first 4 blocks was performed, yielding a partial correlation coefficient of -0.28 (p = 0.12, power = 0.34, see discussion) (Figure 3.5B). After removing two data points with the largest variability (i.e., outliers identified by the studentized residual with a threshold of 3) that may affect this relationship, the partial correlation remains nonsignificant (coefficient = -0.02, p =0.91, power = 0.05). However, the offline learning was significantly correlated with RT variability (coefficient = 0.55, p < 0.005, power = 0.91) (Figure 3.5A). This significant positive correlation remains when those two data points were removed (coefficient = 0.53, p < 0.005, power = 0.87). These results suggest that greater offline learning was associated with larger variability in RT.
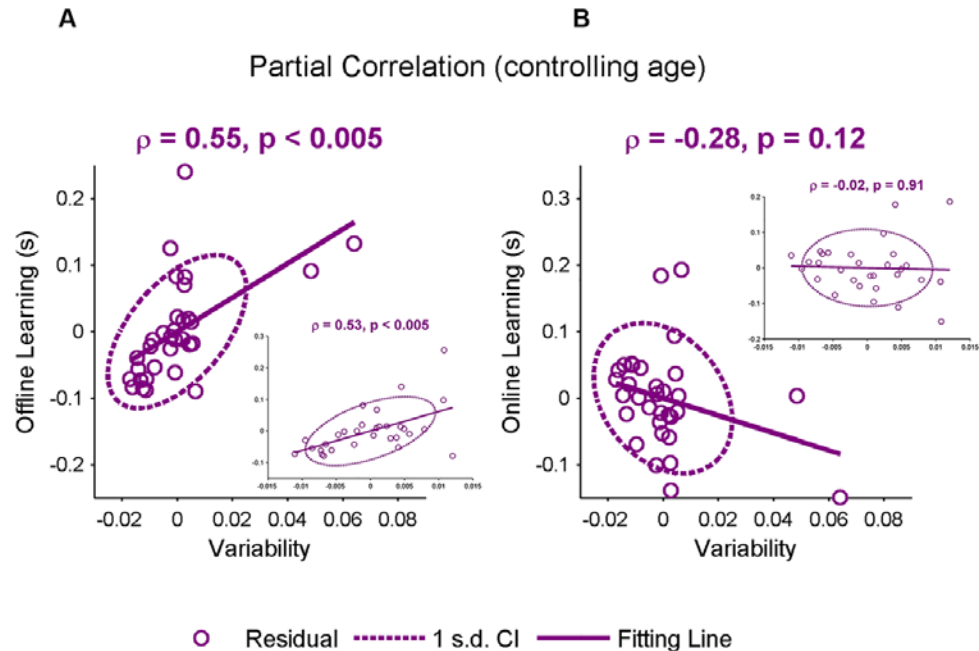
Figure 3.5: RT variability is correlated to offline learning.
(A) The partial correlation between offline learning and RT variability with age effect controlled. Notably, offline learning was positively correlated to RT variability. After removing two data points with the largest variability that may exemplify such a correlation, the result remained (insert); (B) Online learning was not significantly correlated with RT variability. The same result remained after two data points that may drive the relationship were removed.1 s.d. CI = 1 standard deviation confidence interval.

Is offline learning caused by fatigue?

A debate on offline learning is whether the offline improvement in RT results from fatigue (Brawn, et al., 2010; Rickard, et al., 2008a), as fatigue may deteriorate performance with practice, subsequently and misleadingly exaggerating the magnitude of offline learning. As shown in Figure 3.4A, six-year-olds' RT became slower within each block. To exclude fatigue as a potential cause of offline learning observed in 6-year-olds, we further analyzed the RT changes within each individual block. Figure 3.6A illustrates 6-year-olds' online RT changes in the first 50 and last 50 trials of each block (i.e., two phases of block). A two-way (2 phases × 6 blocks)

repeated measures ANOVA revealed that there were significant effects for block ($F_{5,45}$ = 3.72, p<0.01) and block phase ($F_{1,9}$ = 10.93, p<0.01) where more RT was reduced in the first 50 trials than the last 50 trials. Notably, online RT reduction in the first 50 trials was significantly different from zero (mean = 0.111s, p <0.005), while there was no significant RT changes in the last 50 trials (mean = 0.007s, p = 0.8). This result is contrary to the hypothesis that fatigue induces deterioration in performance when more practice was performed within blocks.
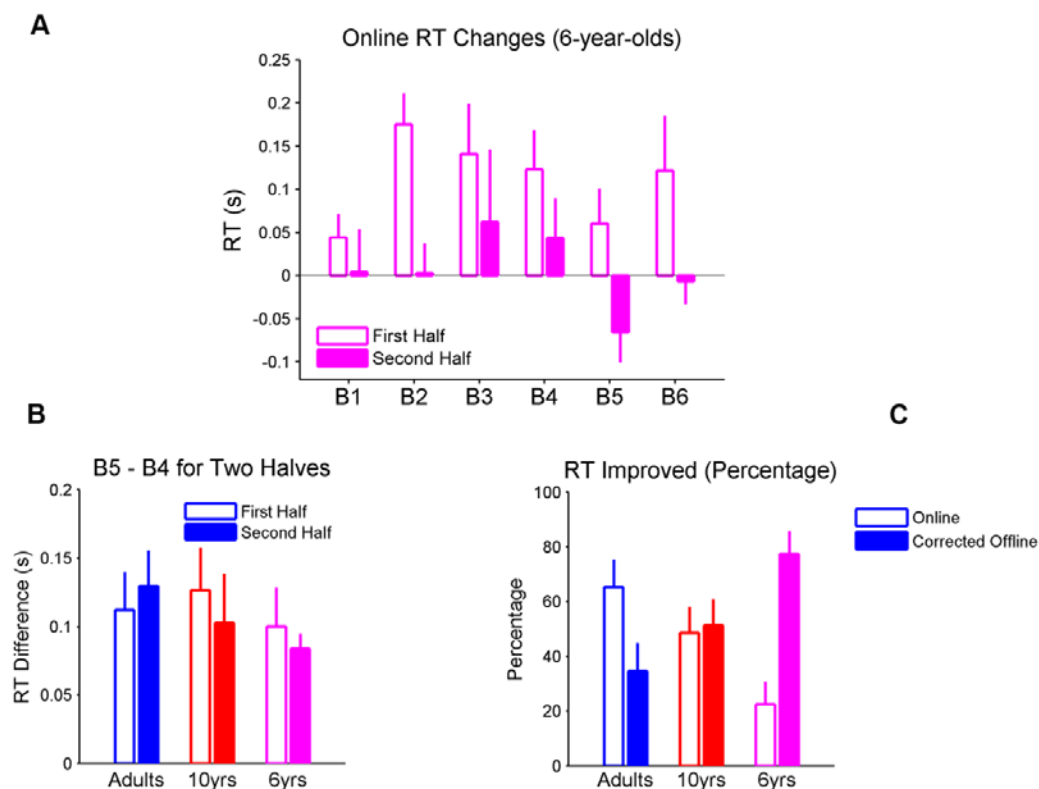
Figure 3.6: Offline learning in six-year-olds was unlikely to be caused by fatigue.
(A) Online RT changes of the first and second halves in each block in 6-year-olds. RT deteriorated in only first halves. However, RT remained constant in the second halves; (B) Learning measured in both the first and second halves. Learning magnitudes were comparable between two halves across all three groups; and (C) Corrected offline learning after removing the RT decrement within the preceding block from the total offline gain. Learning in six-year-olds was dominantly driven by offline improvement in RT. Error bars represent standard errors.

Given that the RT of the last 50 trials in each block did not deteriorate, it could be hypothesized that the RT plateaued due to a ceiling effect for fatigue. To test

this hypothesis, a three-way (3 groups × 6 blocks × 2 phases) repeated measures ANOVA was performed to examine whether mean RT of the last 50 trials improved across blocks. The analysis revealed a significant effect of group ($F_{2, 29} = 35.96$, $p<0.0001$) and the interaction effect between block and block phase ($F_{5, 144} = 2.66$, $p<0.05$). Tukey-Kramer corrected post hoc analyses found that adults had fastest RT, followed by 10-year-olds ($p<0.05$) and then 6-year-olds ($p<0.0001$) regardless of block and the phase of block. All groups decreased RT from block 1 to 4 ($p < 0.005$) and increased RT from block 4 to 5 ($p < 0.0001$) regardless of the whether it was the first or last 50 trials of the block. With regard to the amount of learning measured by changes in mean RT from block 4 to block 5, contrast analyses found no differences between groups in both the first and last 50 trials (all $p > 0.29$) and no differences were found between the first and last 50 trials in each group (all $p > 0.26$) (Figure 3.6B). These results show that the magnitude of learning measured in the last 50 trials of the blocks were comparable to that in the first 50 trials of the blocks in all groups. Most importantly, 6-year-olds, showed the same magnitude of learning as 10-year-olds and adults in both block phases. These results suggest that the non-deteriorated RT across the last 50 trials in each block did not result from the ceiling effect of fatigue.

Finally, we computed corrected offline learning by removing the RT decrement within the preceding block from the total offline gain. The correction on offline learning did not change the age-related difference in offline learning. Specifically, the contribution percentage of corrected offline learning was significantly affected by age ($F_{2, 29} = 5.04$, $p < 0.05$) (Figure 3.6C). Offline learning

contributed more to sequence learning in 6-year-olds than adults (p < 0.05). Indeed, the offline learning contributed more than 50% (mean$_{offline}$ = 77.46%) to motor sequence learning in 6-year-olds (p<0.01), while offline and online improvement contributed equally for 10-year-olds (mean$_{offline}$ = 51.42%, p = 0.87) and adults (mean$_{offline}$ = 34.74%, p = 0.12). Taken together with the fact that offline learning was correlated with learning exploration, these results provide compelling evidence that offline learning observed in this study did not result from fatigue (see discussion below).

Six-year-olds demonstrated less declarative knowledge

Upon completion of the six learning blocks, participants completed two recognition tasks and one recall task (see methods). The non-parametric ANOVA failed to find a significant effect of age on recall scores for all chunk lengths. Nevertheless, there appears a clear downward trend in recall scores with decreasing age (Figure 3.7A). The failure to observe a statistically significant effect may result from a large dispersion of recall performance in 6-year-olds (see S1_data). Unlike the recall test, we found a significant effect of age on scores ($\chi^2_{df=2} = 11.6$, p < 0.005) in the recognition task II where participants were asked to recognized chunks of sequence. Six-year-olds (Figure 3.7B) scored lower than the 10-year-olds and adults. In addition, in recognition task I where sequence A was given along with the other three irrelevant sequences, eight adults (out of 10), nine 10-year-olds (out of 12), but none of 6-year-olds were able to identify the correct sequence.
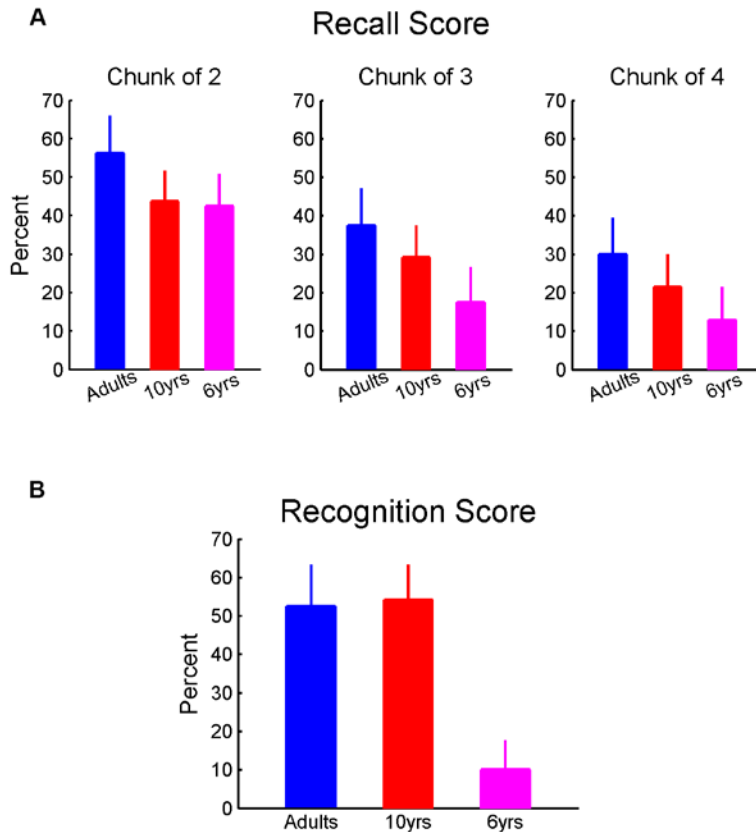
Figure 3.7: Six-year-olds demonstrated less declarative knowledge.
(A) The generation score. There was a tendency that generation scores reduced with age decreasing; (B) Recognition score. Six-year-olds showed lower recognition score compared to adults and 10-year-olds. Error bars represent standard errors.

Discussion

Our results demonstrate that both adults and children learn motor sequences quickly within one learning session not only through online learning (Bornstein & Daw, 2012, 2013; Cleeremans & McClelland, 1991; Verstynen, et al., 2012), but also offline learning. Importantly, the offline and online learning are age-related. Six-year-olds utilize greater offline compared to online learning and greater offline learning than adults, while 10-year-olds and adults utilize the two learning processes equally.

Such age-related differences in offline learning appear to be a function of performance variability rather than fatigue.

Our results reflect similar findings to previous literature on SRT experiments in that both children and adults learn the sequences (Meulemans, et al., 1998; Weiermann & Meier, 2012b). What is new here is the differentiation of two learning processes during one session and their age-related use. It has been reported that learning a motor sequence normally starts with an initial stage known as fast learning (Doyon & Benali, 2005). With a short period of practice (i.e., a single learning session) of a new sequence, fast learning produces considerable improvements in the performance (Honda, et al., 1998; Karni, et al., 1998a; Walker, et al., 2002). After the initial acquisition and short-term memory formation through this fast learning, an early offline "booster" enhances the performance after a 5-30 minutes interval without practice (Albouy, et al., 2006b; Hotermans, et al., 2006; Nettersheim, et al., 2015; Schmitz, et al., 2009). The performance is further strengthened after hours-long rest or sleep through long-term offline learning (Nettersheim, et al., 2015; Robertson, Pascual-Leone, & Press, 2004; Robertson, et al., 2005; Song & Cohen, 2014b; Walker, et al., 2003; Walker, et al., 2002). In contrast, the offline learning we observed here takes place within the fast learning stage and concurrently with online learning, it drives the initial acquisition of motor sequences despite the age of the participants.

Though offline learning is observed in all age groups, it dominantly drives the initial acquisition of motor sequences for six-year-old children in particular. Unlike adults whose RT progressively improves within blocks (i.e., online learning), six-

year-olds' RT worsens within blocks and improves offline between blocks. Such age-related differences imply that the offline learning may be an intrinsic strategy in younger children. Specifically, our results link the offline learning to the exploration mechanism. Exploration is often involved in learning to optimize performance in the long run but may jeopardize current performance (Sutton & Barto, 1998). As in other motor tasks where children and infants show greater exploration at the early phase of learning (Goldfield, et al., 1993; Piek, 2002a; Thelen, et al., 1991), in our task, six-year-old children's RT (i.e., the refined RT after removing time and location effects, see Methods) was more variable than the older children and adults' when they started to learn the sequencing task. This observation suggests that sequence learning in six-year-olds may necessitate greater exploration that negatively impacts their RT performance within blocks (as shown in Fig. 3A). That is, variability may be negatively associated with online learning. Unfortunately, with our limited sample size we cannot provide enough statistical power (lower than 0.4, see results) to detect a negative correlation between online learning and exploration.

Unlike online learning, we found that offline learning was positively correlated to exploration (with statistical power of 0.9, see results). Although the underlying mechanisms bridging offline learning and exploration are unclear, it is possible that offline learning integrates the newly explored knowledge about the sequence. The more information/knowledge explored when the task is performed (i.e., within block), the greater offline learning is required between blocks. In our study, RT variability was naturally produced by children and adults. It is therefore unclear if manipulating the performance variability in a learning task could further

strengthen the offline learning. To understand the relationship between offline learning and exploration, future studies are needed that control the RT variability in both the children and adults.

Besides the correlation between exploration and offline learning, we speculate that offline and online learning that drive the acquisition of a motor sequence over a single learning session may be consonant with procedural and declarative memory. For example, we observed that six-year-old children who showed greater offline learning acquired less declarative knowledge of the sequence as evident by their poor performance in the tasks of recalling and recognizing the sequence. In contrast, 10-year-olds and adults demonstrated greater online learning and also demonstrated more declarative knowledge of the learned sequence. In future studies, modulations of procedural and declarative memory, such as learning a fixed sequence compared to learning a probabilistic sequence, could examine the relationship we propose here. Furthermore, procedural memory was found to be associated with the long-term offline learning that develops hours after a sequence is learned (Brown & Robertson, 2007a). Although it is unclear whether the offline learning that allows initial acquisition of motor sequences is related to offline learning that consolidates the memory of a newly acquired sequence, the association between both types of offline learning and procedural memory leads to an assumption that links the offline learning observed in this study and the long-term offline learning widely found in the literature.

Alternatively, rather than an intrinsic exploration strategy, it could be that specific task constraints cause the offline strategy in young children. For example,

six-year-olds may attempt to learn a sequence online, but the iterative mental computation after each step may interfere with the preparation of the succeeding step due to a limited amount of time available between stimuli. With this line of thinking, offline learning that drives the quick acquisition of a motor sequence may be a strategy forced to emerge by task constraints. Thus, future studies should examine whether the offline learning vanishes in six-year-olds or emerges in adults under certain task constraints such as time between learning trials.

Given that fatigue could result in the large RTs at the end of each block and thus misleadingly exaggerate the magnitude of offline improvement, offline learning may be a byproduct of fatigue. However, analysis of our data precludes fatigue as the prominent factor underlying offline learning in six-year-olds. First, our data show that RT increased as soon as children started to perform the task (i.e., the first 50 steps in the first block). It is not very likely that fatigue caused the worsened RT at the beginning of the first block. Additionally, consistent with previous studies (Meulemans, et al., 1998; Thomas & Nelson, 2001; Weiermann & Meier, 2012b), we observed that like adults, 6-year-olds successfully learned the sequence. If fatigue appeared as soon as children started to perform the task, how could their learning arise quickly and to a comparable level as the adults who did not exhibit fatigue? Second, RT in 6-year-olds increased only in the first 50 trials, while RT remained the same in the last 50 trials, which is contrary to the hypothesis that fatigue causes the decline in performance with extensive practice. The RT in the last 50 trials does not result from the ceiling effect for fatigue, because the RT in the last 50 trials improved as much as the RT in the first 50 trials across blocks. Moreover, the magnitude of

learning as measured by the last 50 trials was comparable between six-year-olds and the other two groups. Third, offline learning, when corrected by removing the RT decrement within the preceding block, displayed the same age-related differences. Taken together, these observations, along with the phenomenon that the magnitude of offline learning was positively correlated to exploration, offers strong evidence that the offline learning observed in this study may be a mechanism underlying motor sequence learning, rather than a by-product of fatigue.

In summary, we found that learning a foot stepping sequences is not only driven by online learning, but also by offline learning that has not previously been reported for sequence learning within a single learning session. These two processes are age-related. The adults and 10-year-olds utilize the online and offline learning strategies equally, whereas the 6-year-olds greatly relied on the latter to acquire the motor sequences. Our results further demonstrated that exploration while performing the sequences, as represented by the variability of RT, may be the mechanism that underlies the offline learning. This result suggests that variability in RT is a principal variable that may provide important insights into motor sequence learning in future studies.

Chapter 4: [†](Study II) Task pacing has no effects on online and offline processes underlying rapid motor sequence learning

Abstract

Implicit sequence learning is ubiquitous in our daily life. However, it is unclear whether the acquisition of sequences results from learning to chunk items (i.e., chunk learning) or learning the first-order transition between elements (i.e., statistical transition learning). By grouping responses with or without a distinct chunk or statistical structure into segments and comparing these responses, previous studies have demonstrated both chunk and statistical transition learning. However, few studies have considered the response sequence as a whole and examined the temporal dependency of the entire sequence, where the order and strength of temporal dependencies could disclose the use of chunk and transition learning. Here, participants performed a serial reaction time (SRT) task under different stimulus interval conditions. We decomposed the response time into reaction time (RT) that reflects mental processing and movement time (MT) that characterizes the movement itself. We found that the improved response time was due to RT and not MT, suggesting sequence learning rather than motor improvements. After examining the temporal dependency of RT and MT, we found notable first-order auto-regressions of RT regardless of stimulus intervals, indicating that the sequence was acquired through learning first-order transitions instead of sequence chunks. Additionally, both RT and MT displayed recursive patterns caused by response locations and sequence

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[†] This manuscript was submitted for publication with the title "New insights into statistical transition learning and chunk learning in implicit sequence acquisition" and it is currently under review. For publication purpose, this submitted manuscript does not include results of online and offline learning. Please see Appendix 1 for the corresponding results.

repetitions, implying that chunk learning found in previous studies may result from biomechanically constrained response tendencies to various response locations. Taken together, our results suggest that implicit sequence learning arises from first-order statistical transition learning rather than chunk learning.

Introduction

The ability to acquire motor sequences is crucial to our daily activities such as riding a bicycle, dressing, and driving a car. These sequences are thought to be learned implicitly where an individual does not have the explicit knowledge of the sequence prior to learning. However, it remains unclear what mechanism underlies the implicit learning of sequences. On one hand, there is evidence that implicit sequence learning results from chunk learning (Koch & Hoffmann, 2000; Stadler, 1993) where a sequence is partitioned into short segments to be learned and the concatenations of segments leads to the acquisition of the sequence. These short segments are grouped by a particular response pattern where a slower response is typically followed by a few quicker responses (Bo & Seidler, 2009; Koch & Hoffmann, 2000). On the other hand, statistical transition learning has been suggested to account for implicit sequence learning (Hunt & Aslin, 2001). Statistical transition learning refers to the process where probabilistic regularities between sequence elements (normally first-order transitions) are learned (Saffran, et al., 1996).

To date, chunk and statistical transition learning have been supported by studies that used the serial reaction time (SRT) task (Nissen & Bullemer, 1987). For example, studies that attempted to demonstrate chunk learning designed a sequence with a certain chunk (Kirsch, Sebald, & Hoffmann, 2010; Koch & Hoffmann, 2000).

Similarly, statistical learning has been demonstrated in studies where sequences had definite statistical structures (Bornstein & Daw, 2012; Hunt & Aslin, 2001). However, there are no studies that compare chunk and statistical transition learning in the same sequence. Therefore, it remains unknown whether chunk or statistical transition learning or both are used to acquire a sequence.

To answer this question, we employed the SRT task and addressed limitations in previous studies that may confound the findings of chunk and statistical transition learning. First, in previous studies, chunk or statistical learning was studied based on response time. Here, we decompose response time into reaction time (RT) that reflects mental processing (Sternberg, 1969b) and movement time (MT) that characterizes the movement itself. Since sequence learning in the SRT task is reflected in RT, but not MT (Moisello, et al., 2009), it is essential to understand whether chunk or statistical learning found in previous studies was contaminated by MT patterns.

Second, in earlier studies, sequence elements with a chunk or statistical structure were separated from the entire sequence. Response improvements in these segmented elements were used to infer chunk or statistical transition learning. However, this is artificial since the sequence is performed as a whole. Segregating the sequence ruins the temporal dependency of the sequence and thus may mislead our understandings of chunk and statistical learning. To circumvent this problem, we examined the time series of RT and MT by fitting them with autoregressive models. The auto-regressions of RT and MT reflected the order and strength of self-dependencies within responses. Given the thesis of chunk learning, higher-order (i.e.,

at least second-order, namely chunks of 3) auto-regressions would be observed. In contrast, first-order auto-regressions would be observed if statistical transition learning is used.

In addition, we asked participants to perform the SRT task under different stimulus interval conditions. The stimulus interval is an important factor in chunk learning (Stadler, 1993). For example, the performer's short-term memory that links successive sequence elements could be impaired when these elements are separated by a long time interval (Frensch & Miner, 1994). Along with this idea, chunk learning would be more noticeable when sequence elements are separated from each other with a short stimulus interval. In contrast, statistical transition learning involves iterative mental computation (Bornstein & Daw, 2012) and thus requires a sufficient amount of time between sequence elements. It is speculated that as long as the stimulus interval is long enough, the stimulus interval would not influence statistical transition learning.

Materials and Methods

Participants

After providing the signed consent form, thirty non-musician adults (age: 20.4 ± 0.29 years, 18 females) without neurological disorders participated in this study. The study was performed in accordance with the guideline approved by the Institutional Review Board at University of Maryland, College Park.

Methods and Procedure

Participants performed a modified serial reaction time (SRT) task. During the task, they were instructed to step to a spatially-matched target as quickly as they could when one of six visual stimuli appeared on the computer screen and then step back to the home position (Figure 4.1A). The distance from the home position to each target was marked at the most comfortable stepping length that was determined prior to the experiment for each individual. A customized program written in Labview (National Instruments, Austin, TX, USA) was used to control the visual stimuli. A Vicon motion capture system (Oxford Metrics, Oxford, UK) recorded the real-time positions of reflective markers attached to the participants' big toes, heels (calcaneus), and the 5th metatarsal on both feet. The sampling frequency was 200Hz. These trajectories were filtered (eighth-order Butterworth filter with a cutoff frequency of 10Hz) before being used in data analyses.

The SRT task was performed under one of three stimulus interval conditions (10 participants were randomly assigned to each condition). In condition I, each stimulus was presented for 700ms prior to its disappearance and the next stimulus appeared after an interval of 600ms (700 + 600ms), yielding a 1300ms-long inter-stimulus-interval (ISI). The time intervals were set as 700 + 200ms for condition II and 300 + 600ms for condition III, both generating a 900ms-long ISI. We chose these three time combinations to control the effect of stimulus appearance or disappearance time that may contaminate the effect of total ISI.

After participants completed a practice block in which the stimuli appeared in a random order, they performed eight learning blocks for their assigned ISI condition.

Specifically, in blocks 1-4, 6, and 8, visual stimuli followed 10 repetitions of sequence A (142315246536). There were no distinct statistical structures in this sequence as each element appeared equal times and each was followed by two other elements with equal likelihoods. In addition, there was no inversion (i.e., 123321) or repetition (i.e., 123123) of any segments that could force the chunking of sequence elements (Jimenez, 2008). Sequence B (146252341356) was repeated 10 times in block 5. In block 7, we used sequence A while replacing two 12-item trials at the middle and end of this block with sequence B as catch trials. Throughout the task, participants were not instructed about the sequence presentation. A three-minute rest was provided after each block.

Data analysis

A customized MATLAB^{TM} (MathWorks, Naticks, MA, USA) script was used to identify the onset and end point of each stepping response. Onset of each stepping response was defined as the first sample when the foot reached 10% maximum movement height. The end point of each stepping response was defined at the time when the foot dropped to the same height as the onset. RT was computed as the time elapsed from the onset of visual stimulus to the onset of foot movement. MT was quantified as the time discrepancy between the onset and the end point of foot movement. The summation of RT and MT formed response time. Response times, RTs, and MTs that corresponded to error steps (i.e., stepping to a wrong target) were discarded (The overall error rate was below 5% despite of ISI conditions). In addition, within each learning block, RTs, MTs, or response times that deviated

beyond or below 2.5 standard deviations from the individual's mean RT, MT, or response time in that block were excluded from further analyses.

To measure performance in the task, the mean response time, RT, and MT was computed for each block. Mean performance differences between blocks 1 and 4, as well as blocks 4 and 5, were used to quantify the learning of sequence A (Robertson, 2007). Mean performance differences between blocks 1 and 8 were computed to represent the learning through the entire task. In addition to the learning quantified by performance differences between blocks, we compared performance between sequence A and catch trials within learning block 7. Specifically, the mean performance on sequence A was computed on 12-step learning trials that preceded the catch trials. To measure the temporal self-dependency of RT/MT, each time series was fitted with autoregressive models (see results for details). Fitted coefficients were subsequently used for statistical analysis.

Statistical analysis

Two-way (8 blocks × 3 groups) mixed effect ANOVAs were used to examine the effects of learning block, ISI group, and their interaction on mean response time/RT/MT and autoregressive coefficients. Tukey-Kramer corrected post hoc tests were conducted following any significant effect. Pre-planned contrast analyses were used to compare mean response time/RT/MT or autoregressive coefficients between blocks 1 and 4, blocks 4 and 5, and blocks 1 and 8. In addition, a two-way (2 types × 3 groups) mixed effect ANOVA was performed to examine the response time/RT/MT difference between learning and catch trials within block 7. The covariance matrix

structure of mixed effect ANOVAs was determined by the Akaike information criterion (AIC). The significance level for statistical analyses was set at $\alpha = 0.05$.

Results

We found a significant effect of block ($F(7,27) = 15.47$, $p < 0.0001$), but no effects for ISI and its interaction with block on response times (Figure 4.1B). Specifically, response times were slower in block 1 compared to blocks 2 to 4 and 6 to 8 (all $p < 0.005$), while response times remained the same between blocks 1 and 5 ($p=0.38$). Response times were faster in block 4 compared to block 5 ($p<0.0001$). Contrast analyses revealed that all groups decreased response time by an equivalent amount from block 1 to 4 (all $p>0.29$) and from block 1 to 8 (all $p>0.74$). However, the response time difference between blocks 4 and 5 was larger when ISI was 300+600ms than 700+600ms ($p<0.05$). It appears that learning is greater under the 300+600ms ISI. However, since the response time change from block 4 to 5 exceeded the improvement from block 1 to 4 under the 300+600 ISI, at least part of the deterioration in response time from block 4 to 5 was not due to a learning effect, but perhaps proactive interference from sequence A to sequence B.

Learning was also demonstrated by the response time difference between learning and catch trials within block 7 (Figure 4.1B). Notably, response time was affected by trial type ($F(1, 27) = 52.51$, $p<0.0001$) but not ISI and its interaction with trial type. Response times were slower in catch trials than learning trials. The response time differences between catch and learning trials were comparable in three groups (all $p > 0.17$). These results together suggest that ISI does not impact sequence learning.
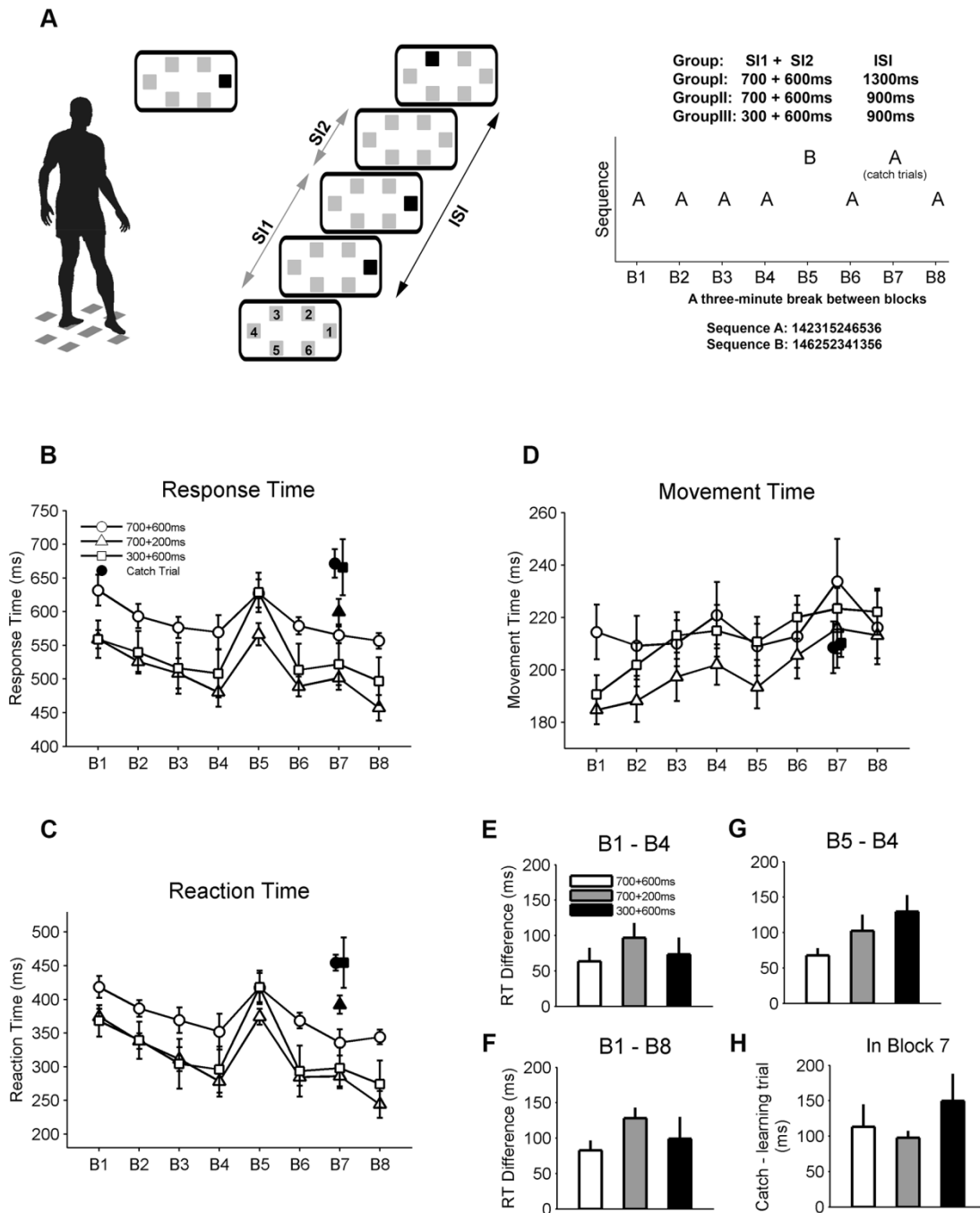
Figure 4.1: Experimental setup and mean performance across blocks.
(A) Experiment procedure. (B) Mean response time across learning blocks. (C) Mean RT across learning blocks. (D) Mean MT across learning blocks. (E) RT difference between blocks 1 and 4. (F) RT difference between blocks 1 and 8. (G) RT difference between blocks 4 and 5. (H) RT difference between learning and catch trials within block 7. Error bars represent standard errors.

When the response time was decomposed into RT and MT, we found that RT exhibited the same pattern as response time (Figure 4.1C). RTs were significantly affected only by block ($F(7,27) = 14.00$, $p < 0.0001$). RTs improved from block 1 to other blocks when sequence A was performed (all $p < 0.005$) and remained the same between blocks 1 and 5 ($p=0.69$). Contrast analyses revealed an equivalent amount of RT improvements from block 1 to 4 (all $p>0.3$) (Figure 4.1E) and from block 1 to 8 (all $p>0.4$) (Figure 4.1F) regardless of ISI groups. Comparable RT changes from block 4 to 5 were found across groups except that between 300+600ms and 700+600ms groups ($p<0.05$) (Figure 4.1G). Like response time, at least part of the RT deterioration from block 4 to 5 was caused by proactive interference from sequence A to sequence B, given that the RT change from block 4 to 5 was larger than that from block 1 to 4. Within block 7, RT was significantly affected by trial type ($F(1, 27) = 41.51$, $p<0.0001$). Learning trials had faster RTs than catch trials, but RT differences between learning and catch trials did not differ across ISI groups (all $p > 0.17$) (Figure 4.1H).

Unlike RT and response time, MT demonstrated a different pattern (Figure 4.1D). There was a significant interaction between ISI group and block ($F(14,27) = 3.08$, $p < 0.01$). It was found that MT became slower as learning progressed. Specifically, MT was faster in block 2 than blocks 7 and 8 under the 700+200ms ISI (both $p < 0.05$). MT was faster in blocks 2 ($p<0.05$) and 3 ($p<0.01$) compared to block 7 under the 700+600ms ISI. Under the 300+600ms ISI, there was a trend that MT was faster in block 2 than blocks 6 and 8 ($p = 0.089$ and $p = 0.096$). Contrast analyses failed to find MT differences between blocks 1 and 4, blocks 1 and 8, and

blocks 4 and 5 among three ISI groups. These results together demonstrate sequence learning rather than motor improvements and suggest that the sequence learning is comparable among all ISI groups.

Given that the response time consisted of RT and MT which behaved differently, we modelled the RT and MT within each block using autoregressive moving average models (i.e., $ARIMA(p, 0, q)$, where p and q are the orders of autoregressive (AR) and moving average (MA) models, respectively). Since MA can be expressed by AR and it is our primary interest to examine the association between successive RTs (i.e., AR) rather than the connection between an RT and its preceding noise (i.e., MA), we set q = 0. Subsequently, the sample autocorrelation (ACF) and partial autocorrelation functions (PACF) of RT and MT time series show that the ACF tailed off in the periodic lags at 12, 24, 36 and so on (Figure 4.2A and 4.2B) while the PACF cut off after lag 12 (Figure 2C and 2D), demonstrating that the same RT or MT pattern recurred every 12 steps that was identical to the length of sequences A and B. Moreover, within each 12 steps, RTs or MTs varied depending on the positions of responses (i.e., from the 1st step to 12th step; Figure 4.3A and 4.3B), suggesting that the periodic RT and MT patterns were caused by repetitions of the sequence. Importantly, the periodic RT and MT exhibited similar chunk patterns demonstrated in previous studies where a slower response was followed by faster responses (Koch & Hoffmann, 2000). These results indicate that chunk learning identified in previous studies is likely to result from biomechanically constrained response tendencies to various response locations (Jimenez, 2008).
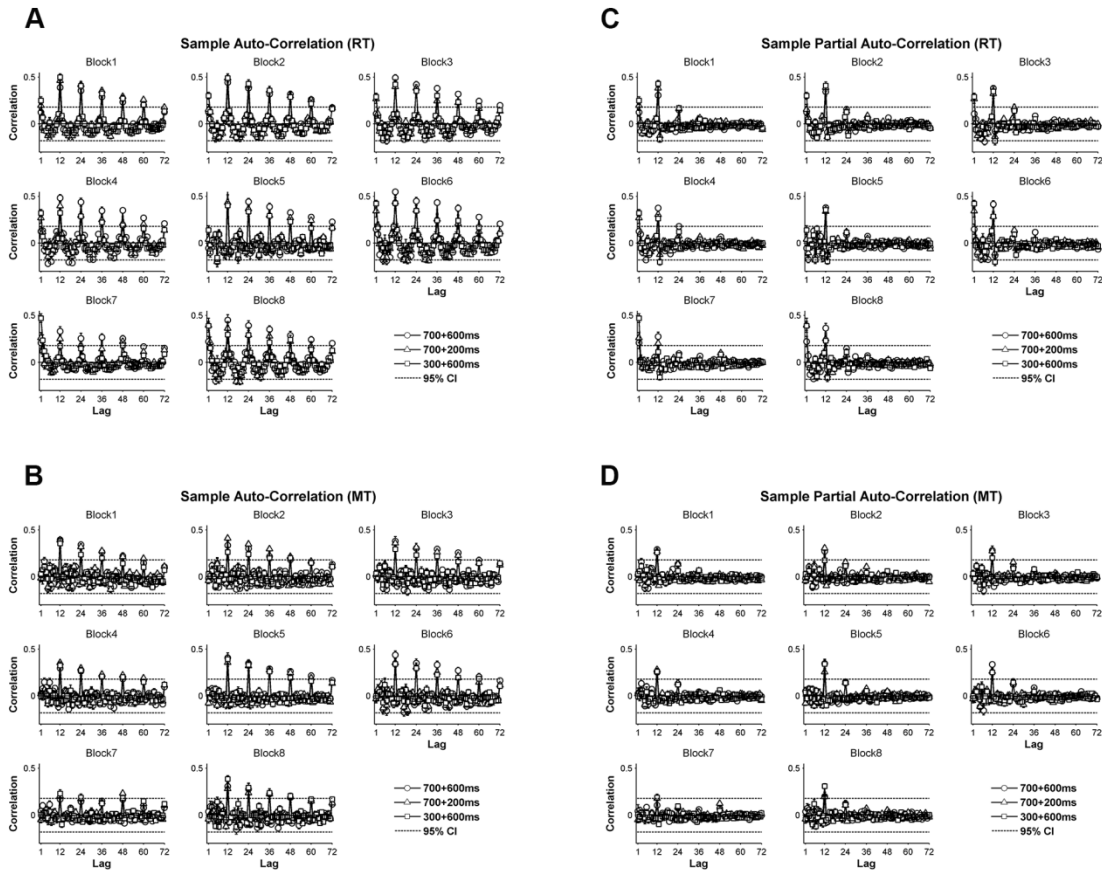
Figure 4.2: Mean sample autocorrelation and sample partial autocorrelation of RT.
(A) Mean sample autocorrelation of RT. (B) Mean sample autocorrelation of MT. (C) Mean sample partial autocorrelation of RT. and (D) Mean sample partial autocorrelation of MT.

In addition to the periodic pattern, the PACF of RT displayed a significant auto-correlation at lag 1 (Figure 4.2C). This auto-correlation together with the periodic component suggests that a seasonal ARIMA$(p, 0, 0) \times (1,0,0)_{12}$ model with p = 1 is appropriate to describe the RT data. However, examinations of individuals' RT revealed that there were individual differences in order p. In addition, the literature has normally demonstrated chunks of 3 in the SRT task (Kirsch, et al., 2010; Koch & Hoffmann, 2000). Thus, we set order p to 1 and 2. Given that each RT time series was fitted by models with a p of order 1 or 2, the AIC was used to select the best model for each RT time series. Because only autoregressive processes were used, we

referred to the models as AR(1) or AR(2) model. Unlike RT, MT did not show significant auto-correlations at lags that were less than order 12 (Figure 4.2D), implying no self-dependencies among MTs. Thus, we did not model MT for further analyses.
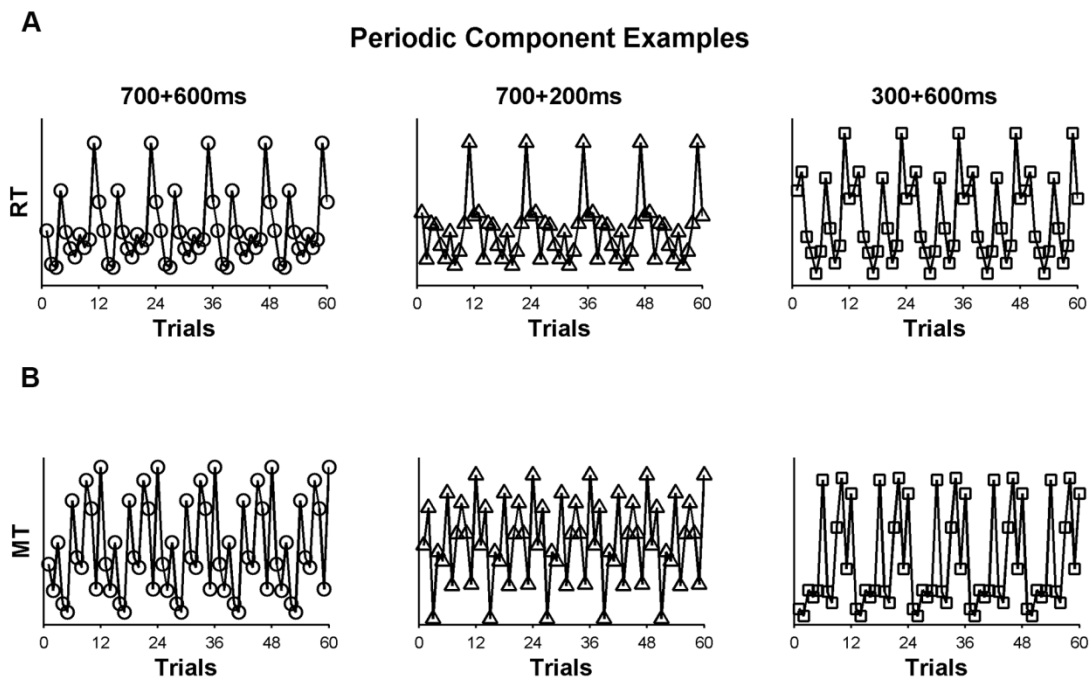
Figure 4.3: Examples of periodic components of RT.
(A) Examples of the periodic components of RT in each group. (B) Examples of the periodic components of MT in each group. As the sequence repeats, the same pattern of RT or MT reoccurs. For display, only first 60 steps in block 1 were illustrated.

Figure 4.4A shows the coefficients of the first-order auto-regressions that were averaged across individuals' coefficients estimated by their favored model. The coefficient magnitude was significantly affected by block ($F(7, 27) = 6.96$, $p < 0.0001$) and ISI ($F(2, 27) = 9.03$, $p < 0.001$). The coefficient was smaller when the ISI was 700+600ms than 700+200ms ($p = 0.05$) and 300+600ms ($p < 0.001$). Additionally, all groups increased the coefficient magnitudes from block 1 to 8 ($p < 0.05$). Student's t-tests revealed that the coefficients were significantly larger than 0

in both blocks 1 and 8 (all p < 0.0001 except for the 1300ms ISI, p = 0.0075 in block 1 and p = 0.0003 in block 8; The significance level was adjusted to $\frac{\alpha}{6} = 0.0083$ to control the familywise error rate given the six simultaneous Student's t-tests), suggesting that the first-order auto-regression strengthened as learning progressed. However, the coefficient changes between blocks 1 and 4, blocks 1 and 8, and blocks 4 and 5 were comparable among three groups (all p > 0.2).
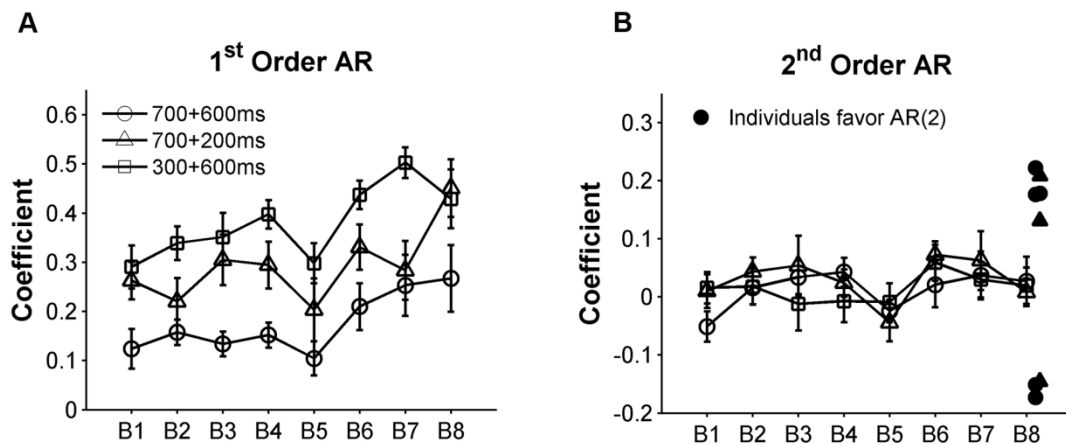
Figure 4.4: Autoregressive Coefficients. First-order autoregressive coefficients were greater than zero.
(A) Mean coefficient of the first-order autoregressive term. (B) Mean coefficient of the second-order autoregressive term and the coefficient of eight individuals whose RT performance prefers AR (2) model. Error bars represent standard errors.

Given that a few participants favored AR(2) model (Figure 4.4B), it was impossible to compare the second-order coefficients using individuals' favored models. Thus, we further fitted all RT time series using AR (2) models. The results of first-order coefficients from the AR(2) model did not qualitatively change from coefficients estimated by individuals' favored models that were shown in Figure 4A. However, there were no effects of ISI, block, and their interaction on the second-order coefficients (Figure 4.4B). Unlike the first-order coefficients, the second-order coefficients were not significantly different from zero in blocks 1 and 8 for three

groups (all p > 0.15, the significant level was adjusted to $\frac{\alpha}{6} = 0.0083$). These results suggest that implicit sequence learning arises through first-order statistical transition learning and it is not affected by stimulus intervals.

Discussion

We demonstrate that in the SRT task, the sequential order rather than the movement itself is learned. The sequence is acquired through learning the first-order transition between sequence elements despite the nature of the stimulus intervals. It is also demonstrated that chunk patterns in both RT and MT appear to result from physical properties of the sequence (i.e., biomechanically constrained response tendencies to various response locations). These results together suggest that the acquisition of sequences results from statistical transition learning rather than chunk learning.

Chunking is suggested as a core mechanism underlying sequence learning (Gobet, et al., 2001). Chunking benefits sequence learning as it attenuates memory loads during learning by segmenting a long sequence into shorter segments (Bo & Seidler, 2009; Wymbs, Bassett, Mucha, Porter, & Grafton, 2012). These shorter segments are normally indicated by a slower response followed by a few quicker responses (Bo & Seidler, 2009; Koch & Hoffmann, 2000). In these studies, slower responses were identified at certain positions in a sequence. However, our data imply that chunks identified in such a way may result from physical properties of sequences such as periodic and spatial effects of response keys. An individual may react and move slower to keys at some locations (e.g. right front vs right back) or keys corresponding to certain effectors (e.g., left vs right foot) due to biomechanical

constraints of the body. The same slow vs fast RT and MT patterns repeat when the sequence recurs. Thus, the resulting RT and MT patterns are likely due to biomechanically constrained response tendencies rather than chunk learning. In sequence learning that involves finger tapping movement, the same conclusion may be true for the various response effectors – index vs middle finger, or right vs left hand (Jimenez, 2008).

Moreover, the observation of the first-order autoregressive pattern in RT does not support chunk learning. Chunk formations often involve at least three sequence elements. However, second-order auto-regressions among RTs were not observed in the majority of participants. Although 8 out of 30 participants demonstrated second-order auto-regressions in RT at the end of learning, more than half of the participants showed positive AR (2) coefficients (Figure 4B). That is, fast (or slow) responses were followed by two other fast (or slow) responses, which is not consistent with the chunking principle. Furthermore, among these 8 participants, five of them were from the longer ISI group. This observation is incompatible with the chunking hypothesis that longer stimulus intervals prevent adjacent stimuli to be present in short-term memory (Frensch & Miner, 1994).

Hence, our results challenge the chunking hypothesis for learning implicit sequences; results that are consistent with recently reported findings. For example, it has been demonstrated that chunks began to form as soon as an individual starts the SRT task, but learning took place without improvements in these chunks (Jimenez, 2008; Song & Cohen, 2014a). In addition, the chunking principle is contradicted with memory consolidation during sequence learning (Robertson, Pascual-Leone, & Miall,

2004). Specifically, there is no time allowed for memory of each chunk to stabilize since chunks are performed continuously. Consequently, chunks would interfere with each other and thus prevent learning of the whole sequence. This converging evidence indicates that chunking appears not to be the mechanism underlying implicit motor sequence learning.

An alternative to chunk learning could arise from the learning of first-order statistical transitions between sequence elements (Bornstein & Daw, 2012; Hunt & Aslin, 2001). Our results favor the statistical learning interpretation given that each RT was found to depend only on the preceding RT. Importantly, given that the magnitudes of first-order auto-regressions increased as practice progressed, the first-order auto-regressions are most likely to result from learning rather than preexisting patterns in the RT. At the end of learning, second-order auto-regressions were found in the RTs of a few participants, especially those who performed the SRT task under a long ISI condition. Although future studies are necessary, this result suggests that as learning progresses, the higher-order statistical structure (i.e., statistical transitions between non-adjacent elements) may also be acquired.

Although our results were observed in a foot stepping SRT task, we surmise that the results and interpretations from the foot stepping task can be generalized to the classic finger-pressing SRT task. The response time observed in this study changed in the same pattern and comparable magnitudes with previous studies using the classic finger-pressing SRT task (Curran & Keele, 1993; Willingham, et al., 1989). In addition, the ISI did not affect sequence learning in the foot stepping SRT task, which is consistent with findings in the finger-pressing SRT task (Destrebecqz

& Cleeremans, 2003; Willingham, et al., 1997). These consistent findings suggest that similar mechanisms may underlie sequence learning in the foot-stepping and finger-pressing SRT tasks, but future studies are necessary for further elucidations.

Conclusion

We found that sequence learning takes place under different stimulus interval conditions. Importantly, using autoregressive models to analyze the reaction time in the SRT task, we found that implicit sequence learning arises from the acquisition of first-order statistical transitions between sequence elements. It is likely that chunk learning observed in previous studies is a byproduct of the physical properties of the sequence. These results suggest that statistical transition learning, rather than chunk learning, is the mechanism that underlies the acquisition of implicit motor sequences.

Chapter 5: (Study III) Acquisition of implicit motor sequences in a self-paced SRT task arises from offline learning in children but online learning in adults

Abstract

Implicit sequence learning, as a fundamental ability in our daily life, has been widely studied in adults. However, our understanding of implicit sequence learning in children is rather limited. In particular, little is known about the age-related differences in learning processes that underlie the acquisition of implicit sequences. Here, we asked young adults and children from 5 to 14 years of age to perform a self-paced serial reaction time (SRT) task and compared their sequence learning that developed during the first learning session. We found that implicit sequence learning, as reflected by reaction time (RT), was comparable across age. Such similar extent of fast sequence learning was expressed by two behavioral processes; progressive changes in RT as the task was performed (i.e., online changes in RT) and offline changes in RT that emerged following short rest. We demonstrated that these two processes were not artifacts of fatigue or reactive inhibition. Rather, they were active learning mechanisms that may result from competitive memory systems of procedural and declarative learning. Importantly, the age-related differences in these two learning processes were divergent. Offline learning attenuated while online learning became stronger as age increased until age 11. After the 11 years of age, online and offline learning remained unchanged. Collectively, our results suggest that offline and online learning driving the initial acquisition of implicit sequences are age-related, where the developmental change is primarily presented before age 11.

Introduction

In the SRT task, a primary window into understanding implicit motor sequence learning (Nissen & Bullemer, 1987), children as young as six years of age demonstrate a comparable capability to learn fixed sequences like adults (Meulemans, et al., 1998), whereas the learning in children and adults may be driven by different learning processes (study I; Chapter 3). In particular, learning in 6-year-old children dominantly relies on an offline process where RT improves after short rest, while offline enhancement and online progressive improvement in RT concurrently drive sequence learning in adults. To date, a unifying mechanisms underlying the age-related online and offline processes remains unclear. A common hypothesis considers the online and offline processes as artifacts of fatigue or reactive inhibition. Although this hypothesis originates from explicit sequence learning, this effect can also be postulated as a dominant source of online and offline processes in implicit sequence learning (Rieth, et al., 2010). This study, therefore, aims to investigate whether the age-related online and offline processes underlying implicit motor sequence learning are artifacts of fatigue or reactive inhibition.

According to the fatigue or reactive inhibition hypothesis, online and offline changes in RT are performance-based. That is to say, rather than being learning-based mechanisms, the online and offline changes in RT may result from the emergence of fatigue or reactive inhibition (Brawn, et al., 2010; Rickard, et al., 2008a). The fatigue or reactive inhibition explanation appears to be more critical in developmental studies as fatigue or reactive inhibition is more likely to accumulate in children than adults when they perform the same task. For example, children's RT worsened within

learning blocks when they performed the SRT task (Study I; Chapter 3). This RT deterioration may be caused by fatigue or reactive inhibition. The effect of fatigue or reactive inhibition dissipated following rest, which resulted in the recovery of RT and consequently led to offline improvements in RT as an artifact of fatigue or reactive inhibition (Brawn, et al., 2010; Rickard, et al., 2008a; Rieth, et al., 2010). Thus, the question raised here is whether offline and online changes in RT observed in children and adults result from fatigue or reactive inhibition.

In addition, the age-related differences in online and offline processes may also be by-products of task constraints (i.e., the task pacing conditions). In study I, children and adults performed the task under the same inter-stimulus-interval (ISI). The same ISI appeared to be relatively shorter in children than adults. Thus, the ISI may prevent children from learning the sequence online as online learning requires iterative mental computations that need an adequate amount of time between stimuli.

In this study, we asked children and adults to perform a self-paced SRT task where the ISI interval was determined by their own response speeds. Such self-determined ISI eliminated the confounding effect of task pacing in study I. According to the fatigue or reactive inhibition hypotheses, fatigue or reactive inhibition accumulates when an individual is practicing the task and thus deteriorate the performance (i.e., reaction time). Previous studies have suggested that fatigue or reactive inhibition could have a detrimental effect on learning (Ammons, 1947; Bourne & Archer, 1956; Denny, et al., 1955) or it could suppress the performance expression without having an effect of learning (Brawn, et al., 2010; Rickard, et al., 2008a). That is to say, the RT deterioration within a block, which is under the

influence of fatigue or reactive inhibition, would yield no effect or negative impact on sequence learning. To determine whether online and offline processes are primarily attributed to learning or the artifacts of fatigue/reactive inhibition, we examined whether the deterioration in performance (i.e., reaction time or RT) that is under the influence of fatigue or reactive inhibition affects sequence learning.

Materials and Methods

This study was performed in accordance with the approval of the Institutional Review Board at the University of Maryland, College Park. Consent forms from adult participants and parents, as well as assent forms from child participants, were received prior to the experiment. Each participant received $15 after the completion of the experiment. In addition, a small toy prize was provided to child participants upon the completion of the Movement Assessment Battery for Children 2 (MABC2) (Henderson, et al., 2007).

Participants

Twenty-seven children (8.78 ± 2.26 years, between 5.39 and 14.37 years, 10 females) were recruited for this study. Prior to the experiment, children completed the MABC2 to exclude those scored below the 15th percentile on the MABC2. One male child was excluded from this study. In addition to child participants, twelve young non-musician adults (20.66 ± 0.5 years, between 18.64 and 25.2 years, 9 females) from the University of Maryland, College Park participated in this study. A neurological health questionnaire was given to each participant to ensure that no participants had neurological impairments or medical conditions that may affect

motor performance.

Experimental task and Procedure

Participants performed a modified SRT task, namely a whack-a-mole game with sequential foot stepping (Figure 5.1A). Participants stood on the home position before starting the task. The home position was surrounded by six stepping targets with two located at the front, two at the side, and the other two at the back of the home position. The distance from the home position to each target was marked at the most comfortable stepping length that was determined prior to the experiment for each individual. Six spatially-matched visual stimuli (i.e., six holes) were presented on a monitor in front of the participants. After the task began, one mouse appeared from one of the six holes. Participants stepped to the corresponding target on the floor as quickly and accurately as they could and then returned to the home position. The mouse did not disappear until participants' feet returned to the home position, as detected by analog signals sent from two electric rubber sensors under the home position. Once participants returned to the home position, the mouse appeared in another hole after an interval of 700ms. Thus, the inter-stimulus-interval (ISI) was self-paced, as determined by the total amount of time spent on stepping to the target and returning to the home position in addition to a 700ms interval. A customized program written in the Labview (National Instruments, Austin, TX, USA) was used to collect the signals from the home position sensors and control the appearance of visual stimuli. Three reflective markers were attached to the participants' big toes, heels, and the 5th metatarsal on both feet. The three-dimensional movement trajectories of these markers were record by a Vicon motion capture system (Oxford

Metrics, Oxford, UK) with a sampling frequency of 200Hz.

Participants first completed a practice block where the stimuli appeared in a random order to understand the task before starting the learning blocks. There were six learning blocks and each consisted of 100 steps. In blocks 1-4 and 6, the visual stimuli followed 10 repetitions of sequence A (i.e., 1423564215; 1 – right side, 2 – right front, 3 – left front, 4 – left side, 5 – left back, and 6 – right back). The order of visual stimuli followed 10 repetitions of sequence B (i.e., 3615425214) in block 5. There was a mandatory three-minute break after each block (Figure 5.1B). During this SRT task, participants were not provided any information about the sequences. Upon completion of the SRT task, participants were asked to complete a posttest to examine whether declarative knowledge of sequence A was acquired. They were first asked to recall the sequence in the SRT task and to write down 10 steps of the sequence (i.e., recall task). They were then given 16 sequence chunks where eight chunks were correct and were asked to choose the ones they thought resembled the sequence they learned in the SRT task (i.e., recognition task).

Data analysis

Response time was used to measure performance in the SRT task (Nissen & Bullemer, 1987). Since changes of response time in the SRT task may be attributed to reaction time (RT) that represents mental processing and/or movement time (MT) that reflects the movement itself (Moisello, et al., 2009), we decomposed response time into RT and MT. RT was computed as the time discrepancy between the onset of visual stimulus and the onset of foot movement. MT was calculated as the time elapsed from the onset of foot movement to the end point of foot movement when

reaching the target. The summation of RT and MT forms response time. To derive these variables, the starting and end points of foot movement were identified from the three-dimension trajectory (filtered by an eighth-order Butterworth filter with a cutoff frequency of 10Hz) of the foot markers using a customized MATLAB^{TM} (MathWorks, Naticks, MA, USA) script. The onset of stepping was defined as the first sample when the foot reached 10% maximum movement height. The end point of stepping was defined at the time when the foot dropped to the same height as the onset. Within each learning block, RTs, MTs, or response times that deviated beyond or below 2.575 standard deviations (i.e., resembles the 99% confidence interval) from the individual's mean RT, MT, or response time in that block were considered as outliers and were excluded from further analyses.

Figure 5.1: Experiment setup and procedure.
(A) The SRT task. (B) Experimental procedure.

Mean response time, RT, and MT were computed for each block. Learning was quantified through the difference in performance (i.e., response time, RT, and MT) between blocks 1 and 4, as well as between blocks 4 and 5. The indicator of sequence learning was whether performance improves from block 1 (sequence A) to block 4 (sequence A) and/or whether performance deteriorates from block 4 (sequence A) to block 5 (sequence B) (Robertson, 2007). Given that RT rather than MT reflected sequence learning (see results below), analyses on online and offline changes in performance were performed only on RT. Online change in RT was defined as the RT change that takes place within block and was computed as the difference between the mean RTs of the first and last 10 steps. Offline change was defined as the RT change after short rest without performing the task. In particular, this change was computed as the difference between the mean RT of last 10 taps in one block and mean RT of first 10 taps in the succeeding block. A positive value of online or offline change indicates RT improvement while a negative value means that RT became slower.

To determine whether online and offline changes in RT are learning-based or due to fatigue/reactive inhibition, we computed the amount of RT deterioration within each block and the magnitude of learning gained through the practice of each block. Specifically, the amount of RT deterioration had the same magnitude as the online change in RT but with opposite sign. For example, a -20ms online change in RT (i.e., RT became slower) was equivalent to 20ms RT deterioration. The magnitude of learning of each block was calculated as the difference in the mean RT of first 10 taps from one block to the succeeding block. Since fatigue/reactive inhibition may occur

within the block, this difference between the RT at the beginning of the block (i.e., mean RT of first 10 taps in the block) and the post-rest RT (i.e., mean RT of first 10 taps in the succeeding block) serves as a better indicator of learning over that block as the effect of fatigue or reactive inhibition is substantially reduced after rest (Brawn, et al., 2010; Rickard, et al., 2008a).

We then conducted a correlation analysis between RT deterioration and the magnitude of learning to examine three hypotheses regarding learning-based or fatigue/reactive inhibition-based online and offline changes in RT. First, previous studies (primarily in explicit sequence learning) that advocated the fatigue/reactive inhibition hypothesis claimed that fatigue/reactive inhibition suppresses performance expression but does not impair learning (Brawn, et al., 2010; Rickard, et al., 2008a). That is to say, the RT deterioration within a block, which is under the influence of fatigue or reactive inhibition, does not impact the magnitude of learning. Therefore, the RT deterioration and magnitude of learning would not be correlated. Second, numerous earlier studies have found the detrimental effect of fatigue/reactive inhibition on procedural skill learning (Ammons, 1947; Bourne & Archer, 1956; Denny, et al., 1955). Notably, learning would be impaired more if a stronger effect of fatigue/reactive inhibition builds, indicating a negative impact of the RT deterioration on the magnitude of learning. Finally, in opposition to the fatigue/reactive inhibition hypothesis, online and offline changes in RT may arise from certain underlying learning mechanisms (Eysenck & Frith, 1977). Under this hypothesis, the online RT deterioration within blocks may take place due to learning itself and may serve as a prerequisite for the offline change in RT following rest. Thus, the amount of RT

deterioration would be positively correlated to the magnitude of learning. In other words, the greater the RT deteriorates before rest, the greater the magnitude of learning would be.

To measure the score in the recall test, we counted the number of correct 2-, 3-, and 4-element chunks in the sequence that participants recalled. The chance levels of recalling 2-, 3-, and 4-element chunks were different. Take the 3-element chunk for an example, given the first element, there were four chunks (i.e., chunks starts from 1, 2, 4, or 5) that could be guessed with a chance level of 0.125 and two chunks (i.e., chunks starts from 3 or 6) that could be guessed with a chance level of 0.0625. Thus, the weighted probability among these six chunks was 0.1042. Given that participants recalled a 10-element long sequence that has eight 3-element chunks, the chance level for recall 3-element chunks in a 10-element long sequence written by an individual was 0.83 (i.e., 8×0.1042). Similarly, the chance levels of recalling 2- and 4-element chunks were 3.38 and 0.29. To assess the recognition performance, we counted the number of correct chunks (i.e., chunks included in sequence A) that participants chose as well as the number of incorrect chunks (i.e., chunks not included in sequence A) that participants did not choose in the recognition task. The sum of these two numbers was used as the recognition score. For example, if a participant chose two correct chunks and did not choose incorrect chunks, the recognition score is 10. The recognition score would be eight if participant chooses all 16 chunks. The chance level is eight for the recognition test.

Statistical analysis

For statistical analyses, we considered age as a continuous variable as well as a categorical variable by clustering participants into three age groups: children younger than eight, children older than eight, and adults. This age cutoff was chosen prior to data collection because the age-related differences in implicit sequence learning have commonly been examined between children younger than eight and adults (Meulemans, et al., 1998). The use of continuous age is because it is unclear whether sequence learning, especially its underlying online and offline processes, develops continuously with age.

A two-way mixed effect ANOVA was used to examine the effects of block and age group (i.e., a categorical variable). Tukey-Kramer corrected *post hoc* tests were conducted following any significant effect. Pre-planned contrast analyses were used to examine the age effect on sequence learning as measured by the performance difference between blocks 1 and 4, as well as between blocks 4 and 5. The same analyses were performed on offline and online changes in RT that developed between blocks 1 and 4. To examine the progressive developmental change in implicit sequence learning, we further modeled sequence learning (as measured by RT differences between blocks 1 and 4 as well blocks 4 and 5), offline, and online RT changes based on the individual's age (i.e., continuous variable). Specifically, we used either a linear model or piecewise linear latent model with unknown knots, which was determined by a likelihood ratio test. To examine whether online and offline changes in RT were artifacts of fatigue/reactive inhibition-based or active learning mechanisms, a partial correlation analysis was performed to confirm the

relationship between the RT deterioration and the magnitude of learning controlling the effect of age and block. Since recall and recognition score were measured by count data, Poisson models were used to examine the effect of age group or continuous age on the recall and recognition score. If over-dispersion was displayed in these two scores, a Poission mixture model (i.e., negative binomial regression) was used instead. The significance level for statistical analyses was set at $\alpha = 0.05$.

Results

It was found that response time was significantly affected by block (F(5,35) = 28.47, p <0.0001) and age group (F(2,35) = 50.35, p <0.0001), but not their interaction (Figure 5.2A). Specifically, response time was slower in children younger than eight compared to children older than eight (p < 0.0001) and adults (p < 0.0001), while the latter two groups had comparable response times (approached significance p = 0.07). Regardless of the age group, response time was slower in block 1 compared to blocks 2-4 and 6 where sequence A was performed (all p < 0.0001) while response time in block 5 in which sequence B was performed was comparable to the response time in block 1 (p = 0.86). Response time in blocks 4 was found to be faster than that in block 5 (p < 0.0001). Pre-planned contrast analyses found comparable improvements in response time from block 1 to block 4 and comparable deteriorations in response time from block 4 to block 5, indicating the same level of learning among age groups. These changes were further modeled with a linear function of an individual's age (i.e., the likelihood ratio test revealed no difference between the linear model and piecewise latent model; $\chi^2_{df=2} = 0.8$, p = 0.67 and $\chi^2_{df=2} = 0.1$, p = 0.95). It was found that there was no significant age effect on the

response time change from block 1 to block 4 (slope = 0.78, p = 0.68) as well as the RT change from block 4 to block 5 (slope = 0.2, p = 0.88). These results suggest that children and adults learned sequence A. Importantly, sequence learning, as measured by the response time, did not depend on age.

After the response time was decomposed into RT and MT, remarkable differences were observed. RT exhibited the same pattern as response time. There were significant effects of block (F(5,35) = 30.82, p <0.0001) and age group (F(2,35) = 56.79, p <0.0001), but no effect of their interaction (Figure 5.2B). Like response time, RT improved from block 1 to blocks 2-4 and 6 (all p < 0.0001) while deteriorated from block 4 to block 5 (p < 0.0001). Children younger than eight had slower RT compared to older children (p < 0.0001) and adults (p < 0.0001). RT in older children was slower than RT in adults (p < 0.05). Contrast analyses revealed the same RT changes from block 1 to block 4 as well as that from block 4 to block 5. Linear function was found to appropriately model these RT changes (the likelihood ratio test: $\chi^2_{df=2} = 0.5$, p = 0.78 and $\chi^2_{df=2} = 3$, p = 0.22), which failed to find a significant age effect on the RT change from block 1 to block 4 (slope = 0.34, p = 0.86)(Figure 5.2C) as well as the RT change from block 4 to block 5 (slope = -0.65, p = 0.68) (Figure 5.2D).
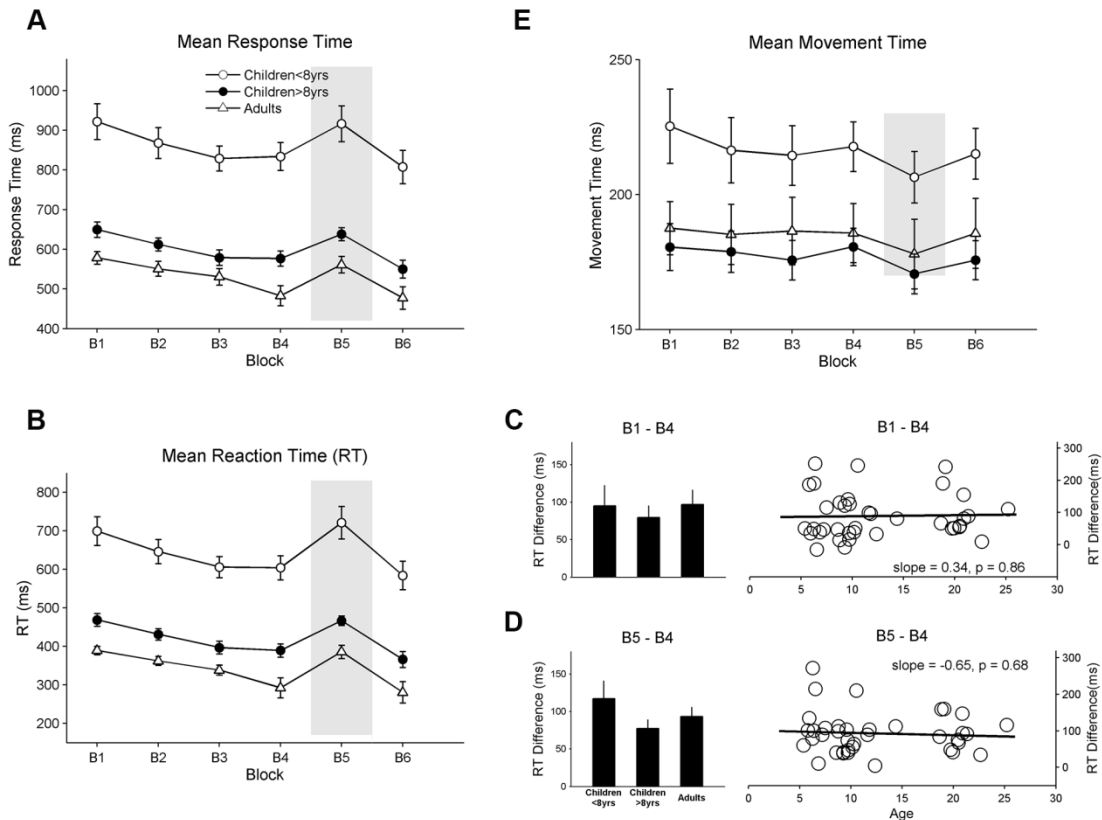
Figure 5.2: Mean performance across learning blocks: No age effect on motor sequence learning.
(A) Mean response time across learning blocks. (B) Mean reaction time (RT) across learning blocks.
(C) Mean RT differences between blocks 1 and 4. (D) Mean RT differences between blocks 4 and 5.
(E) Mean movement time (MT) across learning blocks.

However, MT displayed a different pattern compared to response time and RT (Figure 5.E). There were significant effects of age group ($F_{(2, 35)} = 4.82$, $p < 0.05$) and block ($F_{(5, 175)} = 4.77$, $p < 0.0005$), but no interaction effect on MT. Slower MT was found in children younger than eight compared to children older than eight ($p < 0.05$) and adults (approached significance, $p = 0.07$). MT was the same between the latter two groups. Interestingly, MT did not improve from block 1 to block 4 and 6 when sequence A was performed. However, compared to blocks 1 ($p < 0.005$) and 4 ($p < 0.01$), MT became faster in block 5 where sequence B was performed. Since there was no improvement in MT on sequence A and no perturbation on MT when

sequence B was performed, sequence learning in the SRT task was primarily reflected in RT that represents mental processing rather than MT that characterizes the movement itself.

Since sequence learning was attributed to RT and not MT, further analyses were performed only on RT. Specifically, the offline and online changes in RT from block 1 to block 4 where sequence A was learned were examined. It was found that the RT offline change was not significantly affected by block and its interaction with age. However, the offline change in RT after each rest between blocks 1 and 4 significantly depended on age ($F(2,35) = 7.63$, $p < 0.005$). In particular, the offline change was greater in children younger than eight compared to older children ($p < 0.01$) and adults ($p < 0.005$). Since there was no block effect, the averaged offline change in RT after three rests from block 1 to block 4 was used to further investigate the age-related difference (Figure 5.3A). It was shown in Figure 5.3A that the offline change decreased presumably before a certain age Y and then remained the same after Y. A likelihood ratio test confirmed that fitting the data with a piecewise linear model with Y being a latent variable is superior to a linear model ($\chi^2_{df=2} = 7.7$, $p < 0.05$). This piecewise linear latent model revealed that before age $Y = 10.93$ (SE = 1.68), the offline change in RT decreased with age (slope = -20.8, $p < 0.005$) while remained constant after age $Y = 10.93$ (slope = 1.36, $p = 0.7$). In addition, the magnitude of offline change in RT was estimated to be larger than zero at age 5.39 (114.91ms, $p < 0.0001$) while it diminished to zero (-0.24ms, $p = 0.99$) at age $Y = 10.93$.
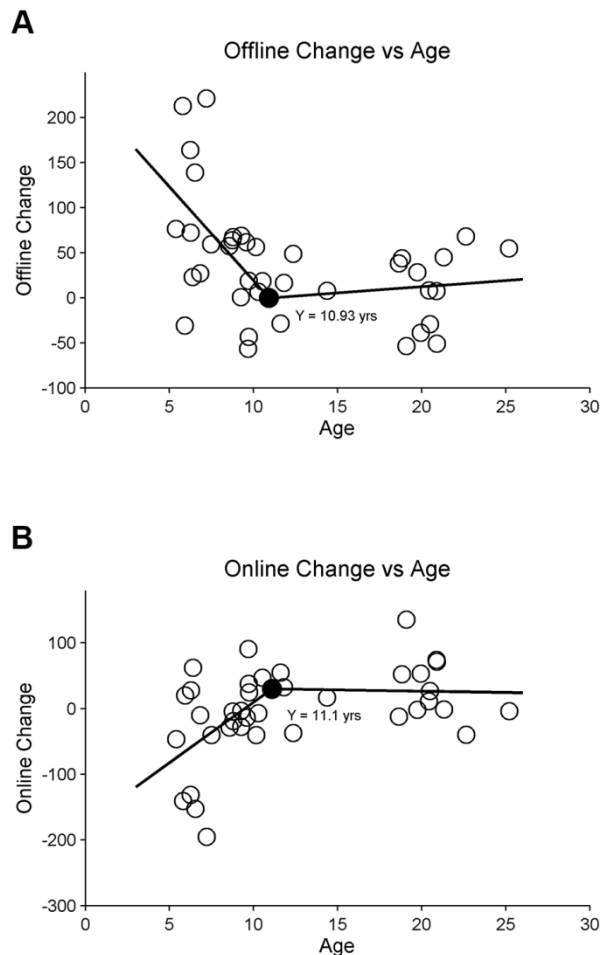
Figure 5.3: Age-related offline and online changes in RT.
(A) Offline changes in RT across age. Empty circles represent individual data. Solid circles represent the estimated age jointing two phases of offline changes development. (B) Online changes in RT across age. Empty circles represent individual data. Solid circles represent the estimated age jointing two phases of online changes development.

Similar to the offline change in RT, the amount of RT that changed online was found to depend only on age ($F(2,35) = 7.82$, p<0.005). There were no effects of block and its interaction with age. In particular, children younger than eight showed significantly less online change than children older than eight (p<0.05) and adults (p < 0.005). The mean online change in RT across block 1 to block 4 was then modeled to examine its dependence on an individual's age. The likelihood ratio test between a linear model and a piecewise latent model approaches significance ($\chi^2_{df=2} = 5.3$, p =

0.07) and thus we used the latter to model the data. It was revealed that before age Y = 11.1 (SE = 2.09), the online change in RT increased with age (slope = 18.44, p < 0.05) while remaining unchanged after age Y = 11.1 (slope = -0.4, p = 0.91). Notably, the offline change was negative at age 5.39 (-75.13ms, p <0.005), suggesting that RT in younger children became slower within blocks (Figure 5.B). Taken together, these results suggest the offline and online changes in RT were age-related. Offline change decreased while online change increased as age increased. More importantly, these age-related differences primarily took place before age 11.
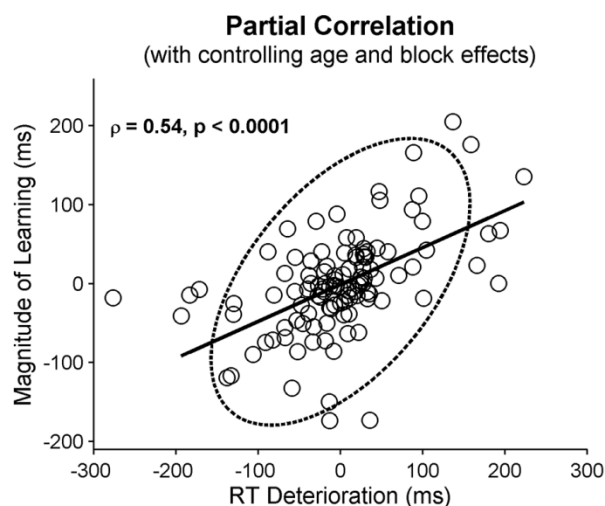
Figure 5.4: Age-related offline and online changes in RT are not caused by fatigue or reactive inhibition.
The partial correlation between RT deterioration and magnitude of learning with controlling age and block effects. Dashed circle represents the 95% confidence interval.

Although children demonstrated greater offline changes in RT, it remains unknown whether the offline enhancement resulted from inhibition/fatigue or arises from learning itself. We examined the partial correlation (controlling the age and block effect) between the RT deterioration within a block and the magnitude of learning that took place over this block (see Methods). It was found that the RT

deterioration was positively correlated to the magnitude of learning ($\rho = 0.54$, p <0.0001, Figure 5.4). This result was contrary to the reactive inhibition/fatigue hypothesis and consistent with the learning-based hypothesis, suggesting that online and offline changes in RT and their development with age are not artifacts of reactive inhibition or fatigue.

In the posttest, we found a significant effect of age group on recalling 2- ($\chi^2_{df=2} = 8.03$, p < 0.05), 3- ($\chi^2_{df=2} = 10.16$, p < 0.01), and 4-element chunks ($\chi^2_{df=2} = 9.7$, p < 0.01). Despite the length of chunks, children younger than eight had lower recall scores than children older than eight (p < 0.05 for 2-element chunk and all p <0.01 for 3- and 4-element chunks) and adults (p < 0.05 for 2-element chunk and all p <0.01 for 3- and 4-element chunks). In addition, children older than eight (p < 0.05 for 2-element chunk and p <0.0001 for 3- and 4-element chunks) and adults (p < 0.05 for 2-element chunk and p <0.0001 for 3- and 4-element chunks) had higher than chance recall for all lengths of chunks, while recall in children younger than eight was at chance for all lengths of chunks (Figure 5.5A). We further examined whether the recall score changed progressively with age (i.e., continuous age). A negative binomial regression failed to find a significant effect of age. In the recognition test, there was a significant effect of age group ($\chi^2_{df=2} = 9.53$, p < 0.01). Specifically, the recognition score was lower in children younger than eight compared to adults (p < 0.0005). Additionally, the recognition score was found to be at chance in children younger than eight but higher than chance in children older than eight (p < 0.05) and adults (p < 0.0005). It was further found that the recognition score progressively increased with age ($\chi^2_{df=1} = 4.46$, p < 0.05) (Figure 5.5B).
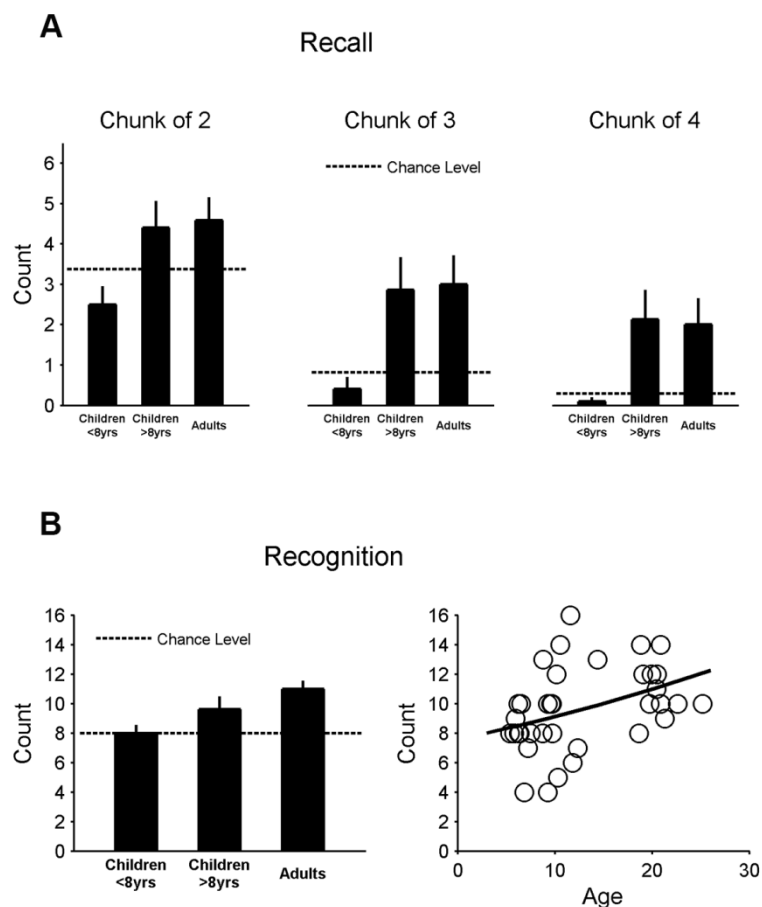
Figure 5.5: Age-related declarative learning.
(A) The recall scores. (B) The recognition score.

Discussion

Our results support the findings in **Study I** (**Chapter 3**) that implicit sequence learning over the first learning session in the SRT task was reflected by two age-related processes in reaction time (RT); offline learning that developed after rest and online learning that occurred when the task was performed. Importantly, compelling evidence enables us to conclude that such online and offline processes are not illusory effects of fatigue or reactive inhibition. Furthermore, we found that the age-related development of online and offline learning took place prior to age 11. In particular, offline learning dominated the implicit acquisition of sequences in children and the

strength of offline learning reduced as age increased to 11. In contrast, online learning was deficient in younger children, but it gradually strengthened until age 11 and remained the same after that.

The similar results that RT worsened online and improved offline in younger children (i.e., age of six) were observed in study I (Chapter 3). But it remains unknown whether the online RT deterioration and offline RT enhancement are learning effects (Du, Prashad, Schoenbrun, & Clark, 2016; Eysenck & Frith, 1977) or illusory phenomenon pertaining to fatigue or reactive inhibition (Brawn, et al., 2010; Rickard, et al., 2008a). In addition, the age-related differences may also be by-products of task constraints. By using a self-paced SRT task to greatly reduce the confounding effect of task pacing and examining the fatigue or reactive inhibition hypotheses, our study demonstrated that the age-related differences in online and offline RT changes were more likely to originate from active learning mechanisms rather than to be artifacts of task constraints or fatigue/reactive inhibition.

One difference between the current study and study I (Chapter 3) is the task pacing condition. In study I (Chapter 3), children and adults performed the task under the same inter-stimulus-interval (ISI). The same ISI appeared to be relatively shorter in children than adults. Thus, the ISI may prevent children from learning the sequence online as online learning requires iterative mental computations that need an adequate amount of time between stimuli. In addition, a shorter ISI in children made the task relatively faster, which may induce greater fatigue or reactive inhibition that slowed down the online RT. To reduce the possible task constraint effect, we used a self-paced SRT task where the ISI was determined by an individual's own response speed.

However, the online RT deterioration and remarkable offline RT enhancement persisted in children in the self-paced SRT task. Although our study did not allow us to statistically compare the magnitudes of RT deterioration under the fixed ISI and self-paced SRT task, the magnitudes were very similar (i.e., about 100ms at the age of six) between the current study and study I (Chapter 3). These results collectively suggest that the pacing condition in the SRT task is not responsible for the differences in online and offline RT changes between children and adults.

Another confounding factor underlying the online RT deterioration and offline RT improvement is the emergence of fatigue or reactive inhibition (Ammons, 1947; Bourne & Archer, 1956; Brawn, et al., 2010; Denny, et al., 1955; Rickard, et al., 2008a; Rieth, et al., 2010). According to this hypothesis, reactive inhibition, elicited by fatigue, motivation, or attentional factors, accumulates when an individual is practicing the task. This effect inhibits the expression of performance and masks the actual learning effect. The reactive inhibition effect dissipated after a rest, yielding artificial offline enhancement effect. Previous studies have suggested that fatigue or reactive inhibition could have a detrimental effect on learning (Ammons, 1947; Bourne & Archer, 1956; Denny, et al., 1955) or it could suppress the performance expression without having an effect of learning. In opposition to the reactive inhibition hypothesis, our data revealed a positive correlation between the RT deterioration and the magnitude of learning. Namely, the larger the RT deteriorated online, the greater the magnitude of learning achieved. This observation is clearly incompatible with the fatigue/reactive inhibition hypothesis and thus suggests that the online RT deterioration did not result from fatigue or reactive inhibition. That is to

say, the offline enhancement in RT was not an illusory effect caused by fatigue or reactive inhibition.

An alternative to being artifacts of task constraints and fatigue/reactive inhibition is that the age-related differences in online and offline learning may arise from certain underlying learning mechanisms. Observation from our data let us propose that the age-related differences originate from the competition between procedural and declarative learning systems (Foerde, Knowlton, & Poldrack, 2006; Poldrack, et al., 2001). In our study, procedural learning dominated in children (especially those younger than eight), as revealed by their chance-level recognition and recall scores. In contrast, adults produced higher than chance scores, suggesting that they acquired declarative knowledge of the sequence in the SRT task; results that are consistent with the literature (Meulemans, et al., 1998; Weiermann & Meier, 2012b). On one hand, the bias to declarative learning in adults may inhibit their offline learning. This inhibition effect was reduced in children as greater procedural learning was involved, yielding a stronger offline learning effect. On the other hand, the greater use of procedural learning may discourage online learning in children, which causes substantial online RT deterioration. Although our data favor the explanation that procedural and declarative learning are responsible for the age-related differences in offline and online learning, it is also plausible that online and offline learning are led by other age-related factors, such as working memory capacity and attentional capacity. Future studies are certainly awaited to examine the effects of these factors on online and offline learning.

In addition to precluding the effects of task pacing and reactive inhibition on online and offline learning, this study extended our understanding of the age-related implicit sequence learning in two other ways. First, in addition to RT, we examined MT. Our results demonstrated that the age-related difference in implicit sequence learning was attributed to the differences in RT, but not MT. Interestingly, unlike RT that was faster to the practiced sequence (sequence A) compared to the novel sequence (sequence B), MT became faster to sequence B than sequence A. Although further evidence is necessary, it is very likely that when an individual responded to sequence B, the feet were moved faster to compensate for the slower RT. Second, study I (Chapter 3) found age-related online and offline processes compared adults to children in certain age groups (10- and 6-year-olds). Here, we further found that offline and online learning developed prior to the age of 11 years. Between age 11 and 20 to 25, offline and online learning remained comparable. This age cutoff is very similar to that found in two previous studies. Implicit sequence learning has been found to be optimal before the age of 12 (Janacsek, et al., 2012a). Before this age, the acquisition of sequence learning was primarily relied on habitual learning, while learning after 12 years of age seemed to be model-based (Nemeth, Janacsek, et al., 2013b). More importantly, the habitual and model-based learning respectively are tied to procedural and declarative memory (Doll, Shohamy, & Daw, 2015), which is consistent with our finding on the age-related differences in the declarative knowledge of sequences acquired through the SRT task. One caveat in our study is that we did not include ages from 14 to 17. Although it is rather unlikely that online and offline learning would differ between this age range and 12 years of age or adults,

the developmental trajectory within this age range needs to be characterized in future studies.

Finally, it is important to emphasize that offline learning in this study occurred within the first learning session where the initial acquisition of the sequences developed. After the initial acquisition stage, learning does not stop. Instead, it continuous to develop at a slow rate and the memory of sequences is consolidated (Doyon & Benali, 2005). In the literature, offline learning has been found as a salient feature underlying sequence learning and observed during the slow learning and consolidation stages (Albouy, et al., 2006b; Brown & Robertson, 2007a; Hotermans, et al., 2006). It is unknown whether the offline learning during the initial acquisition observed in this study is related to offline learning that develops afterwards. One notable parallel between these two types of offline learning is that both of them are related to procedural and declarative memory (Brown & Robertson, 2007a, 2007b; Du, et al., 2016). To further elucidate their relationship, further studies are definitely needed.

In summary, we demonstrated age-related differences in implicit sequence learning that takes place during the first learning session in a SRT task. The age-related differences are reflected by two learning processes; offline learning where reaction time improves following rest and online learning where reaction time progressively improves during practice. The strength of offline learning declines while the strength of online learning increases with age. This development of online and offline learning is present prior to around 11 years of age. After age 11, the online and offline learning remained unchanged between children and adults. Importantly,

we excluded fatigue or reactive inhibition as primary factors underlying these two processes. Instead, we propose that memory systems of procedural and declarative learning are substrates for offline and online learning.

Chapter 6: ‡(Studies IV) Probabilistic motor sequence yields greater offline and less online learning than fixed sequence

Abstract

It is well acknowledged that motor sequences can be learned quickly through online learning. Subsequently, the initial acquisition of a motor sequence is boosted or consolidated by offline learning. However, little is known whether offline learning can drive the fast learning of motor sequences (i.e., initial sequence learning in the first training session). To examine offline learning in the fast learning stage, we asked four groups of young adults to perform the serial reaction time (SRT) task with either a fixed or probabilistic sequence and with or without preliminary knowledge of the presence of a sequence. The sequence and preliminary knowledge were manipulated to emphasize either procedural (probabilistic sequence; no preliminary knowledge) or declarative (fixed sequence; with preliminary knowledge) memory that were found to either facilitate or inhibit offline learning. In the SRT task, there were six learning blocks with a two-minute break between each consecutive block. Throughout the session, stimuli followed the same fixed or probabilistic pattern except in Block 5, in which stimuli appeared in a random order. We found that preliminary knowledge facilitated the learning of a fixed sequence, but not a probabilistic sequence. In addition to overall learning measured by the mean reaction time (RT), we examined the progressive changes in RT within and between blocks (i.e., online and offline learning, respectively). It was found that the two groups who performed the fixed sequence, regardless of preliminary knowledge, showed greater online learning than

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the other two groups who performed the probabilistic sequence. The groups who performed the probabilistic sequence, regardless of preliminary knowledge, did not display online learning, as indicated by a decline in performance within the learning blocks. However, they did demonstrate remarkably greater offline improvement in RT, which suggests that they are learning the probabilistic sequence offline. These results suggest that in the SRT task, the fast acquisition of a motor sequence is driven by concurrent online and offline learning. In addition, as the acquisition of a probabilistic sequence requires greater procedural memory compared to the acquisition of a fixed sequence, our results suggest that offline learning is more likely to take place in a procedural sequence learning task.

Introduction

In the laboratory, studies employing the serial reaction time (SRT) task (Nissen & Bullemer, 1987) have demonstrated that adults can learn a motor sequence quickly within a single training session (i.e., in 4 to 8 practice blocks) (Nissen & Bullemer, 1987; Robertson, 2007; Willingham, et al., 1989). This initial stage of motor sequence learning is referred to as fast learning that leads to the initial acquisition of sequences (Censor, et al., 2012; Dayan & Cohen, 2011; Honda, et al., 1998; Karni, et al., 1998a; Walker, et al., 2002). Fast learning develops over the course of a single training session, where an individual practices a new motor sequence and demonstrates considerable performance improvement. It has been suggested that such improvement in the performance of motor sequences are driven by online learning (Bornstein & Daw, 2012, 2013; Verstynen, et al., 2012), where performance progressively improves as the task is practiced. After the fast learning

stage, performance is strengthened without further practice (i.e., offline learning) by an early offline boost (Hotermans, et al., 2006; Schmitz, et al., 2009) or memory consolidation (Brown & Robertson, 2007a; Nettersheim, et al., 2015; Robertson, Pascual-Leone, & Press, 2004; Robertson, et al., 2005). To date, it is unclear whether offline learning drives the acquisition of motor sequence in the fast learning stage. The purpose of this study, therefore, is to examine whether fast learning of a motor sequence arises from offline learning. Furthermore, given that offline learning in the SRT task has been found to be associated with procedural memory (Brown & Robertson, 2007a, 2007b; Robertson, Pascual-Leone, & Miall, 2004), we further investigate whether a bias towards procedural or declarative memory in the SRT task modulates offline and online sequence learning.

Learning motor sequences in the SRT tasks typically involves both procedural and declarative memory (Brown & Robertson, 2007a; Curran & Keele, 1993; Destrebecqz & Cleeremans, 2001; Reber & Squire, 1994; Robertson, 2007; Willingham & Goedert-Eschmann, 1999; Willingham, et al., 1989). In this task, participants press keys on the keyboard to respond to sequential visual stimuli that are presented in a pattern (e.g., a fixed order). Since participants are not informed of the presence of the sequence, learning in the SRT task requires procedural memory. However, participants may recognize the presence of the sequence after they perform the task and thus form a declarative memory of the sequence (Perruchet, Bigand, & BenoitGouin, 1997; Willingham & Goedert-Eschmann, 1999). This entanglement of procedural and declarative learning suggests the infeasibility of eliminating or isolating either of them from the SRT task. Nonetheless, manipulating the sequence

type and the preliminary knowledge of the sequence can modulate procedural or declarative learning. Particularly, it has been shown that learning a probabilistic sequence favors more procedural memory compared to learning a fixed sequence (Jimenez, et al., 1996; Song, Howard, & Howard, 2007a). In contrast, preliminary knowledge of the sequence facilitates declarative learning (Curran, 1997a; Curran & Keele, 1993; Destrebecqz, 2004).

In this study, we bias the involvement of procedural/declarative memory by manipulating the sequence type and preliminary knowledge of the sequence in the SRT task to examine whether offline or online learning mediate the acquisition of motor sequences in the fast learning stage. Before the experiment, we informed half of the participants that the visual stimuli followed a specific pattern, but no further information was provided about the sequence. No information about the presence of a sequence was provided to the other participants. The participants were further divided into two groups. In one group, the visual stimuli followed a fixed sequence (i.e., ten repetitions of a 12-trial sequence) while in the other group; the visual stimuli followed a probabilistic sequence that was generated by a first-order Markov process. We found that a motor sequence is learned quickly through concurrent online and offline learning. However, the involvement of procedural or declarative memory mediated the use of online and offline learning. Particularly, learning of a fixed sequence arose from greater online learning. In contrast, acquisition of a probabilistic sequence resulted from significant offline learning, regardless of preliminary knowledge. These results suggest that the involvement of procedural and declarative memory modulates how a motor sequence is learned in the fast learning stage.

Materials and Methods

This study was carried out in accordance with the recommendations and approval of the Institutional Review Board at the University of Maryland, College Park. All participants signed consent forms prior to their participation. Each participant received $10 after the completion of the experiment.

Participants

Forty-eight right-handed adults (24 males, see Table 1) were randomly assigned to one of four groups: fixed sequence with preliminary knowledge of the sequence (PK_Fixed; mean age: 21.8 ± 1.91), fixed sequence without preliminary knowledge of the sequence (NPK_Fixed; mean age: 21.5 ± 1.41), probabilistic sequence with preliminary knowledge of the sequence (PK_Prob; mean age: 21.2 ± 0.893), and probabilistic sequence without preliminary knowledge of the sequence (NPK_Prob; mean age; 21.3 ± 0.830). All participants completed a health questionnaire to exclude those with any neurological and motor impairments ,the Edinburgh Handedness Inventory (Oldfield, 1971) to assess that participants were right-handed, and the Global Physical Activity Questionnaire (Armstrong & Bull, 2006) to insure that groups did not differ in their level of physical activity.

Serial reaction time task

Participants were seated in front of a computer monitor (19") and keyboard. Participants placed the middle finger of their left hand on the keyboard's 'D' key, the index finger of their left hand on the 'F' key, the index finger of their right hand on the 'J' key, and the middle finger of their right hand on the 'K' key (see Figure 1A).

At the beginning of each trial, a mouse appeared in one of four squares on the screen and the participant pressed the key that corresponded to the location of the stimulus. After the participant pressed a key, the next stimulus appeared after an interval of 300ms. No visual feedback was provided to participants and a wooden board blocked vision of their finger position. Participants were first randomly assigned to either the preliminary knowledge group or no preliminary knowledge group and were further randomly assigned to either the fixed or probabilistic sequence. The probabilistic sequence was created based on a Markov chain transitional matrix with probabilities associated with each stimulus (Figure 6.1C & 6.1D). The probabilistic sequence was constrained such that the same stimuli were not repeated one after the other and that each stimulus appeared an equal number of times in each block.

There were a total of six blocks for all groups (see Figure 6.1B), each consisting of 120 trials. Prior to the first block, participants practiced a random sequence. These initial trials were included to ensure that participants were able to accurately associate each finger with a corresponding key before the experimental practice blocks commenced. That is, we observed that participants did not produce reaction times (RT: amount of time taken to press the corresponding button after the stimulus was presented) that were slower than 2000m because of incorrect key pressing. After the practice block, participants in the preliminary knowledge groups were informed that a sequence would be present in the subsequent blocks and that they should look for the sequence. No other information about the nature of the sequence was provided. The first four blocks (Blocks 1-4) were the learning blocks consisting of the 120-trial probabilistic sequence or the fixed sequence in which the

sequence was repeated 10 times in each block. Block 5 consisted of 120 trials of stimuli occurring in a random order and block 6 consisted of the assigned probabilistic or fixed sequence (Figure 6.1C & 6.1D). Participants were given a two-minute mandatory break between each block. The participants' RT was recorded for each trial.

All participants completed a posttest after the completion of the six blocks to determine the amount of declarative knowledge of the sequence. Participants were first asked to recall the sequence and attempted to write down the 12 items of the sequence and rated how confident they were that the sequence they wrote was correct. Participants were then asked to complete a recognition task. They were given eight chunks (i.e., four three-element and four four-element chunks where two of each were correct) and were asked to choose the chunks they thought were included in the sequence.

Table 1: Participant demographic information

Group	Age[#] (years)	Sex
PK_Fixed	21.8 ± 1.91	6 female; 6 male
NPK_Fixed	21.5 ± 1.41	6 female; 6 male
PK_Prob	21.2 ± 0.893	6 female; 6 male
NPK_Prob	21.3 ± 0.830	6 female; 6 male

[#] There were no significant differences between the groups in age, $F(3,47) = 0.564$, $p = 0.642$.
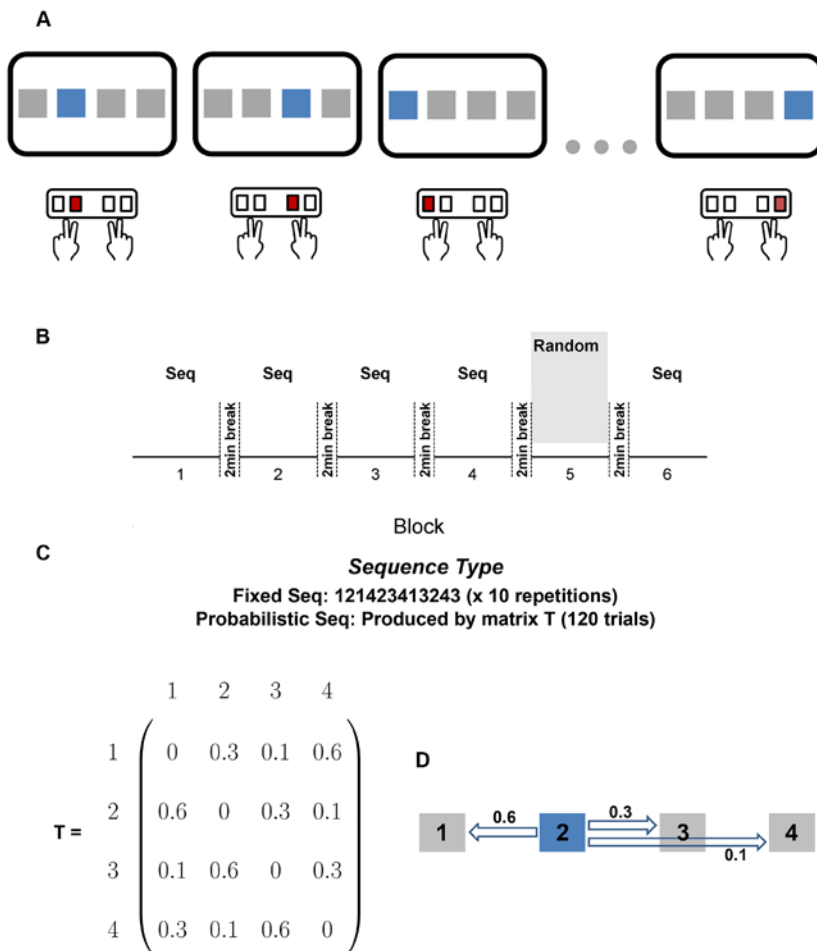
Figure 6.1: Experimental setup and procedure.
A) Experimental setup. At the beginning of each trial, a stimulus appeared in one of four squares on the screen and the participant pressed the key that corresponded to the location of the stimulus. Participants placed the middle finger of their left hand on the keyboard's 'D' key, the index finger of their left hand on the 'F' key, the index finger of their right hand on the 'J' key, and the middle finger of their right hand on the 'K' key. B) Experimental paradigm. Participants performed the learning blocks (blocks 1-4) with either the fixed or probabilistic sequence, followed by randomly ordered stimuli in block 5, and ended with the same sequence in block 6. All blocks consisted of 120 trials. Participants were given a mandatory 2 minute break between each block. C) Sequence Types. Participants were randomly assigned to the fixed sequence group or the probabilistic sequence group. The probabilistic sequence was created using the probabilities defined in the transitional matrix, T. D) Example of how the probabilistic sequence was created using matrix T. If the current stimulus is 2, there is a probability of 0.6 that the next stimulus will be 1, a 0.3 probability that the next stimulus will be 3, and a 0.1 probability that the next stimulus will be 4.

Data analysis

The RTs were trimmed according to the individual participant's mean and standard deviation. Within each block for an individual participant, any RT greater or

less than 2.5 standard deviations was excluded from the analysis (Ratcliff, 1993; Whelan, 2008). Mean RTs were calculated for each block and were averaged across participants in each group. Learning was measured through a decrease in RT from block 1 to block 4 (stimuli in assigned sequence) and an increase in RT from block 4 (stimuli in assigned sequence) to block 5 (stimuli in random order). Online learning was defined as the amount of learning within a block and was determined by performing a linear regression on the 120 RTs within a block. Offline learning was computed as the RT change after a short break without performing the task. Given that the fixed sequence consisted of 10 repetitions of a 12-item long sequence, the difference between mean RT of the last 12 taps in one block and that of the first 12 taps in the succeeding block was used to quantify offline learning. In addition, since participants typically acquire the sequence transitions of higher probabilities in probabilistic sequence learning (Bornstein & Daw, 2012; Howard, Howard, Dennis, Yankovich, & Vaidya, 2004; Hunt & Aslin, 2001), we expect that participants in the two probabilistic sequence groups would only learn sequential stimuli that were associated with transitional probabilities of 0.3 and 0.6 and fail to learn those associated with transitional probability of 0.1. Thus, we computed mean RT, offline- and online-learning of stimuli with transitional probabilities of 0.3 and 0.6 in the two probabilistic sequence groups.

A controversy regarding offline improvement in RT is whether the improvement results from reactive inhibition/fatigue (Brawn, et al., 2010; Rickard, et al., 2008a) or it is driven by active learning mechanisms (i.e., offline learning) (Eysenck & Frith, 1977; Robertson, Pascual-Leone, & Miall, 2004). According to

Eysenck and Frith (1977), in the case of reactive inhibition/fatigue-induced offline improvement, post-rest performance should return to the starting performance level before the rest or so called pre-rest performance, but without improvement over that level. In contrast, post-rest performance is superior to the pre-rest performance if offline improvement arises from offline learning. Given that RT increased (i.e., became slower) within blocks in some participants so that the mean RT of the last 12 taps did reflect the pre-rest performance, we calculated corrected offline learning. Specifically, if RT increased (i.e., became slower) within the previous block, corrected offline learning was calculated by subtracting the amount of RT deterioration (i.e., negative online learning) within the previous block from the amount of offline learning so that the corrected offline learning reflects the difference between the pre-rest and post-rest performance. If RT improved (i.e., became faster) within the previous block, indicating no RT deterioration, corrected offline learning was the same as offline learning, computed as the difference between mean RT of the last 12 taps in the block and that of the first 12 taps in the succeeding block. We expect that all groups should exhibit the same amount of corrected offline learning (none), if offline improvement in RT observed in this study were caused by reactive inhibition or fatigue.

To measure the amount of declarative knowledge of the sequence, we calculated the recognition score as the number of correct chunks that participants chose in the recognition task. The recognition score was normalized by four as there were four correct chunks. To compare the recall score among participants, we calculated the number of three-element chunks that participants could recall. Given

there were 12 three-element chunks in the fixed sequence, the number that a participant recalled was normalized by 12 to compute a percentage. To make the amount of declarative knowledge between probabilistic and fixed sequences comparable, the number of three-element chunks that participants could recall was also used in the two groups who performed the probabilistic sequence. Since participants only learned the stimulus transition with transitional probabilities of 0.3 and 0.6 (see results for details), there were 16 three-element chunks in the probabilistic sequence. Thus, the percent of recalled chunks was normalized by 16 in the two probabilistic sequence groups. Importantly, the chance level for guessing differed between the fixed and probabilistic sequence. Specifically, the chance level for a three-element chunk in the fixed sequence was 18.75% (i.e., given the first element, 75% chance for the second element and 25% chance for the third element) while it was 25% for a three-element chunk in the probabilistic sequence (i.e., given the first element, 50% chance for the second and third elements), we corrected the percentage of recalled chunks by the chance level specific to each sequence group.

Statistical analysis

A three-way (block × knowledge × sequence) repeated measures analysis of variance (ANOVA) was used to compare differences in RT between the blocks and groups. Separate pairwise comparisons were conducted on the priori contrasts of interest (block 1 vs. block 4 and block 4 vs. block 5) to determine any significant differences between the sequenced blocks and the random block. A three-way (block × knowledge × probability) ANOVA was used to compare differences in RT of stimuli with different probabilities in the two probabilistic groups. All repeated

measures ANOVAs were performed in SAS with the MIXED procedure. Thus, the co-variance matrix structures were determined by the Akaike information criterion (AIC). A two-way (knowledge × sequence) ANOVA was employed to examine the effects of preliminary knowledge and sequence type on online, offline learning, and corrected offline learning. A two-way (knowledge × sequence) ANOVA was employed to examine the effects of preliminary knowledge and sequence type on the recall score. Given the violation of the normality assumption, the effects of preliminary knowledge and sequence type on the recognition score was examined by the Scheirer-Ray-Hare test. Tukey-Kramer post hoc tests were used to decompose any significant effects. Student's t-tests/Wilcoxon tests were used to examine whether recall/recognition scores were different from the corresponding chance level for each group. The statistical significance level was set as $\alpha = 0.05$.

Results

Figure 6.2A shows the mean RT across the six blocks. The repeated measures ANOVA reveals a significant interaction between preliminary knowledge, sequence type, and block ($F(5,44) = 2.79$, $p<0.05$). *Post hoc* analyses with the Tukey-Kramer correction found that all four groups produced comparable RTs in all blocks (all $p >0.2$). However, RT in two groups who performed the fixed sequences (i.e., PK_Fixed and NPK_Fixed) improved from blocks 1 to 4 and 6 (all $p <0.0001$). In contrast, RT remained the same from block 1 to 4 in the other two probability sequence groups (i.e., PK_Prob and NPK_Prob, Figure 6.2B) (all $p > 0.1$). Nevertheless, RT was faster in block 6 compared to block 1 in the NPK_Prob group ($p<0.01$) and this improvement approached significance in the PK_Prob group

(p=0.09). In addition, when a random sequence was introduced in block 5, RT in the PK_Fixed and NPK_Fixed groups deteriorated (both p <0.0001) while it remained the same between blocks 4 and 5 in the PK_Prob and NPK_Prob groups (both p = 1) (Figure 6.2C).

The inferior learning in the probabilistic sequence (as expressed in no change in RT from block 1 to 4 and between blocks 4 and 5) is consistent with the hypothesis that probabilistic sequences are harder to learn compared to fixed sequences (Schvaneveldt & Gomez, 1998). However, given our hypothesis that participants typically acquire the sequence transitions of higher probabilities (Bornstein & Daw, 2012; Howard, Howard, Dennis, et al., 2004; Hunt & Aslin, 2001), the marginal learning effect on the probabilistic sequence likely resulted from the difference in RT among stimuli with different transitional probabilities (Figure 6.1C). Thus, we compared RTs between these stimuli (Figure 6.2D) in the probabilistic sequence. A three-way (block × knowledge × probability) repeated measures ANOVA found that preliminary knowledge does not significantly affect RT and there was a significant interaction between block and probability ($F(10,220) = 17.07$, $p<0.0001$). *Post hoc* analyses with the Tukey-Kramer correction revealed that RTs of stimuli with a transitional probability of 0.1 were comparable to that of stimuli with transitional probability of 0.3, while RTs of stimuli with transitional probability of 0.3 were slower than that of probability of 0.6 ($p<0.01$). However, as learning progressed, RTs of stimuli with a transitional probability of 0.1 remained the same. In contrast, RTs improved from blocks 1 to 4 in stimuli with higher transitional probabilities 0.3 ($p<0.01$) and 0.6 ($p<0.0001$), suggesting learning of these higher transitional

probabilities (Figure 6.2E). Additionally, introduction of a random sequence in block 5 did not impair RT of stimuli with transitional probabilities of 0.1 and 0.3, but RTs of stimuli with a transitional probability of 0.6 deteriorated in block 5 ($p<0.0001$) (Figure 6.2F). These results confirm that the participants learned stimulus transitions with higher probabilities, specifically 0.6 and perhaps 0.3.
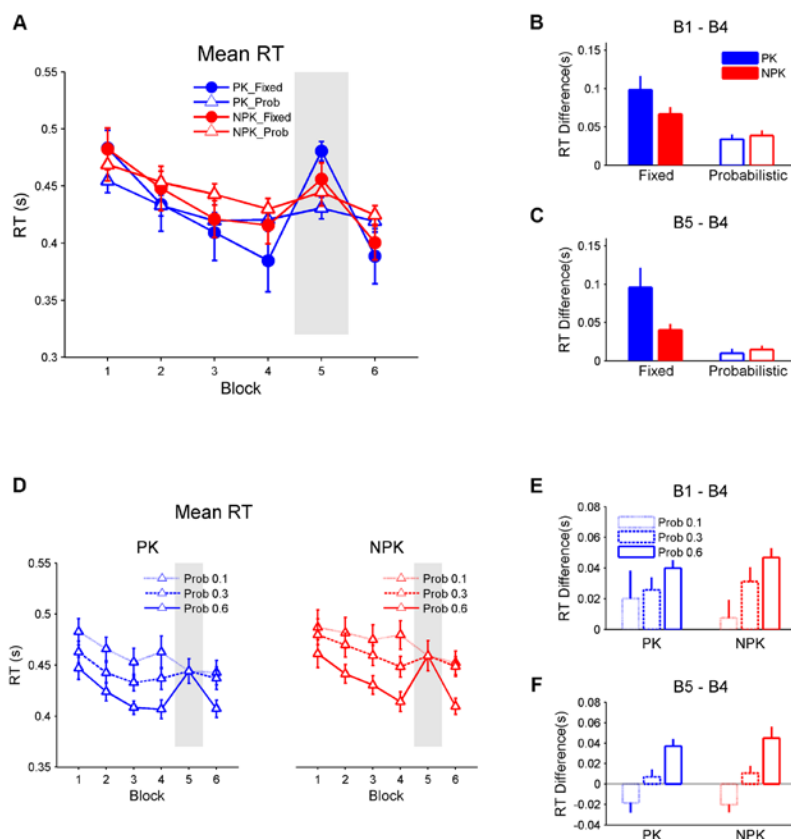
Figure 6.2: Sequence learning of the probabilistic sequence depended on the transitional probability of stimuli.
A) Mean RT and SE bars across the six blocks for all four groups. B) Difference between the RT in block 1 and block 4 to assess whether sequence learning occurred. C) Difference between block 5 and block 4 to assess whether RT increases in block 5 when a random sequence is presented. D) Mean RT and SE bars across the six blocks for only the probabilistic sequence in which the three transitional probabilities (Pro 0.1, Pro 0.3, and Pro 0.6) have been extracted and plotted separately. E) Difference between RT in block 1 and block 4. F) in block 5 and block 4 separated for the 3 transitional probabilities in the probabilistic sequence. PK = preliminary knowledge, NPK = no preliminary knowledge, RT = reaction time, SE = standard error.

Since participants only learned higher transitional probabilities when stimuli followed a probabilistic pattern, we re-compared the learning effects among groups by using RT for stimuli with transitional probabilities 0.3 and 0.6 in PK_Prob and NPK_Prob groups. A repeated measures ANOVA revealed a significant interaction among the effects of block, preliminary knowledge, and sequence ($F(5,44)=3.1$, $p <0.05$). Tukey-Kramer-corrected *post hoc* analyses suggest that all groups had comparable mean RTs across all blocks (Figure 6.3A). In addition, all groups demonstrated improved mean RT from block 1 to 4 (all $p<0.0001$) and deteriorated mean RT from block 4 to 5 (all $p<0.005$). However, contrast analyses showed that the PK_Fixed group had the greatest change in RT from block 1 to 4 compared to the NPK_Fixed ($p<0.05$), PK_Prob ($p<0.0005$), and NPK_Prob groups ($p<0.0005$) (Figure 6.3B), while the latter three groups exhibited the same change in RT. Similarly, the RT change from block 4 to 5 was greater in the PK_Fixed group compared to the other three groups (all $p<0.01$) who had the same RT change (Figure 6.3C). These results suggest that the PK_Fixed group learned better than the other three groups.

Although participants learned either fixed or probabilistic sequences with or without preliminary knowledge of the sequence, learning across trials exhibited different patterns (Figure 6.4A). Specifically, learning of a fixed sequence exhibits decreased RT within blocks while learning of a probabilistic sequence exhibits reduced RT after rest without practice. A two-way (knowledge × sequence) ANOVA found a significant effect of sequence on offline learning ($F(1,44) = 8.84$, $p<0.005$). Particularly, the acquisition of the probabilistic sequence arises from greater offline

learning compared to the acquisition of the fixed sequence (Figure 6.4B). Although sequence type was also found to significantly affect online learning (F(1,44) = 18.72, $p<0.0001$), it was shown that greater online learning was produced when a fixed sequence was learned (Figure 6.4B). Interestingly, when learning a probabilistic sequence, participants did not exhibit online learning. Instead, RT became slower within blocks. We further compared whether online or offline learning contributed more to the acquisition of a motor sequence. A two-way (knowledge × sequence) ANOVA on the RT difference between offline and online learning revealed a significant effect of sequence type (F(1,44)=15.27, $p<0.0005$). Student's t-tests found equal online and offline learning when a fixed sequence is performed ($p=0.59$), while greater offline compared to online learning was found when a probabilistic sequence was performed ($p<0.0001$).
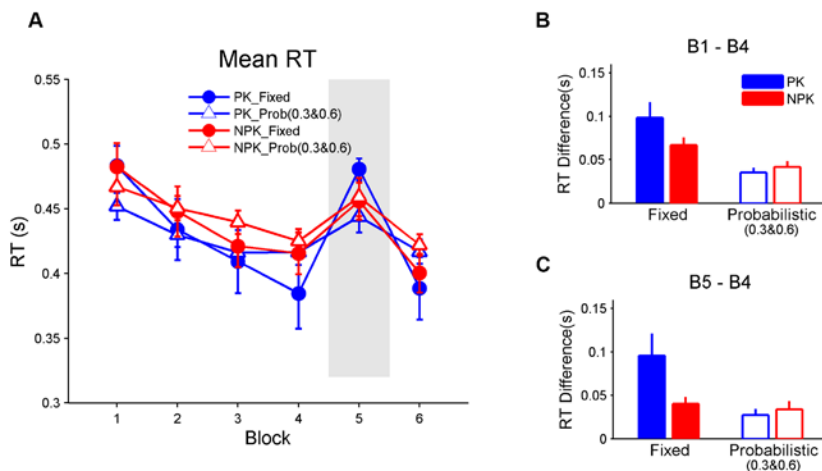
Figure 6.3: Sequence learning is comparable despite the sequence structure and preliminary knowledge.
Mean RT and SE bars to assess learning. Only the RT of stimuli with transitional probabilities of 0.3 and 0.6 were extracted and are shown for the probabilistic sequences. A) Mean RT across the six blocks. B) Difference between the RT in block 1 and block 4 to assess whether sequence learning occurred. C) Difference between block 5 and block 4 to assess whether RT increases in block 5 when a random sequence is presented. PK = preliminary knowledge, NPK = no preliminary knowledge, RT = reaction time, SE = standard error.
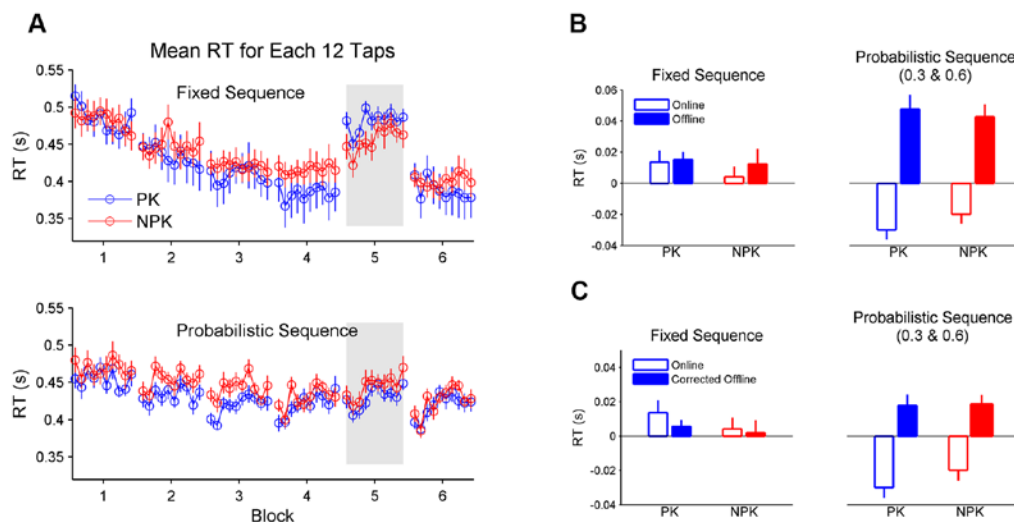
Figure 6.4: Greater offline learning to acquire the probabilistic sequence.
A) Mean RT of each 12 taps to reflect online and offline learning. B) Comparison of online and offline learning between the groups. C) Comparison of online and corrected offline learning between the groups. Error bars represent standard errors. PK = preliminary knowledge, NPK = no preliminary knowledge, RT = reaction time.

We also analyzed the corrected offline learning. The same results were found compared to the original offline learning data (Figure 6.4C). A two-way (knowledge × sequence) ANOVA found a significant effect of sequence ($F(1,44) = 4.99$, $p < 0.05$). Specifically, there was greater corrected offline learning in PK_Prob and NPK_Prob groups compared to PK_Fixed and NPK_Fixed groups. These results suggest that offline learning rather than reactive inhibition/fatigue underlies the offline improvement in RT.

In the posttest, we found that the recognition score did not differ from chance (i.e., 50%) in all four groups and there were no effects of sequence type and preliminary knowledge on the scores. Figure 6.5A shows the percentage of recalled three-element chunks. It is clear that participants in the fixed sequence groups had higher than chance recall, while recall was at chance in the two probabilistic sequence groups. The corrected percentage according to the chance level was shown in Figure

6.5B. A two-way ANOVA found a significant effect of sequence type ($F(1,44)=6.75$, $p<0.01$). Specifically, recall of the fixed sequence was superior compared to that of probabilistic sequence. In addition, using Student's t-tests with an adjusted p level of $\frac{\alpha}{4} = 0.0125$ to control the familywise error rate for the four simultaneous t-tests, the recall in the PK_Fixed was significantly higher than chance level ($p<0.0001$) The recall in the NPK_Fixed did not differ from chance (but approached significance, $p=0.0146$), In contrast, recall in the two groups that performed the probabilistic sequence (i.e., PK_Prob and NPK_Prob) was not significantly different from the chance level (both $p>0.2$)
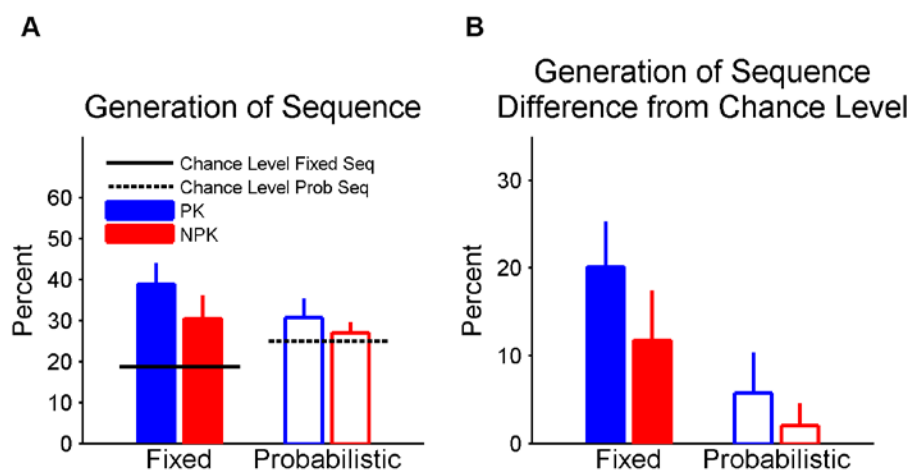
Figure 6.5: Less declarative knowledge of the probabilistic sequence was acquired.
A) Percentage of three-element chunks recalled in the posttest. B) Corrected percentage according to chance level. Error bars represent standard errors. PK = preliminary knowledge, NPK = no preliminary knowledge.

Discussion

In this study, we demonstrated that both fixed and probabilistic motor sequences can be learned quickly (i.e., in one training session). Further, this initial acquisition of a fixed sequence in the fast learning stage arises from both online and offline learning, while acquisition of a probabilistic sequence is driven predominantly by offline learning. Given that learning a probabilistic or fixed sequence requires

greater procedural or declarative memory, respectively, our results suggest that a bias toward procedural or declarative memory modulates how a motor sequence is learned in the fast learning stage.

Offline learning, as a salient feature underlying motor sequence learning (Robertson, Pascual-Leone, & Miall, 2004), can boost the memory of a newly acquired sequence 5 – 30 minutes after the initial acquisition (Albouy, et al., 2006b; Hotermans, et al., 2008; Hotermans, et al., 2006; Nettersheim, et al., 2015; Schmitz, et al., 2009) or consolidate the memory a few hours later without sleep (Brown & Robertson, 2007a, 2007b; Robertson, Pascual-Leone, & Miall, 2004) or after sleep (Censor, et al., 2012; Nettersheim, et al., 2015; Robertson, Pascual-Leone, & Press, 2004; Walker, et al., 2002). Thus, offline learning has been widely considered to occur only after the initial acquisition of sequences that develops over the course of a single training session, referred to as fast learning (Censor, et al., 2012; Dayan & Cohen, 2011; Honda, et al., 1998; Karni, et al., 1998a; Walker, et al., 2002). Unlike the widely-found offline learning that occurs following the fast learning stage, we observed offline learning that drives the fast acquisition of sequences within a first single training session. This result suggests that in addition to online learning (Bornstein & Daw, 2012; Cleeremans & McClelland, 1991), offline learning also contributes to rapid improvements in performance that allow sequences to be learned quickly in a single training session.

The concurrent effect of online and offline learning could be modulated by the involvement of declarative and procedural memory. It is widely accepted that both memory systems cooperate and compete during motor sequence learning (Brown &

Robertson, 2007b; Meulemans, et al., 1998). Remarkably, the presence of declarative memory inhibits offline learning of procedural memory and thus disruption of declarative memory induces offline improvement in procedural skills four hours after the initial acquisition (Brown & Robertson, 2007a). In our study, similar effects of declarative and procedural memory were observed on offline learning in the fast learning stage. The recognition and recall tests were used to measure the engagement of declarative and procedural memory in the SRT task. Although the recognition test shows no differences in the amount of declarative knowledge acquired by participants regardless of the sequence type and preliminary knowledge (see details below), the recall scores reveal that, participants acquired less declarative knowledge of the probabilistic sequence. Notably, participants exhibited greater offline learning when performing probabilistic sequences, suggesting that the offline learning in the fast learning stage was strengthened when greater procedural memory and less declarative memory were required to learn the motor sequences. On the other hand, when greater declarative memory was involved in learning fixed sequences, as indicated by higher recall scores, reduced offline and greater online learning were observed. This inverse relationship between online and offline learning confirms the inhibition effect of declarative memory on offline learning. More importantly, our finding extends our understanding of the competition between multiple memory systems. That is, unlike previous studies that demonstrated this competition after skills are acquired (Brown & Robertson, 2007b; Foerde, et al., 2006; Poldrack, et al., 2001), we demonstrated that the competition begins as soon as learning starts and that declarative and procedural memory may be identified by their distinct behavioral expressions.

The offline learning observed within a single training session (i.e., the fast learning stage) is associated with procedural memory as is offline learning that takes place hours after the initial acquisition and is responsible for memory consolidation. However, it remains unclear whether this offline learning that allows fast initial acquisition of a motor sequence is related to offline learning that consolidates the memory of a newly acquired sequence. It is possible that offline learning that drives the fast acquisition is a precursor of the later occurring memory consolidation, or they may be the same process. To elucidate their relationship, further systematic investigations are needed.

A debate within the offline learning literature is whether offline improvement in performance after rest, referred to as reminiscence (Eysenck & Frith, 1977), results from fatigue or reactive inhibition (Brawn, et al., 2010; Rickard, et al., 2008a) or an active learning mechanism (Eysenck & Frith, 1977; Robertson, Pascual-Leone, & Miall, 2004). It has been suggested that offline learning and reactive inhibition/fatigue are usually combined to lead to reminiscence (Eysenck, 1965), thus making it difficult to determine if reactive inhibition/fatigue is a potential cause of reminiscence. However, observations from our data favor offline learning to reactive inhibition/fatigue as the primary mechanism underlying offline improvement in reaction time or reminiscence observed in the SRT task. Specifically, with the same amount of practice, only participants who performed the probabilistic sequence slowed down their reaction time, while such "fatigue" was not observed when participants performed a fixed sequence. In addition, if fatigue appeared as soon as participants in the probabilistic sequence groups started to perform the task, it would

be unlikely that their learning would arise quickly (i.e., over four learning blocks) and to a comparable level as the participants in the fixed sequence groups who did not exhibit fatigue. Moreover, according to Eysenck and Frith (1977), reminiscence is task-specific. For example, reminiscence that results from reactive inhibition or fatigue usually occurs in a task that does not involve learning, where performance on the task is already perfect when an individual starts to perform the task. In contrast, reminiscence that arises from offline learning usually takes place in a learning task. Obviously, the SRT task involves sequence learning and our data demonstrated that participants learned the sequence. Further evidence supporting offline learning rather than reactive inhibition or fatigue comes from the observation on corrected offline learning. In the probabilistic sequence groups, performance after the short break is superior to the best performance level before the break. Therefore, without fully excluding the effect of reactive inhibition/fatigue, our results favor the statement that the offline improvement in reaction time is driven by offline learning rather than reactive inhibition or fatigue. Meanwhile, we suggest that it is necessary to systematically examine the reactive inhibition or fatigue effects in future sequence learning studies.

Although it appears that offline learning rather than reactive inhibition or fatigue is the primary mechanism underlying the offline improvement in reaction time, the cause of increased reaction time when learning a probabilistic sequence is unclear. One likely reason is the interference of stimuli transitions with a probability of 0.1. It has been found that adults learned a sequence by iteratively updating the internal model of the motor sequence (Bornstein & Daw, 2012, 2013; Cleeremans &

McClelland, 1991; Verstynen, et al., 2012) and our data provide consistent evidence that participants acquired the stimulus transitions with probabilities of 0.3 and 0.6. However, the introduction of stimulus transition governed by a probability of 0.1 may mislead the updating of the internal model (i.e., transitional probability matrix) and thus impair reaction time when the probabilistic sequence was performed.

In addition to the primary findings on online and offline learning, our results provide insights into the learning of probabilistic sequences. Sequence structure plays a critical role in motor sequence learning (Bennett, Howard, & Howard, 2007; Curran & Keele, 1993; Jimenez, et al., 1996; Song, et al., 2007a). To date, a variety of probabilistic sequences have been used in the SRT task, but only a few studies have employed probabilistic sequences that represent the stochastically related events of daily life, such as sequences produced by a finite state grammar (Jimenez, et al., 1996) or a Markov chain. We found that participants acquired stimulus transitions with higher probabilities of 0.3 and 0.6 and the learning of these higher stimulus transitions was comparable to that of the fixed sequence. Moreover, the facilitating effect of preliminary knowledge of a sequence depends on the sequence structure, which is consistent with previous studies (Jimenez, et al., 1996; Stefaniak, Willems, Adam, & Meulemans, 2008). Specifically, preliminary knowledge only facilitates the learning of a simple sequence, such a fixed sequence (Curran, 1997a; Curran & Keele, 1993; Destrebecqz, 2004; Frensch & Miner, 1994; Stefaniak, et al., 2008) and not a sequence with a complex structure.

Finally, one caveat worthy of further study is the measurement of the amount of declarative knowledge. Both recognition and recall tests are most widely used to

examine procedural learning in the SRT task (Destrebecqz & Peigneux, 2005; Shanks & Johnstone, 1999; Wilkinson & Shanks, 2004). In particular, these tests examine whether participants can explicitly recollect the acquired sequence knowledge. However, results from the recognition tests are equivocal in the literature (Perruchet & Amorim, 1992; Reed & Johnson, 1994b; Shanks & Johnstone, 1999; Willingham, Greeley, & Bardone, 1993). Similarly in our study, unlike the recall tests demonstrating the common finding that probabilistic sequence learning favors more procedural memory (Jimenez, et al., 1996; Song, et al., 2007a), the recognition tests reveals no difference in the amount of acquired declarative knowledge despite the sequence type and preliminary knowledge. In addition, the recognition scores in all four groups were not greater than chance. Given that in the recognition test, participants were presented with sequence segments and were asked to determine whether these segments are from the sequence they learned or a new sequence they did not see in the SRT task, it is hard to know whether the chance-level score was due to the participant's inability to explicitly recollect sequence knowledge or that the participant did not learn some segments of the sequence. These two possibilities that may simultaneously account for the chance-level recognition must be addressed by other tests in future studies. Moreover, in our study, only four correct sequence segments were given to participants, while there were more than 10 segments within the learned sequence, the chance-level recognition score was caused possibly because some participants may learn segments other than the four displayed in the recognition test.

In summary, we found that concurrent online and offline learning allows motor sequences to be acquired quickly in the fast learning stage and can be identified by their manifestations in the progressive changes in reaction time. Remarkably, online and offline learning can be mediated by the declarative and procedural memory that are required to learn motor sequences. In addition, the modulation of online and offline learning may reflect the competition between both memory systems during motor sequence learning that begins in the fast learning stage. How the offline learning that drives the initial acquisition of sequences is related to the offline learning that is responsible for memory consolidation occurring hours after the initial acquisition remains to be investigated.

Chapter 7: (Studies IV and V) Generalization of motor sequence learning between children and adults

Abstract

Generation of motor sequence learning is critical in daily life; it allows the flexibility to extend what has been learned in one context to a novel context, which considerably reduces the time to acquire a new motor sequence. It is well known that sequence learning can generalize between effectors and converging evidence has shown that such generalizations are facilitated by fast learning where the memory of sequences is encoded. Compared to the great attention given to the generalization of sequence learning between effectors, little is known about whether learning of one sequence can generalize to another. Here, we asked children and adults to learn sequences in the SRT task and examined whether they are able to generalize the learning of one sequence to a novel sequence that shares the same probabilistic structure with the learned sequence. Given that fast learning in children and adults are driven by two different age-related processes (i.e., online and offline learning), we expect the age-related differences in the generalization of sequence learning. Our results confirmed the hypothesis. Learning in adults primarily relied on online learning and did not generalize to a novel sequence. In contrast, learning sequences in children was driven by offline learning and the learning generalized to a novel sequence. When the learning processes in adults were biased toward offline learning, we surprisingly found that the age-related differences in the learning generalization vanished. Sequence learning in both adults and children are able to generalize and at a comparable level. These results suggest that the generalization of implicit motor sequence learning is age-related and such differences may result from the

involvement of online and offline learning that drive the initial acquisition of sequences.

Introduction

Children and adults learn a sequence quickly, usually over a course of one learning session consisting over 4-8 learning blocks. However, the memory of the newly acquired sequence is fragile. Slow learning and consolidation subsequently occur between multiple learning sessions to stabilize and boost the newly acquired memory. Thus, learning a sequence takes a considerable amount of time. Given there is an enormous amount of sequential actions to perform in daily life, it is certainly beneficial to be able to generalize the learning of a sequence in one context to another novel context, typically referred to as generalization (or transfer) of learning. Generalization, as an indicator of flexibility of sequence learning, has been widely examined through inter-manual transfer (Censor, 2013; Grafton, Hazeltine, & Ivry, 2002). Compared to the greater attention given to inter-manual transfer in the literature, two crucial aspects regarding sequence learning generalization have been neglected. First, little is known whether learning of a sequence is transferrable to another novel sequence that shares the same underlying structure with the learned sequence. Second, a paucity of studies has investigated age-related differences in generalization of sequence learning. The purpose of this study, therefore, is to address these two questions.

In this study, we employed a serial reaction time (SRT) task (Nissen & Bullemer, 1987) that has been a primary window into understanding implicit sequence learning. In the SRT task, stimuli are presented one at a time following a

specific probabilistic structure and participants respond to these stimuli by corresponding actions. An increasing number of studies (together with study II in Chapter 4 and study IV in Chapter 6) have shown that learning a sequence is indeed learning its probabilistic structure (Bornstein & Daw, 2013; Visser, et al., 2007), while little is known whether the acquisition of the probabilistic structure could facilitates the learning of different sequences that share the same structure. Considering the facilitation effect of structural learning on generalization in other motor learning tasks (Acuna & Schrater, 2010; Braun, Mehring, et al., 2010; Braun, Waldert, et al., 2010; Tenenbaum, et al., 2011), we expect to see the generalization of implicit sequence learning in the SRT task. Furthermore, a common notion regarding generalization is that it is facilitated by fast learning where an abstract representation of the task develops (Censor, 2013; Perez, et al., 2007). Evidence from studies I (Chapter 3) and III (Chapter 5) has shown that fast learning in children and adults arise from different processes. Given the distinct fast learning processes that may affect the way in which the memory is encoded, it is very likely that there are age-related differences in the generalization of implicit motor sequence learning.

To investigate our hypotheses, we asked children and adults to perform a modified SRT task. Unlike our previous studies reported earlier, the task in this experiment consisted of eight blocks. The learning sequence was presented in blocks 1-4, 6, and 7. A novel sequence was given in block 5 to assess sequence learning. Generalization of learning was assessed by providing another novel sequence in block 8. This novel sequence was generated by the same structure as the learning sequence.

Experiment 1

Materials and Methods

This study was performed in accordance with the approval of the Institutional Review Board at the University of Maryland, College Park. Consent forms from adult participants and parents of child participants received prior to the experiment. Child participants gave their assent forms before they started the experiment. Each participant received $15 after the completion of the experiment. In addition, a small toy prize was provided to child participants upon the completion of the Movement Assessment Battery for Children 2 (MABC2) (Henderson, et al., 2007).

Participants

Thirty children (8.78 ± 2.51 years, between 4.8 and 12.95 years, 13 females) were recruited for this study. Prior to the experiment, children completed the MABC2 to exclude those scored below the 15th percentile on the MABC2. One male child was excluded from this study owing to his 1st percentile score. In addition to child participants, ten young non-musician adults (19.84 ± 1.08 years, between 18.35 and 21.09 years, 7 females) from the University of Maryland, College Park participated in this study. No participants were excluded owing to neurological impairments or medical conditions that would affect motor performance determined by a neurological health questionnaire.

Experimental task and Procedure

Participants performed the same SRT task as that in study I (Figure 7.1A). Participants stood on the home position before starting the task. The home position

was surrounded by six stepping targets with two located at the front, two at the side, and the other two at the back of the home position. The distance from the home position to each target was marked at the most comfortable stepping length that was determined prior to the experiment for each individual. Six spatially-matched visual stimuli (i.e., six holes) were presented on a monitor in front of the participants. After the task began, one mouse appeared from one of the six holes. Participants stepped to the corresponding target on the floor as quickly and accurately as they could and then returned to the home position. There was a 1500ms inter-stimulus-interval before the next stimulus appeared. A customized program written in the Labview (National Instruments, Austin, TX, USA) was used to collect the signals from the home position sensors and control the appearance of visual stimuli. Three reflective markers were attached to the participants' big toes, heels, and the 5th metatarsal on both feet. The three-dimensional movement trajectories of these markers were record by a Vicon motion capture system (Oxford Metrics, Oxford, UK) with a sampling frequency of 200Hz.

Participants first completed a practice block where the stimuli appeared in a random order to assure that participants understood the task before starting the learning blocks. There were eight blocks and each consisted of 100 steps. Participants were not informed that visual stimuli in these blocks followed specific patterns. In blocks 1-4, 6, and 6, the visual stimuli followed 10 repetitions of sequence A (i.e., 1423564215; 1 – right side, 2 – right front, 3 – left front, 4 – left side, 5 – left back, and 6 – right back). The order of visual stimuli followed 10 repetitions of sequence B (i.e., 3615425214) in block 5. In block 8, transfer sequence A1 was presented. Given

that learning a sequence is typically learning its probabilistic structure (Bornstein & Daw, 2013; Visser, et al., 2007), sequence A1 was generated by the transitional probabilistic matrix *T*. It is important to note that *T* matches with first-order probabilistic structure of sequence A.

$$T = \begin{bmatrix} 0 & 0 & 0 & 0 & 0.47 & 0.63 \\ 0 & 0 & 0 & 1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0.5 & 0 & 0.5 & 0 \\ 0.5 & 0.5 & 0 & 0. & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \end{bmatrix}$$

During the SRT task, there was a mandatory three-minute break after each block (Figure 7.1B). Upon completion of the SRT task, participants were asked to complete a post-test to examine whether declarative knowledge of sequence A was acquired. They were first asked to recall the sequence in the SRT task and to write down 10 steps of the sequence (i.e., recall task). They were then given 16 sequence chunks where eight chunks were correct and were asked to choose the ones they thought resembled the sequence they learned in the SRT task (i.e., recognition task).

Data analysis

The data analysis was similar to that in previous chapters. Response time and its decompositions, reaction time (RT) that represents mental processing and movement time (MT) that reflects the movement itself (Moisello, et al., 2009) were computed. Specifically, RT was computed as the time discrepancy between the onset of visual stimulus and the onset of foot movement. MT was calculated as the time elapsed from the onset of foot movement to the end point of foot movement when reaching the target. The summation of RT and MT forms response time. To derive

these variables, the starting and end points of foot movement were identified from the three dimension trajectory (filtered by an eighth-order Butterworth filter with a cutoff frequency of 10Hz) of the foot markers using a customized MATLAB^{TM} (MathWorks, Naticks, MA, USA) script. The onset of stepping was defined as the first sample when the foot reached 10% maximum movement height. The end point of stepping was defined at the time when the foot dropped to the same height as the onset. Within each learning block, RTs, MTs, or response times that deviated beyond or below 2.575 standard deviations (i.e., resembles the 99% confidence interval) from the individual's mean RT, MT, or response time in that block were considered as outliers and were excluded from further analyses.

Figure 7.1: Experiment setup and procedure.
(A) The SRT task. (B) Experimental procedure.

Mean response time, RT, and MT were computed for each block. The indicator of sequence learning was whether performance improves from block 1

(sequence A) to block 4 (sequence A) and/or whether performance deteriorates from block 4 (sequence A) to block 5 (sequence B) (Robertson, 2007).

Given that RT rather than MT reflected sequence learning (see results below), analyses on generalization of learning, as well as online and offline changes in performance, was performed only on RT. The performance difference from block 7 (sequence A) to block 8 (sequence A1) was considered as a marker of sequence learning generalization. Rather than statistical learning, implicit sequence learning may result from chunk learning (Koch & Hoffmann, 2000; Stadler, 1993) or learning the exact sequence. Therefore, we also measured the generalization of three-element chunks and 10-element sequence (i.e., the exact sequence A) that shared in blocks 7 and 8. Online change in RT was defined as the RT change that takes place within block and was computed as the difference between the mean RTs of the first and last 10 steps. Offline change was defined as the RT change after a short rest without performing the task. In particular, this change was computed as the difference between the mean RT of last 10 taps in one block and mean RT of first 10 taps in the succeeding block. A positive value of online or offline change indicates RT improvement while a negative value means that RT became slower.

A dominant explanation for offline and online changes in RT is that they are illusory effects of reactive inhibition or fatigue. However, studies in previous chapters excluded reactive inhibition or fatigue as the underlying mechanism. Here, we replicated our analyses in study III (Chapter 5). We computed the RT deterioration within each block and the magnitude of learning gained through the practice of each block. Specifically, the amount of RT deterioration had the same magnitude as the

online change in RT but with the opposite sign. For example, a -20ms online change in RT (i.e., RT became slower) was equivalent to 20ms RT deterioration. The magnitude of learning of each block was calculated as the difference in the mean RT of first 10 taps from one block to the succeeding block. Since fatigue/reactive inhibition may occur within the block, this difference between the RT at the beginning of the block (i.e., mean RT of first 10 taps in the block) and the post-rest RT (i.e., mean RT of first 10 taps in the succeeding block) serves as a better indicator of learning over that block as the effect of fatigue or reactive inhibition is substantially reduced after rest (Brawn, et al., 2010; Rickard, et al., 2008a).

We then conducted a correlation analysis between RT deterioration and the magnitude of learning to examine three hypotheses regarding learning-based or fatigue/reactive inhibition-based online and offline changes in RT. First, previous studies (primarily in explicit sequence learning) that advocated the fatigue/reactive inhibition hypothesis claimed that fatigue/reactive inhibition suppresses performance expression but does not impair learning (Brawn, et al., 2010; Rickard, et al., 2008a). That is to say, the RT deterioration within a block, which is under the influence of fatigue or reactive inhibition, does not impact the magnitude of learning. Therefore, the RT deterioration and magnitude of learning would not be correlated. Second, numerous earlier studies have found the detrimental effect of fatigue/reactive inhibition on procedural skill learning (Ammons, 1947; Bourne & Archer, 1956; Denny, et al., 1955). Notably, learning would be impaired more if a stronger effect of fatigue/reactive inhibition builds, indicating a negative impact of the RT deterioration on the magnitude of learning. Finally, in opposition to the fatigue/reactive inhibition

hypothesis, online and offline changes in RT may arise from certain underlying learning mechanisms (Eysenck & Frith, 1977). Under this hypothesis, the online RT deterioration within blocks may take place due to learning itself and may serve as a prerequisite for the offline change in RT following rest. Thus, the amount of RT deterioration would be positively correlated to the magnitude of learning. In other words, the greater the RT deteriorates before rest, the greater the magnitude of learning would be.

To measure the score in the recall test, we counted the number of correct 2-, 3-, and 4-element chunks in the sequence that participants recalled. The chance levels of recalling 2-, 3-, and 4-element chunks were different. Take the 3-element chunk for an example, given the first element, there were four chunks (i.e., chunks starts from 1, 2, 4, or 5) that could be guessed with a chance level of 0.125 and two chunks (i.e., chunks starts from 3 or 6) that could be guessed with a chance level of 0.0625. Thus, the weighted probability among these six chunks was 0.1042. Given that participants recalled a 10-element long sequence that has eight 3-element chunks, the chance level for recall 3-element chunks in a 10-element long sequence written by an individual was 0.83 (i.e., 8×0.1042). Similarly, the chance levels of recalling 2- and 4-element chunks were 3.38 and 0.29. To assess the recognition performance, we counted the number of correct chunks (i.e., chunks included in sequence A) that participants chose as well as the number of incorrect chunks (i.e., chunks not included in sequence A) that participants did not choose in the recognition task. The sum of these two numbers was used as the recognition score. For example, if a participant chose two correct chunks and did not choose incorrect chunks, the recognition score is 10. The

recognition score would be eight if participant chooses all 16 chunks. The chance level is eight for the recognition test.

Statistical analysis

Like study III (Chapter 5), we considered age as a continuous variable as well as a categorical variable by clustering participants into three age groups: children younger than eight, children older than eight, and adults. A two-way mixed effect ANOVA was used to examine the effects of block and age group (i.e., a categorical variable). Tukey-Kramer corrected *post hoc* tests were conducted following any significant effect. To examine the generalization of learning first-order probabilistic transitions, one-way ANOVAs were performed to examine the age group effect on the mean RT differences between blocks 7 and 8. Similarly, the age effect on the generalization of learning three-element chunks or the sequence A itself was examined through the differences in RT of the same three-element chunks or the sequence A between blocks 7 and 8. A two-way mixed effect ANOVA was performed on offline and online changes in RT that developed between blocks 1 and 4. To examine the progressive change in implicit sequence learning with age (i.e., a continuous variable), we further modeled sequence learning (as measured by RT differences between blocks 1 and 4 as well blocks 4 and 5), the generalization of learning (as measured by RT difference between blocks 7 and 8), offline, and online RT changes based on the individual's age. Specifically, we used a linear model, piecewise linear latent model with unknown knots, or nonlinear model, which was determined by likelihood ratio tests and the Akaike information criteria (AIC). To examine whether online and offline changes in RT were fatigue/reactive inhibition-

based or learning-based, a partial correlation analysis was performed to confirm the relationship between the RT deterioration and the magnitude of learning controlling the effect of age and block. Since recall and recognition score were measured by count data, Poisson models were used to examine the effect of age group or continuous age on the recall and recognition score. If over-dispersion was displayed in these two scores, a Poission mixture model (i.e., negative binomial regression) was used instead. The significance level for statistical analyses was set at $\alpha = 0.05$.

Results

There were significant effects of block ($F(7,37) = 33.92$, p <0.0001), age group ($F(2,37) = 28.83$, p <0.0001), and their interaction ($F(14,37) = 5.30$, p <0.0001) on RT (Figure 7.2A). Overall, RT was slower in children younger than eight compared to the other two groups (all p <0.05) that had similar RT. RT improved from block 1 to 4 (all p < 0.01) while deteriorated from block 4 to block 5 (p < 0.01) regardless of age groups, suggesting that all groups learned sequence A at a comparable level. Importantly, RT deteriorated from block 7 to block 8 in adults (p < 0.0001) while RTs in blocks 7 and 8 were not significantly different in two children's groups, suggesting that children, but not adults, generalized the learning of sequence A to sequence A1. However, MT displayed a different pattern compared to RT (Figure 7.2B). There was only a significant effect of block ($F(7, 37) = 14.52$, p < 0.0001) on MT. MT did not improve from block 1 to block 4, 6, and 7 where sequence A was performed. However, compared to blocks 4, 6, and 7 (all p <0.0001), MT became faster in block 5 where sequence B was performed. Since there was no improvement in MT on sequence A and no perturbation on MT when sequence B was

performed, sequence learning in the SRT task was primarily reflected in RT that represents mental processing rather than MT that characterizes the movement itself.
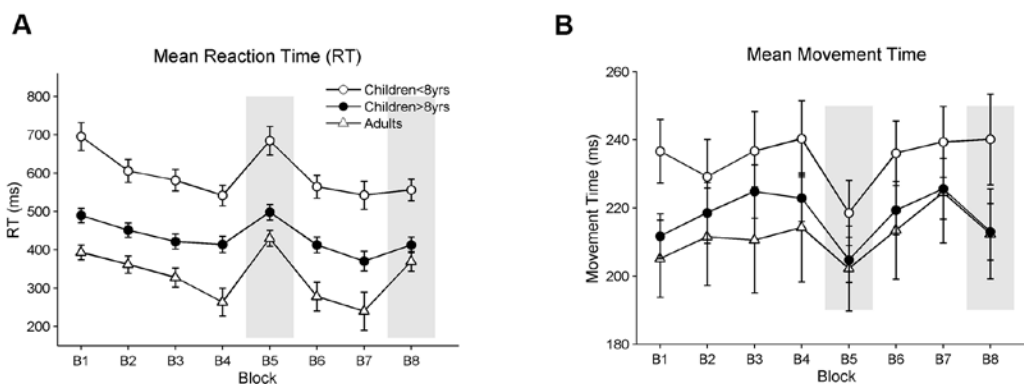
Figure 7.2: Implicit motor sequence learning, as reflected by RT, was comparable between children and adults.
(A) Mean reaction time (RT) across learning blocks. (B) Mean movement time (MT) across learning blocks.

The RT change from block 1 to block 4 as well as from block 4 to block 5 was modeled by a linear function to examine the effect of an individual's age (i.e., continuous variable). These models failed to find significant age effects (slope = -0.3, p = 0.9; slope = 2.55, p = 0.33)(Figure 7.3A & Figure 7.3B).
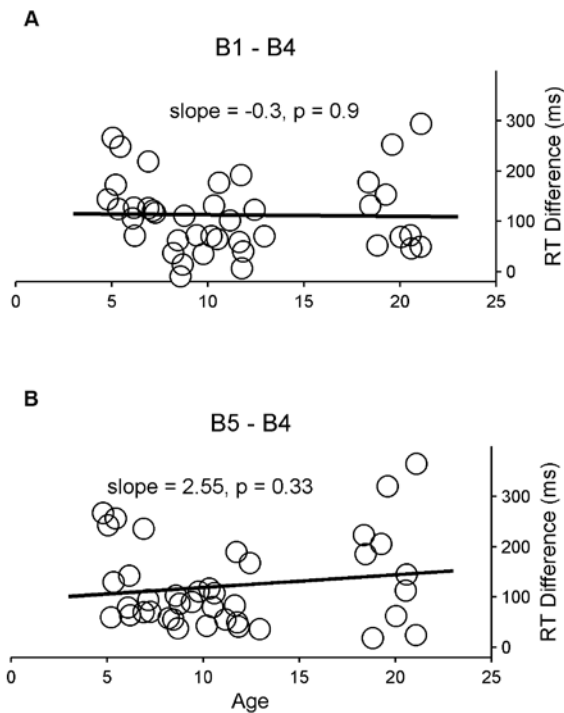
Figure 7.3: Implicit motor sequence learning was not age-related.
(A) Mean RT differences between blocks 1 and 4 across age. (B) Mean RT differences between blocks 4 and 5 across age.

Since sequence learning was attributed to RT and not MT, the generalization of sequence learning was examined only on RT. Our results revealed the significant effect of age group on learning generalization ($F_{(2, 37)} = 4.34$, $p < 0.05$). Specifically, the two children groups showed the comparable generalization of learning while generalization in adults was inferior to the children ($p < 0.05$ for children younger than eight; $p = 0.06$ for children older than eight; Figure 7.4A). When only the triplets ($F_{(2, 37)} = 4.79$, $p < 0.05$; Figure 7.4B) or the 10-element sequence ($F_{(2, 37)} = 3.85$, $p < 0.05$, Figure 7.4C) that shared by sequences A and A1 were compared, the effect of age group remained significant. The linear model on the generalization of learning found that learning generalization was reduced with age despite the length of the sequence (all $p < 0.01$). These results suggest the age-related differences in the

generalization of implicit sequence learning. Notably, the generalization of implicit

motor sequence learning is superior in children compared to adults.
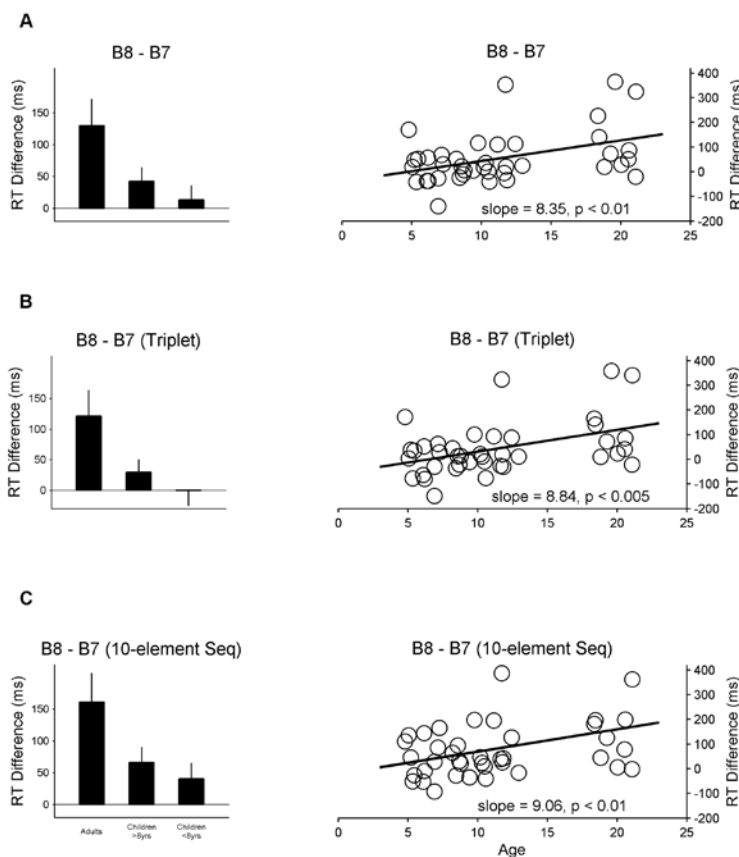
Figure 7.4: Generalization of sequence learning was age-related.
(A) Generalization of first-order probabilistic transitions. (B) Transfer of learned triplets. (C) Transfer of the 10-element sequence A.

The offline and online changes in RT from block 1 to block 4 where sequence

A was learned were examined. It was found that the RT offline change was not

significantly affected by block and its interaction with age. However, the offline

change in RT after each rest between blocks 1 and 4 depended on age ($F(2,37) =$

17.92, $p < 0.0001$). In particular, the offline change was greater in children younger

than eight compared to older children ($p < 0.0005$) and adults ($p < 0.0001$) and

greater in children older than eight compared to adults ($p < 0.05$). Since there was no

block effect, the averaged offline change in RT during three breaks from block 1 to block 4 was used to further investigate the age-related difference (Figure 7.5A). As shown in Figure 7.5A, the offline change decreased presumably before a certain age Y and then remained the same after Y. A likelihood ratio test confirmed that fitting the data with a piecewise linear model with Y being a latent variable is superior to a linear model ($\chi^2_{df=2}$ = 15, p < 0.001). This piecewise linear latent model revealed that before age Y = 7.25 (SE = 0.61), the offline change in RT decreased with age (slope = -71.84, p < 0.005) while rate of change decreased after age Y = 7.25 (slope = -5.8, p < 0.05). In addition, the magnitude of the offline change in RT was estimated to be larger than zero at the age of 4.8 (247.03ms, p <0.0001) while it diminished to zero (-2.86ms, p =0.87) at age 20.
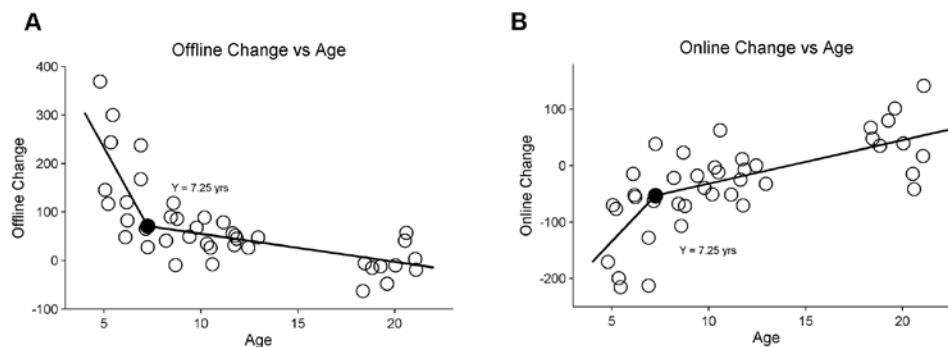
Figure 7.5: Age-related offline and online changes in RT.
(A) Offline changes in RT across age. Empty circles represent individual data. Solid circle represent the estimated age jointing two phases of offline changes development. (B) Online changes in RT across age. Empty circles represent individual data. Solid circle represent the estimated age jointing two phases of online changes development.

Similar to the offline change in RT, the amount of RT that changed online was found to depend only on age (F(2,37) = 16.87, p<0.0001). There were no effects of block and its interaction with age. In particular, children younger than eight showed significantly less online change than children older than eight (p<0.01) and adults (p

< 0.0001). In addition, less online change was shown in children older than eight compared to adults (p<0.05). The mean online change in RT across block 1 to block 4 was then modeled to examine its dependence on an individual's age. The likelihood ratio test between a linear model and a piecewise latent model was not significant ($\chi^2_{df=2} = 4$, p = 0.13). We reported results of both the linear model and piecewise linear model. The linear model found that the online change in RT was negative at the age 4.8 (-97.63ms, p < 0.0001) and increased with age (slope = 10.04, p < 0.0001). The piece-wise latent model revealed that before age Y = 7.25 (SE = 1.29), the online change in RT enhanced with age (approached significance; slope = 36.17, p = 0.09) while keeping increasing with a slower rate after age Y = 7.25 (slope = 7.72, p < 0.001). Notably, the online change was negative at age 4.8 (-141.64ms, p <0.0001) while it increased to be positive (45.45ms, p < 0.05) at age 20, suggesting that RT in younger children became slower within blocks (Figure 7.5B). Taken together, these results suggest the offline and online changes in RT were age-related. Offline change decreased while online change increased as age increased.
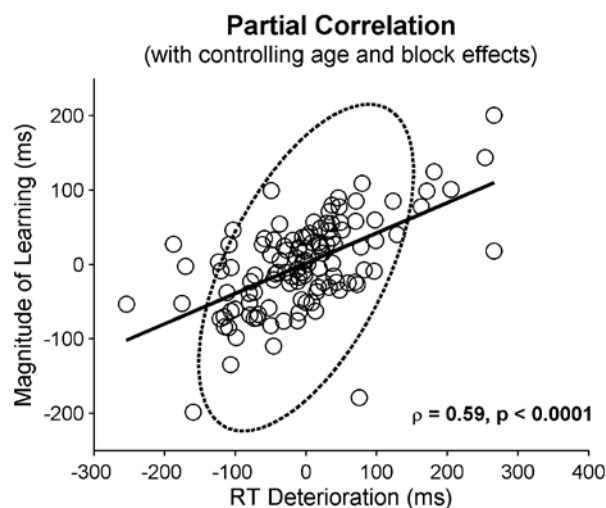
Figure 7.6: Age-related offline and online changes in RT are not caused by fatigue or reactive inhibition.

The partial correlation between RT deterioration and magnitude of learning with controlling age and block effects. Dashed circle represents the 95% confidence interval.

Although children demonstrated greater offline changes in RT, it remains unknown whether the offline enhancement resulted from inhibition/fatigue or arises from learning itself. We examined the partial correlation (controlling the age and block effect) between the RT deterioration within a block and the magnitude of learning that took place over this block (see Methods). It was found that the RT deterioration was positively correlated to the magnitude of learning ($\rho = 0.59$, p <0.0001, Figure 7.6). This result was contrary to the reactive inhibition/fatigue hypotheses and consistent with the learning-based hypothesis, suggesting that online and offline changes in RT and their development with age were not artifacts of reactive inhibition or fatigue.

In the posttest, we found a significant effect of age group on recalling 2- ($\chi^2_{df=2} = 15.31$, p < 0.0001), 3- ($\chi^2_{df=2} = 11.77$, p < 0.01), and 4-element chunks ($\chi^2_{df=2} = 10.23$, p < 0.01). Despite the length of chunks, the two child groups had lower recall scores than adults (p < 0.0001 for 2-element chunk and all p <0.01 for 3- and 4-element chunks). In addition, adults (p < 0.05 for 2-element chunk and p <0.0001 for 3- and 4-element chunks) had higher than chance recall for all lengths of chunks, while recall in children younger than eight was at chance for all lengths of chunks (Figure 7.7A). A negative binomial regression confirmed the age effect (i.e., continuous age). Specifically, the recall score became higher as age increased (all p <0.001, not graphically shown here). In the recognition test, there was a significant effect of age group ($\chi^2_{df=2} = 8.12$, p < 0.05). Specifically, the recognition score was

lower in children compared to adults (both p < 0.001). Additionally, the recognition score was found to be at chance in children but higher than chance in adults (p < 0.0005). It was further found that the recognition score progressively increased with age ($\chi^2_{df=1} = 6.03$, p < 0.05) (Figure 7.7B).
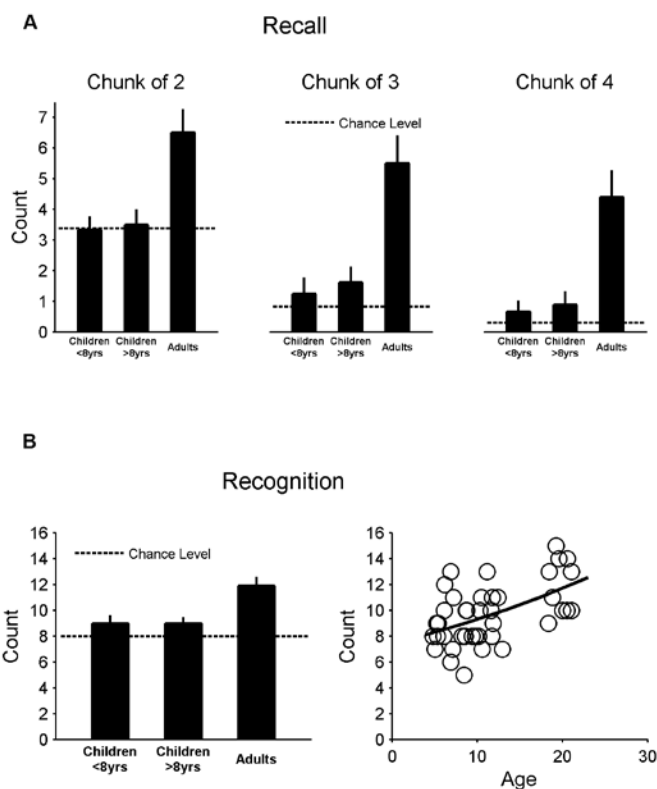
Figure 7.7: Age-related declarative learning.
(A) The recall scores. (B) The recognition score.

Discussion

This study replicated the results in previous chapters that implicit sequence learning over the first learning session in the SRT task was reflected by two age-related processes in reaction time (RT); offline learning that developed after a rest and online learning that occurred when the task was performed. Such online and offline processes are not illusory effects of fatigue or reactive inhibition. Most importantly, we found the age-related differences in the generalization of implicit

sequence learning. In particular, the generalization of implicit sequence learning reduced with age. That is to say, learning generalization is better in children compared to adults.

Given that generalization is affected by the way in which memory is encoded during fast learning (Censor, 2013; Clark & Ivry, 2010; Sagi, 2011), we propose that the age-related differences in generalizing implicit sequence learning may be related to the age-related online and offline learning. Since offline learning is very likely to correspond to procedural memory (Du, et al., 2016), the age-related differences in the generalization may vanish in sequence learning that demands greater procedural memory. To test this hypothesis, in experiment 2, we asked children and adults to learn a probabilistic sequence that favors more procedural memory compared to learning a fixed sequence (Du, et al., 2016; Jimenez, et al., 1996; Song, Howard, & Howard, 2007b).

Experiment 2

Materials and Methods

This study was performed in accordance with the approval of the Institutional Review Board at the University of Maryland, College Park. Consent forms from adult participants and parents of child participants received prior to the experiment. Child participants gave their assent forms before they started the experiment. Each participant received $15 after the completion of the experiment. In addition, a small toy prize was provided to child participants upon the completion of the Movement Assessment Battery for Children 2 (MABC2) (Henderson, et al., 2007).

Participants

Twenty six children (9.46 ± 2.07 years, between 6.03 and 13 years, 5 females) were recruited for this study. Prior to the experiment, children completed the MABC2 to exclude those scored below the 15th percentile on the MABC2. Ten young non-musician adults (20.17 ± 0.84 years, between 19.2 and 21.5 years, 5 females) from the University of Maryland, College Park participated in this study. No participants were excluded owing to neurological impairments or medical conditions that may affect motor performance determined by a neurological health questionnaire.

Experimental task and Procedure

Participants performed the same SRT task as that in experiment 1. The only difference was the sequence that visual stimuli followed in each block. In blocks 1-4, 6, and 7, the visual stimuli followed sequence A that was generated by the transitional matrix *T*. The order of visual stimuli followed a random sequence B in block 5. In block 8, generalization sequence A1 was presented. Sequence A1 was different from sequence A but it was also created by the transitional probabilistic matrix *T*. There were 102 stimuli in each block to constrain that each stimulus appeared an equal number of times in each block.

$$T = \begin{bmatrix} 0.01 & 0.05 & 0.5 & 0.3 & 0.09 & 0.05 \\ 0.09 & 0.01 & 0.05 & 0.05 & 0.5 & 0.3 \\ 0.05 & 0.5 & 0.01 & 0.05 & 0.3 & 0.09 \\ 0.05 & 0.3 & 0.09 & 0.01 & 0.05 & 0.5 \\ 0.3 & 0.09 & 0.05 & 0.5 & 0.01 & 0.05 \\ 0.5 & 0.05 & 0.3 & 0.09 & 0.05 & 0.01 \end{bmatrix}$$

Data analysis

Data analysis was identical to that in experiment 1 except one difference. It has been found that learning a probabilistic sequence involves the acquisition of sequence transitions of higher probabilities. Thus, instead of analyzing the mean RT of total 102 steps, we analyzed the mean RTs of steps to stimuli with higher and lower probabilities respectively. We considered the probabilities below 0.09 as low probabilities as in the entire sequence of 102 steps, they appeared less than 20 times.

Statistical analysis

The analyses were identical to that in experiment 1 with one difference. When the mean RT was analyzed, a three-way mixed effect ANOVA was used. That is, in addition to the effects of block and age group, the effect of probability was examined as well.

Results

There were significant effects of block ($F(7,231) = 14.51$, $p < 0.0001$), age group ($F(2,33) = 35.65$, $p < 0.0001$), and probability ($F(1,33) = 9.65$, $p < 0.005$) (Figure 7.8). RT was slower in children younger than eight compared to the other two groups ($p < 0.01$ for children older than eight; $p < 0.0001$ for adults). Children older than eight had slower RT than adults ($p < 0.0001$). RT to lower transitional probability stimuli were slower compared to that to stimuli with higher transitional probability ($p<0.005$). RT in all groups improved from block 1 to 4 (all $p < 0.005$) while deteriorated from block 4 to block 5 ($p < 0.005$), suggesting that all groups learned sequence A to a comparable level. Importantly, RTs in blocks 7 and 8 were

not significantly different despite age groups, suggesting that both children adults

generalized learning of sequence A to sequence A1. Linear functions that modeled

the RT change from block 7 to block 8 (i.e., generalization) failed to find significant

age effects (slope = 0.33, p = 0.66 for high probability stimuli; slope = -0.7, p = 0.64

for low probability stimuli) (Figure 7.9). Similar to the results in previous chapters,

MT was not found to reflect sequence learning as there was not effects of block, age

group, and probability on MT.
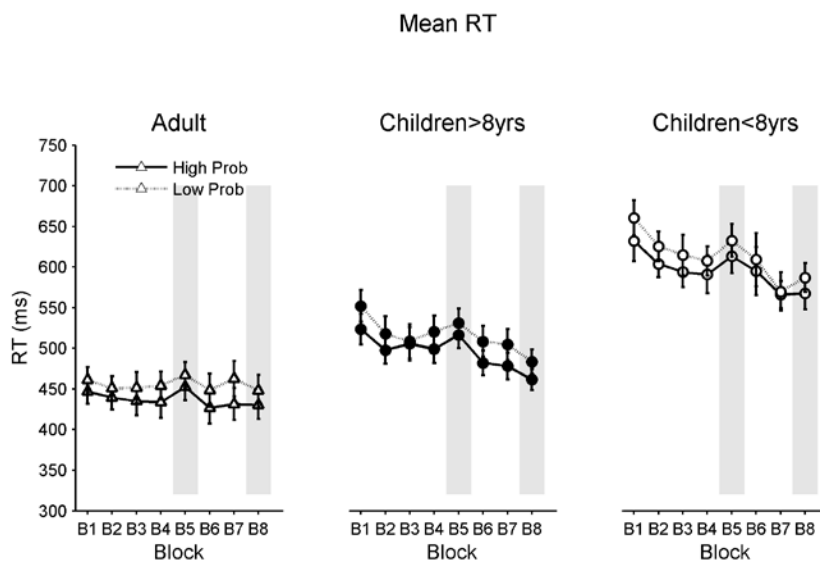
Figure 7.8: Mean RT across learning blocks when adults and children learned a probabilistic sequence, revealing no age effect on probabilistic sequence learning.
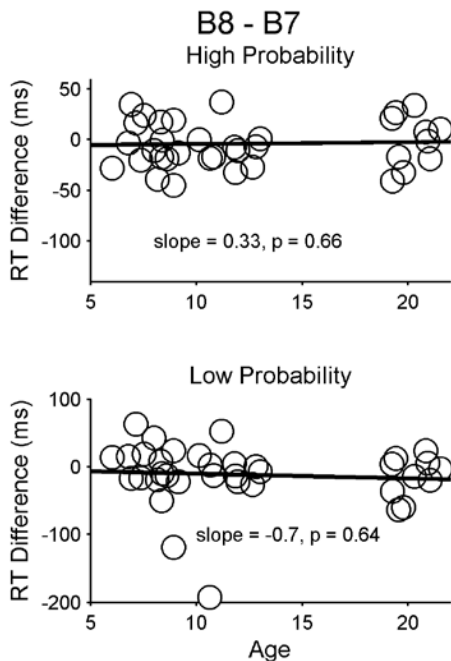
Figure 7.9: Generalization of probabilistic sequence learning is not age-related.

The offline and online changes in RT from block 1 to block 4 where sequence A was learned were examined. It was found that the RT offline change was not significantly affected by block and its interaction with age. However, the offline change in RT after each rest between blocks 1 and 4 significantly depended on age ($F(2,33) = 4.1$, $p < 0.05$). In particular, the offline change was greater in children younger than eight compared to adults ($p < 0.05$). Since there was no block effect, the averaged offline change in RT during three rests from block 1 to block 4 was used to further investigate the age-related difference (Figure 7.10A). According to AIC, we modeled the offline change in RT using an exponential function $b_1 e^{b_2(Age-6.03)}$ (i.e., 6.03 was the minimum age). This nonlinear regression revealed a significant effect of age ($b_2 = -0.06$, p<0.01). In addition, the magnitude of offline change in RT was estimated to be larger than zero at age 6.03 ($b_1 = 120.69$ms, p <0.0001). Unlike

previous chapters where adults had no offline changes in RT, we found that offline

change in RT was 37.08ms (p < 0.01) estimated at age 20.
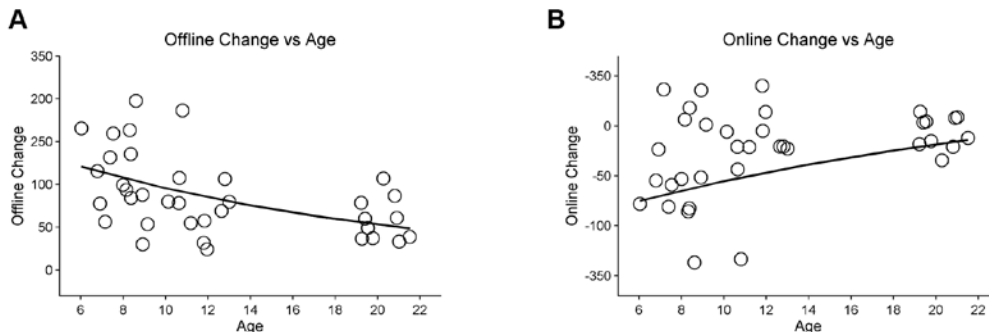
Figure 7.10: Age-related offline and online changes in RT.
(A) Offline changes in RT across age. Empty circles represent individual data. (B) Online changes in RT across age. Empty circles represent individual data.

Similar to the offline change in RT, the amount of RT that changed online was

found to depend only on age (F(2,33) = 4.39, p<0.05). There were no effects of block

and its interaction with age. In particular, children younger than eight showed

significantly less online change than adults (p < 0.05). The mean online change in

RT across block 1 to block 4 was then modeled to examine its dependence on an

individual's age (Figure 7.10B). The exponential function $b_1 e^{b_2(Age-6.03)}$ was chose

according to AIC. It was revealed that the age effect approached significance

($b_2 = -0.04$, p=0.05). Notably, the online changes were estimated to be negative at

age 6.03 (b_2 = -96.83ms, p <0.0001) and 20 (40.9ms, p < 0.01). Taken together, these

results suggest that although there are age-related differences in online and offline

learning changes in RT when adults and children learn a probabilistic sequence,

probabilistic sequence learning in both children adults is primarily driven by offline
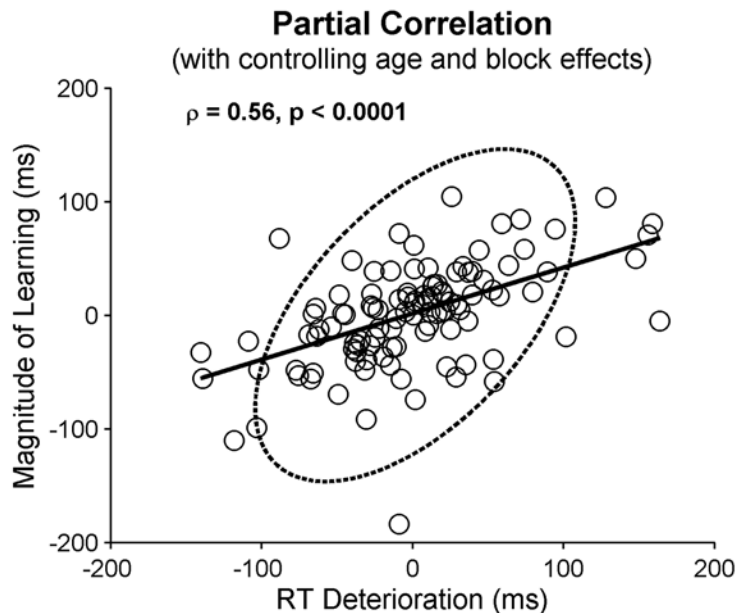
learning.

Figure 7.11: Age-related offline and online changes in RT are not caused by fatigue or reactive inhibition.
The partial correlation between RT deterioration and magnitude of learning with controlling age and block effects. Dashed circle represents the 95% confidence interval.

Children demonstrated greater offline changes in RT, but it remains unknown whether the offline enhancement resulted from inhibition/fatigue or arises from learning itself. We examined the partial correlation (controlling the age and block effect) between the RT deterioration within a block and the magnitude of learning that took place over this block (see Methods). It was found that the RT deterioration was positively correlated to the magnitude of learning ($\rho = 0.56$, p <0.0001, Figure 7.11). This result was contrary to the reactive inhibition/fatigue hypothesis and consistent with the learning-based hypothesis, suggesting that online and offline changes in RT and their development with age were not artifacts of reactive inhibition or fatigue.

In the posttest, there was no age effect on the declarative knowledge of sequence A acquired during the SRT task (not graphically shown).

Discussion

In this study, we found that the generalization of implicit motor sequence learning was superior in children compared to adults. However, when greater offline learning was involved in the SRT task, which was mediated by the involvement of procedural memory required by the learning sequence, the age-related differences in learning generalization disappeared. The age-related differences in offline and online learning can, at least partially, explain the age effect on the generalization of implicit sequence learning.

Generalization has been suggested to be affected by the way in which the memory of sequence is encoded. Thus, what is generalized from one sequence to another depends on the sequence structure that is encoded into memory. An increasing amount of studies have suggested statistical transition learning to account for sequence learning (Bornstein & Daw, 2012, 2013; Hunt & Aslin, 2001). Statistical transition learning refers to the process where probabilistic regularities between sequence elements (normally first-order transitions) are learned (Saffran, et al., 1996). However, when the learning sequence (i.e., fixed sequence in experiment 1) and novel sequence shared the same statistical transitional structure, adults failed to generalize sequence learning to the novel sequence. In contrast, learning generalized to the novel sequence in children. These results demonstrate the age-related differences in the generalization of implicit sequence learning.

The underlying mechanisms of the age-related differences remain unclear. One explanation is that implicit sequence learning in adults results from chunk learning (Koch & Hoffmann, 2000; Stadler, 1993) rather than statistical learning. In

chunk learning, a sequence is partitioned into short segments to be learned and the concatenations of segments leads to the acquisition of the sequence. Thus, learning in adults is likely to generalize to the same chunks shared between the learning and generalization sequences. However, our results preclude this possibility. When only three-element chunks were compared between the learning and generalization sequences, RT of chunks in the generalization sequence was slower compared to those in the learning sequence, indicating that adults failed to generalize sequence chunks learned in sequence A. An alternative to the chunk learning hypothesis is the concept of over-fitting. That is to say, if adults learned the entire sequence itself, a small variation in the sequence would result in reduced generalization (Censor, 2013; Clark & Ivry, 2010; Sagi, 2011). This explanation was incompatible with our results. In the generalization sequence, there were about 40 stimuli that followed sequence A. When RTs of these steps in the generalization sequence were compared to the learning sequence, we failed to observed learning generalization in adults.

Surprisingly, the age-related differences in the generalization prevailed only when the learned sequence was fixed. Learning of a probabilistic sequence generalized to a novel sequence in both children and adults and at a comparable level. The chief difference between fixed and probabilistic sequence learning in adults is that greater offline learning contributed to acquire probabilistic sequences while fixed sequence learning was dominated by online learning. Learning a probabilistic sequence favors more procedural memory compared to learning a fixed sequence (Jimenez, et al., 1996; Song, et al., 2007b), which yields greater offline learning (study IV, Chapter 6). Considering the connection between procedural memory and

offline learning, as well as between declarative memory and online learning (study IV, Chapter 6), these results suggest that the generalization of sequence learning is possibly governed by procedural memory encoding, but not declarative memory encoding. We suggest that future studies systematically examine this hypothesis by comparing the generalization in adults with modulating the procedural and declarative memory that were required in sequence learning.

Another explanation for the age-related generalization of fixed sequence learning is the memory stability. It has been found that the high-level representation of the learned sequence that enables generalization is only formed when the memory of sequence is unstable. It is possible that memory encoded by online learning is more stable compared to that encoded by offline learning, which results in inferior generalization of fixed sequence learning in adults. To provide a unifying explanation, further systematical research is needed.

Conclusion

We confirmed the common notion that the generalization of sequence learning depends on the way in which the memory is encoded. When offline learning dominated the fast acquisition of sequences, sequence learning generalized to a novel sequence. In contrast, sequence learning failed to generalize to a novel sequence when online learning dominated the acquisition of sequences. In addition, we demonstrated that the generalization of fixed but not probabilistic sequence learning was age-related. We propose that the age effect on the generalization of implicit sequence learning can be, at least partially, explained by the age-related differences in offline and online learning.

General Discussions

The initial acquisition of motor sequences usually develops over a course of one learning session and is known as fast learning (Doyon & Benali, 2005; Nissen & Bullemer, 1987; Willingham, et al., 1989). Previous studies have consistently found the comparable ability in fast sequence learning between children as young as six-year-old and adults (Meulemans, et al., 1998; Weiermann & Meier, 2012b). Given that distinct memory may be encoded during fast learning in children and adults (Meulemans, et al., 1998; Nemeth, Janacsek, et al., 2013b), age-related learning processes are likely to differentially contribute to the fast acquisition in children and adults. The dissertation programmatically examined the behavior expressions of learning processes underlying implicit motor sequence learning in the serial reaction time (SRT) task (or its modified version). The results of study I demonstrated the comparable ability between children as young as 6-year-old and adults to quickly learn motor sequences, as revealed by reaction time (RT) that represents mental processing. Interestingly, the comparable fast sequence learning was expressed by two age-related processes; an online process as reflected by progressive changes in RT as the sequence was practiced and offline process as indicated by enhanced RT following short rests without physical practice on the sequence. Studies II to IV demonstrated that these two processes were neither illusory effects of fatigue or reactive inhibition nor by-products of task pacing. Rather, they were likely to be active learning mechanisms that may mirror to memory systems of procedural and declarative learning. Given the age-related differences in procedural and declarative learning (Meulemans, et al., 1998; Nemeth, Janacsek, et al., 2013b), the development

of online and offline learning are divergent. Offline learning reduced as age increased and impaired around the age 20. In contrast, online learning strengthened with age and dominated sequence learning in adults. Furthermore, results of studies V and VI revealed that online and offline learning and their underpinning memory systems did not only affect the acquisition of sequence, but also had significant effects on the generalization of implicit motor sequence learning. Notably, the generalization of sequence learning appeared to be facilitated by offline learning rather than online learning. Taken together, this dissertation demonstrated that age-related learning processes, offline and online learning, drive the fast acquisition of motor sequences. These two learning processes are likely to be responsible for the age-related differences in the generalization of implicit motor sequence learning.

The consistent observation in this dissertation was that RT in children, especially around the age of six, deteriorated as they were practicing the SRT task, which was subsequently followed by remarkable RT enhancements after a short rest. This deteriorated online RT makes it unclear whether the online and offline changes in RT are active learning mechanisms or illusory effects of fatigue or reactive inhibition. For example, children's RT worsened within learning blocks when they performed the SRT task, presumably because of the accumulation of fatigue or reactive inhibition. The effect of fatigue or reactive inhibition dissipated following a rest, which resulted in the recovery of RT and consequently led to offline improvements in RT (Ammons, 1947; Bourne & Archer, 1956; Brawn, et al., 2010; Denny, et al., 1955; Rickard, et al., 2008a; Rieth, et al., 2010). It has been suggested that fatigue or reactive inhibition and offline learning itself are usually combined to

result in offline changes in RT, making it challenging to determine whether the offline enhancement in RT results from active learning or fatigue/reactive inhibition.

Results from this dissertation provided insights into this problem and suggest that online and offline processes observe during fast learning of motor sequences are functional mechanisms of learning rather than illusory effects of fatigue or reactive inhibition. It has been suggested that whether offline enhancement in performance (i.e., RT) is caused by fatigue or reactive inhibition depends on the task (Eysenck & Frith, 1977). When a motor task does not require learning, indicating that performance on the task is already perfect as soon as the task starts, fatigue or reactive inhibition is likely to be primary cause of offline changes in performance. In addition, in the non-learning task, post-rest performance usually returns to the pre-rest performance level without improvement. In contrast, post-rest performance should be superior compared to pre-rest performance if offline changes in performance results from learning rather than fatigue or reactive inhibition. Results in study I provide supporting evidence for the learning-based offline process. However, the possibility of fatigue or reactive inhibition could not be fully excluded given that online RT did remarkably deteriorated when children were practicing the task.

To further determine whether online RT deterioration and its subsequent offline RT enhancement arise from fatigue or reactive inhibition, the hypotheses related to fatigue or reactive inhibition were examined through studies III to VI. Reactive inhibition, elicited by fatigue, motivation, or attentional factors, accumulates when an individual is practicing the task. Previous studies have suggested that the fatigue or reactive inhibition could have a detrimental effect on learning (Ammons,

1947; Bourne & Archer, 1956; Denny, et al., 1955) or could suppress the performance expression without impairing learning (Brawn, et al., 2010; Rickard, et al., 2008a; Rieth, et al., 2010). Remarkably, the results in studies III to VI revealed a facilitation effect of RT deterioration on sequence learning. This observation is clearly incompatible with the fatigue or reactive inhibition hypotheses. It further suggests that the online RT deterioration and subsequent offline RT improvement are rather likely to be functional mechanisms of learning.

Although the underlying mechanisms of online and offline learning remain unclear, we propose that they originate from the interactive memory systems of declarative and procedural learning. In this dissertation, procedural learning dominated in children, as revealed by their chance-level recognition and recall scores. In contrast, adults produced higher than chance scores, suggesting that they acquired declarative knowledge of the sequence in the SRT task; results that are consistent with the literature (Meulemans, et al., 1998; Weiermann & Meier, 2012b). On one hand, the greater use of procedural learning may discourage online learning in children, which causes substantial RT deterioration. On the other hand, the bias to declarative learning in adults may inhibit their offline learning. This inhibition effect is reduced in children as greater procedural learning is involved, yielding stronger offline learning effect. Supporting evidence comes from studies IV and VI in which online learning was inhibited and offline learning strengthened when the SRT required greater procedural learning (i.e., learning a probabilistic sequence).

Generalization of sequence learning is facilitated by fast learning (Censor, 2013; Clark & Ivry, 2010; Sagi, 2011). This dissertation suggests that the age-related

differences in online and offline learning that contribute to the initial acquisition of sequences may lead to children and adult different abilities in generalizing the learning of one sequence to a novel sequence. Indeed, results show that the ability in the generalization of fixed sequence learning reduced as age increased, perhaps because offline learning, rather than online learning, enabled to encode the memory of abstract sequence presentation (i.e., the probabilistic structure). When learning in adults was dominated by greater offline learning, the age-related differences in generalization vanished. Considering the connection between procedural memory and offline learning, as well as between declarative memory and online learning, these results suggest that the generalization of implicit motor sequence learning is possibly governed by procedural memory encoding, but not declarative memory encoding. In addition, a recent study has demonstrated that unstable rather than stable memory has a positive effect on learning generalization (Mosha & Robertson, 2016). It could also be posited that memory formed by offline learning is more fragile compared to that formed by online learning. To further elucidate this hypothesis, future studies are awaited.

In the literature, developmental studies usually compared sequence learning among distinct age groups, for example 10- and 6-year old children and adults in study I. Studies III, V, and VI in this dissertation attempted to further characterize the developmental landscape of implicit motor sequence learning. It was found that offline and online learning developed in early age while the developmental rate reduced as age increased. This developmental trend is very similar with that found in two previous studies. Implicit sequence learning has been found to be optimal before

the age of 12 (Janacsek, et al., 2012a). Before this age, the acquisition of sequence learning was primarily relies on habitual learning, while learning after 12 years of age seems to be model-based (Nemeth, Janacsek, et al., 2013b). More importantly, the habitual and model-based learning are respectively mirrored to procedural and declarative memory (Doll, et al., 2015), which is consistent with our finding on the age-related differences in the declarative knowledge of sequences acquired through the SRT task.

In addition to identifying two age-related learning processes, this dissertation extended our understanding of the age-related implicit sequence learning in another aspect. Traditionally, the SRT task is performed much like a typing or a keyboarding task with participants seated. But as Sherrington (1906) argued, "posture follows movement like a shadow", and the vast majority of our daily life motor sequence learning involves whole body movement that is not required in typing or keyboarding. The postural control requirements of the whole body sequence task used here could be a "rate limiter" to performance and perhaps to learning (Thelen, 1989; Thelen, et al., 1989). This dissertation employed a modified SRT task where finger pressing was replaced by foot stepping to incorporate postural control into the task. Although this dissertation did not directly compare sequence learning in finger-pressing and foot-stepping SRT tasks, it replicates the results for learning finger sequences by children and adults (Meulemans, et al., 1998; Thomas & Nelson, 2001; Weiermann & Meier, 2012a) despite the age-related nature of the required postural control for our whole body foot-stepping task (Bair, Kiemel, Jeka, & Clark, 2007).

These results suggest that postural control has little effect on motor sequence learning.

This dissertation extends our understanding into the development of motor sequence learning and also raises several questions to be addressed in future research. First, the developmental landscape of motor sequence learning as well as its underlying learning processes should be further studied. This current research attempted to characterize the landscape, but one caveat is that this research did not include ages from 14 to 17. Although it is unlikely that online and offline learning would differ from the age of 12 and 20, the developmental trajectory within this age range needs to be explored in future studies.

This current research suggests that the interactive memory systems of procedural and declarative learning are substrates for offline and online learning processes. Future studies are needed to systematically test this hypothesis. Numerous studies has demonstrated that the cooperative and competitive memory systems of procedural and declarative learning concurrently contribute to implicit learning (Borragán, Slama, Destrebecqz, & Peigneux, 2016; Brown & Robertson, 2007a, 2007b; Foerde, et al., 2006; Poldrack, et al., 2001) and the interaction starts as soon as learning begins (study IV, Chapter 6). Previous studies have demonstrated that interrupting declarative memory positively affect procedural learning (Brown & Robertson, 2007a; Filoteo, Lauritzen, & Maddox, 2010; Keisler & Shadmehr, 2010a; Nemeth, et al., 2012), however, little is known about the behavior expressions of the positive effect on procedural learning. According to this dissertation, greater offline learning is expected to be seen when the declarative memory is impaired. Given that

the interactive memory system is mediated by the dorsal lateral prefrontal cortex (Cohen & Robertson, 2011; Diekelmann, et al., 2011), the disruption of the dorsal lateral prefrontal cortex could allow to examine online and offline learning as well (Cohen & Robertson, 2011).

It is important to emphasize that offline learning in this study occurred within the first learning session where the initial acquisition of sequences developed. After the initial acquisition stage, learning does not terminate. Instead, it continuous to develop with a slow rate and the memory of sequences is consolidated (Doyon & Benali, 2005). In the literature, offline learning has been found as a salient feature underlying sequence learning and observed during the slow learning and consolidation stages (Nettersheim, et al., 2015; Robertson, Pascual-Leone, & Miall, 2004). Specifically, the memory of sequences can be boosted a few minutes after its acquisition (Eysenck & Frith, 1977; Hotermans, et al., 2008; Hotermans, et al., 2006). Later on, the memory is further strengthened following a few hours with (Brown & Robertson, 2007a, 2007b) or without sleep (Nettersheim, et al., 2015; Robertson, Pascual-Leone, & Press, 2004; Walker, et al., 2002; Wilhelm, Diekelmann, & Born, 2008; Wilhelm et al., 2011; Wilhelm, et al., 2012). The relationship between the offline learning during the initial acquisition observed in this dissertation and the offline boost and learning that develop afterwards remains to be examined in future studies. One notable parallel between these two types of offline learning is that both of them are related to procedural and declarative memory (Brown & Robertson, 2007a, 2007b; Du, et al., 2016).

Findings in this current research should be extended to the classic finger-pressing SRT task (Curran & Keele, 1993; Nissen & Bullemer, 1987; Willingham, et al., 1989). It is expected that similar age-related differences in learning process and learning generalization will be identified. The major difference between these two paradigms is that foot stepping requires longer movement time (MT) while finger-pressing does not. However, results of studies through II to VI demonstrated that sequence learning was reflected by RT as a marker of mental processing, but not MT that characterizes the movement itself. In addition, results of the task pacing effect on sequence learning found in the current dissertation replicated previous findings in the classic finger-pressing SRT task (Destrebecqz & Cleeremans, 2003; Meulemans, et al., 1998; Weiermann & Meier, 2012b; Willingham, et al., 1997). Most importantly, it is hypothesized that online and offline learning, as well as the generalization of implicit motor sequence learning, is attributed to the interactive procedural and declarative memory systems. It is, therefore, not surprising to see offline and online learning processes in all tasks that require both procedural and declarative learning (e.g., the classic finger-pressing SRT task).

It has been widely found that postural control interacts with cognitive loads in both adults (Ramenzoni, Riley, Shockley, & Chiu, 2007; Riley, Baker, Schmit, & Weaver, 2005) and children (Olivier, Cuisinier, Vaugoyeau, Nougier, & Assaiante, 2007; Schmid, Conforto, Lopez, & D'Alessio, 2007). Yet, little is known about the interaction of motor sequence learning and postural control. Compared to the finger-pressing SRT task, the foot stepping task offers a window to study the interaction between postural sway and the cognitive process (i.e., RT). More importantly,

studying the temporal profile of the interaction could provide insights into the dynamics of the cognitive-motor coupling during sequence learning, which could help us better understand the learning of sequential motor actions in adults, children, and also children with movement or cognitive disabilities.

In summary, studies in this dissertation are the first to identify two age-related learning processes, namely online and offline learning, underlying the initial rapid acquisition of motor sequences. The two learning processes are, at least partially, responsible for the age-related differences in the generalization of implicit motor sequence learning. In addition, these two processes are likely to be attributed to the competition between procedural and declarative memory. Overall, this dissertation extends our understanding into the development of implicit motor sequence learning. Future studies are needed to further probe the underlying memory mechanisms and characterize the developmental landscape of these two learning processes.

Summary and Overall Significance

The set of experiments in this dissertation programmatically investigated whether there are age-related differences in implicit motor sequence learning. Specific Aim 1 demonstrated the age-related differences in the processes underlying the fast learning of motor sequences. Specifically, learning in six-year-old children dominantly relied on an offline process where RT improves after a short rest, while offline enhancement and online progressive improvement in RT concurrently drove sequence learning in 10-year-old children and adults. This is the first study reporting online and offline processes during the fast learning stage of implicit motor sequence acquisition. These results serve as a foundation for the subsequent experiments.

Specific Aims 2 and 3 investigated whether online and offline processes are illusory effects of fatigue or reactive inhibition or by-products of task pacing. The results excluded fatigue, reactive inhibition, and task pacing as factors leading to online and offline processes. Rather, Specific Aim 4 examined the effect of procedural and declarative memory on these two processes and the results suggest that age-related online and offline processes are active learning mechanisms that may be tied to declarative and procedural memory systems. Specific Aims 5 and 6 build on aims 1 to 4 by investigating if the generalization of implicit motor sequence learning is age-related and whether generalizing sequence learning is related to online and offline processes. Results indicate that children outperformed adults in generalizing learning of one sequence to a novel sequence when offline learning was greater in children compared to adults. When learning in adults and children resulted from comparable offline learning, the age-related differences in generalization were no longer observed. Collectively, these studies characterized the age-related differences in two learning processes (i.e., online and offline learning) that drive the fast implicit sequence acquisition and demonstrated that the age-related online and offline learning may lead to a superior ability in generalizing motor sequence learning in children compared to adults.

These experiments taken together are significant as they offer insights into the mechanisms underlying the development of implicit motor sequence learning from childhood to adulthood. Particularly, this line of research is unique in its ability to characterize the nature of the age effect in implicit motor sequence learning. Compared to previous studies that used aggregate outcomes (mean RT) to assess age-

related learning, this dissertation focuses on the learning process underlying implicit motor sequence acquisition, the understanding of which is a crucial aspect of research in motor development (Clark & Whitall, 1989). A clear picture about how typically developing children and typically developed adults learn implicit motor sequences may serve as a theoretical foundation to develop interventions for children and adults with learning disabilities. In addition to better understanding sequence learning in the motor domain, the results of these studies may offer suggestions regarding learning in other domains such as cognition where the learning of sequences is also critical. Most importantly, it provides potential insights into the question of why childhood is an optimal period for learning.

Appendices I: Supplementary Results for Study II (Chapter 4) Task pacing has no effects on online and offline processes underlying rapid motor sequence learning

Results in study I (Chapter 3) demonstrate that fast sequence learning in children and adults are expressed by two different processes. In particular, learning in six-year-old children dominantly relies on an offline process where RT improves after short rests, while offline RT enhancement and online progressive improvement in RT concurrently drive the sequence learning in 10-year-olds and adults. The age-related differences in online and offline processes may also be by-products of task pacing conditions. In study I, children and adults performed the task under the same inter-stimulus-interval (ISI). The same ISI was relatively shorter in children than adults. Thus, the ISI may prevent children from learning the sequence online as online learning demands iterative mental computation that needs an adequate amount of time between stimuli. In addition, a shorter ISI in children made the task relatively faster, which may induce greater fatigue or reactive inhibition that slow down the online RT.

To examine the effects of task pacing on online and offline processes underlying the fast motor sequence learning, in study II, we asked adults to perform the SRT task under different ISI conditions. A one-way ANOVA failed to find a significant effect of ISI on offline changes in RT ($F(2,27) = 0.95$, $p=0.4$) and online changes in RT ($F(2,27) = 2.31$, $p = 0.12$) (Figure A.1A). We also analyzed the corrected offline learning as that in study I. The same results were found compared to the original offline learning data (Figure A.1B). The corrected offline changes in RT

were not affected by ISI (F(2,27) = 0.41, *p*=0.67). Interestingly, online changes in RT were negative under the 300 + 600ms ISI. This result indicates that offline RT changes observed under this ISI condition were likely to result from fatigue or reactive inhibition. Taken together, the results suggest that the task pacing has little effect on online and offline changes in RT.
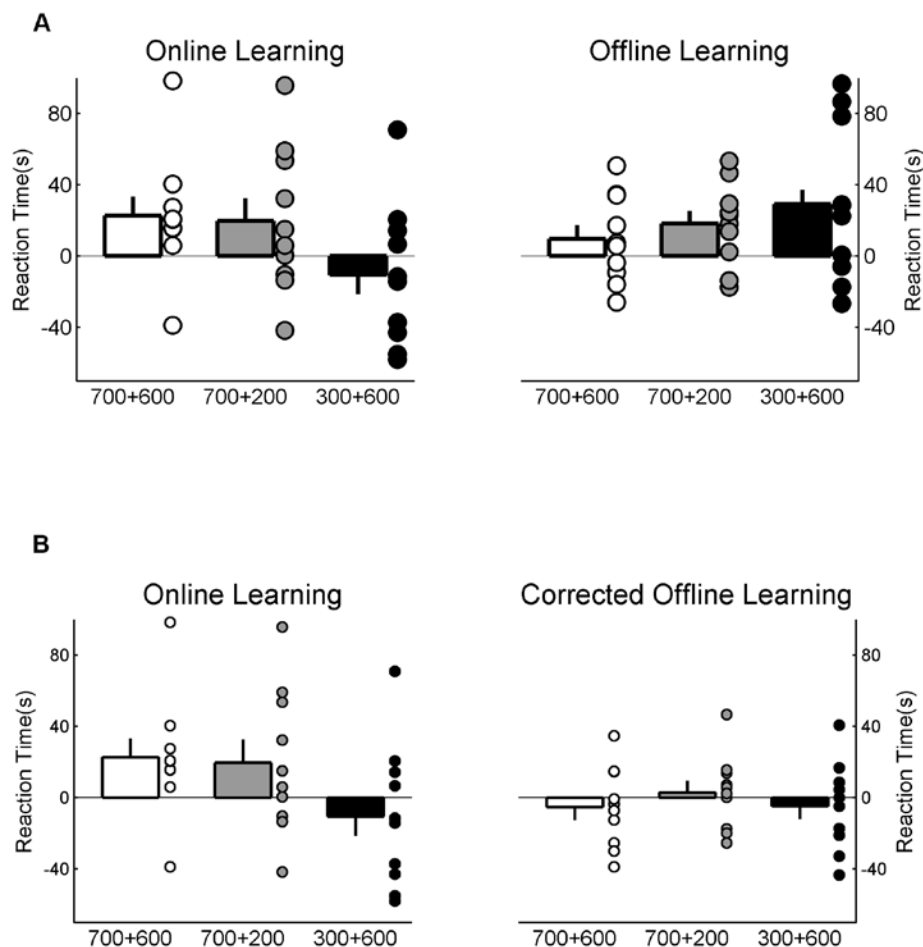
Figure A.1. ISI does not affect online and offline learning.
(A) Online and offline learning. (B) Online and Corrected offline learning.

To examine whether declarative knowledge of sequence A was acquired during the SRT task, we used the process dissociation procedure (Destrebecqz & Cleeremans, 2001; Destrebecqz & Cleeremans, 2003). Specifically, upon completion of the SRT task, participants were asked to generate two sequences under either the

inclusion or exclusion condition. Under the inclusion condition, participants were asked to recall the sequence in the SRT task and to write down 60 steps of the sequence (i.e., inclusion). Under the exclusion condition, they generated another 60-step sequence in which they were instructed to avoid the sequence they experienced during the SRT task (i.e., exclusion). To measure the score in the recall test, we counted the number of correct 2-, 3-, and 4-element chunks in the sequences that participants generated. The chance levels of recalling 2-, 3-, and 4-element chunks were different. The chance level for a two-element chunk in the sequence was 50% (i.e., given the first element, 33.33% chance for the second element); the chance level for a three-element chunk in the sequence was 5.56% (i.e., given the first element, 33.33% chance for the second element and 16.78% chance for the third element); and the chance level for a four-element chunk in the sequence was 0.94% (i.e., given the first element, 33.33% chance for the second element, 16.78% chance for the third element, and 16.78% chance for the fourth element). Given that participants recalled a 60-element long sequence that has 59 2-element chunks, the chance level for recalling 2-element chunks in a 60-element long sequence written by an individual was 19.65 (i.e., 59×0.333). Similarly, the chance level was 3.25 (i.e., 58×0.056) for recalling 3-element chunks and 0.54 (i.e., 57×0.009) for recalling 4-element chunks in a 60-element long sequence written by an individual.

Since recall score were measured by count data, Poisson models were used to examine the effect of ISI and recall conditions (inclusion vs exclusion). If over-dispersion was displayed in the recall score, a Poission mixture model (i.e., negative binomial regression) was used instead.

In the recall test, we found a significant effect of recall condition on recalling 2- ($\chi^2_{df=1} = 10.06$, p < 0.001), 3- ($\chi^2_{df=1} = 9.05$, p < 0.01), and 4-element chunks ($\chi^2_{df=1} = 7.15$, p < 0.01, Figure A.2). There was no effect of ISI and its interaction with the recall condition. Despite the ISI and length of chunks, participants recalled more chunks under the inclusion compared to the exclusion condition. In addition, recall was higher than chance under the inclusion condition despite the ISI (all p <0.001). Recall of all length of chunks under the exclusion condition was higher than chance when the ISI was 700+200ms and 300+600ms. For participants who performed the SRT task under the 700+600ms ISI, recall under the exclusion condition was higher than chance for recalling 2- and 3-element chunks but was at chance for recalling 4-element chunks. These results suggest that participants acquired declarative knowledge of sequence A but they had little control over the knowledge they acquired, especially if they learned the sequence under a shorter ISI.
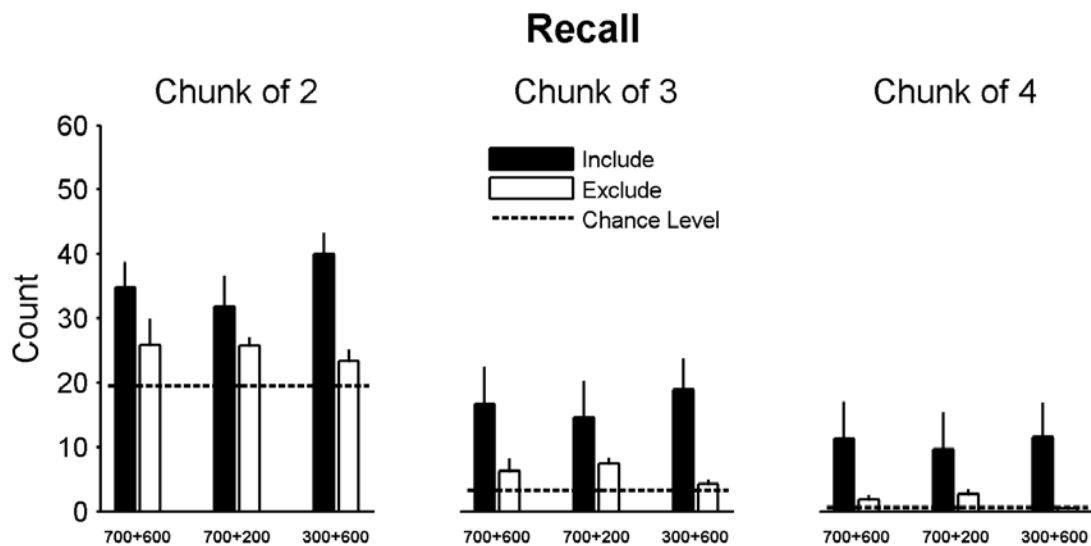
Figure A.2: ISI does not affect the declarative knowledge of the sequence acquired during the SRT task.

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