ABSTRACT

Title of Dissertation: THE EFFECTS OF SEQUENCE STRUCURE, AGE-RELATED IMPAIRMENTS, AND PARKINSON'S DISEASE ON MOTOR SEQUENCE LEARNING

Shikha Prashad, Doctor of Philosophy, 2015

Dissertation directed by: Dean and Professor, Dr. Jane E. Clark
Department of Kinesiology
Graduate Program in Neuroscience and Cognitive Science

Parkinson's disease (PD) is a neurodegenerative movement disorder that affects over one million individuals in the US with approximately 60,000 new diagnoses every year. While characterized as a movement disorder, the effect of PD and aging on learning new motor skills has yielded equivocal results. Thus, the broad objective of this dissertation is to investigate the influence of PD on motor sequence learning. We begin by examining different sequence structures and how they are affected by age before investigating the effects of PD. To address the inadequacies of previous studies using fixed order sequences, we used probabilistic sequences, in which stimuli are linked by statistical associations. The first study directly compared the learning of probabilistic sequences to fixed sequences and randomly ordered stimuli in typical young adults (18-23 years) using a modified serial reaction time (SRT) paradigm. The results suggest that both fixed and probabilistic sequence groups exhibited learning, but the underlying learning processes were different in employing online and offline learning strategies. In the second and third studies,

electroencephalography (EEG) was recorded from typical young adults (18-23 years), typically aging adults (55-75 years), and patients with PD (55-75 years) while they performed the same modified SRT task. We characterized the developmental landscape of 55-75 year old adults and found that cluster analysis separated typically aging adults into groups that provided a clearer understanding of their impairments. By unraveling movement and cognitive deficits and matching participants based on functional characteristics, we found that some typically aging adults and those with PD learned the fixed sequence, but not the probabilistic sequence, indicating age-related impairments in probabilistic motor sequence learning. We found cortical activations indicative of learning, even in the absence of behavioral indications suggesting that some adults may require more practice to learn the sequence, and possible compensatory mechanisms in patients with PD. Novel applications of these techniques prove effective for a deeper understanding of the dynamic motor learning process and provide evidence that impairments observed in patients with PD may be related more to the aging process than to Parkinson's disease.

THE EFFECTS OF SEQUENCE STRUCURE, AGE-RELATED IMPAIRMENTS, AND PARKINSON'S DISEASE ON MOTOR SEQUENCE LEARNING

By

Shikha Prashad

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2015

Advisory Committee:

Professor Jane E. Clark, Chair
Professor Bradley D. Hatfield
Assistant Professor Donald J. Bolger
Assistant Professor Rodolphe Gentili
Associate Professor Jeffrey R. Harring

Acknowledgements

I would like to thank everyone who has helped me through this process, especially:

- Dr. Jane Clark for "adopting" me midway through my graduate career and providing me the freedom to pursue my research interests. You are the best advisor I could have asked for and I am grateful for your mentorship, advice, and friendship.
- Drs. Hatfield, Gentili, Bolger, and Harring for serving on my committee, being incredibly encouraging, and answering my many questions.
- Dr. Marcio Oliveira for being a great mentor, providing advice, and helping me develop my teaching.
- Dr. José Contreras-Vidal for teaching me everything I know about data collection, working with patients, signal processing, and EEG.
- Yue Du for being extremely helpful and inspiring me to think about data in novel ways.
- Stephanie Youlios, Catherine Robinson, Natasha Kumar, Matthew Lee, Devin Kanach, Alex Gurton, Ilana Schoenbrun, and Hannah Haruni for their hard work in assisting with the data collection and analysis. I could not have collected these data without you.
- All of my participants for "lending" me their brains and without whom none of this would have been possible.
- The NACS program (especially Pam Komarek) and the Department of Kinesiology (especially Dr. Roth, Polly, Regina, Jessica, Bianca, and Joanna) for their support.

- The sources of funding that made it possible to attain a world-class education for very little cost: Department of Kinesiology, Sally Phillips Dissertation Fellowship, School of Public Health Dean's Fellowship, and Graduate School Summer Research Fellowship.
- The Department of Kinesiology Graduate Research Initiative Project (GRIP) for providing the funding to conduct the studies in this dissertation.
- My current and previous CogMo labmates: Li-Chuan, Kyle, Hyuk, Andrew, Harsha, Anusha, and Alessandro for providing advice and assistance, tolerating my whining, having deep discussions about random topics, and for being wonderful friends.
- Elisa and Hannah for being incredible friends for almost 15 years.
- Ayush for being a solid rock of support throughout this process and for always lifting me up when I am down.
- My parents and brothers for their patience, inspiration, unconditional love, and for always pushing me to excel and follow my dreams.

My deepest gratitude to all of you for your support and friendship. This important milestone would not have been possible without you.

Table of Contents

List of Tables

List of Figures

Chapter 1 : Introduction

Parkinson's disease (PD) is a neurodegenerative movement disorder that affects over one million individuals in the US with approximately 60,000 new diagnoses every year. However, a large number of cases may be undetected and it is estimated that there are up to 10 million people with PD worldwide. In addition, PD has an estimated 4% diagnosis rate before the age of 50 years and is a common disorder in adults over the age of 80 years (National Institute of Neurological Disorders and Stroke, 2015; Parkinson's Disease Foundation, 2015). Thus, the incidence of PD will likely increase as a larger proportion of the population ages and life expectancies increase. Further, by the time symptoms surface and PD is diagnosed, there is 60-80% dopamine depletion (Fahn & Jankovic, 2007), leaving a small window for treatment of the disease. PD has been characterized as a movement disorder, owing primarily to the movement impairments that are associated with the disease. However, the effect of PD on learning new motor skills has demonstrated equivocal results. It is therefore the purpose of this dissertation to characterize the potential impairments in motor sequence learning in patients with PD.

Motor sequence learning is fundamental to performing complex motor behaviors that emerge from simpler movements produced in a particular order. From brushing our teeth, getting out of a car, typing on the computer keyboard, and speaking, our actions follow a sequence of movements performed at specific times in a specific order. Sequences can be learned explicitly, in which there is a conscious effort to learn the sequence (e.g., learning how to type or play the piano), or they can be learned implicitly, in which there is no conscious knowledge that a sequence is being learned (e.g., learning to ride a bicycle).

Both types of learning play an important role in daily functioning throughout the life span. Thus, it is not only important to study motor sequence learning in young adults, but also to characterize the influence of aging and diseases on this important motor capacity.

While previous studies have shown functional abnormalities associated with the execution of movement sequences, little is known about the neural correlates of motor sequence learning in PD. Specifically, there are inconsistent findings on whether patients with PD are impaired in implicit sequence learning (e.g., Shin & Ivry, 2003; Siegert et al., 2006; Wilkinson & Jahanshahi, 2007; Wilkinson et al., 2009). The pathology exhibited in PD (such as bradykinesia, rigidity, and postural instability) is due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta, a nucleus that is a part of the basal ganglia (Fahn & Jankovic, 2007). Dopamine plays an important role in the regulation of movement and its depletion causes increased inhibitory outflow in the basal ganglia and thus causes slow movement (bradykinesia) and difficulties in the initiation of movement (Fahn & Jankovic, 2007). The effects of dopamine depletion are widespread in the brain due to the various cortico-striatal loops and the depletion in these loops plays a role in higher cognitive functions (Middleton & Strick, 2000a, 2000b). Thus, the regions that are affected in PD are not just the basal ganglia, but also cortical motor regions that play an important role in motor skill learning and areas related to cognition (Middleton & Strick, 2000b).

Neuroimaging studies using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have focused on determining the anatomic neural correlates of motor sequence learning. Many have suggested that the basal ganglia and associated cortical areas play an important role in implicit motor sequence learning

(Doyon, 2008; Doyon et al., 2009a; Doyon & Benali, 2005; Doyon & Ungerleider, 2002; Jueptner, Frith, Brooks, Frackowiak, & Passingham, 1997; Mentis et al., 2003; Penhune & Doyon, 2002; Ungerleider, Doyon, & Karni, 2002). The subcortical nuclei in the basal ganglia form loops with cortical regions through the thalamus and facilitate the flow and processing of motor information. These loops include specific motor regions in the cortex, such as the primary motor cortex (M1), supplementary motor area (SMA), and premotor cortex (PM) as well as other areas related to cognitive function, such as the prefrontal cortex (PFC) (Middleton & Strick, 2000a, 2000b). Further evidence supporting the role of the basal ganglia in motor skill learning also comes from impairments found in patients with basal ganglia dysfunction, such as patients with PD.

The presence of PD surely is a major contributor to the patients' impairment; however, these individuals are also aging. Thus, it is important to disentangle the effects of aging from those of PD. Aging is known to have a detrimental effect on learning and memory. However, research findings on learning motor sequences in typically aging adults have been equivocal (D'Esposito, Zarahn, Aguirre, & Rypma, 1999; Daselaar, Rombouts, Veltman, Raaijmakers, & Jonker, 2003; Wu & Hallett, 2005). Particularly, it is unclear whether implicit learning is affected by aging. Since motor skill learning is an important skill throughout the lifespan, there is a need for a clearer understanding of the effect of aging on motor sequence learning. The investigation of the influence of aging on motor sequence learning is also important for the characterization of the developmental landscape of the behavioral and neural correlates of implicit motor sequence learning upon which the profile of those with PD can be compared.

Thus, this dissertation research begins by investigating the effect of sequence structure on learning and performance in typical young adults. On this foundation, the effect of sequence structure in typically aging adults and patients with PD will be investigated to answer critical questions about the influence of aging and PD on implicit motor sequence learning. The importance of the proposed set of studies lies not only in examining the impairment of motor sequence learning in typically aging adults and patients with PD behaviorally using novel methods, but also in investigating the neural correlates of motor sequence learning; specifically the role of the basal ganglia and cortex. Results from these studies will help obtain a better understanding of motor sequence learning through the lifespan and in disease, while also furthering research in the brain dynamics of those with PD.

Research strategy

The most commonly used paradigm to assess implicit motor sequence learning in the laboratory is the serial reaction time (SRT) task (Nissen & Bullemer, 1987). In this task, participants respond to stimuli on a computer screen by pressing the corresponding key as quickly and accurately as possible. However, the participants are unaware that the stimuli are presented in a repeating sequence. Learning is observed as a progressive reduction in the reaction time (RT) during the learning blocks and by a reduction in the number of errors.

While many studies have used the SRT task, there are important knowledge gaps that are yet to be addressed. Traditionally, a fixed structure has been used to create the sequences in which the stimuli occur. The sequence most commonly consists of 10 or 12

items that repeat 10 times in a block, making it very likely that participants will eventually recognize the presence of a sequence. Other types of sequences may be better suited to study implicit motor sequence learning, but little is known about other types of sequence structures. Probabilistic sequences are an example of an alternate type of sequence that do not have a fixed structure and are created based on probabilistic associations between the items, hence making the sequence less likely to be detected and therefore explicit to the learner.

Previous neuroimaging studies involving motor sequence learning focus on changes in activation of select cortical areas rather than connectivity between these areas. Electroencephalography provides a useful technique for investigating cortico-cortical activations with excellent temporal resolution within the SRT paradigm (Doyon, et al., 2009a; Jin, Lin, Auh, & Hallett, 2011; Jin, Lin, & Hallett, 2011).

In summary, the following specific aims describe the goals and hypotheses that form the basis of this dissertation in which the differences between typical young adults, typically aging adults, and patients with Parkinson's disease are investigated while performing a motor sequence learning task with sequences of different structures.

Specific aims (SA)

Before investigating the primary goal of understanding the differences in typical young adults, typically aging adults, and patients with Parkinson's disease while performing a motor sequence learning task, preliminary studies were needed to better understand how typical young adults learning sequences with fixed and probabilistic structures.

SA 1 (Study 1). To determine the effect of sequence structure on the learning and performance of motor sequences in typical young adults.

The performance patterns of fixed sequences (e.g., 10 repetitions of 3412432142) have been well characterized in the SRT task with an expected decrease in response times during the learning blocks and an increase in response time when stimuli are displayed in a random order. To our knowledge, the performance pattern of probabilistic sequences generated by a first-order transitional probabilistic structure has not yet been characterized.

In this study, typical young adults were randomly assigned a fixed, probabilistic, or random sequence. Response times were analyzed to determine whether stimuli occurring in fixed and probabilistic are significantly different from those occurring in a random order as well as learning within and between blocks.

Hypothesis 1: Young adults will be able to learn the probabilistic sequence and will exhibit a similar performance pattern as fixed sequences. The performance patterns of both fixed and probabilistic sequences will be different from that of the random sequence, which will only exhibit a decrease in response time in the first few blocks.

SA 2 (Studies 2 & 3). To determine whether the sequence structure has a differential effect on reaction time, movement time, and response time.

Previously, SRT studies have used reaction and response times interchangeably. For example, if the task requires a choice between four buttons, the participants place one finger on each of the buttons and press the button that corresponds to the location of the stimulus. However, in this design, the reaction time and movement time cannot be

distinguished because the amount of movement is very small. This difference may be an important distinction and may provide insight into differences between sequence structures. The modified SRT task used in the current set of studies allows for the decomposition of the response time into the reaction time and movement time by increasing the amount of movement required in the task, making it possible to separate out the movement time from the reaction time.

Hypothesis 2.1: In both typical young and aging adults, RT will decrease with the learning of the sequence and will increase when random stimuli are presented. However, MT will remain constant. Typically aging adults will exhibit slower RTs and MTs than the typical young adults.

Hypothesis 2.2: Patients with PD with exhibit slower RTs and MTs than the typically aging adults. Due to the movement deficits in PD, MT will play a more critical role in the overall response time in the patients with PD.

SA 3 (Study 2). To determine whether sequence structure has a differential effect in typically aging adults compared to typical young adults.

Previous studies have shown inconsistent findings on the effect of aging on motor sequence learning. However, given the cognitive decline and impaired memory in typically aging adults, there are likely to be deficits in performance. In addition, it is not known whether typically aging adults will exhibit the same differences in performance patterns between fixed and probabilistic sequences as seen in young adults in Study 1.

Hypothesis 3: Typically aging adults will display a decrease in reaction time during the learning blocks and an increase in reaction time when stimuli occur randomly for both

fixed and probabilistic sequences (i.e. typically aging adults will exhibit similar differences in performance patterns between fixed and probabilistic sequences as seen in young adults). However, typically aging adults will exhibit significantly slower reaction times compared to typical young adults.

SA 4 (Study 2). To characterize the developmental landscape of 55-75 year old adults with respect to the learning of fixed and probabilistic sequences using cluster analysis.

Previous studies investigating the effects of aging on motor sequence learning have often combined older adults into one group, despite large age ranges, to compare with young adults. It is important to characterize the developmental landscape of aging since different age groups may perform differently (e.g., 55-year-old adults may perform differently from 75-year-old adults).

Reaction time measurements have been demonstrated to have high variability both between and within individuals. Thus, statistical methods that not only emphasize the performance of the individual, but also explore population level effects. Cluster analysis is one such method that may provide insights into how aging influences motor sequence learning by grouping participants based on all their reaction time trials through all the blocks, rather than age.

Hypothesis 4: Typically aging adults will display differential rates of change in reaction time. Those in the lower age range (55-63 years) will display a faster change in reaction time with practice and will be clustered separately than those in the higher age range (67-75 years).

SA 5 (Study 2). To characterize the brain dynamics of typical young and aging adults while performing a motor sequence learning task measured through EEG.

Most neuroimaging studies investigating implicit motor sequence learning have been conducting using fMRI as it provides excellent spatial resolution. Given that reaction time (measured in milliseconds) is the variable used to infer learning, we propose that electroencephalography (EEG) is better suited to identify cortical activations and cortico-cortical connectivity associated with learning and impairments, as EEG provides excellent temporal resolution.

Hypothesis 5: While performing a motor sequence learning task, typically aging adults will exhibit lower levels of cortical activation and connectivity in the alpha and beta bands compared to typical young adults.

SA 6 (Study 3). To determine whether individuals with Parkinson's disease can learn sequences with fixed and probabilistic structures.

Previous studies have shown inconsistent findings on the effect of Parkinson's disease on motor sequence learning. Implicit learning is thought to be mediated by the cortico-striatal circuit and given that the basal ganglia have vast connections throughout the cortex, it seems likely that basal ganglia deficits will influence the learning of both fixed and probabilistic sequences. In addition, the basal ganglia have been implicated in probabilistic learning and patients with PD have been shown to be impaired in certain probabilistic tasks. Studying motor sequence learning in those with PD provides a unique window to understand not only the neural correlates of implicit learning, particularly the relationships of the cortex and basal ganglia, but also insight into the disorder.

Hypothesis 6: Patients with Parkinson's disease will exhibit an impaired performance while learning the fixed sequence and will exhibit no learning of the probabilistic sequence. They will have significantly slower reaction and movement times compared to typically aging adults.

SA 7 (Study 3). To characterize the brain dynamics of individuals with Parkinson's disease and typically aging adults while performing a motor sequence learning task measured through EEG.

As described above, the temporal resolution provided by EEG is better suited to identify cortical activations and cortico-cortical connectivity associated with learning and impairments in the SRT task where learning is inferred through reaction times measured in milliseconds.

Hypothesis 7: While performing a motor sequence learning task, patients with Parkinson's disease will exhibit lower levels of cortical activation and connectivity in the alpha and beta bands compared to typically aging adults.

Overall significance

The set of research studies that comprise this dissertation will have a significant impact on the understanding of motor sequence learning in typical young adults, typically aging adults, and patients with Parkinson's disease. These studies will be the first to determine whether probabilistic sequences are an effective means to better assess implicit learning. Since probabilistic sequences more accurately reflect learning acquired in daily life, the use of probabilistic sequences will provide more ecological validity to the SRT

framework. In addition, the influence of age and Parkinson's disease will be examined on the learning of these different sequence types. By decomposing response time into reaction and movement times and using cluster analysis, these studies will delve deeper into the effects of sequence learning on reaction and movement times while clustering typically aging participants based on participants' reaction times rather than their chronological age to attain a clearer understanding of performance differences that may or may not be age-related. Thus, these studies will address methodological and analytical problems in current SRT studies and address knowledge gaps regarding the effects of aging and PD on motor sequence learning in both behavioral performance and cortical dynamics.

Learning complex behaviors is a requirement throughout the lifespan, making it imperative to study the influence of aging and PD on motor sequence learning. As a larger proportion of the population ages and the incidence of Parkinson's disease increases, our understanding of the influence of aging and PD is crucial to add to our knowledge of the motor system and development of interventions for movement and cognitive deficits.

Organization of the dissertation

This dissertation is divided into six chapters. Chapter 1 summarizes the overall purpose, specific aims, and significance of the research strategy for this dissertation. Chapter 2 reviews the relevant literature including the theoretical framework for motor sequence learning, neural correlates, and neuroimaging of motor sequence learning in typical young adults, typically aging adults, and patients with Parkinson's disease. Chapter 3 details study 1 (SA1) that investigated whether there are differences in the learning and performance patterns of fixed, probabilistic, and random sequences in typical young adults.

Chapter 4 reports on study 2 that examined the influence of aging on learning fixed and probabilistic sequences at the behavioral and cortical dynamics level. Chapter 5 describes study 3 that explored the influence of Parkinson's disease on the learning of the two sequence types. Chapters 3-5 are written as separate manuscripts that will be submitted for publication. Chapter 6 discusses the major findings of the three studies, their implications, and paths for future research.

Chapter 2 : Review of Literature

This chapter will provide a basis for the research in this dissertation. It will start by elucidating the difference between explicit and implicit motor sequence learning and will describe a theoretical framework for motor sequence learning. The next section lays the foundation for the serial reaction time task, which is the paradigm that is most commonly used for measuring motor sequence learning in the laboratory and a modified version of which was used in this research. Next, it will delve into the neural correlates underlying motor sequence learning in young adults. The next sections describe impairments in motor sequence learning in typically aging adults and patients with Parkinson's disease, respectively, as well as how electroencephalography (EEG) can be used for studying cortical dynamics. Finally, the last section summarizes the knowledge gaps and how this dissertation attempts to address them.

Motor sequence learning

Implicit and explicit motor sequence learning

Motor sequences can be acquired through explicit or implicit learning. Explicit learning occurs when there is a conscious awareness of the sequence, while implicit learning occurs when the sequence is learned unconsciously (A. S. Reber, 1967b, 1989b). Both types of learning are essential to learning complex motor skills throughout the lifespan. Previous studies have suggested that explicit and implicit learning have distinct neural substrates with the cortico-limbic circuit involved in explicit learning and cortico-striatal circuit involved in implicit learning (Knowlton, Mangels, & Squire, 1996; P. J. Reber & Squire, 1994; Squire & Zola, 1996). This dissertation will focus on implicit motor

sequence learning as participants will not be informed that there is an underlying sequence in the task.

Theoretical framework for motor sequence learning

Doyon & Benali (2005) propose that motor sequence learning is acquired in five stages: 1) an early learning stage in which performance improves significantly at a fast rate; 2) a later learning stage in which performance further improves, but at a slower rate and over multiple learning sessions; 3) a consolidation stage that takes place over a break of 4-6 hours after which performance increases; 4) an automatic stage that occurs after further practice and requires few cognitive resources to execute the skill; 5) a retention stage that does not require any more practice to perform the skill even after extended breaks (Doyon & Benali, 2005). This dissertation will focus on the first stage of motor sequence learning. We expect participants to exhibit significant improvements in performance in one session and will investigate the dynamic functional connectivity changes during this early learning stage.

Figure 2.1: Stages of motor sequence learning (adapted from Doyon & Benali, 2005)

The serial reaction time task

Implicit motor sequence learning has traditionally been examined by using a serial reaction time (SRT) task (Nissen & Bullemer, 1987). In this task, participants are presented with four squares on a computer screen. Each square corresponds to a button on a response

box. On a given trial, a stimulus appears in one of the squares and the participant must press the corresponding button as quickly and accurately as possible. Participants are unaware that the stimuli are presenting in a repeating sequence. The paradigm generally consists of six blocks: four learning blocks (B1-B4) in which the sequence is repeated 10 times each, one block (B5) in which the stimuli appear in a random order, and a final block (B6) consisting of the repeating sequence again. Learning is inferred through the progressive reduction in the reaction time (RT) during B1-4, an increase in RT in B5, since the stimuli are occurring in random order, and another decrease in RT in B6 (Robertson, 2007). The RT indicates the amount of time spent processing the information and is used as a measure of cognitive learning (Laming, 1968). Learning is also assessed by a reduction in the number of errors.

Sequence structure

The SRT task has traditionally used fixed sequences consisting of 10 or 12 items with four locations where the stimulus can appear. These sequences follow a rigid structure and the order remains the same in every repetition of the sequence. However, fixed sequences are not a practical model to assess the adaptive learning that occurs in real life.

After four learning blocks with a total of 40 repetitions of the sequence, it is very likely that participants become consciously aware of the presence of a sequence, thus changing the nature of learning from implicit to explicit (D. V. Howard et al., 2004; J. H. Howard & Howard, 1997; Nissen & Bullemer, 1987; Reed & Johnson, 1994; Song, Howard, & Howard, 2007b). This change can occur at different points during the learning process for different participants, further contaminating implicit motor sequence learning performance. Studies have tried to circumvent this issue by using modified versions of the

SRT task, such as incorporating a random-ordered stimuli in alternating trials (D. V. Howard, et al., 2004), employing dual task methodology (Reed & Johnson, 1994; Schvaneveldt & Gomez, 1998; Seidler et al., 2005), intermixing fixed sequences and random sequences within a block (Curran, 1997) or embedding probabilities within fixed sequences (J. H. Howard, Howard, Dennis, & Kelly, 2008; Wilkinson & Jahanshahi, 2007). The approach used in these studies to avoid explicit learning during the task has been three fold: 1) to increase the number of stimuli to eight, instead of four; 2) to increase the size of the sequence to 16; and 3) to use more complex sequences.

Second order conditional sequences

Second order conditional (SOC) sequences are a type of fixed sequence in which the response on a trial can only be determined by the past two trials (Reed & Johnson, 1994). These sequences are better suited to assess implicit learning in SRT tasks as no stimulus has more responses than another and no transition between stimuli occurs more often than others. This ensures that participants are improving their RT performance by learning the sequence rather than learning patterns within the sequence (DeCoster & O'Mally, 2011b).

Probabilistic sequences

In order for learning to remain implicit, the underlying stimulus structure must be complex enough to escape conscious awareness (A. S. Reber, 1989b). This does not seem to hold true for fixed sequences as they have a simplistic structure that can be uncovered at the conscious level. Previous studies have used complex stimuli such as finite state grammars in artificial grammar learning (A. S. Reber, 1967b, 1989b; P. J. Reber & Squire, 1999a) to assess implicit learning. Reber has shown that when participants memorize

seemingly random strings of letters generated by a finite state grammar, they unconsciously learned the underlying rules and were able to differentiate between novel strings that violate a rule and those that follow the rules (A. S. Reber, 1967b). However, these paradigms do not provide insight into the learning process. The SRT task can facilitate the understanding of the progression of learning by examining the RT in the individual blocks.

In the current set of studies, the probabilistic sequences were generated by a first-order transitional probabilistic structure in which the present state influences what the next state will be based on probabilities between the states. These transitional probabilities are defined in a transition matrix containing the probabilities associated with each pair of states. Probabilistic sequences do not follow a rigid structure, but rather follow a probabilistic rule and are more complex. The rule is not deterministic and in any given repetition of the sequence, the order of the stimuli may be different. Over numerous trials, the participant is expected to unconsciously detect the probabilistic rule underlying the sequence (e.g., 2 is most likely to be followed by 6) and exhibit a decreased RT.

Thus, while fixed sequences have been used extensive in the implicit motor sequence learning literature, they are not conducive to understanding how learning occurs in real life, where we continuously make statistical associations between events unconsciously (Cleeremans & McClelland, 1991; Cleeremans, Servan-Schreiber, & McClelland, 1989; A. S. Reber, 1989b) and learn probabilistic orders that can change in a dynamic environment.

Motor sequence learning in young adults

Neural correlates of motor sequence learning

Human neuroimaging studies suggest that various cortical and subcortical areas, including primary motor cortex (M1), premotor cortex (PM), prefrontal cortex (PFC), supplementary motor area (SMA), basal ganglia, and cerebellum are activated during the early learning stage (Doyon, et al., 2009a; Doyon & Benali, 2005; Doyon & Ungerleider, 2002; Hikosaka, Nakamura, Sakai, & Nakahara, 2002; Jueptner, et al., 1997; Mentis, et al., 2003; Penhune & Doyon, 2002; Sakai et al., 1998; Ungerleider, et al., 2002). The interaction between two circuits, the cortico-striatal and the cortico-cerebellar circuits, underlies the activations of these cortical and subcortical areas in relation to motor sequence learning (Doyon, 2008; Doyon & Benali, 2005; Doyon & Ungerleider, 2002; Middleton & Strick, 2000a, 2000b; Ungerleider, et al., 2002). Doyon et al. further suggest that the interactions between these two circuits are critical in order to create the motor routines to learn the new sequence. Impairments in patients with striatal (Parkinson's or Huntington's disease), cerebellar, or frontal cortical dysfunction further suggest a role of these areas in motor skill learning (Doyon, 2008; Doyon et al., 1997; Mentis, et al., 2003), including a possible role for compensation for striatal dysfunction via the cortico-cerebellar loop (see below for further discussion of compensatory mechanisms in patients with Parkinson's disease). Additional evidence has been provided by animal studies (White, 1997) in rodents (McDonald & White, 1993), cats (Milak, Shimansky, Bracha, & Bloedel, 1997), and non-human primates (Lu, Hikosaka, & Miyachi, 1998).

While widespread activations in the cortical and subcortical areas are particularly found in the early earning stage of the motor sequence learning, studies suggest that

dissociation between these two loops occurs in the later stages of motor learning. The cortico-cerebellar loop appears to be involved in the early learning stage (Doyon & Benali, 2005; Doyon, Penhune, & Ungerleider, 2003; Jenkins, Brooks, Nixon, Frackowiak, & Passingham, 1994; Ungerleider, et al., 2002), but the activation decreases with practice and as the skill becomes automatic. However, the cortico-striatal loop activation does not decrease and remains the same during the consolidation and retention of learned sequences (Doyon, et al., 2009a; Doyon, et al., 2003; Jueptner, et al., 1997; King, Fogel, Albouy, & Doyon, 2013; Penhune & Doyon, 2002; Ungerleider, et al., 2002; Willingham, 1998), suggesting that the cerebellum is not necessary for long term retention of sequences, but the basal ganglia are. The basal ganglia have also been shown to be activated during incremental learning of associations and stimulus incidence over time that leads to automatization of the skill (Knowlton, et al., 1996; Rieckmann & Backman, 2009) (see Figure 2.2).

Penhume & Doyon (2002) used positron emission tomography (PET) to determine differences in the neural correlates of learning a sequence and recall of the sequence. The study took place over multiple weeks in which participants were scanned on three days. On day 1 (early learning), participants were explicitly taught the sequence and then scanned while they performed the sequence during one block. On day 5, after five days of practicing the sequence, the participants were again scanned for one block. Lastly, participants were scanned after four weeks, with no further practice. The neuroimaging data demonstrated that the cerebellum was activated during the early learning stage. However, by day 5, the cerebellar activity decreased, while the basal ganglia activity increased. During recall, increased activation was seen in the M1, PM, and parietal lobe, but not in the cerebellum

or basal ganglia, suggesting that recall involves a primarily cortical network. Further evidence for the role of the cerebellum in the early learning stage comes from patients with cerebellar lesions who do not demonstrate any sequence learning in the SRT task (Pascual-Leone et al., 1993; Shin & Ivry, 2003).

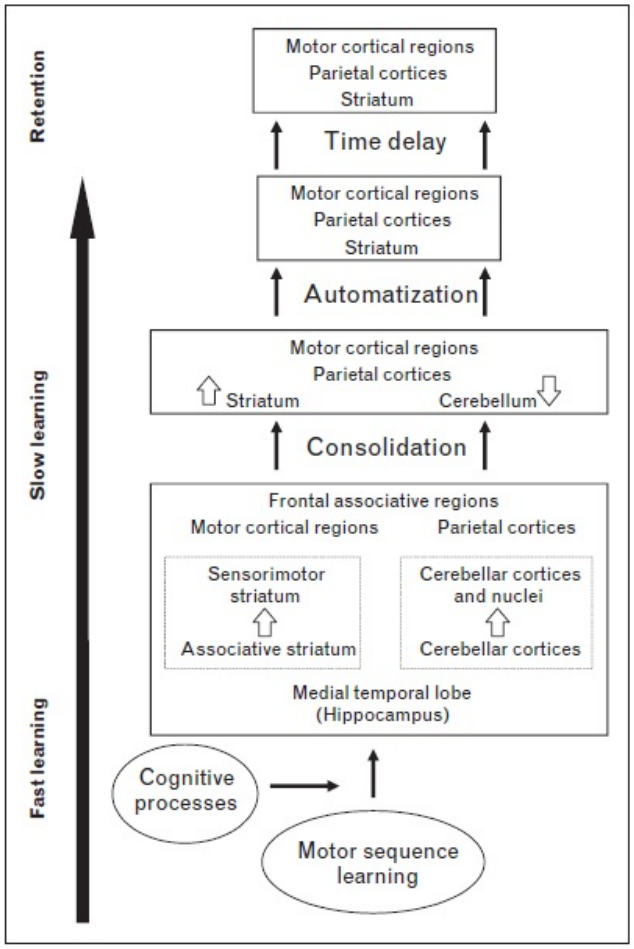

Figure 2.2: Doyon et al. (2002, 2003, 2005, 2008) have suggested a model in which dynamical interactions occur between cortico-striatal and cortico-cerebellar circuits during the early learning stage of motor sequence learning. As consolidation and automatization take place, the striatum plays a greater role in the process, while activation in the cerebellum decreases. This dissertation will focus on the early learning stage of the model (Motor sequence learning and movement disorders by J. Doyon, 2008, *Current Opinion in Neurology, 21*, p. 479).

Role of the basal ganglia in motor learning and cognition

The basal ganglia are a group of subcortical structures consisting of the striatum, caudate, putamen, globus pallidus (GP), subthalamic nucleus (STN), and substantia nigra (SN). The classical view of the basal ganglia consisted of circuits that only involved the motor cortex. It was thought that the only areas of the cerebral cortex that were targets of basal ganglia output were those that are involved in the generation and control of movement: the basal ganglia receives information from other cortical areas, such as the prefrontal cortex (PFC), parietal, and temporal lobes, and integrated these inputs in the subcortical nuclei, which were then sent to the primary motor cortex (M1) (Dum & Strick, 2009). However, this view was challenged by Alexander et al. (1986), who described five basal ganglia loops, of which only two were motor loops and the others were involved with higher-order cognitive processes. These five loops are: skeletomotor, oculomotor, dorsolateral prefrontal, lateral orbitofrontal, and anterior cingulate circuits (Alexander, Delong, & Strick, 1986).

Strick and colleagues have conducted various studies using neurotropic viruses as transneuronal traces in the CNS of primates to disentangle the paths of the circuits. When the virus was injected into portions of the M1, it was found that the M1 is richly innervated by the output of the basal ganglia nuclei, with the densest projections from the internal globus pallidus (GPi) and less dense projections from parts of the substantia nigra pars reticulata (SNr). However, these projections originated from only 15% of the GPi, which suggests that the majority of the output is directed to other cortical areas (Dum & Strick, 2009; Kelly & Strick, 2000; Strick & Card, 1992). The GPi also projects to multiple areas of the premotor cortex (PM) and supplementary motor area (SMA). When injected into the

PFC, the labeled neurons in the GPi were different from those labeled from injections to the motor areas. This suggests that there are separate motor and nonmotor domains in the GPi. Evidence of the separation of motor and cognitive output areas in the GPi also comes from pallidotomies of patients with PD in which lesions to anteromedial GPi (origin of output to PFC) leads to cognitive impairments, while lesions to intermediate GPi (origin of output to motor areas) has little effect on cognition (Dum & Strick, 2009). Subfields within PFC areas that are related to working memory and thought to guide behavior based on transiently stored information had projections from the GPi and SNr as well. In addition, GPi and SNr projections were also found in the posterior parietal cortex, which may be the basis of visuospatial deficits observed in patients with basal ganglia lesions. Area TE of the inferotemporal cortex plays an important role in the visual recognition & discrimination of objects. This area also receives projections from the SNr. These areas of the SNr have also been found to be responsive to the presentation of visual stimuli. Taken together, the basal ganglia are extensively connected to vast regions of the cerebral cortex, such as motor, premotor, prefrontal, posterior parietal, and inferotemporal areas (Dum & Strick, 2009; Packard & Knowlton, 2002), and are thus involved in higher-order cognitive processing.

Computational model of learning in the cerebral cortex, basal ganglia, and cerebellum

The interactions between the cortico-striatal and cortico-cerebellar loops have been computationally modelled by Doya (2000). According to this model, the basal ganglia, cerebellum, and cerebral cortex are involved in different types of learning (Doya, 2000). The basal ganglia is hypothesized to be involved in reward-based reinforcement learning via modulation of the dopaminergic pathways, while the cerebellum performs error-based

supervised learning via the convergence of climbing fibers on the Purkinje cells. Through unsupervised learning, the cerebral cortex organizes inputs from the basal ganglia and cerebellum via the cortico-striatal and cortico-cerebellar circuits. Learning occurs as a result of this interaction of the basal ganglia, cerebellum, and cerebral cortex (Doya, 1999, 2000; Hikosaka, et al., 2002; Houk & Wise, 1995) and has been supported by animal models in which dopamine modulated reward processing has also been found in cortical neurons. For example, single cell electrophysiology studies in monkeys, have shown differential reward processing in the orbitofrontal cortex and striatum (Schultz, Tremblay, & Hollerman, 2000).

Motor sequence learning in typically aging adults

Studies investigating the influence of aging on motor sequence learning have found that typically aging adults exhibit similar levels of performance on the SRT task as young adults if the sequence is not a complex higher order sequence (Bennett, Howard, & Howard, 2007; Daselaar, et al., 2003; Dennis, Howard, & Howard, 2006; Feeney, Howard, & Howard, 2002; Fraser, Li, & Penhune, 2009; Nemeth & Janacsek, 2010; Seidler, 2007; Weiermann & Meier, 2012). Differences may arise, however, when learning more complex sequences with typically aging adults exhibiting an impairment in learning higher order sequences (Bo & Seidler, 2010; Dennis, et al., 2006). These overall conclusions, however, do not always hold (Bennett, et al., 2007; J. H. Howard & Howard, 2013). For example, Curran (1997) investigated typically aging adults while learning sequences with different underlying structures. Both were fixed sequences, but one was a first-order conditional (FOC) sequence while the other was a more complex second-order conditional (SOC)

sequence. Curran found that typically aging adults only exhibited learning in the SOC sequence, but not the FOC sequence (Curran, 1997). This is surprising as FOC sequences are less complex and thus should be more likely to be learned than the more complex SOC sequences. It should be noted that the design used by Curran was not the typical block design used in SRT tasks in which four learning blocks of the repeating sequence are followed by a block containing stimuli in a random order. Instead, he used an intermixed block design in which sequenced and randomly ordered stimuli occurred within a block.

In contrast, while Dennis et al. (2006) also investigated the learning of FOC and SOC sequences in typically aging adults in an auditory SRT task, they found conflicting results in that typically aging adults were able to learn both FOC and SOC sequences. In addition, they also assessed learning of sequences described as higher-order probabilistic sequences, however there are no probabilities associated with each stimulus. Instead, an alternating serial reaction time (ASRT) task was used in which sequence trials alternated with random trails (e.g., 3*r*2*r*4*r*1 where the sequence is 3241 and *r* is a random trial that could be any of the four stimuli). Thus, a fixed sequence exists, but there is an interference by random trials, rather than a probabilistic sequence with underlying probabilities between the different stimuli. The authors found that typically aging adults were unable to learn the sequence in the ASRT task, concluding that aging impairs the learning of higher order sequences. However, it still remains unclear whether typically aging adults can learn probabilistic sequences. The differences they found may also be attributed to the task being described as an auditory task and thus more perceptual, however there was still a motor component as subjects responded to the stimuli with the middle and index finger of each hand. To further complicate any conclusions, another study found that typically aging

adults were in fact able to learn an even more complex sequence in an ASRT task in which a sequence trial was followed by two random trials (e.g., 3*rr*2*rr*4*rr*1) (Bennett, et al., 2007).

These differences in the results may also in part be due to the statistical analyses conducted. Most SRT studies assess learning by comparing the mean or median of each block across the age groups using ANOVA. However, means only provide a cursory assessment of the performance, rather than providing insight into how the learning occurs, which may provide an understanding of differences between age groups. Furthermore, reaction time measurements are highly variable between and within individuals and general linear models, such as ANOVA, do not adequately capture this variability. It is important to use statistical methods that not only emphasize the performance of the individual, but also explore population level effects. One such statistical method is random coefficient modeling that lends itself to analysis of data like that of the SRT task (Cudeck & Harring, 2007, 2010) and has been used to detect differences between typically developing children and those with developmental coordination disorder that the general linear model failed to detect (King, Harring, Oliveira, & Clark, 2011).

Neuroimaging studies have suggested that while the behavioral impairments may be small, there are larger underlying neurological changes and the impairments may be explained through cognitive deficits in typically aging adults. For example, it has been suggested that declines in working memory may result in impaired motor sequence learning, particularly in the early stage (Bo, Jennett, & Seidler, 2011, 2012; Ghilardi, Eidelberg, Silvestri, & Ghez, 2003; Hedden & Gabrieli, 2004; Seidler, Bo, & Anguera, 2012). Seidler and colleagues have further suggested differential effects of different types of working memory, in which verbal working memory may compensate for declines in

visuospatial working memory (Bo, et al., 2012). Neurochemical changes and a loss in striatal volume leading to degrading cortico-striatal networks may be additional factors related to impairments in learning more complex sequences (King, et al., 2013; Rieckmann & Backman, 2009; Seidler et al., 2010). Further cognitive declines may be caused by decreased function in the prefrontal cortex (Aizenstein et al., 2006; Daselaar, et al., 2003) that may be modulated by the dopamine projections from the striatum to the prefrontal cortex (Braver & Barch, 2002; Braver et al., 2001), however some studies have found no differences in brain activations between young and typically aging adults (Daselaar, et al., 2003). In addition to an increased cognitive load when learning higher order sequences, studies have also found that providing instructions to explicitly search for a sequence hinders implicit learning in typically aging adults (D. V. Howard & Howard, 2001), but not young adults (Willingham & Goedert-Eschmann, 1999). This may suggest that in typically aging adults, explicit knowledge pushes the processing capacity to its limit, thus manifesting in impairments in implicit learning (Rieckmann & Backman, 2009; Salthouse, 1996).

Thus, behavioral and neuroimaging studies on the influence of aging on motor sequence learning collectively lead to ambiguous conclusions. It is important to elucidate these findings to characterize the relationship between typical aging and motor learning.

Motor sequence learning in individuals with Parkinson's disease

Motor and cognitive deficits in Parkinson's disease

Parkinson's disease (PD) is a progressive neurodegenerative disease exhibiting both motor and cognitive symptoms. The pathology present in PD, such as bradykinesia,

rigidity, and postural instability, is due to the degeneration of dopaminergic neurons in the substantia nigra pars compacta, a nucleus that is a part of the basal ganglia. Dopamine plays an important role in the regulation of movement and its depletion causes increased inhibitory outflow in the basal ganglia and thus causes slow movement (bradykinesia) and difficulties in the initiation of movement (Fahn & Jankovic, 2007). The effects of dopamine depletion are widespread in the brain due to the various cortico-striatal loops (Alexander, et al., 1986; Middleton & Strick, 2000a, 2000b). Thus, the regions that are affected in PD are not just the basal ganglia, but also cortical regions that play an important role in motor skill learning and areas related to cognition. For example, studies assessing cognitive control suggest that patients with PD are impaired at response inhibition (Aron & Poldrack, 2006; Aron, Poldrack, & Wise, 2009; Mendes et al., 2012), category learning (Ashby & Ell, 2001b; Ashby & Maddox, 2005, 2011; Ashby & O'Brien, 2005; Filoteo & Maddoz, 2007; Keri, 2003; Knowlton, et al., 1996) , and spatial working memory (Owen, Doyon, Dagher, Sadikot, & Evans, 1998).

However, the relationship between dopamine and cognition is a complex one that is further complicated by dopaminergic medications. Studies have shown that a complex modulatory relationship exists between dopamine and performance in higher cognitive tasks, specifically through modulation of the prefrontal cortex (Braver & Barch, 2002; Seger, 2006b). It has been suggested that the relationship between dopamine and cognitive performance is an inverted-U, in which an optimum level of dopamine is required for optimum performance and excessive or insufficient levels impair performance (Cools, 2011; Cools & D'Esposito, 2006; Fallon et al., 2015). Thus, cognitive impairments in patients with PD can be explained by the intake of dopamine medications. The depletion

of dopamine in PD is not prevalent throughout the basal ganglia (Fahn & Jankovic, 2007; Seger, 2006b), causing greater levels of dopamine with the intake of levodopa medications in certain areas than is typical. Thus, if the area that the task is associated with has a depleted or increased level of dopamine, performance in the task is expected to be impaired (Argyelan et al., 2008; Feigin et al., 2003; Fuhrer et al., 2014; Kwak, Mueller, Bohnen, Dayalu, & Seidler, 2010, 2012; Seo, Beigi, Jahanshahi, & Averbeck, 2010; Shohamy, Myers, Geghman, Sage, & Gluck, 2006). Surgical interventions through deep brain stimulation further confound the differential effects of dopamine on higher cognitive functions (Carbon & Eidelberg, 2006; Mure et al., 2012).

Impairments in motor sequence learning in Parkinson's disease

Studies investigating impairments in motor sequence learning in patients with PD have found equivocal results possibly due to differences in methodologies, sequence types, disease severity, and effect of medications. However, the general conclusion is that implicit motor sequence learning is impaired in patients with PD (Fukuda, Edwards, & Eidelberg, 2001; Gamble et al., 2014; Jackson, Jackson, Harrison, Henderson, & Kennard, 1995; Ruitenberg, Duthoo, Santens, Notebaert, & Abrahamse, 2015; Siegert, Taylor, Weatherall, & Abernethy, 2006; Wilkinson & Jahanshahi, 2007; Wilkinson, Khan, & Jahanshahi, 2009), including SRT tasks without the motor component (Westwater, McDowall, Siegert, Mossman, & Abernethy, 1998), and under certain conditions, such as more complex sequences (Shin & Ivry, 2003; Smith & McDowall, 2006), perhaps in part due to a reduced working memory (Gabrieli, Singh, Stebbins, & Goetz, 1996). It has also been found that there is a trend towards degradation in performance and neural activity in PD as the disease progresses (Carbon, Reetz, Ghilardi, Dhawan, & Eidelberg, 2010) and thus impairment

may be a function of disease severity where those in the early stages of PD are relatively spared from impairment (Muslimovic, Post, Speelman, & Schmand, 2007; Stephan, Meier, Zaugg, & Kaelin-Lang, 2011). Results are further confounded by differential effects of dopamine on learning and activation of cortical regions (Argyelan, et al., 2008; Cools, 2011; Cools & D'Esposito, 2006; Feigin, et al., 2003; Kwak, et al., 2010, 2012; Seo, et al., 2010; Tremblay et al., 2010) and surgical interventions through deep brain stimulation (Carbon & Eidelberg, 2006; Mure, et al., 2012).

However, other studies have reported no impairments in the SRT task and artificial grammar (Hayes et al., in press; Helmuth, Mayr, & Daum, 2000; Nagy et al., 2007; P. J. Reber & Squire, 1999a; Smith, Siegert, & McDowall, 2001; Wilkinson & Jahanshahi, 2007) as long as patients with PD are provided more time to learn, which may be a result of compensation. To investigate possible compensatory mechanisms, Mentis et al. (2003) conducted a PET study in which early stage patients with PD and typically aging adults performed a center out task consisting of a sequence that participants determined through trial and error. In order to prevent potential confounds from differing levels of performance, the patients with PD and control participants were matched based on performance level. Over time, the patients with PD were able to perform at a level similar to that of typically aging adults, but the PET results indicated that patients with PD exhibited four times greater activation of the cerebellum to reach the same level of performance as typically aging adults (Mentis, et al., 2003). This suggests that in certain conditions (e.g., short fixed sequences) and given enough time, early stage patients with PD can achieve greater performance levels through a compensation mechanism via the cortico-cerebellar system. PD have also exhibited increasingly greater activation in premotor cortex, parietal cortex,

and SMA while performing more complex sequential finger movements suggesting that patients with PD compensate for degradations in cortico-striatal circuits by engaging more cortical regions (Catalan, Ishii, Honda, Samii, & Hallett, 1999; Fukuda, et al., 2001; Nakamura et al., 2001). Further evidence to support compensations via the cerebellum is from studies that show direct connections between the basal ganglia and cerebellum (Bostan, Dum, & Strick, 2010; Bostan & Strick, 2010). Additional compensatory mechanisms have been suggested via the hippocampus (Carbon, et al., 2010).

Thus, studying PD provides a unique opportunity to gain an understanding of the influence of an impaired cortico-striatal circuit on motor sequence learning. Determining whether certain mechanisms are used to compensate for striatal dysfunction can help uncover strategies that can be facilitated through new treatments. In addition, by developing a global network perspective on functional interactions, the connectivity between brain regions can be explored to further the understanding of neural underpinnings of motor sequence learning and the nature of impairment in aging and Parkinson's disease to develop novel strategies for interventions.

Understanding brain dynamics using EEG

Electroencephalography (EEG) is a non-invasive and relatively inexpensive neuroimaging technique that records electrical brain activity at the cortical level. It allows for the recording of cortical activity in various environmental and task constraints, while not being excessively taxing on participants, a particularly important consideration for clinical populations. Excellent temporal resolution makes EEG a valuable technique for

characterizing brain dynamics during motor sequence learning, by tracking changes occurring during early and late learning.

EEG in aging and Parkinson's disease

The alpha band is thought to reflect cognitive and memory performance and is synchronized in the resting state, but is attenuated when engaging in a cognitive task. The synchronization is a result of a large number of neurons oscillating in the same phase and frequency that is disrupted during a cognitive task in which different networks oscillate at different frequencies, resulting in suppression of the alpha band. Alpha power can be influenced by a number of factors, such as skull thickness, cerebrospinal fluid volume, methodology and data acquisition, arousal, and age. In adults over the age of 50 years, there is a slowing of alpha, a general increase in frequencies of less than 7Hz and decrease in frequencies greater than 7 Hz, and the suppression of alpha tends to decrease with age (Bonstrup, Hagemann, Gerloff, Sauseng, & Hummel, 2015; Klimesch, 1999; Polich, 1997; Rossini, Rossi, Babiloni, & Polich, 2007). Similar results have been found in patients with neurological disorders.

Patients with PD exhibit an abnormal pattern of synchronization that appears to underlie symptoms such as tremor and bradykinesia (P. Brown, 2003; Schnitzler & Gross, 2005). Studies have found abnormally high oscillations in the globus pallidus externus (GPe), the subthalamic nucleus (STN), and the globus pallidus internus (GPi) that were found in patients exhibiting tremor, but not in those without tremor (Levy, Hutchison, Lozano, & Dostrovsky, 2000). These results are consistent with the alleviation in symptoms seen in participants after deep brain stimulation (DBS) surgery in which electrodes provide high frequency stimulation of the GPi or STN. This stimulation may

eliminate or at least reduce the high oscillatory activity in the basal ganglia. Local field potential (LFP) recordings from patients with PD suggest that dopamine depletion causes abnormal oscillations of certain frequencies with suppression of those that facilitate movement and enhancement of those that suppress movement. Frequencies that are less than 10 Hz project to the cortex with movement suppression area pathologically enhanced, frequencies between 11-30 Hz, that fall in the beta band, are projected from the cortex to the STN and suppress movement are also enhanced, and frequencies greater than 70 Hz (gamma band) facilitate movement, but are suppressed (Hutchison et al., 2004; Wichmann, Bergman, & DeLong, 1994). These findings are supported by studies that have found that with dopamine medication, patients with PD had greater power in the STN and greater coherence between the STN and GPi with movement facilitating frequencies of 70-85 Hz (Cassidy et al., 2002; Williams et al., 2002). Interestingly, this relationship was seen in the coherence between the STN and cortical EEG as well, suggesting a functional network between STN, GPi, and cortex that facilitates movements. An increase in beta activity is thought to interfere with the execution of movement, leading to suppression of voluntary movements in PD (Schnitzler & Gross, 2005). The symptom of tremor also appears to be related to abnormally synchronized oscillations that includes the cortico-striatal, cortico-cerebellar, and primary motor cortex (Ahn, Zauber, Worth, Witt, & Rubchinsky, 2015; Bergman & Deuschl, 2002; Hellwig et al., 2000; Timmermann et al., 2003).

Summary of knowledge gaps

Presently, most motor sequence learning studies using the SRT task use fixed sequences; however, fixed sequences do not reflect the motor sequence learning involved

in performing complex motor behaviors in our everyday lives. These motor behaviors are subject to changing individual, task, and environmental constraints that one must adapt to. Probabilistic sequences are a better approximation of the type of motor sequence learning we are required to do outside of the laboratory. Thus, our first step is to determine the learning and performance of probabilistic sequences in typical young adults within the SRT paradigm. Our next step is to determine whether typically aging adults are impaired at learning probabilistic sequences and whether they differ from learning fixed sequences. Lastly, we determine whether basal ganglia dysfunction has an effect on learning probabilistic and fixed sequences by assessing patients with Parkinson's disease and investigate cortico-cortical relationships via electroencephalography recordings.

Generally, motor sequence learning studies assess learning by calculating the mean or median for each block and compare these values across the age groups using analyses of variance. This ignores the dynamic changes that occur within each block, which could provide insight into how learning occurs and whether the learning process is different between the groups. Simply considering the mean reaction times only provides a cursory view of the learning and performance. To address this issue, we took a closer look at the changes in response time that occur within and between blocks and how they differed between sequence type and between age groups. In addition, a deeper look at the statistical analyses are also important. Most studies conduct ANOVAs on the means across blocks and groups without delving deeper into the contrasts of interest. This only produces a global score that may not reflect differences between blocks and groups. Furthermore, additional statistical methods may prove useful in capturing both within and between individual differences and in characterizing age-related differences.

Finally, this dissertation also addresses knowledge gaps in the changes in brain dynamics while learning a sequence in young adults, typically aging adults, and patients with Parkinson's disease. While the neural correlates have been extensively studied, few studies have looked at the time course of motor sequence learning. Thus, there is a need for further research into changes in cortico-cortical connectivity while performing a motor sequence learning task using a technique that provides the temporal resolution necessary to provide greater insight into both behavioral differences and neural underpinnings of motor sequence learning.

Chapter 3 (Study 1): Probabilistic sequences offer a unique window on motor sequence learning[1]

Introduction

Motor sequences are typically acquired implicitly, such that there is no conscious knowledge that a sequence is being learned (A. S. Reber, 1967a, 1989a; Seger, 1994; Stadler & Frensch, 1998). The implicit motor sequence learning literature has prominently used fixed sequences with deterministic structures; however, these are not conducive to understanding how learning occurs in real life where we continuously make statistical associations between events unconsciously that change in a dynamic environment (Cleeremans & McClelland, 1991; Cleeremans, et al., 1989; A. S. Reber, 1989a). Thus, fixed sequences are inadequate to assess the adaptability required to learn motor skills.

The most commonly used paradigm to assess implicit motor sequence learning in the laboratory is the serial reaction time (SRT) task (Nissen & Bullemer, 1987). In this task, participants respond to the location of a stimulus on a computer screen by pressing the corresponding key as quickly and accurately as possible. Participants are unaware that the stimuli are presented in a pre-determined repeating sequence. Learning is inferred from a progressive reduction in the reaction time during the learning blocks (Nissen & Bullemer, 1987; Robertson, 2007). Traditionally, a fixed order has been used to create the sequences of 10 or 12 items that repeat 10 times in a block (e.g., 10 repetitions of 3412432142). This repetition contributes to the likelihood that participants become consciously aware of the presence of a sequence, thus changing the nature of learning from implicit to explicit (D. V. Howard, et al., 2004; J. H. Howard & Howard, 1997; Nissen & Bullemer, 1987; Reed

[1] This study will be submitted for publication with the following authors: Prashad, S., Du, Y, & Clark, J. E.

& Johnson, 1994; Song, et al., 2007b). In order for learning to remain implicit, the underlying stimulus structure must be complex enough to escape conscious awareness (A. S. Reber, 1989a), a condition that does not appear to hold for fixed sequences.

Previous studies have suggested that sequence structure plays a critical role in learning (Bennett, et al., 2007; Cleeremans, et al., 1989; Curran, 1997; DeCoster & O'Mally, 2011a; Dennis, et al., 2006; D. V. Howard, et al., 2004; Jimenez, Mendez, & Cleeremans, 1996; A. S. Reber, 1967a, 1989a; P. J. Reber & Squire, 1999b; Reed & Johnson, 1994), but few have used complex probabilistic sequences (Cleeremans & McClelland, 1991; Peigneux et al., 2000; Schvaneveldt & Gomez, 1998; Song, et al., 2007b; Stadler, 1992). Cleeremans and colleagues (1991) used a finite state grammar to create the probabilistic sequence for their SRT task and found that participants had a significantly faster reaction time for predictable trials compared to unpredictable trials, suggesting learning of the finite state grammar rules (Cleeremans & McClelland, 1991; Jimenez & Mendez, 1999; Jimenez, et al., 1996; Peigneux, et al., 2000). Schvaneveldt and colleagues (1998) used a different approach in which two four-item sequences appeared with a probability of either 80% or 20% and found that reaction times were faster for the probable transitions compared to the improbable transitions. Howard and colleagues have modified the SRT task into a more complex alternate serial reaction time (ASRT) task in which each item of a fixed sequence occurs in alternation with a random item (e.g., a sequence 1-2-3-4 would appear as 1-*r*-2-*r*-3-*r*-4, where *r* is randomly picked from one of the four items) and found that higher frequency triplets have a faster reaction time compared to lower frequency triplets (Feeney, et al., 2002; D. V. Howard & Howard, 2001; J. H. Howard & Howard, 1997; Song, Howard, & Howard, 2007a; Song, et al., 2007b).

Although different types of probabilistic sequences were used in these studies, the results indicate that participants are sensitive to probabilities between stimuli as inferred from faster reaction times to stimuli occurring with greater probability, but it is unclear how probabilistic sequences compare directly with fixed and randomly ordered stimuli in the SRT paradigm. In addition, to our knowledge, no studies in the SRT paradigm have characterized the learning processes of sequences or investigated whether participants are able to transfer their learning, an essential component of assessing motor learning that provides an evaluation of the effectiveness of learning and whether performance can be maintained in a different context or variation of the skill (Newell, 1991; Newell & Shapiro, 1976).

Thus, the aims of this study are to: 1) directly compare fixed and probabilistic sequences in a modified SRT task; 2) characterize the underlying learning processes of the two sequence types; and, 3) investigate the transfer of learning from the learned sequence to a novel sequence. For this task, we generated sequences using a first-order transitional probabilistic structure, in which the present state influences the next state based on transitional probabilities between the states that are defined in a transitional matrix containing the probabilities associated between each pair of states. Over numerous trials, the participant is expected to unconsciously learn the probabilistic rules underlying the sequence (e.g., 2 is most likely to be followed by 6) and exhibit a decreased reaction time. A completely randomized sequence condition was included to characterize performance changes that would result from the motor component of the task independent of learning the sequence structure. Thus, the experimental design compared learning performance between a fixed, a probabilistic, and a completely random sequence across four blocks of

learning trials followed by a block in which stimuli occurred in a random order and a transfer block to assess transfer of learning to a novel sequence.

Materials and methods

Participants

Thirty female right-handed adults were randomly assigned to one of three groups: fixed sequence (FX; mean age: 20.0 ± 1.18), probabilistic sequence (PB; mean age: 20.5 ± 1.25), and randomly ordered stimuli (RD; mean age: 20.2 ± 1.37). All participants completed the Global Physical Activity Questionnaire (Armstrong & Bull, 2006), a spatial version of the *n*-back test to assess working memory (Jaeggi, Buschkuehl, Jonides, & Perrig, 2008), and a computer skills questionnaire to assess their familiarity with the number pad on the computer keyboard (see Table 1). Participants were also screened for neurological and motor impairments through a health questionnaire. No significant differences were found between the groups in age ($F(2,29) = 0.40$, $p = 0.7$), physical activity ($F(2,29) = 0.91$, $p = 0.4$), or *n*-back score ($F(2,29) = 0.86$, $p = 0.4$).

Serial reaction time task

Participants were seated in front of a computer monitor (21”) and keyboard (keys size 13x15mm, keys are 6mm apart vertically and horizontally and 8mm apart diagonally). A modified SRT task was used that consisted of nine white squares in a 3×3 matrix on the computer screen (37x37mm each). Participants placed the index finger of their right hand on the center button on the number pad of the keyboard. The relationship between the squares on the screen and the buttons on the number pad was spatially compatible, i.e., the top right square corresponded to the top right button. At the beginning of each trial, one of

the eight squares turned blue and the participant pressed the key that corresponded to the location of the stimulus and then returned to the home position. After the participant pressed a key, a response-to-stimulus interval between 300-1000ms was selected randomly for each trial to prevent participants from anticipating the appearance of the subsequent stimulus as well as to prevent any confounding effects from the length of the response-to-stimulus interval (Willingham, Greenberg, & Thomas, 1997). No visual feedback was given to participants as a wooden board blocked vision of their finger position (see Figure 3.2).

Participants were randomly assigned to either a fixed (FX) 16-item second order conditional sequence (Reed & Johnson, 1994), probabilistic sequence (PB), or were presented with stimuli in a random order (RD). The probabilistic sequence was created based on a first-order transitional probabilistic structure with underlying probabilities associated with each stimulus. The transitional matrix was created such that the generated sequence resembled a deterministic, but not repeated, sequence (e.g., if stimulus 2 occurs, there will be a 60% probability that the next stimulus will be 6, a 30% probability that the next stimulus will be 8, and a 2% probability that the next stimulus will be 1, 3, 4, 7, or 9). Participants were not informed that a sequence existed regardless of which group they assigned to. The probabilistic and randomly ordered stimuli were 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 (20 times per block).

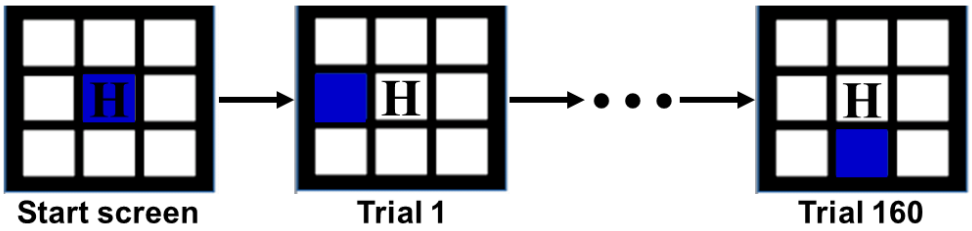

Figure 3.1: The modified serial reaction time (SRT) task. Participants placed their right index finger on the home position (H). On a given trial, one of the 8 locations turned blue and the participant pressed the corresponding button on the number keypad and then returned to the home position.

Figure 3.2: Experimental Setup. Participants were seated in front of a computer monitor with their hand placed on the number keypad. Participants did not receive any visual feedback and could not see their hands. Participants' right hand was wrapped with athletic pre-wrap to prevent the use of the other fingers.

There were a total of eight blocks for all groups, each consisting of 160 trials (see Figure 3.3). The first block was a baseline block (B0), consisting of 160 trials in which the stimuli appeared in a random order. The next four blocks (B1-4) were the learning blocks consisting of the fixed or probabilistic sequence in which the sequence was repeated 10 times each. Block 5 (B5) consisted of 160 trials of stimuli occurring in a random order and Block 6 (B6) consisted of 10 repetitions of the assigned sequence. An increase in response time in B5 and decrease in B6 would indicate learning (Robertson, 2007). Lastly, Block 7 (B7) consisted of 10 repetitions of a different sequence that was constructed from the same underlying structure as the learned sequence to assess transfer of learning. If the response

times decrease from B5 to B7, it would suggest that participants were able to transfer their learning. A unique sequence was assigned to each participant to ensure that the results are not intrinsic to the sequence used, but can be generalized to all sequences (DeCoster & O'Mally, 2011a). In the RD group, stimuli occurred in a random order in all eight blocks. Participants were given a two-minute mandatory break between each block. The experiment was performed using Presentation® software (Version 18.1, www.neurobs.com).

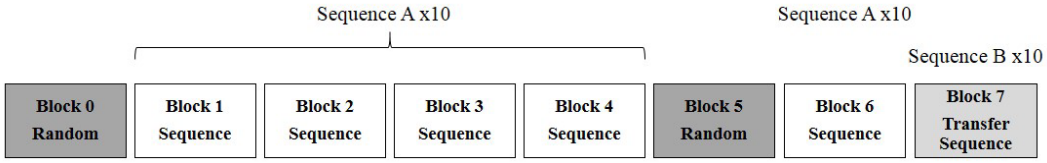

Figure 3.3: The experimental paradigm used for the three groups. All groups started with a baseline (B0), then the fixed and probabilistic groups performed the learning blocks (B1-4) and ended with a random block (B5) followed by another sequence block (B6) and a transfer block (B7). Each block consisted of 160 trials. Participants were given a two-minute break between each block. Participants in the fixed and probabilistic groups were given a unique fixed or probabilistic sequence, respectively. Participants in the random group were presented with stimuli in a random order for all blocks.

The participants' response time (amount of time taken to press the corresponding button after the stimulus was presented) and accuracy were recorded for each trial. It is important to emphasize that response times, and not reactions times, were recorded. Thus, the movement time (amount of time taken to move the index finger from the home position to the corresponding button after initial reaction) was embedded in the recorded response time.

Posttest

All participants completed a posttest after the completion of the eight blocks to determine if learning was implicit. First, participants were asked the following question: "The stimulus movement is best described as:" with the following options: "a) Random; b) Some positions occurred more often than others; c) The movement was often predictable; d) The same sequence of movements would often appear; and e) The same sequence of movements occurred throughout the entire experiment" (Curran, 1997).

Second, participants completed a recognition test to assess explicit recall of the sequence (Destrebecqz & Cleeremans, 2001) consisting of two parts: in the first part, participants were presented with six-item chunks from their assigned sequence as well as random chunks and were asked to rate how confident they were that they had seen that chunk before from a scale of 1-5 (where 1 was "Confident that I *have not* seen it before" and 5 was "Confident that I *have* seen it before"). In the second part, participants were presented with the entire 16-item sequence as well as other random sequences and they were asked to rate them on the same scale.

Data analysis

The response times were trimmed according to the individual participant's mean and standard deviation to eliminate any outliers. Any response times greater or less than 2.5 standard deviations were excluded from the analysis (Ratcliff, 1993; Whelan, 2008). Mean response times were calculated for each block and were averaged across participants in each group. Learning was measured through a decrease in response time from B1 to B4, an increase from B4 to B5 (stimuli in random order) and a decrease from B5 to B6 (stimuli

in assigned sequence). Transfer of learning was inferred if there was a significant decrease between B5 and B7 (stimuli in different sequence of same structure as assigned sequence).

Variability was calculated within-subject around the individual participant's mean. Overall variability was calculated by collapsing the standard deviations for all the blocks for each subject and then averaging for each group.

Mean response times for each sequence repetition within a block were also calculated and averaged across participants to uncover dynamic changes in response time within and between blocks. The amount of learning within a block was determined by performing a linear regression on the mean sequence repetitions. A negative slope (reducing response times) within blocks indicated online learning and between blocks indicated offline learning. Mean response times for each stimulus location also were calculated for each block and averaged across participants in each group to determine if stimulus location had an effect on the response time.

A mixed factorial analysis of variance (ANOVA) was used to compare differences in response time and variability between the blocks and groups. One-way ANOVAs were used to compare differences between online and offline learning. Bonferroni *post-hoc* tests were used to decompose any significant effects. Separate pairwise comparisons were conducted on the contrasts of interest (B1 vs. B4, B4 vs. B5, B5 vs. B6, and B5 vs. B7) to assess learning and transfer. Statistical significance was defined at $p < 0.05$. The data were processed using custom scripts written in MATLAB version 8.4 (Mathworks, Natick, MA) and SPSS Statistics 22 (IBM, Armonk, NY).

Results

Accuracy

All groups exhibited high levels of accuracy with 2% or fewer errors. Thus, accuracy cannot be used as a measure of learning in this task and was not analyzed further. Low error rates are consistent with findings from previous studies (Robertson, 2007; Willingham, Nissen, & Bullemer, 1989).

Mean response time

A two-way mixed factorial (3 x 8) ANOVA on Sequence Type (Fixed, Probabilistic, Random) x Block (0-7) on the response times with Block as the within subject variable indicated only a main effect for Block, $F(7,189) = 37.0$, $p < 0.001$. There was no main effect of Sequence Type, $F(2, 27) = 2.4$, $p = 0.1$, and no significant interaction, $F(14,189) = 0.73$, $p = 0.7$. Pairwise comparisons between contrasts that were determined *a priori* revealed significant differences between B1 and B4 in FX ($p = 0.001$), PB ($p < 0.001$), and RD ($p = 0.03$) suggesting a significant improvement in the motor component of the task. There were also significant differences between B4 and B5 in FX ($p = 0.002$), but not in PB ($p = 0.8$) or RD ($p = 0.2$). This indicates that in FX, the occurrence of random stimuli resulted in increased response times, while the response times in the PB and RD groups stayed the same. However, significant differences appeared between B5 and B6 in FX ($p = 0.004$) and approaching significance in PB ($p = 0.06$), but not RD ($p = 0.2$) suggesting that in both FX and PB, the response times decreased when the stimuli occurred in the learned sequence, indicating that sequence learning did occur in PB (see Figure 3.4). These results suggest that both FX and PB groups were able to learn their assigned

sequence, but there was a significant motor component in this task as exhibited by the RD group.

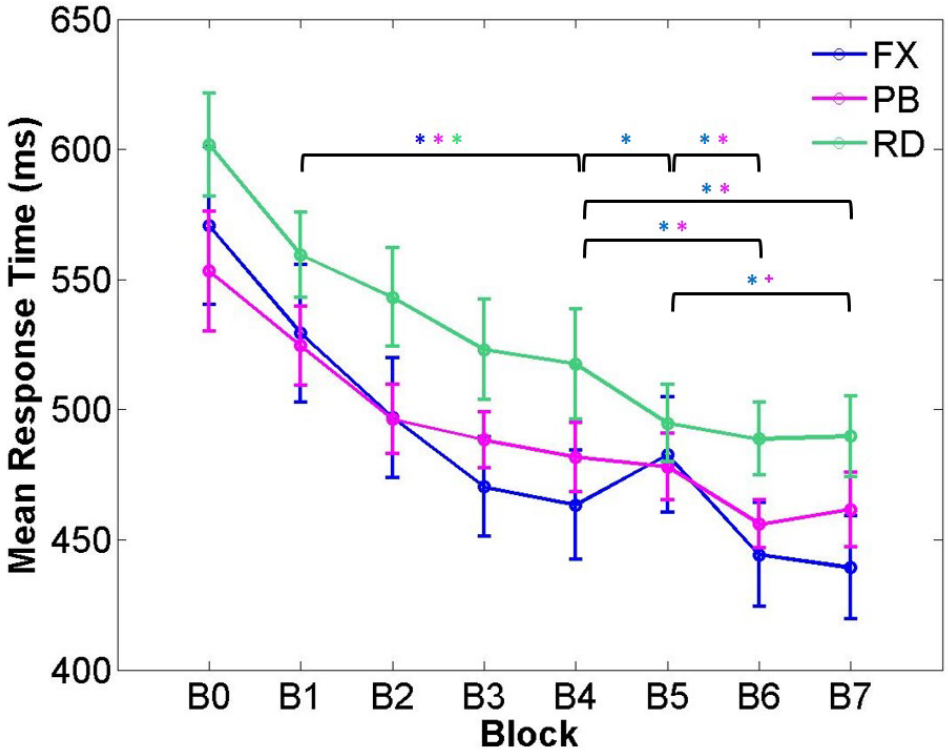

Figure 3.4: Mean response time of each block for all three groups. Error bars indicate standard error. The FX and PB groups exhibit learning of the sequences and the RD group exhibits a significant improvement in the motor component of the task.
* Indicates significance level of $p < 0.05$; + indicates significance level of $p < 0.08$.

Transfer of learning

Transfer of learning was assessed by comparing B5 (stimuli in a random order) and B7 (stimuli in a novel sequence created using the same underlying structure as the learned sequence). Response times in B5 were significantly slower than in B7 in FX ($p = 0.03$), approaching significance in PB ($p = 0.06$), and not significant in RD ($p = 0.1$). This indicates that the FX and PB groups were able to transfer their learning to a new sequence as the response times decreased when the transfer sequence was presented.

Variability in response time

Overall variability was calculated by collapsing all the blocks for each group. There was no significant difference between the groups in overall variability as determined by a one-way ANOVA ($F(2,27) = 1.09$, $p = 0.4$). However, the measure of overall variability does not provide insight on how performance variability changes with learning. A reduction in variability is an important characteristic of motor learning (R. G. Cohen & Sternad, 2009; Wulf & Schmidt, 1997), but to our knowledge, no studies have examined changes in variability in the SRT task.

A two-way mixed factorial (3 x 8) ANOVA on Sequence Type (Fixed, Probabilistic, Random) x Block (0-7) on the standard deviations of the response times with Block as the within subject variable indicated a main effect of Block, $F(7,189) = 13.4$, $p < 0.001$, but no main effect of Sequence Type, $F(2,27) = 1.09$, $p = 0.4$ or interaction, $F(14,189) = 0.41$, $p = 1.0$. Pairwise comparisons between contrasts that were determined *a priori* revealed significant differences between B1 and B4 in FX ($p = 0.03$) and PB ($p = 0.05$), but not in RD ($p = 0.6$). This reduction in variability for the fixed and probabilistic sequence groups, but not the random group is consistent with previous literature that variability in motor performance decreases with learning. Since there was no sequence to be learned in the random group, the variability did not change. In addition, significant differences were found between B5 and B6 in FX ($p = 0.03$), but not for PB ($p = 0.1$) or RD ($p = 0.8$). No other significant differences were found for any of the groups for pairwise comparisons between B4 and B5.

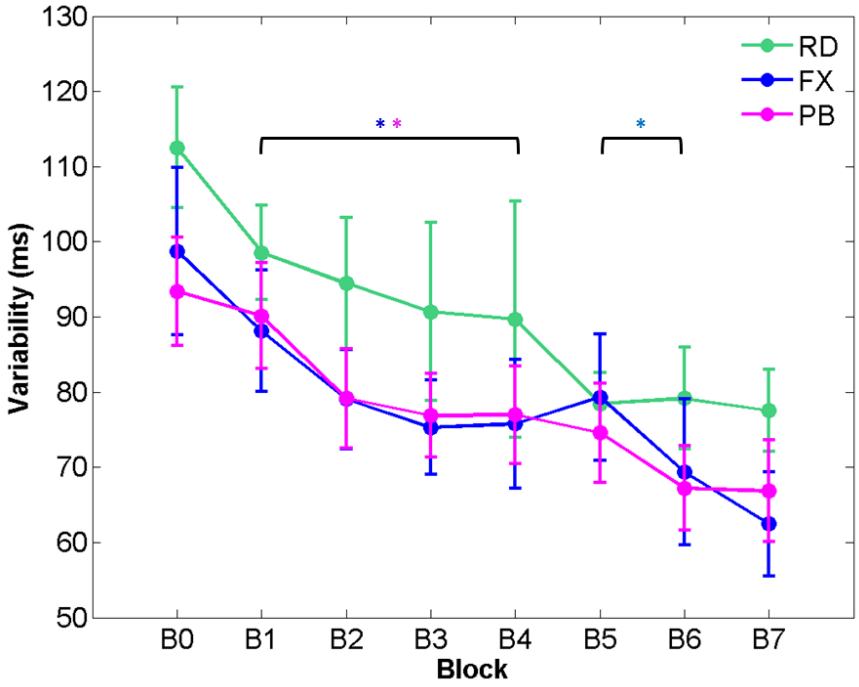

Figure 3.5: Within subject variability across blocks for the fixed (FX), probabilistic (PB), and random (RD) groups. Error bars indicate standard error. The FX and PB groups exhibited a significant decrease in variability in the learning blocks, but the RD group did not.
* Indicates significance level of $p < 0.05$.

Dynamic changes in response time within and between blocks

The mean response time of each sequence repetition within each block was calculated to investigate the dynamic changes in the response times within and between blocks. Since the sequence is repeated 10 times in each block, this analysis yielded 10 points per block (see Figure 3.6). Both FX and PB groups exhibited within and between block trends. Firstly, within each block in the FX group, the performance stayed constant overall (response times were about the same at the beginning and end of block) for B1-7 (all $p > 0.1$). B0 exhibited a decrease in response times ($p = 0.03$) that may be related to practice effects. However, the PB group exhibited deteriorating performance within some blocks as they started at a lower response time at the beginning of the block, but ended at

a higher response time at the end of the block (B2-3, both $p < 0.05$, B4, $p = 0.09$, and B7, $p = 0.08$), suggesting that participants in the PB group were unable to maintain their performance within some blocks. Secondly, for both FX and PB, blocks start with better performance (lower response time) than that of the end of the previous block. This trend is greater for the PB group (all $p < 0.05$) than FX (B1-2, $p = 0.089$, B2-3, $p = 0.01$, B5-6, $p = 0.08$). This suggests that the learning continued during the breaks and resulted in better performance at the beginning of the next block, particularly in the PB group. No overall trends were found in the RD group.

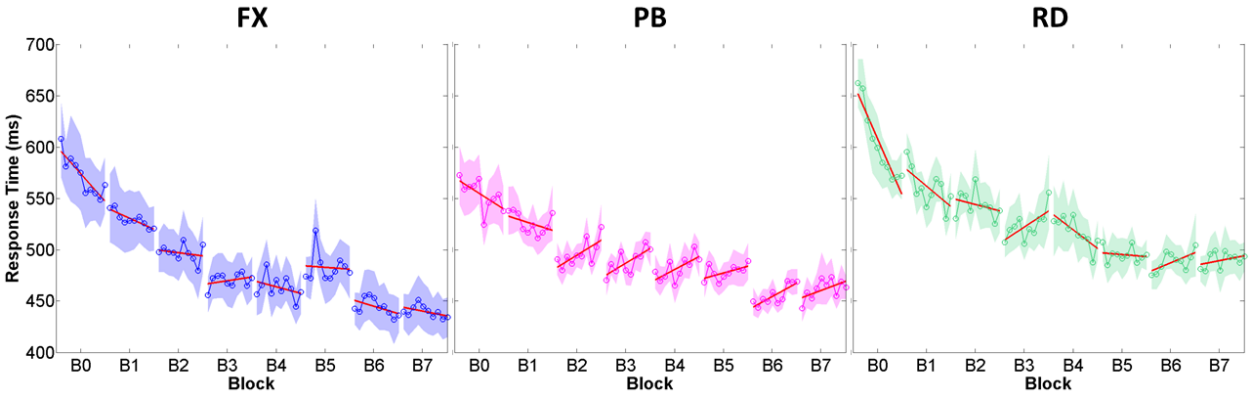

Figure 3.6: Mean response time of each sequence repetition for the three groups. Shading represents standard error. The FX group stays at a similar performance level within each block; however, the PB group deteriorates in performance. In addition, for both FX and PB, each block begins at a better performance level than the level that the previous block ended at. The RD group does not exhibit any overall trends.

Online and offline learning

To further investigate and quantify online (within block) learning, a linear regression was performed on the mean response times for each sequence repetition for each block (see red linear fit lines in Figure 3.6). In addition, a linear fit was performed on the last five sequence repetitions and the first five sequence repetitions of the subsequent block

to investigate offline (between block) learning. The averages of the online and offline learning slopes were calculated for each group (see Figure 3.7). A negative slope (reduction in response times) indicates learning.

There was a significant difference in online learning between the groups as determined by a one-way ANOVA on the slopes, $F(2,27) = 5.19$, $p = 0.012$. A Bonferroni post-hoc test revealed that the PB groups exhibited less online learning than the FX group (approaching significance at $p = 0.063$) and the RD group ($p = 0.016$), suggesting that different processes underlie the learning of the fixed and probabilistic sequences. This difference may be due to the deteriorating performance observed within blocks exhibited in the PB group. There were no differences between online learning in the FX and RD groups ($p = 1.00$).

There were no significant differences in offline learning between the groups as determined by a one-way ANOVA ($F(2,27) = 1.79$, $p = 0.2$). However, the mean slopes of the FX group and the PB group were significantly different from a slope of zero (both $p < 0.01$), while those of the RD group were not ($p = 0.4$). These findings suggest that both the FX and PB groups exhibited offline learning, but the RD group did not.

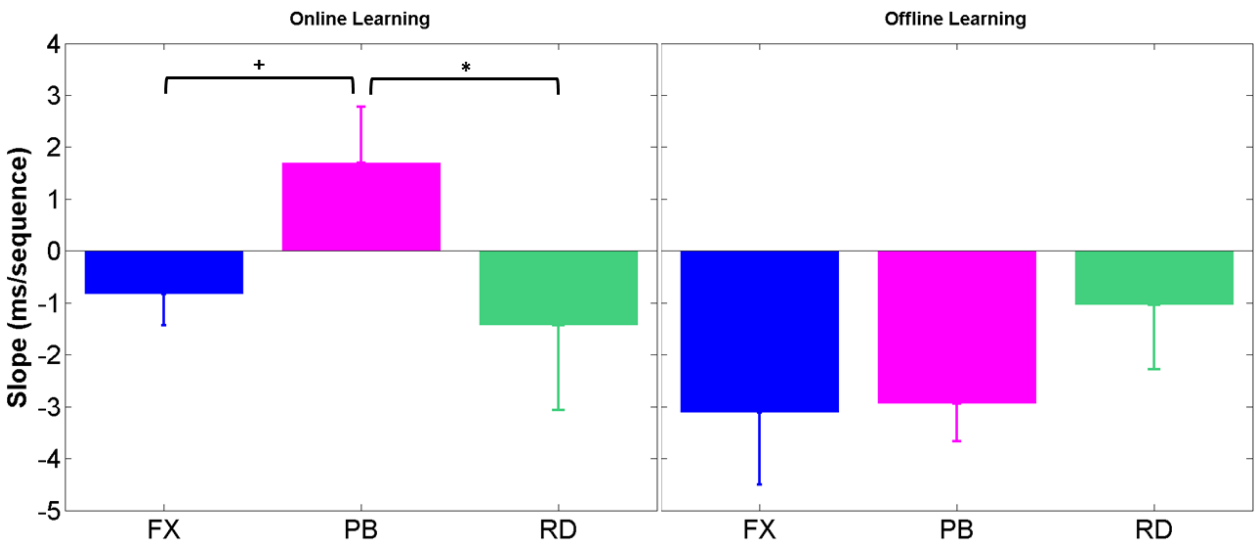

Figure 3.7: The learning slopes for online and offline learning. Error bars indicate the SE. Negative values indicate greater learning (greater reduction in response time). There were significant differences in online learning between the PB and RD groups (p = 0.02) and approached significance between the PB and FX groups (p = 0.06). There were no significant differences between the groups in offline learning.

Effect of stimulus location on mean response times

In order to determine whether the stimulus had an effect on performance, the response times were parsed based on the location of the stimulus. Indeed, the stimuli occurring diagonally from the home position (the four corners) had greater response times than those that were straight up and down or side-to-side (non-diagonal locations). To investigate this further, the mean across the diagonal locations and the mean across the non-diagonal locations were calculated for each group (see Figure 3.8). A two-way repeated measures ANOVA on Stimulus Location (Diagonal, Non-diagonal) x Block (0-7) on the response times was conducted on each group separately.

In the FX group, there was a main effect of Block, $F(7,63) = 28.6$, $p < 0.001$, a main effect of Stimulus Location, $F(1,9) = 28.7$, $p < 0.001$, and a significant interaction, $F(7,63)$, $p = 0.05$. A *post hoc* analysis using the Bonferroni correction revealed significant differences between B1 and B4 (p = 0.001), B4 and B5 (p = 0.007), B5 and B6 (p = 0.001),

and B5 and B7 ($p < 0.001$) for the diagonal locations. However, for the non-diagonal locations, there was a significant difference between B1 and B4 ($p = 0.001$), B5 and B6 ($p < 0.001$), and B5 and B7 ($p < 0.001$), but not between B4 and B5 ($p = 0.1$), suggesting that stimulus location has a differential effect on the response times and the diagonal locations highlighted the differences between the blocks, particularly B4 and B5.

In the PB group, there was a main effect of Block, $F(7,63) = 13.4$, $p < 0.001$ and a main effect of Stimulus Location, $F(1,9) = 87.6$, $p < 0.001$, but no significant interaction, $F(7,63) = 1.02$, $p = 0.4$, suggesting that while response times in the diagonal locations were significantly higher than those in the non-diagonal locations, there was no differential effect of location on the blocks. As expected, a *post hoc* analysis using the Bonferroni correction revealed a significant difference between B1 and B4 ($p = 0.002$), B5 and B6 ($p = 0.008$), and approaching significant difference between B5 and B7 ($p = 0.07$), but not between B4 and B5 ($p = 0.7$).

The RD group also exhibited a main effect of Block, $F(7,63) = 15.1$, $p < 0.001$ and a main effect of Stimulus Location, $F(1,9) = 35.0$, $p < 0.001$, but no significant interaction, $F(7,63) = 0.755$, $p = 0.6$. A *post hoc* analysis using the Bonferroni correction revealed a significant difference only between B1 and B4 ($p = 0.04$). There were no significant differences between B4 and B5 ($p = 0.274$), B5 and B6 ($p = 0.2$), or B5 and B7 ($p = 0.3$).

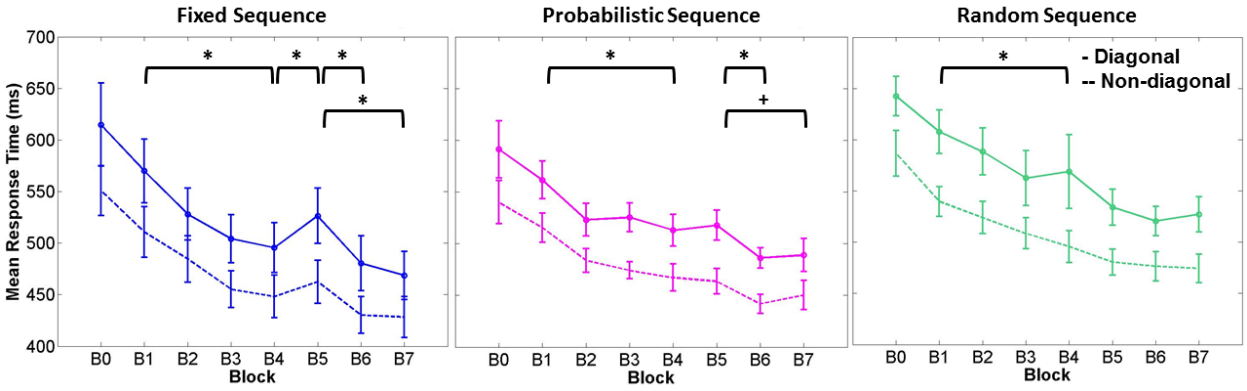

Figure 3.8: Mean response times for diagonal stimulus locations and non-diagonal locations. In the FX group, there was no significant main effect of location, but there was a significant main effect of location for both the PB and RD groups.

Posttest

After the completion of the task, participants were asked how they would describe the stimulus movement in the task (see Methods for question). No significant differences were found in the responses between the groups, $F(2,29) = 0.231$, $p = 0.8$.

The posttest also required participants to rate their confidence on a scale of 1-5 whether they had seen the presented chunk in any of the blocks. Some of the chunks presented to participants were from the assigned sequence and some were random chunks. In the FX group, there was no significant difference between the rating for the chunks from the sequence (mean rating = 2.95) and random chunks (mean rating = 2.95; $F(1,19) = 0.00$, $p = 1.00$). Similar results were found for the PB group (sequenced chunk mean rating = 3.20; random chunk mean rating = 3.25; $F(1,19) = 0.087$, $p = 0.8$). A statistical analysis between chunks from the assigned sequence and random chunks was not run for the RD group because there was no assigned sequence for this group (all stimuli occurred in a random order).

Participants were also shown entire sequences, one of which was their assigned sequence. Here, differences emerged between the rating for the entire sequence and random sequences. In the FX group, the difference between the rating scale for the assigned (mean rating = 3.50) and the random sequences (mean rating = 2.67) approached significance, $F(1,19) = 4.00$, $p = 0.06$. For the PB group, there was no significant difference between the assigned (mean rating = 3.4) and the random sequence rating (mean rating = 3.17), $F(1,19) = 0.34$, $p = 0.6$. This suggests that participants who were assigned probabilistic sequences were not able to determine differences between their assigned sequence and other random sequences, but participants assigned to fixed sequences were able to differentiate between their sequence and other random sequences. Thus, the probabilistic structure is more likely to ensure implicit sequence learning and prevent contamination of the implicit motor sequence-learning paradigm by explicit learning.

Discussion

By directly comparing probabilistic and fixed sequence structures, we demonstrated that both groups exhibited learning indicated by significant decreases in response time and variability. Both online and offline learning played a role in the learning of the fixed sequences; however, only offline learning contributed to the learning of the probabilistic sequence. Additionally, the stimulus location only influenced the response times for the probabilistic sequence. These results suggest that the probabilistic structure can be learned in the SRT paradigm, but is learned differently from fixed sequences.

Probabilistic sequences are representative of adaptive motor learning

The probabilistic sequences used in this study were generated by a first-order transitional probabilistic structure. To our knowledge, these types of probabilistic sequences have not been used in the SRT task and it was unclear whether they would provide a useful alternative to the traditionally used fixed sequences and whether their use would reveal unique insights into sequence learning. As expected, the fixed sequence group exhibited a significant decrease in response time during the learning blocks (B1 and B4), an increase from B4 to B5, and a decrease from B5 to B6. This is consistent with results from previous SRT studies and indicates that sequence learning occurred. In addition, there was no significant difference between B6 and B7, indicating that the learning was transferred to a new sequence with the same underlying structure. The probabilistic sequence group also exhibited a decrease in response time through the learning blocks (B1 to B4), a significant decrease from B5 to B6, and approaching significant difference between B5 and B7 indicating both learning of the sequence and transfer to a new sequence. However, the probabilistic sequence group did not exhibit a significant increase in response time from B4 to B5, suggesting that participants were not perturbed by the appearance of randomly ordered stimuli in B5. Previous studies have suggested that interference in performance may be due to incompatibility between task requirements (Bock, Schneider, & Bloomberg, 2001) and varied training experiences result in a greater rate of learning (Seidler, 2007, 2010). Similarly, the learning of probabilistic sequences provides a more varied experience than fixed sequences and thus may represent learning that is more resilient to interference.

Interestingly, the random sequence group also displayed a continuous decrease in response times in B1 to B4, but did not exhibit significant differences between B4 and B5 or B5 and B6 indicating no sequence learning. Since there was no sequence to learn, this is not altogether surprising, but what was interesting was the appearance of learning as performance improved over the "learning" blocks (B1 to B4). Clearly, the modified SRT task we used here included a significant motor component that improved with practice contributing to the reduction in response time. This is an important insight into how we assess "learning" in an SRT task. To assess learning, it is imperative to disentangle it from performance (Newell, 1991; Newell & Shapiro, 1976; Wulf & Schmidt, 1997). Learning is best assessed using retention or transfer tasks (Sanchez, Yarnik, & Reber, 2014) as well as decomposing response times into reaction and movement time.

Different processes underlie the learning of fixed and probabilistic sequences

While mean block times provide overall trends of the response time, they do not reflect dynamic changes within a block. These dynamic changes are important to examine the underlying learning processes. As our data demonstrate, online learning emerged as an important learning process in the fixed sequence group, but not in the probabilistic sequence group. Online learning is comprised of a stimulus-by-stimulus update of the sequence and is more computationally expensive than offline learning. In the probabilistic sequence group, online learning would require a continuous update of the estimation of the transitional probabilities between the items while performing the task (Laming, 1969). Since the sequence was complex, online learning and maintenance of performance during the block could be too computationally expensive and may explain the performance deterioration within the blocks. It is important to note that this deterioration does not appear

to be due to fatigue since performance in the fixed and random conditions would decline as well if fatigue were the cause. This decrement in performance also suggests that no online learning occurred in the probabilistic sequence group.

The presence of offline learning in both the fixed and probabilistic groups suggests that the learning process continues to occur during the breaks and manifests as better performance in the subsequent block. Offline learning has previously been found to occur between sessions that are four hours apart (R. M. Brown & Robertson, 2007; Robertson, Pascual-Leone, & Miall, 2004). However, few studies have explored offline learning during breaks lasting minutes (Hotermans, Peigneux, Maertens de Noordhout, Moonen, & Maquet, 2006; Schmitz et al., 2009), but our results indicate that offline learning may occur even when the intervals between blocks are only two minutes long. Thus, the learning process underlying changes in the probabilistic sequences was offline learning, while the fixed sequences were learned via both online and offline processes.

Spatial location of stimulus is important in probabilistic sequence learning

It has previously been suggested that motor sequence learning may be better characterized by the learning of a sequence of response locations, rather than a sequence of stimuli (Willingham, 1999; Willingham, Wells, Farrell, & Stemwedel, 2000). This is particularly important for our modified SRT task as it had a greater spatial aspect than the traditional SRT task, and in turn, a greater motor component. The stimulus location had a differential effect on response time in the blocks for the fixed sequence group, but there was no significant interaction between stimulus location and blocks in the probabilistic or random groups. However, all groups exhibited a main effect of stimulus location, suggesting that additional parameters can be used to characterize learning. This result is

consistent with previous literature on SRT learning that participants must not only learn the sequence of stimuli perceptually, but also the sequence of motor responses, and the stimulus-response pairing of the sequence (Song, Howard, & Howard, 2008; Willingham, 1999; Willingham, et al., 2000). Locations that are harder to reach (i.e., the diagonal locations) may be more important in uncovering differences between groups, particularly when studying aging or clinical populations.

The differences in response time based on location highlight another factor prevalent in SRT studies. Most studies use multiple fingers for the response, with one finger on each response location; however, each finger may have different response times. We attempted to avoid this articulator effect in the modified version of the SRT task by requiring participants to use only their right index finger throughout the task. Due to the use of one finger, there was a strong motor component in the task. Since movement time appears to play an important role (Moisello et al., 2009), it is critical to decompose the response time into the reaction and movement times in future studies.

Variability is an important measure to assess learning

While previous studies have focused on comparing response time means to assess learning, to our knowledge, no studies have analyzed the change in within-subject variability of response times. A reduction in motor performance variability has been a hallmark of motor learning (R. G. Cohen & Sternad, 2009; Wulf & Schmidt, 1997). Our results are consistent with previous findings as both fixed and probabilistic sequence groups exhibited a significant reduction in variability over the learning blocks, and the random group's within-subject variability did not change as would be predicted if reduced variability were due to sequence learning.

Implications for future SRT studies

Probabilistic sequences are not only more representative of the adaptive motor learning process that occurs in daily life, but they also allow for the investigation of implicit learning processes that are less likely to be contaminated by explicit awareness of the sequence. Both of these characteristics offer a compelling reason to use probabilistic sequences for the study of motor skill learning while addressing methodological problems with wide implications for future SRT studies.

The posttest questionnaire indicated that participants who were assigned probabilistic sequences were less likely to differentiate the assigned sequence from other sequences. This suggests that the knowledge of the sequence did not become explicit and that learning remained implicit throughout the task. This is particularly significant as participants can become aware of the sequence at different times in the learning process, thereby contaminating implicit learning in an unquantifiable manner. This contamination is particularly problematic when applying neuroimaging methods to the SRT framework to study the neural correlates of implicit learning since it is difficult to separate explicit and implicit learning using fixed sequences. Thus, probabilistic sequences also provide a method to better assess the neural underpinnings of implicit motor sequence learning.

Conclusion

These results suggest that probabilistic sequences may be more effective than fixed sequences to assess the adaptive learning required in learning motor skills in everyday life. This is an important finding that addresses a specific methodological problem that has wide implications for future SRT studies. While previous studies have used alternate methods to generate probabilistic sequences, such as a finite-state grammar, (Jimenez & Mendez,

1999; Jimenez, et al., 1996) and the alternate serial reaction time task (Feeney, et al., 2002; D. V. Howard & Howard, 2001; J. H. Howard & Howard, 1997; Song, et al., 2007a, 2007b), no other studies have used first-order transitional probabilistic structures that result in more complex and entirely probabilistic sequences. The ability to manipulate probabilities and determine the effects of different types of sequences on motor sequence learning may be useful in more deeply understanding the learning processes. In addition, probabilistic sequences more accurately reflect the learning acquired in daily life, since ultimately our aim is to better understand motor skill learning that is adaptive to changes in the environment.

To our knowledge, this is the first time that probabilistic sequences and fixed sequences have been directly compared, their learning processes have been analyzed, and transfer to a novel sequence has been assessed. Studying these underlying learning processes may be critical in understanding what types of sequences are learned best and how learning changes developmentally, with age, and in clinical populations. This paper represents an essential starting point towards a deeper understanding of this dynamic motor learning process.

Chapter 4 (Study 2): Typically aging adults are impaired at probabilistic motor sequence learning[2]

Introduction

Motor sequence learning is a ubiquitous process that pervades our activities of daily living in which our actions follow a sequence of movements performed with specific timing and order. As life expectancies increase, it is essential to investigate the relationship between motor learning and aging in order to enhance our understanding of the motor system, its age-related impairments, and the basis for interventions that address cognitive and motor deficits.

The serial reaction time (SRT) task (Nissen & Bullemer, 1987) is the paradigm used most frequently in studying motor sequence learning and is particularly well suited to the study of those with potential motor impairments. In this task, participants respond to the location of a stimulus on a computer screen by pressing the corresponding button as quickly and accurately as possible. Participants are unaware that the stimuli are presented in a pre-determined repeating sequence. Learning is inferred from a progressive reduction in the reaction time during the learning blocks (Nissen & Bullemer, 1987; Robertson, 2007) and an increase in reaction time to stimuli that occur in a random order. A repeating fixed sequence is most commonly used in the SRT paradigm, but is an inadequate reflection of learning in daily life, where fixed sequences are rarely part of our daily motor repertoire. Rather our motor behavior is adaptive and is dependent on statistical associations between

[2] This study will be submitted upon revision for publication with the following authors: Prashad, S., Du, Y, & Clark, J. E.

events that are often made unconsciously and vary across task and environmental constraints (Cleeremans, et al., 1989; A. S. Reber, 1989b).

We have shown previously, in Study 2 of this dissertation, that typical young adults are able to learn probabilistic sequences created using a first-order transitional probabilistic structure, in which the present state influences the subsequent state based on defined transitional probabilities between each pair of states. Over numerous trials, the participants unconsciously learned the probabilistic rules underlying the sequence (e.g., 2 is most likely to be followed by 6) and exhibit a decreased reaction time. These types of sequences have not been used in other studies and it is unclear whether typically aging adults would be able to learn these realistic, but complex sequences.

Numerous studies have attempted to understand the effects of aging on learning motor sequences, but the results have been largely equivocal. Many studies have found that that typically aging adults exhibit similar levels of performance on the SRT task as young adults if the sequence is a simple sequence and not a complex higher order sequence (Bennett, et al., 2007; Bo & Seidler, 2010; Daselaar, et al., 2003; Dennis, et al., 2006; Feeney, et al., 2002; Fraser, et al., 2009; J. H. Howard & Howard, 2013; Nemeth & Janacsek, 2010; Seidler, 2007; Weiermann & Meier, 2012). However, this overall conclusion does not always hold (Bennett, et al., 2007). Curran (1997) investigated typically aging adults while learning sequences with different underlying structures. Both were fixed sequences, but one was a first-order conditional (FOC) sequence while the other was a more complex second-order conditional (SOC) sequence. Curran found that typically aging adults only exhibited learning in the SOC sequence, but not the FOC sequence

(Curran, 1997). This is a surprising finding as FOC sequences are less complex and thus should be more likely to be learned than the more complex SOC sequences.

In contrast, while Dennis et al. (2006) also investigated the learning of FOC and SOC sequences in typically aging adults in an auditory SRT task, they found conflicting results in that typically aging adults were able to learn both FOC and SOC sequences. In addition, they also assessed learning of higher-order sequences with a somewhat probabilistic association, called the alternating serial reaction time (ASRT) task. In the ASRT, sequence trials alternated with random trails (e.g., 3*r*2*r*4*r*1 where the sequence is 3241 and *r* is a random trial that could be any of the four stimuli). The authors found that typically aging adults were unable to learn the sequence in the ASRT task, suggesting that age-related impairments in the learning of higher order sequences. To further confound any conclusions, another study found that typically aging adults were in fact able to learn an even more complex sequence in an ASRT task in which a sequence trial was followed by two random trials (e.g., 3*rr*2*rr*4*rr*1) (Bennett, et al., 2007).

These differences in the results may, in part, be due to the statistical analyses conducted. Most SRT studies assess learning by comparing the mean or median of each block across the age groups using ANOVA; however, means only provide a cursory assessment of the performance. In addition, there appears to be a very lenient definition of aging adults, with studies using large age ranges representing this age group. This presents two problems: 1) If a large age range, such as 60-80 years old is used within a study and the reaction times for these individuals is averaged to determine a group mean, the assumption is that a 60-year-old adult and an 80-year-old adult would perform at the same level and this assumption is likely to be incorrect, and 2) Studies may use different age

ranges, making it difficult to compare and assimilate results between studies. These factors may contribute to the large variability in results that exists in the implicit motor sequence learning and aging literature. In order to overcome these issues, we used cluster analysis to group the typically aging adults based on their reaction times, thus dividing participants on their behavior, rather than their age – a representation merely of their years since birth.

A clearer understanding may also be attained from neuroimaging techniques that glimpse into the neural correlates of motor sequence learning. Neuroimaging studies have suggested that while the behavioral impairments may be small, there are larger underlying neurological changes that may explain the impairments through cognitive deficits in typically aging adults. For example, it has been suggested that declines in working memory with age may result in impaired motor sequence learning, particularly in the early stage (Bo, et al., 2011, 2012; Ghilardi, et al., 2003; Seidler, et al., 2012). Seidler and colleagues have further suggested differential effects for different types of working memory, in which verbal working memory may compensate for declines in visuospatial working memory (Bo, et al., 2012). Neurochemical changes and a loss in striatal volume leading to degrading cortico-striatal networks may be additional factors related to impairments in learning more complex sequences in older individuals (J. H. Howard & Howard, 2013; King, et al., 2013; Rieckmann & Backman, 2009; Seidler, et al., 2010). Further cognitive declines may be caused by decreased function in the prefrontal cortex (Aizenstein, et al., 2006; Daselaar, et al., 2003) that may be modulated by the dopamine projections from the striatum to the prefrontal cortex (Braver & Barch, 2002; Braver, et al., 2001). In addition to an increased cognitive load when learning higher order sequences, studies have also found that providing instructions to explicitly search for a sequence hinders implicit learning in

typically aging adults (D. V. Howard & Howard, 2001), but not young adults (Willingham & Goedert-Eschmann, 1999). This may suggest that in typically aging adults, explicit knowledge pushes the processing capacity to its limit, thus manifesting impairments in implicit learning (Rieckmann & Backman, 2009; Salthouse, 1996). Most of the neuroimaging studies investigating implicit motor sequence learning have been conducted using functional MRI which provides excellent spatial resolution, but poor temporal resolution. Given that reaction time (measured in milliseconds) is the variable used to infer learning, we propose that electroencephalography (EEG) is better suited to identify cortical activations and cortico-cortical connectivity associated with learning and impairments, as EEG provides excellent temporal resolution.

Thus, the aims of this study are to: 1) determine whether typically aging adults can learn fixed and probabilistic sequences in our modified SRT task; 2) apply cluster analysis to reaction time series data to separate typically aging adults into groups based on their performance; and, 3) investigate the cortical dynamics of motor sequence learning using EEG to assess learning and any impairments.

Methods

Participants

Twenty typical young adults (TY; mean age: 20.9 ± 1.18) and 42 typically aging adults (TA; mean age: 64.7 ± 7.36) were randomly assigned to either a fixed (FX) or a probabilistic (PB) sequence. All participants completed the Global Physical Activity Questionnaire (Armstrong & Bull, 2006), a spatial version of the *n*-back test to assess working memory (Jaeggi, et al., 2008), the Wisconsin Card Sorting Test to assess set-

shifting (Grant & Berg, 1948; Mueller, 2010), and a computer skills questionnaire to assess familiarity with the number pad on the computer keyboard. Participants were also screened for neurological and motor impairments through a health questionnaire. The typically aging adults completed the Mini Mental State Exam (Folstein, Folstein, & McHugh, 1975) to screen for cognitive impairments. All participants were right handed.

Serial reaction time task

Participants were seated in front of a computer monitor (21") and keyboard (keys size 13x15mm, keys are 6mm apart vertically and horizontally and 8mm apart diagonally). A modified SRT task was used that consisted of nine white squares in a 3x3 matrix on the computer screen (37x37mm each). Participants placed the index finger of their right hand on the center button on the number pad of the keyboard. The relationship between the squares on the screen and the buttons on the number pad was spatially compatible, i.e., the top right square corresponded to the top right button. At the beginning of each trial, one of the eight squares turned blue and the participant pressed the key that corresponded to the location of the stimulus and then returned to the home position. After the participant pressed a key, a response-to-stimulus interval between 300-1000ms was selected randomly for each trial to prevent participants from anticipating the appearance of the subsequent stimulus as well as to prevent any confounding effects from the length of the response-to-stimulus interval (Willingham, et al., 1997). No visual feedback was given to participants as a wooden board blocked vision of their finger position (see Figure 4.2).

Table 4.1: Participant information for all groups.

Sequence Type	Group	Age (Mean ± S.D.)	Sex	Physical Activity Level [#]	2-back (% correct)	Wisconsin Card Sorting Test				MMSE
						Correct (%)	Perservative Response (%)	Perservative Errors (%)	Non-perservative Errors (%)	
FX	TY	20.7 ± 1.12	5 female; 5 male	6 high; 4 moderate	65.1 ± 19.4	85.7 ± 2.29	33.7 ± 3.10	9.81 ± 1.38	4.49 ± 2.27	-
	TA	64.8 ± 7.27	14 female; 8 male	8 high; 8 moderate; 5 low	35.2 ± 19.7	69.1 ± 14.8	31.3 ± 15.6	14.6 ± 10.0	16.4 ± 15.0	29.4 ± 1.07
PB	TY	21.0 ± 1.24	5 female; 5 male	8 high; 2 moderate	58.3 ± 17.8	81.3 ± 9.13	32.6 ± 6.35	10.6 ± 1.68	8.19 ± 9.60	-
	TA	64.5 ± 7.45	13 female; 7 male	7 high; 7 moderate; 6 low	44.6 ± 23.1	72.2 ± 13.0	34.1 ± 4.33	13.8 ± 3.22	12.1 ± 9.33	29.7 ± 0.571

[#] Physical activity levels were determined based on the GPAQ calculated from the number of days and amount of time spent engaged in physical activity.
FX – fixed sequence group; PB – probabilistic sequence group; TY – typical young adults; TA – typically aging adults.

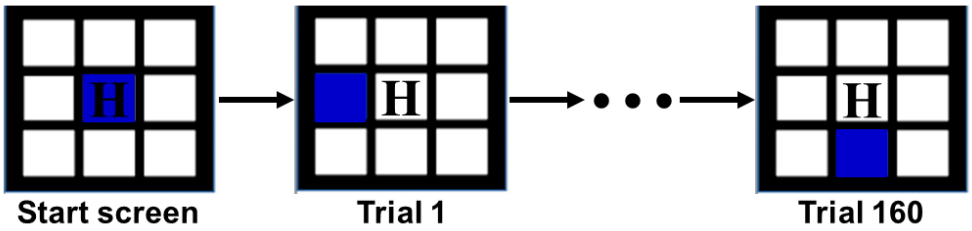

Figure 4.1: The modified serial reaction time (SRT) task. Participants placed their right index finger on the home position (H). On a given trial, one of the 8 locations turned blue and the participant pressed the corresponding button on the number keypad and then returned to the home position.

Figure 4.2: Experimental Setup. Participants were seated in front of a computer monitor with their hand placed on the number keypad. Participants did not receive any visual feedback and could not see their hands. Participants' right hand was wrapped with athletic pre-wrap to prevent the use of the other fingers.

There were a total of eight blocks for all groups, each consisting of 160 trials (see Figure 4.3). The first block was a baseline block (B0), consisting of 160 trials in which the stimuli appeared in a random order. The next four blocks (B1-4) were the learning blocks consisting of the fixed or probabilistic sequence in which the sequence was repeated 10 times each. Block 5 (B5) consisted of 160 trials of stimuli occurring in a random order and Block 6 (B6) consisted of 10 repetitions of the assigned sequence. An increase in response time in B5 and decrease in B6 would indicate learning (Robertson, 2007). Lastly, Block 7 (B7) consisted of 10 repetitions of a different sequence that was constructed from the same underlying structure as the learned sequence to assess transfer of learning. If the response times decrease from B5 to B7, it would suggest that participants were able to transfer their

learning. A unique sequence was assigned to each participant to ensure that the results are not intrinsic to the sequence used, but can be generalized to all sequences (DeCoster & O'Mally, 2011a). In the RD group, stimuli occurred in a random order in all eight blocks. Participants were given a two-minute mandatory break between each block. The experiment was performed using Presentation® software (Version 18.1, www.neurobs.com).

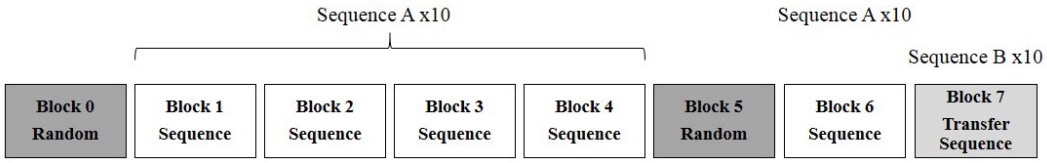

Figure 4.3: The experimental paradigm used for the three groups. All groups started with a baseline (B0), then the fixed and probabilistic groups performed the learning blocks (B1-4) and ended with a random block (B5) followed by another sequence block (B6) and a transfer block (B7). Each block consisted of 160 trials. Participants were given a two-minute break between each block. Participants in the fixed and probabilistic groups were given a unique fixed or probabilistic sequence, respectively. Participants in the random group were presented with stimuli in a random order for all blocks.

The participants' reaction time (RT), movement time (MT), and accuracy were recorded. At the beginning of each trial, participants pressed the home button. The participants' RT (time taken to release the home button after the stimulus was presented), MT (time between release of home button and pressing of the corresponding button), and accuracy were recorded for each trial (see Figure 4.4). Both RT and MT were recorded to address limitations in previous studies in which only the response times were recorded.

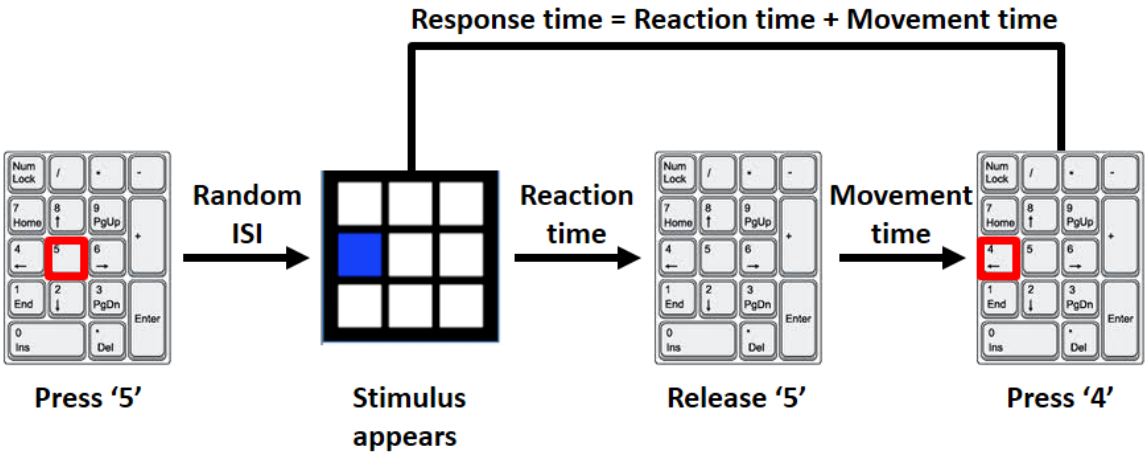

Figure 4.4: Diagram depicting reaction time (RT) and movement time (MT) recordings in the modified SRT task. At the start of a trial, the participant presses the home button ('5' on the number keypad). After a random interval (300-1000ms), a stimulus will appear on the screen (one of the eight locations will turn blue). The RT is the amount of time taken to release the home button and the MT is the time from the release of the home button to the pressing of the button that corresponds to the stimulus. The RT and MT are added to calculate the response times.

Posttest

All participants completed a posttest after the completion of the eight blocks to determine if learning was implicit. First, participants were asked the following question: "The stimulus movement is best described as:" with the following options: "a) Random; b) Some positions occurred more often than others; c) The movement was often predictable; d) The same sequence of movements would often appear; and e) The same sequence of movements occurred throughout the entire experiment" (Curran, 1997).

Second, participants completed a recognition test to assess explicit recall of the sequence (Destrebecqz & Cleeremans, 2001) consisting of two parts: in the first part, participants were presented with six-item chunks from their assigned sequence as well as random chunks and were asked to rate how confident they were that they had seen that chunk before from a scale of 1-5 (where 1 was "Confident that I *have not* seen it before" and 5 was "Confident that I *have* seen it before"). In the second part, participants were

presented with the entire 16-item sequence as well as other random sequences and they were asked to rate them on the same scale.

EEG recording and pre-processing

EEG data were recorded from 64 electrodes mounted on an actiCAP and BrainVision actiCHamp Amplifier (Brain Products, LLC) using the international 10-20 system. The sampling frequency was 1000Hz. The reference electrodes were placed on the left and right mastoids and AFz was the ground electrode. Channel impedances were kept below 10kΩ.

Prior to the SRT task, four resting states were recorded from each participant. Participants were asked to sit as motionless as possible with their eyes open and then with their eyes closed for one minute each. Participants were also asked to view the task as the stimuli appeared but did not respond. Lastly, participants pressed each of the response buttons their right index finger in the clockwise direction at their preferred speed without any visual stimulus.

Behavioral data analysis

The RT and MT were trimmed according to the individual participant's mean and standard deviation. Any values greater or less than 2.5 standard deviations were excluded from the analysis (Ratcliff, 1993; Whelan, 2008). Mean RT and MT were calculated for each block and were averaged across participants in each group. Learning was measured through a decrease in RT from B1 to B4, an increase from B4 to B5 (stimuli in random order) and a decrease from B5 to B6 (stimuli in assigned sequence). Transfer of learning was inferred if there was a significant decrease in RT between B5 and B7 (stimuli in different sequence of same structure as assigned sequence).

Cluster analysis

Cluster analysis is a technique used to structure large complex data sets into relatively homogenous groups without any predetermined criteria (Lloyd, 1982; MacQueen, 1967). Specifically, *k*-means clustering creates groups in which objects are as close to other objects in the same group as possible, while being as far away as possible from objects in other groups. In order to create a developmental landscape of the typically aging adults that was not based on age, the entire RT time series for the 42 typically aging adults were included in the *k*-means cluster analysis (each participant had 160 trials in 8 blocks for a total of 1280 trials). The algorithm starts with initial estimates of the means of the *k* clusters, then categorizes each subject into the cluster with the closest mean and calculates new mean for each cluster. This is repeated until each subject is in a cluster with a minimum distance from the other subjects in the same cluster and the maximum distance from subjects in other clusters. Cluster analysis is a popular technique used in image analysis, including neuroimaging (Balslev et al., 2002) and bioinformatics, but to our knowledge, has not been used for behavioral data such as reaction time.

EEG data analysis

The EEG data were preprocessed using EEGLAB (Delorme & Makeig, 2004). The data were re-referenced to the average of the mastoid electrodes (M. X. Cohen, 2014). Data were filtered using a FIR low-pass filter (cut off frequency: 55Hz, roll off 24dB/octave) to eliminate electrical noise. Independent component analysis (ICA) was used to remove eye artifacts, such as eye blinks, eye movements, and muscle artifacts.

Spectral power. Following preprocessing, the data were exported into MATLAB version 8.4 (Mathworks, Natick, MA). Data were segmented in one-second intervals with

respect to the RT (250ms before and 750ms after the RT) and Fast Fourier transforms (FFT) were applied in the alpha (8-12Hz) and beta (13-30Hz) bands for each of the experimental blocks. Relative alpha and beta power were standardized to the baseline block (B0) in which the stimuli occurred in a random order. Data from the a subset of electrodes were extracted for the following regions: frontal left (F7, F5, F3, and F1), frontal right (F8, F6, F4, and F2), central left (C5,C3, and C1), central right (C6, C4, and C2), parietal left (P7, P5, P3, and P1), parietal right (P8, P6, P4, and P2), parieto-occipital left (PO7, PO3, and O1), and parieto-occipital right (PO8, PO4, O2).

Coherence. Coherence is a measure of the amount of cortical communication between two electrical sites (ranging from 0 to 1) (Fries, 2005; Nunez, 2000; Srinivasan, Nunez, & Silberstein, 1998). The electrode pairings used here between the Fz electrode and frontal, motor, temporal, parietal, and occipital regions. The specific electrode pairings used for the left hemisphere were: Fz-F3, Fz-C3, Fz-T3, Fz-P3, and Fz-O1 and those for the right hemisphere were: Fz-F4, Fz-C4, Fz-T4, Fz-P4, and Fz-O2.

Statistical analysis

Behavioral data. A mixed factorial analysis of variance (ANOVA) was used to compare the differences in average response time, RT, and MT between the Group (TY, TA) x Sequence Type (FX, PB) x Block (0-7) with Block as the within subject variable. Bonferroni *post-hoc* tests were used to decompose any significant effects. Separate pairwise comparisons were conducted on the contrasts of interest (B1 vs. B4, B4 vs. B5, B5 vs. B6, and B5 vs. B7) to determine whether learning occurred and whether learning was transferred to a novel sequence created using the same underlying structure.

EEG analysis. Separate mixed factorial analyses of variance (ANOVA) were used to compare differences in average spectral power and coherence in the alpha and beta bands between Group (TY, TA) x Region (Frontal, Central, Parietal, Occipital) x Hemisphere (Left, Right) x Block (B1, B4, B5, B7) with Block as the within subject variable. Bonferroni *post-hoc* tests were used to decompose any significant effects. Separate pairwise comparisons were conducted on the contrasts of interest (B1 vs. B4, B4 vs. B5, and B5 vs. B7) to determine differences in early, late, and transfer of learning.

Statistical significance was defined at $p < 0.05$. The data were processed using custom scripts written in MATLAB version 8.4 (Mathworks, Natick, MA) and SPSS Statistics 22 (IBM, Armonk, NY).

Results

Accuracy

All groups exhibited high levels of accuracy with 7% or fewer errors. Thus, accuracy cannot be used as a measure of learning in this task and was not analyzed further. Error rates have also been demonstrated to be low in previous studies (R. M. Brown & Robertson, 2007; Willingham, et al., 1989).

Mean response time confounds performance related to learning and movement

A three-way mixed factorial (2 x 2 x 8) ANOVA on Group (TY, TA) x Sequence Type (FX, PB) x Block (0-7) on the response times with Block as the within subject variable indicated a main effect of Block, $F(7,406) = 26.7$, $p < 0.001$ and Group, $F(1,58) = 25.0$, $p < 0.001$. In addition, there was a significant two-way interaction between Block x Group, $F(7, 406) = 2.54$, $p = 0.01$, suggesting that the response time was influenced

differently depending on the block and group. Bonferroni's *post-hoc* analysis revealed that for all blocks, the TY was significantly faster than the TA group (all $p < 0.001$). However, differences emerged between blocks for each group. For the TY group, there were no significant differences between the blocks, but for the TA group, the response time significantly decreased from B1 to B4, from B5 to B6, from B5 to B7, and from B6 to B7 (all $p < 0.002$). This suggests that when collapsing across sequence type, the differences between blocks are masked in the TY group, but not in the TA group.

Fixed sequence. Pairwise comparisons between contrasts that were determined *a priori* (see Figure 4.5) revealed significant differences between B1 and B4 for both groups (both $p < 0.001$). Differences between B4 and B5 were approaching significance in the TY group learning the FX sequence ($p = 0.10$) and were significant in TA group learning the FX sequence ($p = 0.004$) as well as differences between B5 and B6 (TY, $p = 0.10$; TA, $p = 0.070$), suggesting that both TY and TA adults were able to learn the FX sequence. In addition, a significant decrease was found from B5 to B7 in the TY ($p < 0.001$) and TA ($p = 0.05$), indicating that both groups were able to transfer their learning to a novel sequence.

Probabilistic sequence. The response time decreased significantly from B1 to B4 for both groups (both $p < 0.004$). As found in our previous study (Prashad, Du, & Clark), no significant differences were found between B4 and B5 (both, $p = 0.7$). The decrease between B5 and B6 approached significance for both groups (TY, $p = 0.07$; TA, $p = 0.08$), suggesting possible learning of the probabilistic sequence by both groups. A significant decrease was exhibited between B5 and B7 in both groups (both $p < 0.002$), suggesting that the young and typically aging adults were able to transfer their learning to a novel sequence.

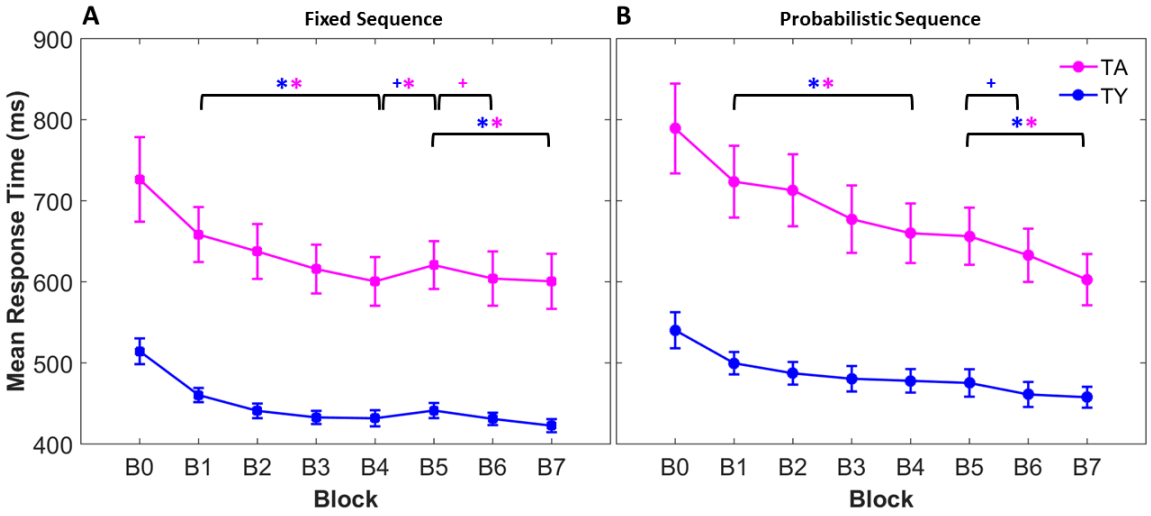

Figure 4.5: Mean response time. (A) Both groups exhibited a decrease in response time from B1 to B4, an increase from B4 to B5, and a decrease from B5 to B7 in the fixed sequence groups. (B) TY exhibited a decrease from B1 to B4, B5 to B6, and B5 to B7 in the probabilistic sequence groups. The TA groups exhibited a decrease from B1 to B4 and B5 to B7.
*Indicates significance level of $p < 0.05$; +indicates significance level of $p < 0.10$. Error bars indicate standard error.

Mean reaction time (RT) is a better assessment of learning

A three-way mixed factorial (2 x 2 x 8) ANOVA on Group (TY, TA) x Sequence Type (FX, PB) x Block (0-7) on the RT with Block as the within subject variable indicated a main effect of Block, $F(7,406) = 50.5$, $p < 0.001$ and Group, $F(1,58) = 28.1$, $p < 0.001$ and no significant interactions. Bonferroni's *post hoc* analysis on Group revealed that the TY group had significantly faster RTs than the TA group.

Fixed sequence. Pairwise comparisons between contrasts that were determined *a priori* revealed significant differences decrease from B1 to B4 for both groups (both $p < 0.001$), increase from B4 to B5 (both $p < 0.05$), and decrease from B5 to B6 (both $p < 0.004$), further bolstering evidence from the response time that both typical young and aging adults were able to learn the fixed sequence. Furthermore, there was a significant

decrease from B5 to B7 in both groups (both $p = 0.001$), suggesting that both typical young and aging adults were able to transfer their learning to a novel fixed sequence (see Figure 4.6).

Probabilistic sequence. There was a significant decrease from B1 to B4 in both groups (both $p < 0.008$), no significant increase from B4 to B5 (both $p > 0.05$), and a significant decrease from B5 to B5 only in the TY group ($p = 0.04$). The lack of change in RT from B4 to B5 was expected based on results from our previous study (Prashad, et al.); however, no difference from B5 to B6 in the TA group suggests these participants were unable to learn the sequence. Both groups did show a significant decrease from B5 to B7 (both $p < 0.05$), suggesting transfer of learning to a novel sequence. Thus, it appears that the typical young adults learned the probabilistic sequence, but it is unclear whether the typically aging adults learned the probabilistic sequence.

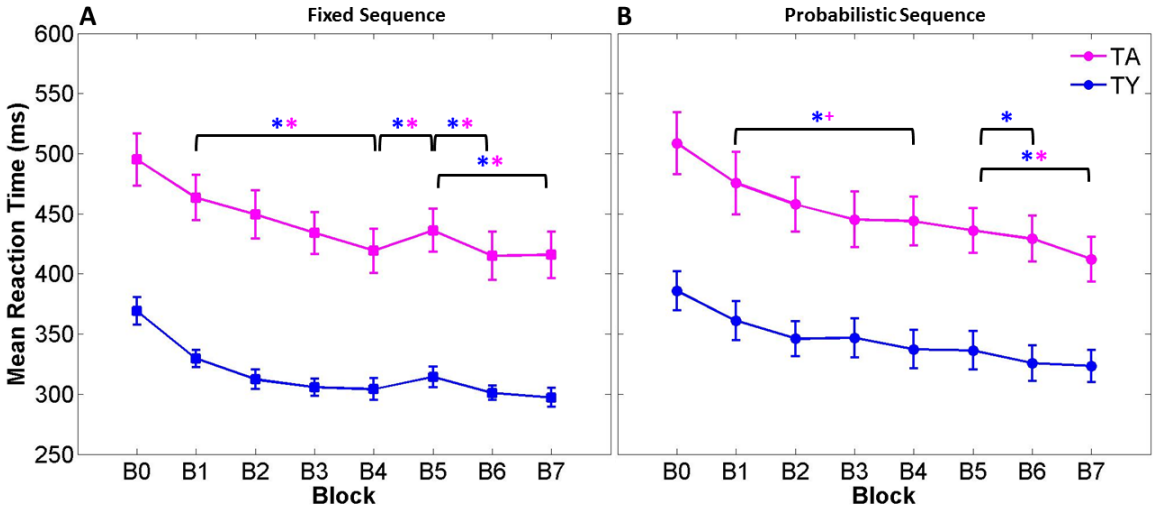

Figure 4.6: Mean reaction time. (A) Both groups exhibited a decrease in response time from B1 to B4, an increase from B4 to B5, and a decrease from B5 to B6 and B7 in the fixed sequence groups. (B) TY exhibited a decrease from B1 to B4, B5 to B6, and B5 to B7 in the probabilistic sequence groups. The TA groups exhibited a marginal decrease from B1 to B4 and B5 to B7.
* Indicates significance level of $p < 0.05$; + indicates significance level of $p < 0.10$. Error bars indicate standard error.

Mean movement time (MT) may be significant for complex sequences

A three-way mixed factorial (2 x 2 x 8) ANOVA on Group (TY, TA) x Sequence Type (FX, PB) x Block (0-7) on the RT with Block as the within subject variable indicated a main effect of Block, $F(7,406) = 4.50$, $p < 0.001$ and Group, $F(1,58) = 10.7$, $p = 0.002$. There were no significant interactions. A Bonferroni *post-hoc* analysis on Group revealed that the TY group was significantly faster than the TA group.

Fixed sequence. As expected, pairwise comparisons between contrasts that were determined *a priori* only revealed no significant differences for either group.

Probabilistic sequence. No differences were found for the TY group, but differences were found for the TA group between B1 and B4 ($p = 0.02$), B5 and B6 ($p = 0.04$), and B5 and B7 ($p = 0.03$). These results were surprising as MT is not expected to change between blocks.

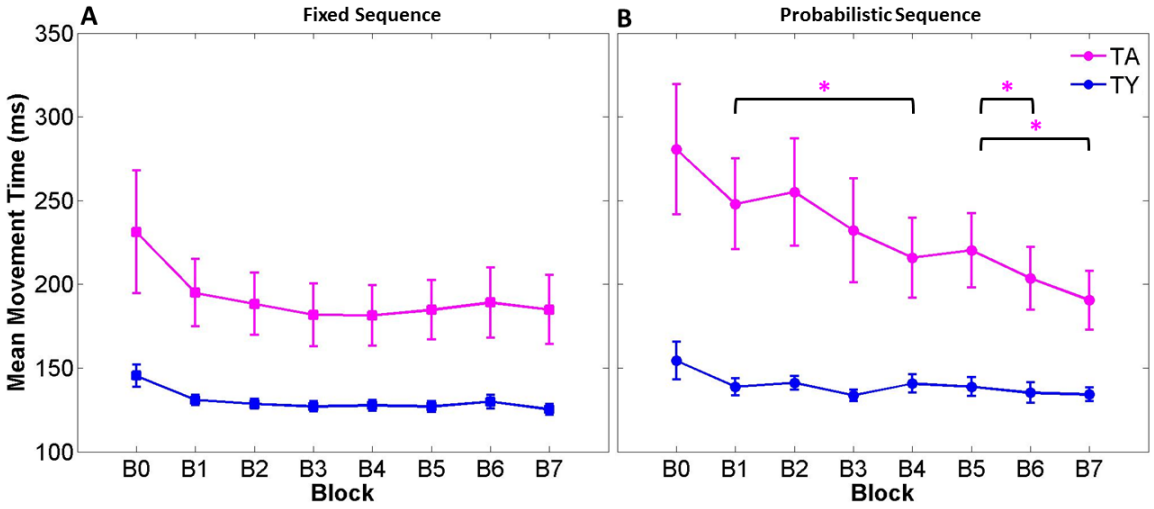

Figure 4.7: Mean movement time. (A) No significant differences were observed in MT in the fixed sequence groups. (B) TA exhibited a significant decrease in MT from B1 to B4 and B5 to B6 and B7. Error bars indicate standard error.

Thus, the results suggest that typical young adults learned both fixed and probabilistic sequences and the typically aging adults learned the fixed sequences, but the results were not conclusive whether they were able to learn the probabilistic sequences. In order to better understand the effect of aging on motor sequence learning, we divided the typically aging adults into three groups using cluster analysis on their RT.

Cluster analysis reveals functional groups separated by mean RT and working memory

A *k*-means cluster analysis with three clusters was conducted on the 42 typically aging adults. The entire RT time series for each participant was included in the cluster analysis (160 trials in 8 blocks for a total of 1280 trials). The algorithm separated the participants into three clusters (TA1, TA2, and TA3; see Table 4.2 for demographic information on the three clusters). Separate one-way ANOVAs revealed no significant differences between the groups in age, $F(2,41) = 1.77$, $p = 0.2$ (see Figure 4.8A), the Wisconsin Card Sort Task, $F(2,41) = 0.514$, $p = 0.6$, or physical activity level, $F(2,41) = 0.311$, $p = 0.7$. However, significant differences were found in the overall percent correct in the *n*-back task, $F(2,41) = 3.81$, $p = 0.03$ as well as the overall mean RT collapsed across blocks, $F(2,41) = 107.0$, $p < 0.001$ (see Figure 4.8B and 4.8C). *Post hoc* analysis using the Bonferroni correction revealed that TA1 had a significantly higher *n*-back score than TA3 ($p = 0.03$) and a significantly faster overall mean RT ($p < 0.001$). There were no other significant differences between TA1 and TA2 in the *n*-back score, but the overall mean RT for the TA2 cluster was significantly faster than the TA3 cluster ($p < 0.001$). Thus, it appears that working memory is inversely correlated with mean RT and may explain impairments in motor sequence acquisition (see Figure 4.9). This result is consistent with previous studies that suggest that working memory capacity is correlated to sequence

learning performance (Bo, Borza, & Seidler, 2009; Bo, et al., 2011, 2012) and may provide a mechanism for impaired motor sequence learning in aging and clinical populations.

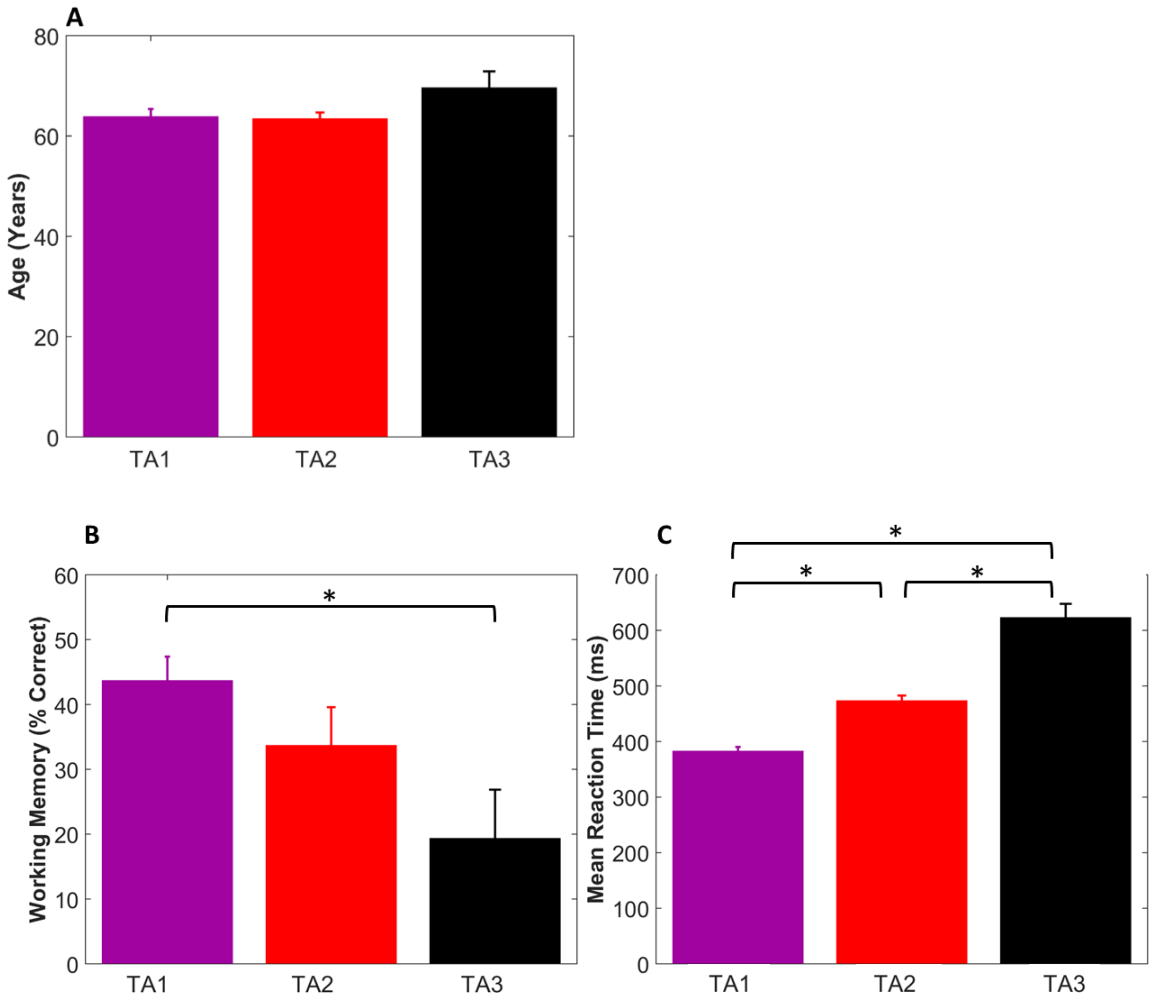

Figure 4.8: Characteristics of the three typically aging clusters. A) Age was not significantly different between the three TA clusters. B) Working memory, as assessed by percent correct in the *n*-back test, was significantly higher in the TA1 cluster than the TA3 cluster. C) The overall mean RT of the TA1 cluster was significantly faster than the TA2 and TA3 clusters.

Table 4.2: Demographic information of the different clusters from the typically aging group.

Cluster	Age (Mean ± S.D.)	Sex	Physical Activity Level [#]	2-back (% correct)	Wisconsin Card Sorting Test				MMSE
					Correct (%)	Perservative Response (%)	Perservative Errors (%)	Non-perservative Errors (%)	
TA1	63.9 ± 1.45	15 female; 8 male	10 high; 6 moderate; 7 low	43.7 ± 3.64	68.9 ± 14.4	29.7 ± 15.6	13.6 ± 9.27	17.5 ± 15.4	29.5 ± 1.03
TA2	63.5 ± 1.15	9 female; 4 male	4 high; 5 moderate; 4 low	33.7 ± 5.84	73.9 ± 12.0	36.0 ± 9.24	16.0 ± 8.34	10.2 ± 6.44	29.7 ± 0.49
TA3	69.7 ± 3.19	3 female; 3 male	1 high; 4 moderate; 1 low	19.4 ± 7.46	69.8 ± 14.4	38.6 ± 11.0	18.9 ± 11.0	11.2 ± 8.42	29.5 ± 0.84

[#] Physical activity levels were determined based on the GPAQ calculated from the number of days and amount of time spent engaged in physical activity.
FX – fixed sequence group; PB – probabilistic sequence group; TA1 – typically aging adults in cluster 1, TA2 – typically aging adults in cluster 2, TA3 – typically aging adults in cluster 3.

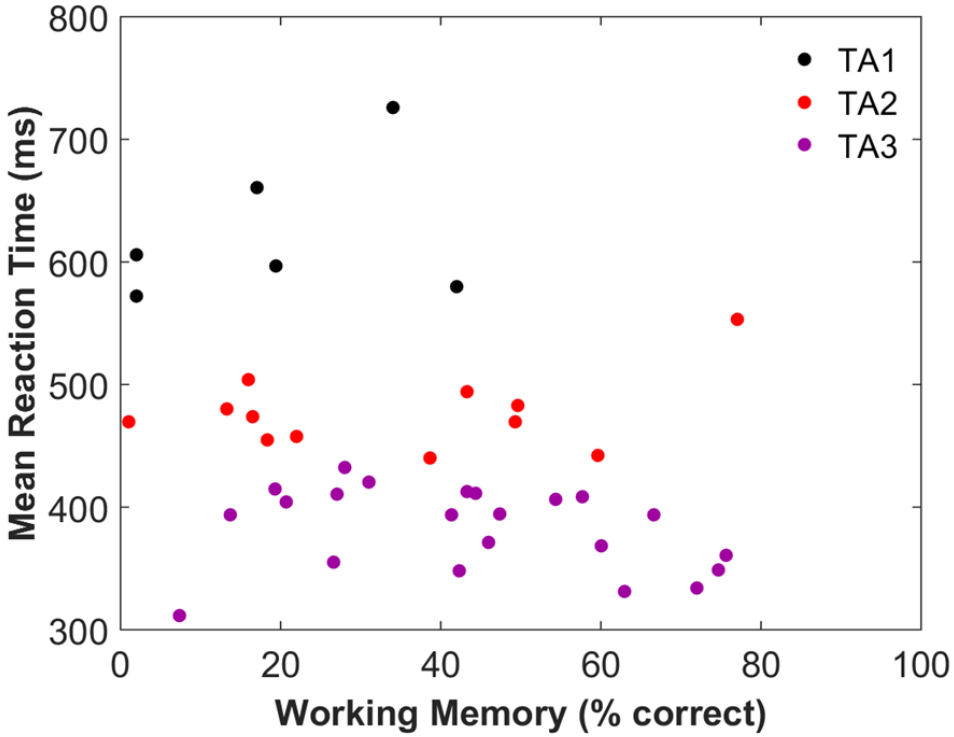

Figure 4.9: Visualization of the three typically aging adult clusters. A) *k*-means cluster analysis revealed three clusters for the typically aging group separated by overall mean reaction time and working memory.

Mean reaction times of the typically aging clusters

A three-way mixed factorial (4 x 2 x 8) ANOVA on Group (TY, TA1, TA2, TA3) x Sequence Type (FX, PB) x Block (B0-7) on the RT with Block as the within subject variable indicated a main effect of Block, $F(7,378) = 71.0$, $p < 0.001$, Group, $F(3,54) = 105.5$, $p < 0.001$ and significant interactions between Block x Group, $F(21,378) = 2.74$, $p < 0.001$, Block x Sequence, $F(7,378) = 3.18$, $p = 0.003$, and Block x Sequence x Group, $F(21,378) = 2.46$, $p < 0.001$. Simple effects analysis revealed that there were no significant differences between the RT of the two sequences types. In addition, within the fixed sequence, for each block, TY had the fastest RT ($p < 0.002$ for all blocks), TA1 had the second fastest RT ($p < 0.001$ for all blocks), TA2 had the third fastest RT ($p < 0.001$ for all

blocks), and TA3 had the slowest RT ($p < 0.001$ for all blocks). However, the RTs were not as clearly different for the groups' learning the probabilistic sequence. In B1, the RT of TY and TA1 was not significantly different ($p = 0.1$), but both were significantly faster than TA2 (both $p < 0.001$) and TA2 was significantly faster than TA3 ($p < 0.001$). However, by B4 TY was significantly faster than TA1 ($p = 0.03$) and remained significantly faster than TA2 and TA3 (both $p < 0.001$). For B5-7, all groups were significantly different from each other (all $p < 0.05$).

Fixed sequence. Pairwise comparisons between contrasts that were determined *a priori* revealed significant differences between B1 and B4 ($p = 0.001$), B4 and B5 ($p = 0.010$), B5 and B5 ($p < 0.001$), and B5 and B7 ($p = 0.002$) in TA1, suggesting learning of the sequence. However, only a significant decrease was found between B1 and B4 for TA2 ($p = 0.03$) and no significant differences were found for TA3, suggesting that these two clusters were unable to learn the fixed sequence.

Probabilistic sequence. Significant differences were found for a decrease from B5 to B7 in TA1 and TA2 (both $p < 0.04$) and from B1 to B4 in TA3 ($p = 0.04$), suggesting impairment in the learning of the probabilistic sequence in the typically aging group.

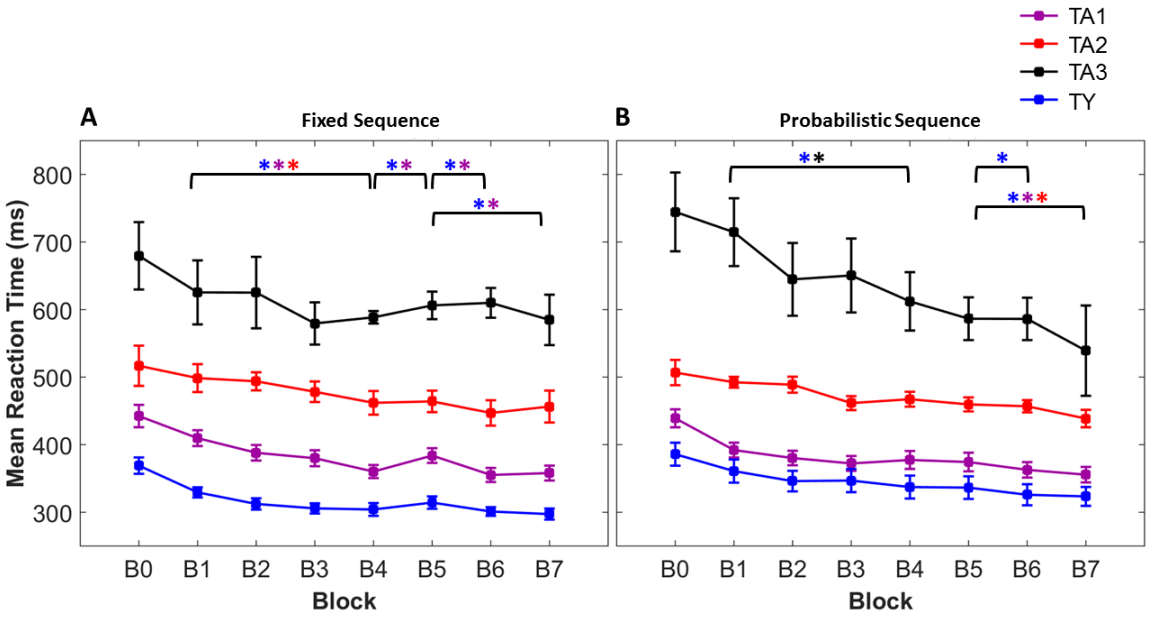

Figure 4.10: Mean RT for the typical young and aging adults. A) In the fixed sequence groups, TY and TA1 exhibited a decrease in RT from B1 to B4, an increase from B4 to B5, and a decrease from B5 to B6 and B7. TA2 exhibited a decrease from B1 to B4 and TA3 exhibited no changes. B) In the probabilistic sequence groups, TY exhibited a decrease from B1 to B4 and B5 to B6 and B7. TA1 and TA2 exhibited a decrease from B5 to B7. TA3 exhibited a decrease from B1 to B4.
* Indicates significance level of $p < 0.05$. Error bars indicate standard error.

In summary, the typically aging group consisted of three distinct clusters separated by mean RT and working memory capacity, but not age. Cluster analysis provided an objective method for separating typically aging adults based on functional characteristics and afforded an approach for more deeply understand age-related changes in implicit motor sequence learning. The mean RT of all the typically aging adults did not provide clear results on whether typically aging adults learned the probabilistic sequence, but separating the participants through cluster analysis indicated that they were unable to learn the probabilistic sequence, and only the TA1 cluster learned the fixed sequence.

Relative spectral power

Separate ANOVAs were conducted on the alpha and beta bands for each sequence type. The typical young adults were compared to the typically aging clusters.

Fixed sequences

Alpha. A four-way mixed factorial (4 x 4 x 2 x 4) ANOVA on Group (TY, TA1, TA2, TA3) x Region (Frontal, Central, Parietal, Occipital) x Hemisphere (Left, Right) x Block (B1, B4, B5, B7) on the alpha band with Block as the within subject variable indicated a main effect of Hemisphere, $F(1,27) = 4.16$, $p = 0.05$ and significant interactions between Region x Block, $F(9,243) = 2.10$, $p = 0.03$, Region x Hemisphere x Block, $F(9,243) = 4.72$, $p < 0.001$, and Region x Hemisphere x Block x Group, $F(27,243) = 1.53$, $p = 0.05$.

Simple effects analysis revealed that in B4, there was significantly lower alpha power in TY than TA2 in the right frontal and central regions and bilaterally in the occipital region (all $p < 0.05$). TA1 also exhibited significantly lower alpha power than TA2 in the right central, parietal, and occipital regions (all $p < 0.05$). Interestingly, TA3 exhibited significantly lower alpha power than TA2 in the right parietal area and bilaterally in the occipital region (all $p < 0.05$). In B7, TY and TA1 had significantly lower alpha power than TA2 in the right parietal and bilateral occipital regions (all $p < 0.05$) and TA3 exhibited significantly lower alpha power than TA2 in the right central region ($p = 0.05$) and bilateral occipital region (both $p < 0.05$).

Although TA2 did not exhibit learning of the fixed sequence behaviorally, this group exhibited differences in cortical activations between regions, but the other groups did not. In B1, there was greater alpha power in the left central region than left occipital

and frontal regions (both $p < 0.03$), as well as greater alpha power in the right parietal region than the right occipital region ($p = 0.007$). In B4, there was marginally greater alpha power in the left central region than the left frontal ($p= 0.07$) and occipital regions ($p = 0.05$). In B7, there was greater alpha power in the left frontal area than the parietal area ($p = 0.05$) and greater alpha power in the left central area than the parietal and occipital regions (both $p < 0.05$).

Pairwise comparisons between contrasts that were determined *a priori* revealed significant differences in the TY group. In the left and right frontal cortical areas, alpha power decreased as participants learned the fixed sequenced, as exhibited by greater alpha power in B1 than B4 (left, $p = 0.04$ and right, $p = 0.02$) and increased power during the random block, B5 (left, $p = 0.005$ and right, $p = 0.03$). A similar activation pattern was exhibited in the parietal region, with a marginally significant decreasing alpha power from B1 to B4 (left, $p = 0.05$ and right, $p = 0.09$) and an increase in B5, but only in the left hemisphere ($p = 0.06$). The central and occipital regions only exhibited a decrease in power with learning (left central, $p = 0.084$; right central, $p = 0.01$; left occipital, $p = 0.04$; and right occipital, $p = 0.05$), but no changes in B5. The TA1 cluster did not exhibit these cortical activations, but did exhibit greater alpha power in B7 than B5 that approached significance in the left frontal region ($p = 0.08$) and left central region ($p = 0.06$). The TA2 and TA3 clusters did not exhibit any differences in blocks in the alpha band.

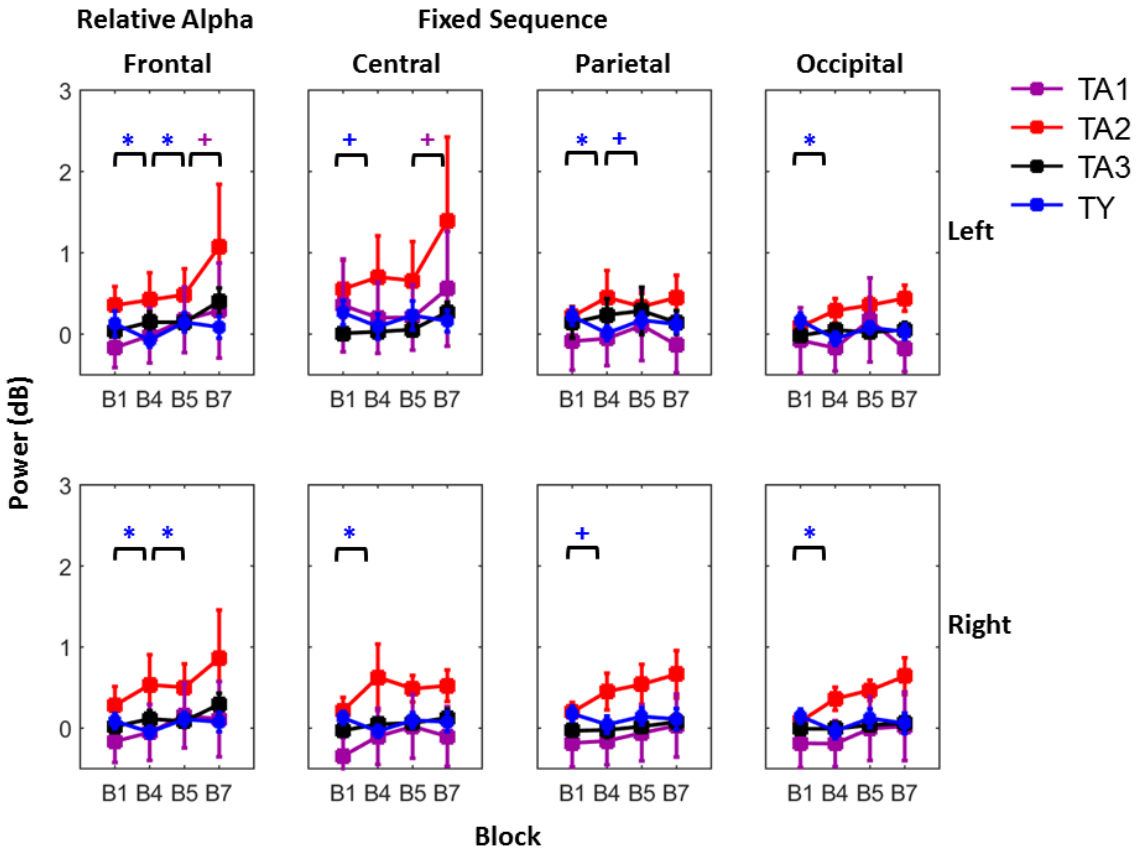

Figure 4.11: Relative alpha power for B1 (early learning), B4 (late learning), B5 (random – no sequence), and B7 (transfer of learning) for the fixed sequence groups.

Beta. In the beta band, only the interaction between Region x Hemisphere x Block was significant ($F(9,243) = 1.93$, $p = 0.05$. Across the groups, there was greater beta power in the left central region compared to the left frontal ($p = 0.03$) and occipital areas ($p = 0.005$) in both B1 and B4 (both $p < 0.05$). In the right hemisphere, the frontal area had greater beta power than the central, parietal, and occipital regions (all $p < 0.05$) in B1, but by B4, the occipital area had significantly lower beta power than the frontal and central regions (both $p = 0.04$). There were no differences in beta power between regions in B5. In B7, there was greater beta power in the left frontal and central areas than the left parietal

and occipital areas (all $p < 0.05$) and greater beta power in the right frontal area than the right central and occipital areas (both $p < 0.04$).

At the hemisphere level, there were few significant differences in the central and occipital regions. There was greater beta power in the left hemisphere in the central region (both $p < 0.01$) in both B1 and B7, but greater beta power in the right hemisphere in the occipital region ($p = 0.04$) in B7.

At the block level, there was marginally greater beta power in B4 than B1 in the right central region ($p = 0.07$) and significantly greater beta power in B7 than B5 in the left and right frontal region (both $p < 0.04$) and left central region ($p = 0.01$).

Pairwise comparisons revealed similar activation patterns as the alpha band in the TY group. There was a decrease in beta power as learning occurred and B4 had significantly lower beta power than B1 in the left and right parietal regions (both $p < 0.03$), left and right occipital regions (both $p < 0.03$), and right central region ($p = 0.03$). In addition, there was a significant decrease in beta power in B5 compared to B4 in the left parietal region ($p = 0.009$) and approaching significance in the left and right occipital regions (both $p = 0.07$). In TA1, there was significantly greater beta power in B7 than B5 in the left and right frontal regions (both $p < 0.005$) and left central region ($p = 0.02$). As in the alpha band, no significant differences were found in the TA2 and TA3 clusters.

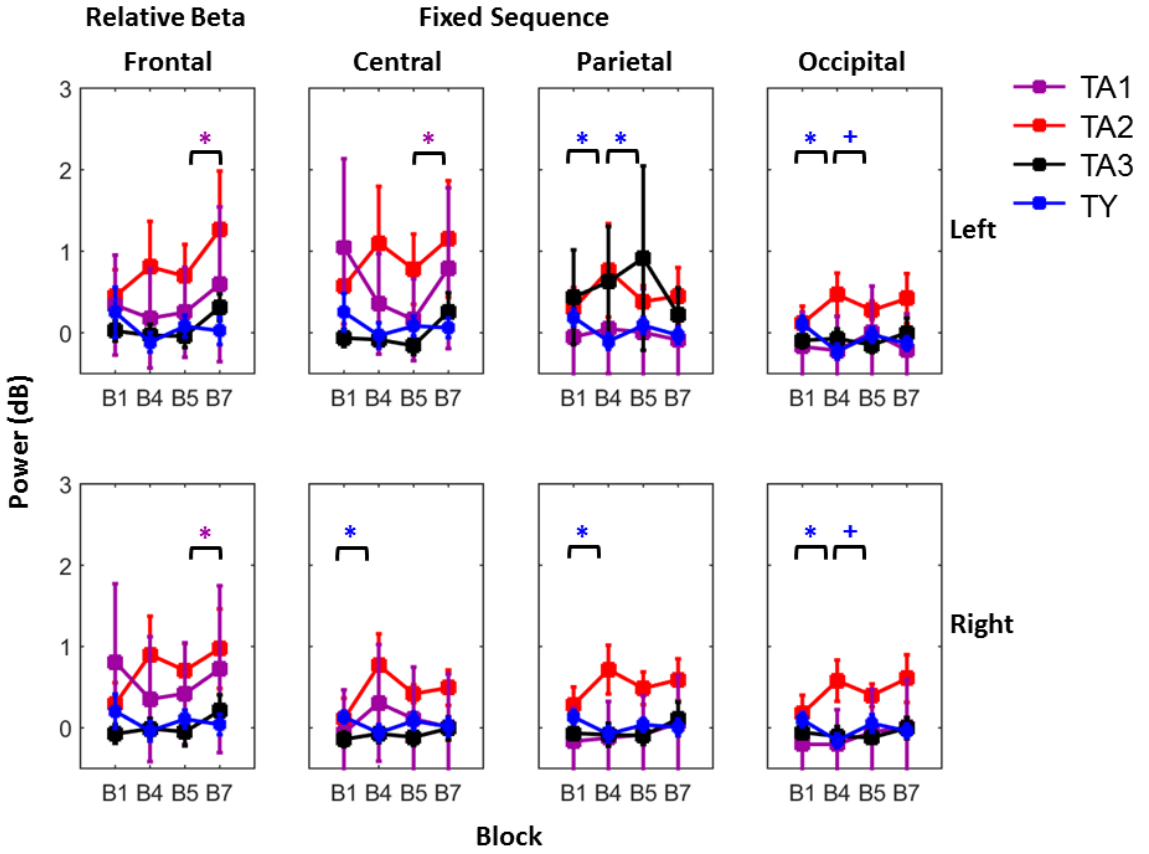

Figure 4.12: Relative beta power for B1, B4, B5, and B7 for the fixed sequence groups.

Probabilistic sequences

Alpha. A four-way mixed factorial (4 x 4 x 2 x 4) ANOVA on Group (TY, TA1, TA2, TA3) x Region (Frontal, Central, Parietal, Occipital) x Hemisphere (Left, Right) x Block (B1, B4, B5, B7) on the alpha band with Block as the within subject variable indicated a main effect of Region, $F(3,75) = 7.26$, $p < 0.001$) and a significant interaction between Region x Hemisphere x Block x Group, $F(27,225) = 1.54$, $p = 0.05$. TA3 exhibited greater alpha power compared to TY, TA1, and TA2 in B1 in the left frontal (all $p < 0.05$) and central regions (all $p < 0.01$) and greater alpha power in the right frontal region

compared to TY ($p = 0.04$). TA3 also exhibited greater alpha power than TA1 in the left frontal region in B4 ($p = 0.05$) and B5 ($p = 0.03$).

In addition, TA3 exhibited greater power in the left frontal and central regions compared to the left parietal and occipital areas (all $p < 0.05$) in B1. In B4, TY exhibited greater alpha power in the occipital region than the frontal and parietal areas (both $p < 0.05$) in the left hemisphere, but greater alpha power in the frontal region than the parietal and occipital regions in the right hemisphere (both $p < 0.05$). Similarly, in B7, TY exhibited greater alpha power in right frontal region compared to the parietal and occipital areas (both $p < 0.05$), suggesting that similar cortical activations are exhibited in late learning and transfer.

Pairwise comparisons between contrasts that were determined *a priori* revealed an increase in alpha power in B5 compared to B4 in the left and right (both $p < 0.04$) parietal regions and approaching significance in the left and right (both $p = 0.06$) occipital regions in the TY group. The TA1 and TA2 clusters did not exhibit any differences. However, the TA3 cluster exhibited a greater alpha power in B4 than B1 in the left occipital region that approached significance ($p = 0.07$). Additionally, there was significantly greater alpha power in B5 than B4 in the left central region ($p = 0.05$) and approaching significance in the right frontal region and right parietal region (both $p = 0.07$).

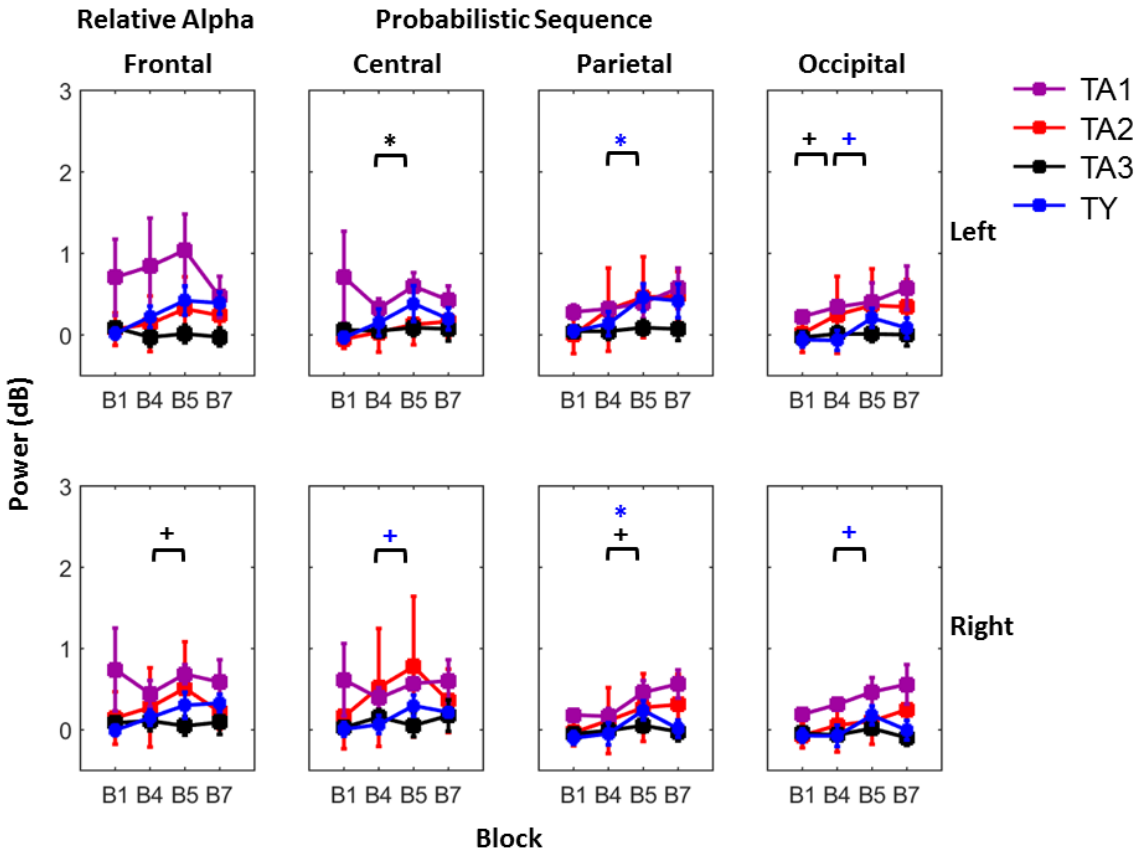

Figure 4.13: Relative alpha power for B1, B4, B5, and B7 for the probabilistic sequence groups.

Beta. In the beta band, there was a main effect of Region, $F(3,75) = 5.87$, $p = 0.001$ and significant interactions between Region x Group, $F(9,75) = 2.39$, $p = 0.02$, Region x Block, $F(9,225) = 3.78$, $p < 0.001$, Region x Block x Group, $F(27,225) = 2.10$, $p = 0.002$, Hemisphere x Block x Group, $F(9,75) = 2.19$, $p = 0.03$, Region x Hemisphere x Block, $F(9,225) = 2.00$, $p = 0.04$, and Region x Hemisphere x Block x Group, $F(27,225) = 1.59$, $p = 0.04$.

Simple main effects analysis of the four-way interaction revealed that, similar to the alpha band, TA3 exhibited greater beta power than TY, TA1, and TA2 in the frontal region bilaterally in B1, B4, and B5 (all $p < 0.05$). Additionally, TA3 exhibited greater beta

power bilaterally in the frontal and central regions compared to parietal and occipital regions in B1 (all $p < 0.05$), B4 (all $p < 0.05$), and B5 (all $p < 0.02$).

Pairwise comparisons revealed that beta power increased with learning in TY in the left frontal region ($p = 0.009$) and approached significance with an increase in B5 compared to B4 ($p = 0.07$). The TA clusters did not exhibit any differences between blocks.

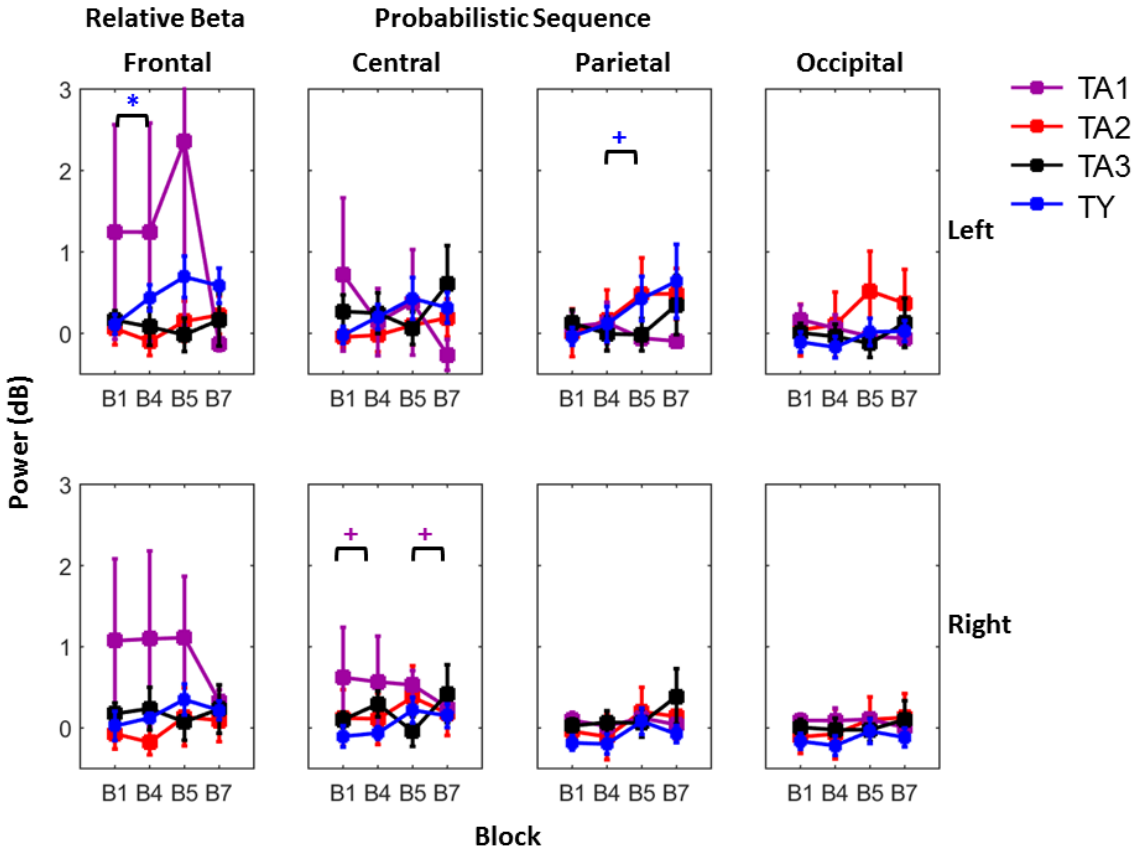

Figure 4.14: Relative beta power for B1, B4, B5, and B7 for the probabilistic sequence groups.

Coherence analysis

Fixed sequences

Alpha. A four-way mixed factorial (4 x 5 x 2 x 4) ANOVA on Group (TY, TA1, TA2, TA3) x Region (Fz pairing with each of the following: Frontal, Central, Temporal, Parietal, Occipital) x Hemisphere (Left, Right) x Block (B1, B4, B5, B7) on the alpha band

with Block as the within subject variable indicated a main effect of Region, $F(4,108) = 134.9$, $p < 0.001$. *Post-hoc* analysis with Bonferroni correction on Region revealed significantly greater cortical connectivity in the frontal region than the other regions (all $p < 0.001$). In addition, there was greater connectivity in the central region than the temporal, parietal, and occipital regions (all $p < 0.001$); greater connectivity in the temporal and parietal regions than occipital region ($p < 0.001$), but no difference between the temporal and parietal regions ($p = 0.8$).

Pairwise comparisons between contrasts that were determined *a priori* revealed significantly greater fronto-temporal connectivity in the left hemisphere in B4 than B1 ($p = 0.001$) and greater frontal connectivity in the right hemisphere in B5 than B7 ($p = 0.03$) in TY. In TA2, there was greater fronto-temporal connectivity in the right hemisphere in B4 than B1 ($p = 0.05$). No differences were found in TA1 or TA3.

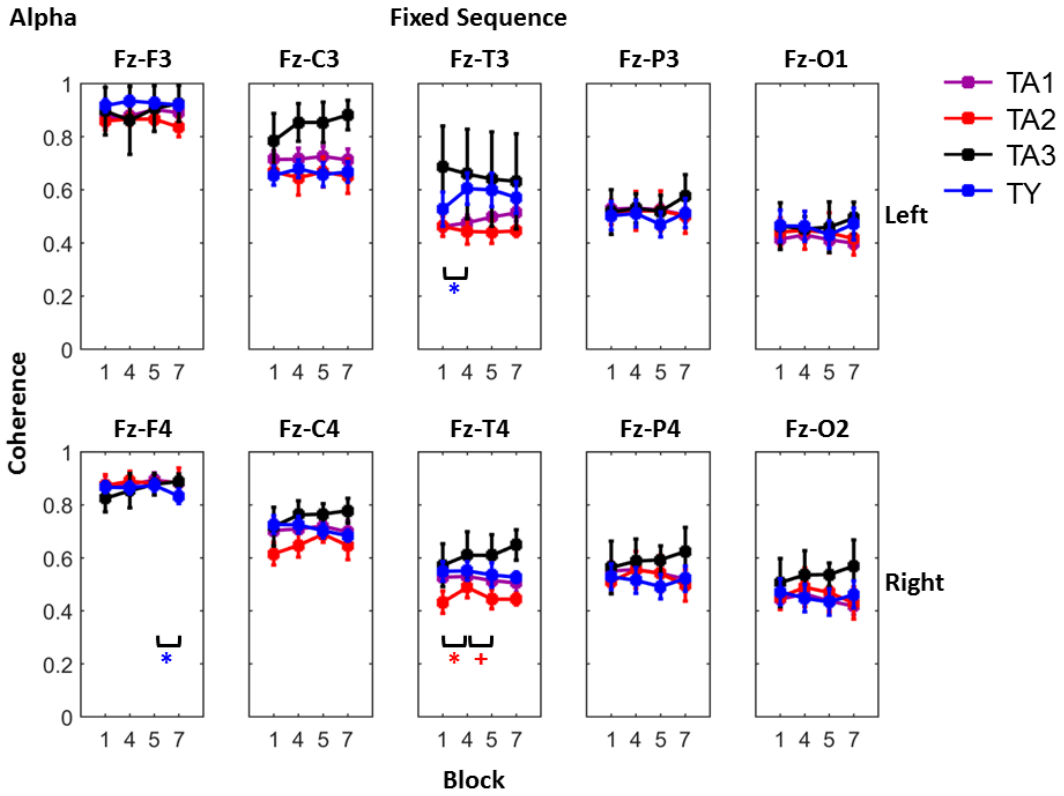

Figure 4.15: Coherence in the alpha band for B1, B4, B5, and B7 for the fixed sequence groups.

Beta. In the beta band, there was also a main effect of Region, $F(4,108) = 119.5$, $p < 0.001$ that followed the same patterns as found in the alpha band.

Pairwise comparisons found significantly greater left fronto-temporal connectivity in B4 compared to B1 ($p = 0.04$) in TY and greater right fronto-central connectivity in B5 than B7 approaching significance ($p = 0.06$) in TA1. In TA2, there was greater right fronto-temporal connectivity in B4 than B1 ($p = 0.03$) and B5 than B4 ($p = 0.005$), greater fronto-parietal connectivity in B5 than B7 in both the left ($p = 0.04$) and right ($p = 0.05$) hemispheres and greater fronto-occipital connectivity in B5 than B7 approaching significance in both the left ($p = 0.06$) and right ($p = 0.07$) hemispheres. In TA3, right fronto-central connectivity was greater in B5 than B7 ($p = 0.01$).

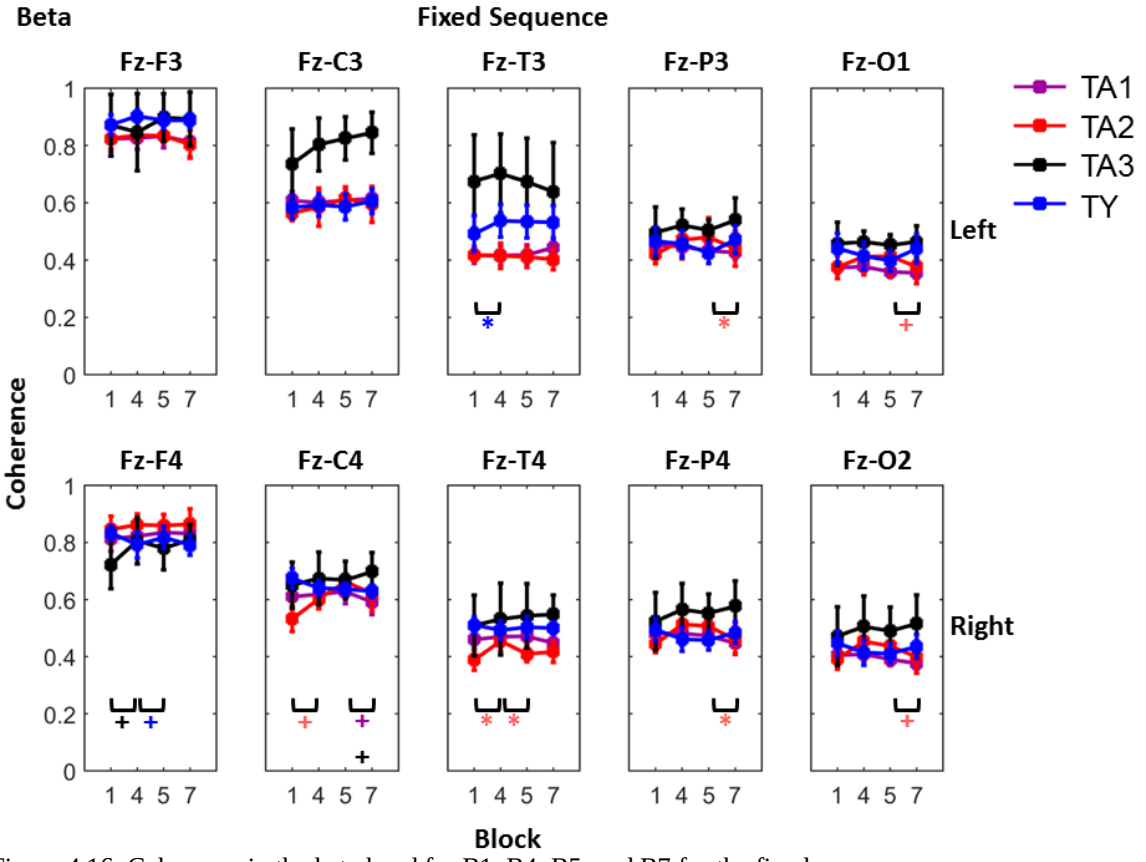

Figure 4.16: Coherence in the beta band for B1, B4, B5, and B7 for the fixed sequence groups.

Probabilistic sequences

Alpha. There was a main effect of Region, $F(4,104) = 98.6$, $p < 0.001$ and Hemisphere, $F(1,26) = 6.52$, $p = 0.02$, as well as a significant interaction between Region x Hemisphere, $F(4,104) = 2.55$, $p = 0.04$. Simple effects analysis at the hemisphere level revealed greater connectivity in the right hemisphere in the temporal ($p = 0.01$), parietal ($p = 0.05$), and occipital ($p = 0.002$) regions.

Pairwise comparisons between contrasts of interest that were determined *a priori* revealed marginally greater connectivity in B1 than B4 in left parietal ($p = 0.07$) and left occipital ($p = 0.08$) areas in the TY group. In addition, there was marginally greater connectivity in B4 than B5 in the right temporal area ($p = 0.08$). In TA1, only B1 revealed significantly greater connectivity than B4 in the left occipital area ($p = 0.004$). There was significantly greater connectivity in B4 than B5 in the left frontal area in TA2 ($p = 0.02$). In TA3, there was marginally greater connectivity in B1 than B4 in the left occipital area ($p = 0.06$), as well as significantly greater connectivity in B7 than B5 in the right parietal area ($p = 0.03$) and marginally in the right central area ($p = 0.07$).

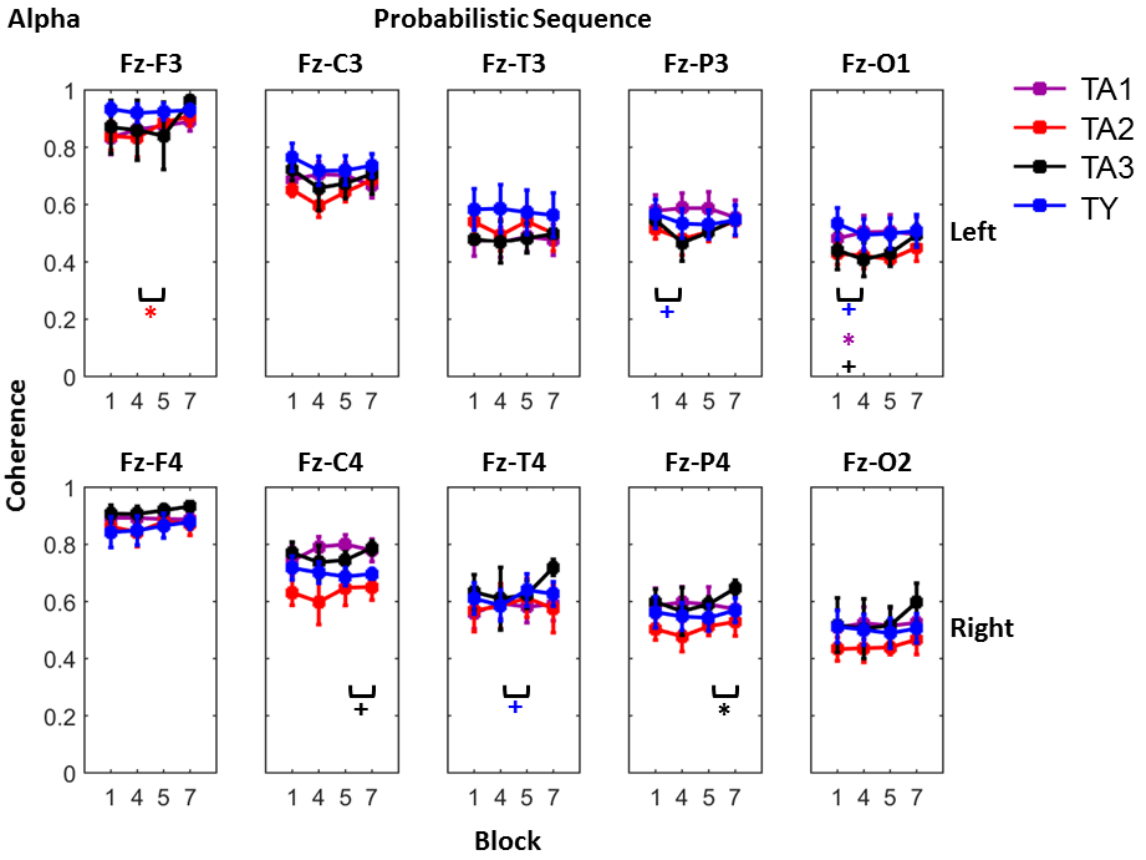

Figure 4.17: Coherence in the alpha band for B1, B4, B5, and B7 for the probabilistic sequence groups.

Beta. In the beta band, there was a main effect of Region, $F(4,104) = 99.0$, $p < 0.001$ and Hemisphere, $F(1,26) = 6.63$, $p = 0.02$ that followed the same patterns as those in the alpha band, as well as significant interactions between Region x Block, $F(12,312) = 2.15$, $p = 0.01$, and Region x Hemisphere x Block, $F(12,12) = 2.40$, $p = 0.006$. Simple main effects analysis revealed greater connectivity in the right hemisphere than the left hemisphere in B4, B5, and B7 in the central (all $p < 0.05$), parietal (all $p < 0.01$), and occipital (all $p < 0.04$) regions. Additionally, there was significantly greater connectivity in B5 than B4 ($p = 0.05$) and B7 than B5 ($p = 0.005$) in the left frontal area.

Pairwise comparisons found no significant differences for TY. There was significantly greater right frontal connectivity in B5 than B7 in TA1 ($p = 0.01$), greater

connectivity left frontal in B5 than B4 in TA2 (p = 0.004), and marginally greater left fronto-occipital connectivity in B4 than B5 in TA3 (p = 0.08).

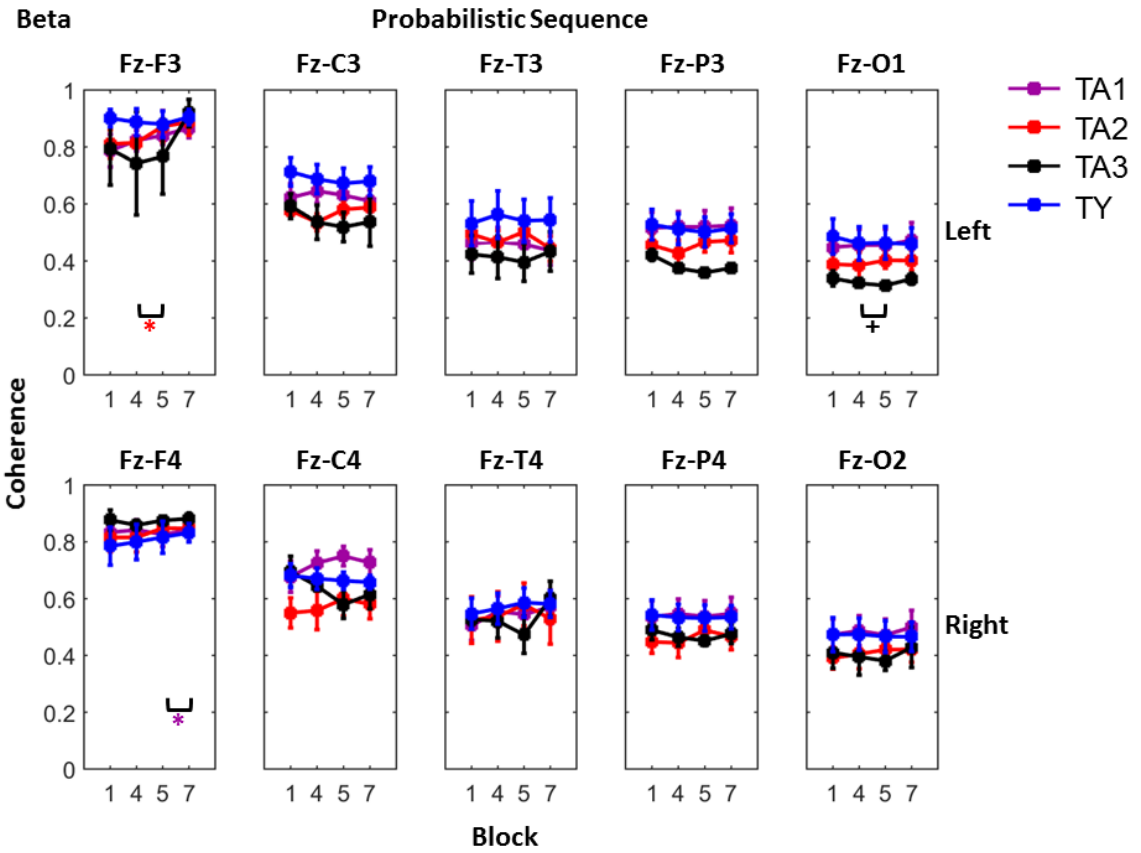

Figure 4.18: Coherence in the beta band for B1, B4, B5, and B7 for the probabilistic sequence groups.

Posttest

The posttest required participants to rate their confidence on a scale of 1-5 whether they had seen the presented chunk in any of the blocks. Some of the chunks presented to participants were from the assigned sequence and some were random chunks. Participants were also shown entire sequences, one of which was their assigned sequence.

Fixed sequence. In TY, there was a significant difference between the rating for the chunks from the sequence and random chunks (p = 0.007), however when they were shown entire sequences, there was no difference between their rating of their assigned sequence

and other sequences (p = 0.1). In TA1, there was a marginally significant difference between the chunks (p = 0.06) and no difference between entire sequences (p = 0.3). Surprisingly, there was a significant difference in TA2 for chunks (p = 0.05), even though they did not exhibit learning of the sequence, but not for the entire sequence (p = 0.4). There was no difference for chunk or sequence in TA3 (p > 0.1).

Probabilistic sequence. There was no difference between the chunks or sequences in any of the groups (all p > 0.05), indicating that participants in the probabilistic group were unable to recognize the chunks or their assigned sequence.

Discussion

In this study, we have demonstrated that typically aging adults are unable to learn a probabilistic motor sequence. We used cluster analysis to separate the typically aging adults into functional groups and found an inverse relationship between mean reaction time and working memory. These clusters elucidated that while some typically aging adults learned the fixed sequence, none learned the probabilistic sequence. This is consistent with previous studies that despite reduced working memory compared to young adults, performance can be maintained to a certain level (Bo, et al., 2012).

Decomposing response time

As expected, both response and reaction times indicated that young adults were faster than typically aging adults. Young adults and some aging adults learned the fixed sequence, but only the young adults learned the probabilistic sequence. This finding is consistent with our previous study in that young adults were able to learn probabilistic sequences within the SRT framework (Study 2 of this dissertation). The results are also

consistent with previous studies that indicate that typically aging adults are able to learn simple sequences (Bennett, et al., 2007; Bhakuni & Mutha, 2015; Bo, et al., 2011, 2012; Daselaar, et al., 2003; Dennis, et al., 2006; Feeney, et al., 2002; Fraser, et al., 2009; Gaillard, Destrebecqz, Michiels, & Cleeremans, 2009; D. V. Howard, et al., 2004; J. H. Howard & Howard, 2013; King, et al., 2013; Lin, Wu, Udompholkul, & Knowlton, 2010; Seidler, 2007; Wu & Hallett, 2005), but not complex probabilistic sequences (Dennis, et al., 2006; D. V. Howard, et al., 2004; J. H. Howard & Howard, 1997, 2013). Importantly, the results highlight a potential factor contributing to the inconsistencies in the literature. Most paradigms do not distinguish between response time and reaction time since participants' fingers are placed on the buttons themselves and are pressed after the presentation of a stimulus. The modified SRT task used in this study required participants to place their right index finger on the home button and move to a different button that corresponded to the location of the stimulus. In this way, we were able to record reaction time and movement time separately. The movement time remained constant for all groups except the typically aging adults learning the probabilistic sequence, suggesting that for more complex sequences being presented to aging or clinical populations, movement time may be an important variable to record and analyze (Moisello, et al., 2009), but is often overlooked in SRT studies. Thus, the decomposition of response time into reaction and movement time is critical and provides more nuanced insights into the differential effect of sequence structure on reaction and movement time as well as age- and disease-related impairments.

Characterizing the developmental landscape of 55-75 year old adults

Previous studies investigating the effects of aging on motor sequence learning have combined older adults into one group, despite large age ranges, to compare with young adults or age-matched with clinical populations. It is important to characterize the developmental landscape of aging because adults in different age groups most likely perform at different levels, i.e., a 55-year-old may not perform at the same level as a 75-year-old.

Here, we found that one cluster of typically aging adults learned the fixed sequence, but were unable to learn the probabilistic sequence. Previous studies have found inconsistent results where some have found that typically aging adults are impaired at learning higher-order sequences (Dennis, et al., 2006; Feeney, et al., 2002; J. H. Howard & Howard, 1997, 2013), while others have found that they are not (Bhakuni & Mutha, 2015; D. V. Howard & Howard, 2001; Simon, Howard, & Howard, 2010). The *k*-means cluster analysis separated the typically aging adults into three clusters (TA1, TA2, and TA3). These clusters were not significantly different in age, but had significantly different overall mean reaction times and *n*-back scores. Specifically, TA1 had the highest *n*-back score and the fastest reaction time, while TA3 had the lowest *n*-back score and the slowest reaction time. Interestingly, we found that the TA1 cluster was able to learn the fixed sequence, but not the probabilistic sequence. The TA2 and TA3 clusters were impaired at learning both fixed and probabilistic sequences. These results further suggest that working memory plays an important role in motor sequence acquisition. This is consistent with previous studies that have found that visuospatial working memory capacity is related to both explicit (Bo, et al., 2009) and implicit (Bo, et al., 2011, 2012; Seidler, et al., 2012)

motor sequence learning. In addition studies have found that while a typically aging adults exhibit reduced working memory (Li et al., 2008), their performance levels are similar to those of young adults (Bo, et al., 2012). Since the TA2 and TA3 clusters were unable to learn the fixed sequence and none of the of the TA clusters were able to learn the probabilistic sequence, there may be a threshold at which performance may be maintained even with reduced working memory that the probabilistic sequences have surpassed because of their complexity.

These results suggest that typically aging group samples should be more tightly controlled based on functional characteristics to assess differences and impairments within this large age range and variability found in typically aging adults. These characteristics may be different based on the task requirements. Additionally, rather than age-matching clinical populations with control groups, it is important to characterize the clinical population and match controls according to these functional characteristics in order to attain a clearer understanding of impairments related to aging and/or disease. Statistical methods, such as cluster analysis, have the potential to offer a clearer understanding of age-related differences where task and individual variability can greatly confound conclusions.

Distinct cortical activations may indicate learning

The young adults and the TA1 cluster in the fixed sequence groups exhibited less alpha power than the TA2 cluster in B4. Since both these groups learned the sequence, they were expected to exhibit attenuated alpha power, suggesting that learning can be inferred not only from behavior, but also from cortical activations. Across the groups, B4 exhibited greater beta power than B1 in the right central region and B7 exhibited greater beta power

than B5 in the left central and bilateral frontal regions, suggesting that these activations reflect both learning and transfer of learning.

The young adults learning the fixed sequences exhibited greater relative alpha in the first learning block (B1) and which was reduced significantly in B4 (last learning block) bilaterally in the frontal, central, parietal, and occipital areas, consistent with learning the sequence. Similar bilateral activity has been seen in previous studies in young adults learning a fixed sequence (Poldrack et al., 2005). B4 also exhibited greater cortico-cortical connectivity than B1 in the left temporal area. Surprisingly, beta power also decreased from B1 to B4, in the right central and bilateral parietal and occipital areas, but exhibited greater cortico-cortical connectivity in B4 in the left temporal area. Alpha power increased when presented with stimuli in a random order (B5) bilaterally in the frontal region and left parietal region and beta power also increased in left parietal and bilateral occipital areas. No differences were exhibited in the transfer block (B7) in alpha or beta power, but B5 exhibited greater connectivity in the right frontal area than B7 in the alpha band, suggesting greater task-related frontal activation for B7 related to learning of a novel sequence. For young adults learning the probabilistic sequence, differences were only seen in B5 in the right central region and bilaterally in the parietal and occipital areas for alpha and in the left frontal and parietal regions in the beta band, indicating fewer distinct cortical activations while learning a probabilistic sequence.

The typically aging adults in cluster TA1 learning a fixed sequence exhibited greater power in B5 than B7 in the alpha band in the left frontal and central areas that may reflect working memory access from the prefrontal cortex. The beta band exhibited the opposite, with greater power in B7 than B5 bilaterally in the frontal region and in the left

central region, but exhibited greater connectivity in B5 than B7 in the right central area, which together reflect the use of the right hand to perform the task. Since TA1 was able to successfully transfer their learning of the fixed sequence, it was expected that alpha power decreased and beta power increased when a novel sequence was learned, particularly in the frontal and central areas. No differences were exhibited in alpha power when learning a probabilistic sequence, but B4 exhibited greater cortico-cortical connectivity than B1 in the left occipital region. In addition, greater beta power was exhibited in B4 compared to B1 and B7 compared to B5 in the right central region. Thus, distinct cortical activations, such as attenuated alpha and increased beta in the frontal and central regions may reflect both learning and transfer of learning.

Cortical activations may indicate learning before it is reflected behaviorally

Interestingly, in the fixed sequence groups, TA3 exhibited similarly attenuated alpha power as the young adults and TA1 cluster and significantly less than TA2. This suggests that TA3 may be attempting to learn the sequence, or at least forming the visuo-spatial relationships between the locations on the screen and the physical buttons, but may require more learning blocks in order to learn the sequence. It may also be that since the difference between the two clusters was found in the parietal and occipital areas, they were attempting to integrate the visuo-spatial aspects of the task, which is consistent with previous studies indicating that greater parietal, temporal, and occipital activation is associated with visuo-spatial perception of a sequence (Poldrack, et al., 2005; Seidler, et al., 2005), particularly one that has a high spatial mapping between stimulus and response such as the modified SRT task used here.

Although the TA1 cluster did not exhibit learning of the probabilistic sequence behaviorally, the increase in beta power through the learning blocks and in the transfer blocks suggests that perhaps with more learning blocks, the typically aging adults may be able to learn the sequence. No differences were found in the other two typically aging clusters, consistent with their behavioral data. However, cluster TA2 did exhibit greater cortico-cortical connectivity in the alpha band in B4 compared to B1 and in B4 than B5 in the right temporal region, as well as greater connectivity in B4 than B1 in the beta band in the right central and temporal areas and greater connectivity in B5 than B7 bilaterally in the parietal and occipital areas. This suggests that participants in this group were attempting to integrate the spatial and visual aspects of the task, but were unable to learn the sequence. These reduced cortical activations have been shown in previous studies (Aizenstein, et al., 2006; D'Esposito, et al., 1999; King, et al., 2013) and is often accompanied by an increased activation in the striatum (Rieckmann, Fischer, & Backman, 2010). However, some studies have shown no differences between young and typically aging adults (Daselaar, et al., 2003), although in other studies, typically aging adults required more practice to achieve similar performance levels as young adults (Wu & Hallett, 2005).

Impaired learning may be reflected by greater alpha power

The TA2 cluster in the fixed sequence groups exhibited greater alpha power than the young adults, TA1 cluster, and TA3 cluster in B4 as well as greater alpha power in the frontal area than the parietal and occipital areas, indicating an impairment in learning the fixed sequence. Similarly, in the probabilistic sequence groups, TA3 exhibited greater alpha power than TY, TA1, and TA2 in the left frontal and central regions and greater alpha power than TY in the right frontal region. Since both the TA2 in the fixed sequence groups

and TA3 in the probabilistic sequence groups exhibited greater alpha power than the other groups and exhibited impaired learning of their respective sequences and previous studies have suggested that increased alpha is an indication of impaired cognitive performance (Bonstrup, et al., 2015; Klimesch, 1999).

Conclusion and limitations

We have demonstrated that the learning of fixed sequences is spared in some typically aging adults, but not in others. We suspect this difference is due, in part, to working memory capacity. We also demonstrated that typically aging adults are unable to learn probabilistic sequences. We created a developmental landscape to better understand the role of aging in motor sequence learning and propose that these methods can provide a clearer understanding of disease-related impairments in older adults. We also found distinct cortical activations reflecting both learning and transfer of learning even in the absence of behavioral indications of learning, suggesting that some adults may require more learning blocks to exhibit a decrease in reaction time.

The differences exhibited in some groups are consistent with previous neuroimaging studies suggesting that greater parietal, temporal, and occipital activation is associated with visuo-spatial perception of a sequence (Poldrack, et al., 2005; Seidler, et al., 2005) and involvement of the prefrontal cortex in working memory (Braver & Barch, 2002; Braver, et al., 2001) and particularly the importance of working memory while learning a sequence (Bo, et al., 2009; Bo, et al., 2011, 2012). Activation in the motor and temporal areas is related to detection and encoding of the pattern (Seidler, et al., 2005). Activity in the parietal and occipital regions is also unsurprising, given the importance of visuo-spatial integration in the task and the importance of encoding spatial locations. In

addition, the motor cortex has been implicated in early learning as well as consolidation (Muellbacher et al., 2002). However, studies have also found no changes between pre- and post-training blocks or during sequenced and random blocks (Poldrack, et al., 2005). It is important to remember that EEG provides excellent temporal resolution and the effects seen here are immediate, whereas the effects seen in studies using fMRI are more downstream.

An important consideration is that due to changes in alpha frequency in aging and neurological disorders, the use of fixed frequency bands may not be ideal. An alternative is to define each participant's alpha band by determining the dominant frequency that attenuates during a task and using this personalized narrow band to calculate alpha power (Klimesch, 1999). In typically aging participants, who have lower peak alpha, calculating alpha using fixed ranges may omit a portion of their real alpha power. Conversely, alpha power may be contaminated by theta, which increases during engagement in a cognitive task, thus canceling out any changes in alpha. Separating the alpha band to low and high alpha may also provide greater insight into differences in the learning process as well as between groups.

The participants that were unable to behaviorally exhibit learning, but displayed cortical activations that indicating learning, such as the TA1 cluster in the probabilistic sequence group, may be able to learn less complex probabilistic sequences. Probabilistic sequences created using a first-order transitional probabilities can be manipulated to change the level of complexity. Future studies can gradually change the complexity of the sequence to determine whether these adults can learn simpler probabilistic sequences and if so, whether they can learn more complex sequences if the complexity level is increased

gradually. This may be a potential intervention for impairments in motor sequence learning.

Consistent with our previous study (Study 1 of this dissertation), the posttest suggests that participants who were assigned probabilistic sequences were not able to differentiate between their assigned sequences, or chunks from their assigned sequence, and other random sequences/chunks, but participants assigned to fixed sequences were able to differentiate between their sequence/chunks and other random sequences/chunks. Thus, the probabilistic structure is more likely to ensure implicit sequence learning and prevent contamination of the implicit motor sequence-learning paradigm by explicit learning. A surprising finding was that the TA2 cluster in the fixed sequence groups were able to differentiate between chunks from their assigned sequence and those that were not, further bolstering evidence from the alpha power that even though this cluster did not exhibit learning via reaction time, perhaps with more learning blocks, they would have been able to do so.

The separation of individuals using cluster analysis has the potential to have profound effects on the way aging studies are conducted as well as how controls are matched with clinical populations. Statistical analyses are critical in order to gain a clearer understanding of the complicated processes underlying aging- and disease-related effects on cognition and motor learning.

Chapter 5 (Study 3): Patients with Parkinson's disease and typically aging adults with similar age-related impairments are comparable in motor sequence learning[3]

Introduction

Parkinson's disease (PD) is a neurodegenerative movement disorder that affects over one million individuals in the US with approximately 60,000 new diagnoses every year. PD has an estimated 4% diagnosis rate before the age of 50 years and is a common disorder in adults over the age of 80 years (National Institute of Neurological Disorders and Stroke, 2015; Parkinson's Disease Foundation, 2015). The incidence of PD will likely increase as a larger proportion of the population ages and life expectancies continue to increase. PD has been characterized as a movement disorder, owing primarily to the movement impairments that are associated with the disease. However, the effect of PD on learning new motor skills has demonstrated equivocal results.

One of the most commonly studied motor learning tasks is the learning of a motor sequence. Motor sequence learning is fundamental to performing complex motor behaviors that emerge from simpler movements produced in a particular order. From brushing our teeth, getting out of a car, typing on the computer keyboard, and speaking, our actions follow a sequence of movements performed in a specific order. Given the importance of this motor capacity on motor learning and quality of life, it is important to characterize the influence of Parkinson's disease on motor sequence learning to better understand the cognitive and motor deficits and develop interventions.

[3] This study will be submitted upon revision for publication with the following authors: Prashad, S., Du, Y, & Clark, J. E.

The most commonly used paradigm to assess motor sequence learning is the serial reaction time (SRT) task (Nissen & Bullemer, 1987). In this task, participants respond to the location of a stimulus on a computer screen by pressing the corresponding button as quickly and accurately as possible. Participants are unaware that the stimuli are presented in a pre-determined repeating sequence. Learning is inferred from a progressive reduction in the reaction time during the learning blocks (Nissen & Bullemer, 1987; Robertson, 2007) and an increase in reaction time to stimuli that occur in a random order. A repeating fixed sequence is most commonly used in the SRT paradigm, but obviously is not an accurate reflection of learning in daily life, in which our motor behavior is dependent on statistical associations between events that are often made unconsciously and vary in dynamic task and environmental constraints (Cleeremans, et al., 1989; A. S. Reber, 1989b).

We have shown previously, in Study 1 of this dissertation that typical young adults are able to learn probabilistic sequences created using first-order transitional probabilities within the SRT framework. These transitional probabilities define statistical associations between each pair of states and subsequent states change based on these associations. After many trials, the participants unconsciously learned the probabilistic rules underlying the sequence (e.g., 4 is most likely to be followed by 2) and exhibited a decreased reaction time. In Study 2 of this dissertation, we found that typically aging adults were unable to learn these probabilistic sequences and did not exhibit a decrease in reaction time. Sequences with this unique structure have not been used in other studies and it is unclear whether patients with PD would be able to learn these complex sequences. In addition, it remains unclear whether patients with PD are impaired at learning fixed sequences.

Studies investigating impairments in motor sequence learning in patients with PD have found equivocal results possibly due to differences in methodologies, sequence types, disease severity, and effect of dopaminergic medications. Despite these differences, the general conclusion is that implicit motor sequence learning is impaired in patients with PD (Fukuda, et al., 2001; Gamble, et al., 2014; Jackson, et al., 1995; Siegert, et al., 2006; Wilkinson & Jahanshahi, 2007; Wilkinson, et al., 2009) including SRT tasks without the motor component (Westwater, et al., 1998), and when attempting to learn more complex sequences (Shin & Ivry, 2003; Smith & McDowall, 2006). It is thought that as in typically aging adults, these impairments are, at least partly, due to a reduced working memory (Braver & Barch, 2002; Braver, et al., 2001; Cools, 2011; Cools & D'Esposito, 2006; Gabrieli, et al., 1996; Owen, et al., 1998). It has also been found that there is a trend towards degradation in performance and neural activity in PD as the disease progresses (Carbon, et al., 2010) and thus impairment may be a function of disease severity where those in the early stages of PD are relatively spared from impairment (Muslimovic, et al., 2007; Stephan, et al., 2011). Results are further confounded by differential effects of dopamine on learning and activation of cortical regions (Argyelan, et al., 2008; Cools, 2011; Cools & D'Esposito, 2006; Feigin, et al., 2003; Kwak, et al., 2010, 2012; Seo, et al., 2010; Tremblay, et al., 2010) and surgical interventions through deep brain stimulation (Carbon & Eidelberg, 2006; Mure, et al., 2012).

Other studies, however, have reported no impairments in the SRT task and artificial grammar (Helmuth, et al., 2000; Nagy, et al., 2007; P. J. Reber & Squire, 1999a; Smith, et al., 2001; Wilkinson & Jahanshahi, 2007) as long as patients with PD are provided more time to learn, which may be a result of compensation. To investigate possible compensatory

mechanisms, Mentis et al. (2003) conducted a PET study in which early stage patients with PD and typically aging adults performed a center out task consisting of a sequence that participants determined through trial and error. To prevent potential confounds from differing levels of performance, the patients with PD and control participants were matched based on performance level. Over time, the patients with PD were able to perform at a level similar to that of typically aging adults, but the PET results indicated that patients with PD exhibited four times greater activation of the cerebellum to reach the same level of performance as typically aging adults (Mentis, et al., 2003). This suggests that in certain conditions (e.g., short fixed sequences) and given enough time, early stage patients with PD can achieve greater performance levels through a compensation mechanism via the cortico-cerebellar system. PD have also exhibited increasingly greater activation in premotor cortex, parietal cortex, and SMA while performing more complex sequential finger movements suggesting that patients with PD compensate for degradations in cortico-striatal circuits by engaging more cortical regions (Catalan, et al., 1999; Fukuda, et al., 2001; Nakamura, et al., 2001).

Most of the neuroimaging studies investigating implicit motor sequence learning have been conducted using functional MRI as it affords excellent spatial resolution; however, it provides poor temporal resolution. Given that reaction time, measured in milliseconds, is the variable used to infer learning, we propose that electroencephalography (EEG), which provides excellent temporal resolution, is better suited to identify cortical activations and cortico-cortical connectivity associated with learning and impairments.

An additional source of variability is from matching control participants with patients based on age. In our previous study (Study 2 of this dissertation), we demonstrated

that grouping participants based on functional characteristics, instead of age, results in a clearer understanding of deficits in typically aging adults and differences among the groups. We propose that it may prove beneficial to compare patients with PD to those functional groups in order to understand the deficits in PD. In this study, we compare the patients with PD with the typically aging adults in two ways: 1) by selecting a subset of typically aging adults that are age-matched with the patients, and 2) by comparing the patients with the functional groups created in Study 2. Furthermore, we decomposed response time to reaction and movement times to unravel movement and cognitive deficits in patients with PD.

Thus, the purpose of this study is to directly compare the learning of fixed and probabilistic sequences in a modified SRT task by patients with PD by comparing them to the developmental landscape and functional groups of typically aging adults characterized in Study 2 of this dissertation. Studying PD provides a unique opportunity to gain an understanding of the influence of an impaired cortico-striatal circuit on motor sequence learning and allows for the expansion of our understanding of neural underpinnings of motor sequence learning and the nature of impairment in Parkinson's disease to develop novel strategies for interventions.

Methods

Participants

Forty-two typically aging adults (TA; mean age: 64.7 ± 7.36), and 10 patients with Parkinson's disease (PD; mean age: 64.5 ± 5.15) were randomly assigned to either a fixed (FX) or a probabilistic (PB) sequence. All participants completed the Global Physical

Activity Questionnaire (Armstrong & Bull, 2006), a spatial version of the *n*-back test to assess working memory (Jaeggi, et al., 2008), the Wisconsin Card Sorting Test to assess set-shifting (Grant & Berg, 1948; Mueller, 2010), and a computer skills questionnaire to assess familiarity with the number pad on the computer keyboard. Participants were also screened for neurological and motor impairments through a health questionnaire and the Mini Mental State Exam (Folstein, et al., 1975) to screen for cognitive impairments. Additionally, motor impairments in patients with PD were assessed via the motor section of the updated Movement Disorder Society-sponsored Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (Goetz et al., 2007; Goetz et al., 2008) and the Hoehn & Yahr scale (Hoehn & Yahr, 1967). A subset of the typically aging adults (TA-age) was randomly selected based on age in order to provide an age-matched control group for the patients with PD (see Table 5.1). All participants were right-handed.

Serial reaction time task

Participants were seated in front of a computer monitor (21") and keyboard (keys size 13x15mm, keys are 6mm apart vertically and horizontally and 8mm apart diagonally). A modified SRT task was used that consisted of nine white squares in a 3x3 matrix on the computer screen (37x37mm each). Participants placed the index finger of their right hand on the center button on the number pad of the keyboard. The relationship between the squares on the screen and the buttons on the number pad was spatially compatible, i.e., the top right square corresponded to the top right button. At the beginning of each trial, one of the eight squares turned blue and the participant pressed the key that corresponded to the location of the stimulus and then returned to the home position. After the participant pressed a key, a response-to-stimulus interval between 300-1000ms was selected randomly

for each trial to prevent participants from anticipating the appearance of the subsequent stimulus as well as to prevent any confounding effects from the length of the response-to-stimulus interval (Willingham, et al., 1997). No visual feedback was given to participants as a wooden board blocked vision of their finger position (see Figure 5.2).

Participants were randomly assigned to either a fixed (FX) 16-item second order conditional sequence (Reed & Johnson, 1994) or a probabilistic sequence (PB). The probabilistic sequence was created based on a first-order transitional probabilistic structure with underlying probabilities associated with each stimulus, e.g., if stimulus 2 occurs, there will be a 60% probability that the next stimulus will be 6, a 30% probability that the next stimulus will be 8, and a 2% probability that the next stimulus will be 1, 3, 4, 7, or 9. Participants were not informed that a sequence existed regardless of which group they were assigned to. The sequences were constrained such that the same stimulus was not repeated one after the other and that each stimulus appeared an equal number of times in each block (20 times per block).

Table 5.1 Participant information for the age-matched subset from the typically aging adults and those with PD

Sequence Type	Group	Age (Mean and SD)	Sex	Physical Activity Level [#]	2-back (% correct)	Wisconsin Card Sorting Test				MMSE	MDS-UPDRS (III)	Hoehn & Yahr	Years Since Diagnosis
						Correct (%)	Perser-vative Response (%)	Perser-vative Errors (%)	Non-perser-vative Errors (%)				
FX	TA-age	66.3 ± 5.39	8 female; 5 male	5 high; 5 moderate; 3 low	38.6 ± 21.1	70.9 ± 9.35	30.93 ± 15.2	13.7 ± 15.2	15.4 ± 10.3	29.5 ± 0.820	-	-	-
	PD	65.0 ± 3.89	3 female; 2 male	3 high; 1 moderate 1 low	50.5 ± 26.5	75.6 ± 4.62	30.5 ± 4.90	11.72 ± 2.30	12.7 ± 4.34	29.2 ± 1.10	30.4 ± 12.4	1.30 ± 0.273	6.18 ± 5.09
PB	TA-age	64.7 ± 5.97	8 female; 5 male	5 high; 3 moderate; 5 low	38.8 ± 23.2	74.8 ± 10.8	37.3 ± 6.47	16.3 ± 6.54	8.88 ± 5.95	29.9 ± 0.277	-	-	-
	PD	63.6 ± 6.86	3 female; 2 male	3 high; 1 moderate 1 low	40.1 ± 13.8	63.4 ± 11.8	37.2 ± 6.53	22.2 ± 5.78	14.4 ± 6.41	28.6 ± 1.14	26.8 ± 10.6	1.20 ± 0.447	6.00 ± 4.90

[#] Physical activity levels were determined based on the GPAQ calculated from the number of days and amount of time spent engaged in physical activity.
FX – fixed sequence group; PB – probabilistic sequence group; TA-age – age-matched subset from the typically aging adults; PD – patients with PD.

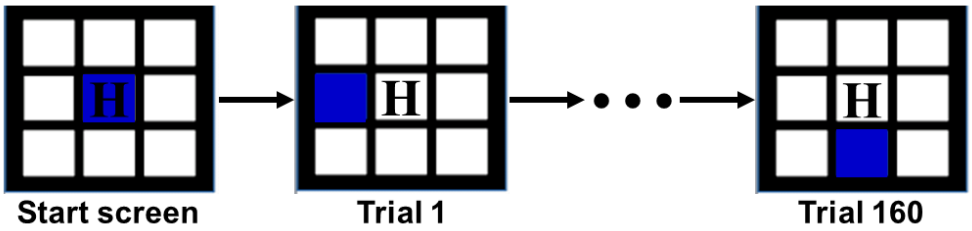

Figure 5.1: The modified serial reaction time (SRT) task. Participants placed their right index finger on the home position (H). On a given trial, one of the 8 locations turned blue and the participant pressed the corresponding button on the number keypad and then returned to the home position.

Figure 5.2: Experimental Setup. Participants were seated in front of a computer monitor with their hand placed on the number keypad. Participants did not receive any visual feedback and could not see their hands. Participants' right hand was wrapped with athletic pre-wrap to prevent the use of the other fingers.

There were a total of eight blocks, each consisting of 160 trials (see Figure 5.3). The first block was a baseline block (B0), consisting of 160 trials in which the stimuli appeared in a random order. The next four blocks (B1-4) were the learning blocks consisting of the fixed or probabilistic sequence in which the sequence was repeated 10 times each. Block 5 (B5) consisted of 160 trials of stimuli occurring in a random order and Block 6 (B6) consisted of 10 repetitions of the assigned sequence. An increase in response time in B5 and decrease in B6 would indicate learning (Robertson, 2007). Lastly, Block 7 (B7) consisted of 10 repetitions of a different sequence that was constructed from the same underlying structure as the learned sequence to assess transfer of learning. If the response times decrease from B5 to B7, it would suggest that participants were able to transfer their

learning. A unique sequence was assigned to each participant to ensure that the results are not intrinsic to the sequence used, but can be generalized to all sequences (DeCoster & O'Mally, 2011a). Participants were given a two-minute mandatory break between each block. The experiment was performed using Presentation® software (Version 18.1, www.neurobs.com).

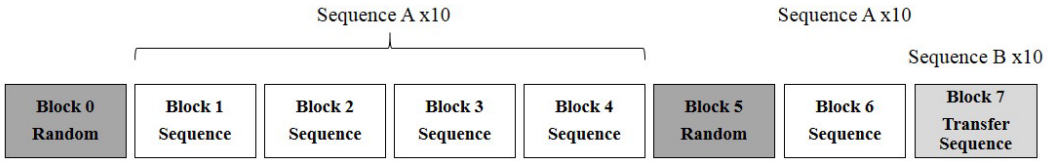

Figure 5.3: The experimental paradigm used for the three groups. All groups started with a baseline (B0), then the fixed and probabilistic groups performed the learning blocks (B1-4) and ended with a random block (B5) followed by another sequence block (B6) and a transfer block (B7). Each block consisted of 160 trials. Participants were given a two-minute break between each block.

The participants' reaction time (RT), movement time (MT), and accuracy were recorded. At the beginning of each trial, participants pressed the home button. The participants' RT (time taken to release the home button after the stimulus was presented), MT (time between release of home button and pressing of the corresponding button), and accuracy were recorded for each trial (see Figure 5.4).

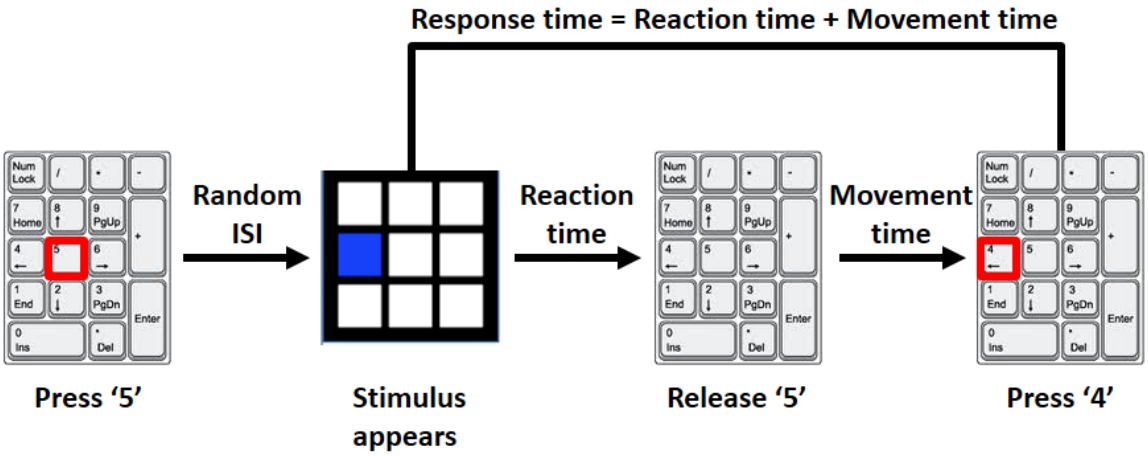

Figure 5.4: Diagram depicting reaction time (RT) and movement time (MT) recordings in the modified SRT task. At the start of a trial, the participant pressed the home button ('5' on the number keypad). After a random interval (300-1000ms), a stimulus appeared on the screen (one of the eight locations turned blue). The RT is the amount of time taken to release the home button and the MT is the time from the release of the home button to the pressing of the button that corresponded to the stimulus. The RT and MT were added to calculate the response times.

Posttest

All participants completed a posttest after the completion of the eight blocks to determine if learning was implicit. First, participants were asked the following question: "The stimulus movement is best described as:" with the following options: "a) Random; b) Some positions occurred more often than others; c) The movement was often predictable; d) The same sequence of movements would often appear; and e) The same sequence of movements occurred throughout the entire experiment" (Curran, 1997).

Second, participants completed a recognition test to assess explicit recall of the sequence (Destrebecqz & Cleeremans, 2001) consisting of two parts: in the first part, participants were presented with six-item chunks from their assigned sequence as well as random chunks and were asked to rate how confident they were that they had seen that chunk before from a scale of 1-5 (where 1 was "Confident that I *have not* seen it before" and 5 was "Confident that I *have* seen it before"). In the second part, participants were

presented with the entire 16-item sequence as well as other random sequences and they were asked to rate them on the same scale.

EEG recording and pre-processing

EEG data were recorded from 64 electrodes mounted on an actiCAP and BrainVision actiCHamp Amplifier (Brain Products, LLC) using the international 10-20 system. The sampling frequency was 1000Hz. The reference electrodes were placed on the left and right mastoids and AFz was the ground electrode. Channel impedances were kept below 10kΩ.

Prior to the SRT task, four resting states were recorded from each participant. Participants were asked to sit as motionless as possible with their eyes open and then with their eyes closed for one minute each. Participants were also asked to view the task as the stimuli appeared but did not respond. Lastly, participants pressed each of the response buttons their right index finger in the clockwise direction at their preferred speed without any visual stimulus.

Behavioral data analysis

The RT and MT were trimmed according to the individual participant's mean and standard deviation. Any values greater or less than 2.5 standard deviations were excluded from the analysis (Ratcliff, 1993; Whelan, 2008). Mean RT and MT were calculated for each block and were averaged across participants in each group. Learning was measured as a decrease in RT from B1 to B4, an increase from B4 to B5 (stimuli in random order) and a decrease from B5 to B6 (stimuli in assigned sequence). Transfer of learning was inferred if there was a significant decrease in RT between B5 and B7 (stimuli in different sequence of same structure as assigned sequence).

EEG data analysis

The EEG data were preprocessed using EEGLAB (Delorme & Makeig, 2004). The data were re-referenced to the average of the mastoid electrodes (M. X. Cohen, 2014). Data were filtered using a FIR low-pass filter (cut off frequency: 55Hz, roll off 24dB/octave) to eliminate electrical noise. Independent component analysis (ICA) was used to remove eye artifacts, such as eye blinks, eye movements, and muscle artifacts.

Spectral power. The data were exported into MATLAB version 8.4 (Mathworks, Natick, MA) after preprocessing. Data were segmented into one-second intervals with respect to the RT (250ms before and 750ms after the RT) and Fast Fourier transforms (FFT) were applied in the alpha (8-12Hz) and beta (13-30Hz) bands for each block. Relative alpha and beta power were standardized to the baseline block (B0) in which the stimuli occurred in a random order. Data from the a subset of electrodes were extracted for the following regions: frontal left (F7, F5, F3, and F1), frontal right (F8, F6, F4, and F2), central left (C5,C3, and C1), central right (C6, C4, and C2), parietal left (P7, P5, P3, and P1), parietal right (P8, P6, P4, and P2), parieto-occipital left (PO7, PO3, and O1), and parieto-occipital right (PO8, PO4, O2).

Coherence. Coherence is a measure of the amount of cortical communication between two electrical sites (ranging from 0 to 1) (Fries, 2005; Nunez, 2000; Srinivasan, et al., 1998). The electrode pairings used here between the Fz electrode, which overlies the premotor region, and frontal, motor, temporal, parietal, and occipital regions. The specific electrode pairings used for the left hemisphere were: Fz-F3, Fz-C3, Fz-T3, Fz-P3, and Fz-O1 and those for the right hemisphere were: Fz-F4, Fz-C4, Fz-T4, Fz-P4, and Fz-O2.

Statistical analysis

Behavioral data. A mixed factorial analysis of variance (ANOVA) was used to compare the differences in average response time, RT, and MT between the Group (TA-age, PD) x Sequence Type (FX, PB) x Block (0-7) with Block as the within subject variable. Bonferroni *post hoc* tests were used to decompose any significant effects. Separate pairwise comparisons were conducted on the contrasts of interest (B1 vs. B4, B4 vs. B5, B5 vs. B6, and B5 vs. B7) to determine whether learning occurred and whether learning was transferred to a novel sequence created using the same underlying structure.

EEG analysis. Separate mixed factorial analyses of variance (ANOVA) were used to compare differences in average spectral power and coherence in the alpha and beta bands between Group (TA1, TA2, TA3, PD) x Region (Frontal, Central, Parietal, Occipital) x Hemisphere (Left, Right) x Block (B1, B4, B5, B7) with Block as the within subject variable. Bonferroni *post hoc* tests were used to decompose any significant effects. Separate pairwise comparisons were conducted on the contrasts of interest (B1 vs. B4, B4 vs. B5, and B5 vs. B7) to determine differences in early, late, and transfer of learning.

Statistical significance was defined at $p < 0.05$. The data were processed using custom scripts written in MATLAB version 8.4 (Mathworks, Natick, MA) and SPSS Statistics 22 (IBM, Armonk, NY).

Results

Accuracy

All groups exhibited high levels of accuracy with 7% or fewer errors. Thus, accuracy cannot be used as a measure of learning in this task and was not analyzed further.

Error rates have also been demonstrated to be low in previous studies (R. M. Brown & Robertson, 2007; Willingham, et al., 1989).

Mean response time

A three-way mixed factorial (2 x 2 x 8) ANOVA on Group (TA-age, PD) x Sequence Type (FX, PB) x Block (0-7) on the response times with Block as the within subject variable indicated a main effect of Block, $F(7,224) = 16.9$, $p < 0.001$ and Group, $F(1,32) = 5.27$, $p = 0.03$. Bonferroni's *post hoc* analysis on Group revealed that TA-age was significantly faster than PD and on Block revealed that the response time was significantly faster in B4 compared to B1 ($p = 0.001$) and B5 ($p = 0.01$).

Fixed sequence. Pairwise comparisons between contrasts that were determined *a priori* revealed that in TA-age, response time was significantly faster in B4 than B1 ($p = 0.002$) and B5 ($p = 0.001$) and significantly slower response time in B5 compared to B6 ($p < 0.001$) and B7 ($p = 0.006$). No differences between blocks were found in PD.

Probabilistic sequence. TA-age exhibited significantly faster response time in B4 than B1 ($p = 0.008$) and significantly slower response time in B5 compared to B7 ($p = 0.006$). Similarly, a significant decrease in B4 compared to B1 ($p = 0.04$) was found in PD.

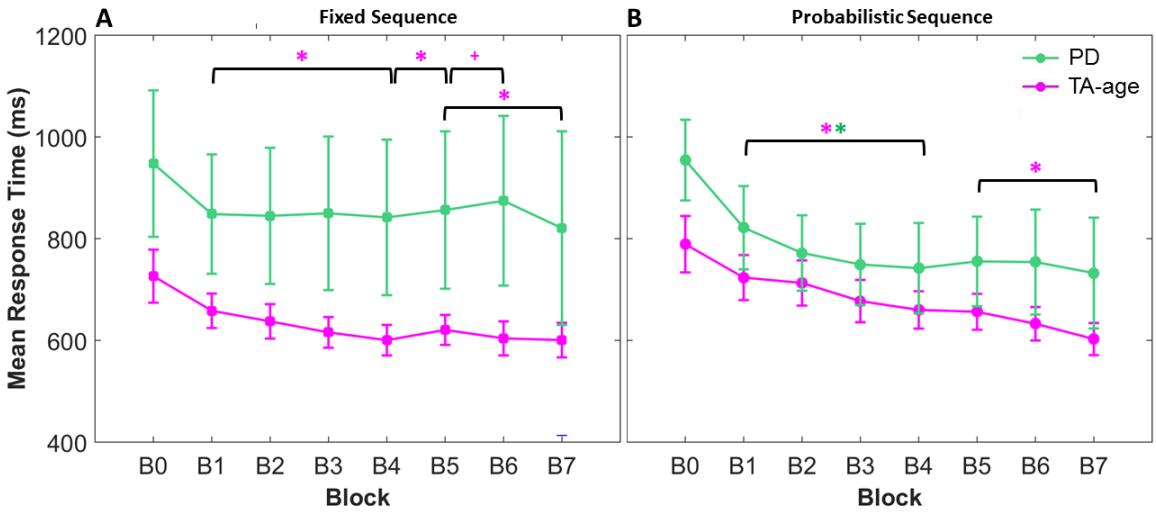

Figure 5.5: Mean response time. (A) TA-age exhibited a decrease in response time from B1 to B4, an increase from B4 to B5, and a decrease from B5 to B6 and B5 to B7. PD exhibited no changes in response time. (B). TA-age exhibited a decrease in response time from B1 to B4 and a decrease from B5 to B7, while PD exhibited a decrease from B1 to B4.
* Indicates significance level of $p < 0.05$; + indicates significance level of $p < 0.10$. Error bars indicate standard error. TA-age – age-matched subset from the typically aging adults; PD – patients with PD

Mean reaction time (RT)

A three-way mixed factorial (2 x 2 x 8) ANOVA on Group (TA-age, PD) x Sequence Type (FX, PB) x Block (0-7) on the RT with Block as the within subject variable indicated only a main effect of Block, $F(7,224) = 35.6$, $p < 0.001$ and no significant interactions. *Post hoc* analysis using a Bonferroni correction on Block indicated that RT was significantly faster in B4 than B1 ($p < 0.001$) and B5 ($p = 0.05$) and significantly slower in B5 than B6 and B7 (both $p = 0.05$). The lack of a main effect of Group indicated that the RT of TA-age was not significantly different from that of PD ($p = 0.4$). This is an important finding because the response time for TA-age was significantly faster than PD, but the RT was not. Thus, response time and RT are not necessarily interchangeable, particularly when testing clinical populations and RT is a more accurate indicator of sequence learning. The equivocal results in the literature may be due to this lack of distinction.

Fixed sequence. In TA-age, B4 exhibited a faster RT than B1 (p = 0.003) and B5 exhibited a slower RT than B4 (p = 0.001), B6 (p < 0.001), and B7 (p = 0.02), indicating both learning and transfer. In PD, B4 exhibited a faster RT than B1 (p = 0.001) and marginally faster RT than B5 (p = 0.08), but no difference between B5 and B7 (p = 0.5).

Probabilistic sequence. There was a marginally significant decrease in RT from B1 to B4 in TA-age (p = 0.08) and a significant decrease from B5 to B7 (p = 0.02). Similarly, in PD, there was a significant decrease from B1 to B4 (p = 0.05).

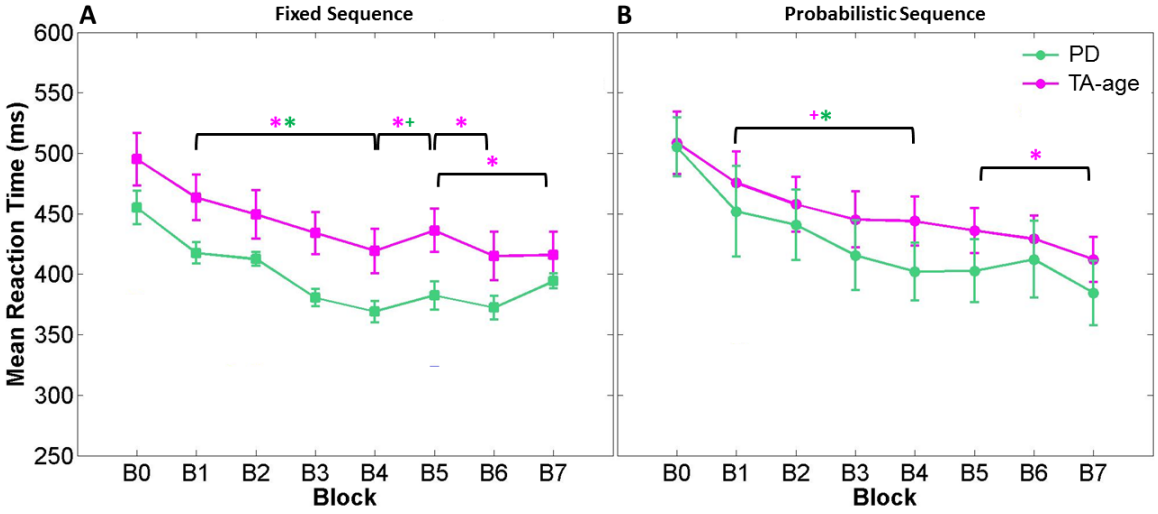

Figure 5.6: Mean reaction time. The reaction time exhibits a more nuanced inference of motor sequence learning. (A) In the fixed sequence groups, both TA-age and PD exhibited a decrease in RT from B1 to B4 and B4 to B5. TA-age exhibited a decrease in RT from B5 to B6 and B5 to B7. (B) In the probabilistic sequence groups, TA-age exhibited a marginal decrease from B1 to B4 and a decrease from B5 to B7, while PD exhibited a decrease from B1 to B4.
* Indicates significance level of p < 0.05; + indicates significance level of p < 0.10. Error bars indicate standard error.

Mean movement time (MT)

A three-way mixed factorial (2 x 2 x 8) ANOVA on Group (TA-age, PD) x Sequence Type (FX, PB) x Block (0-7) on the RT with Block as the within subject variable indicated a main effect of Block, $F(7,224) = 4.31$, p < 0.001 and Group, $F(1,32) = 10.6$, p

= 0.003. A Bonferroni *post hoc* analysis on Group revealed that TA-age exhibited a faster MT than PD, suggesting that MT was driving the differences in response time, since the RT of TA-age and PD were not significantly different.

For both fixed and probabilistic sequence groups, no differences were found in the MT in TA-age or PD.

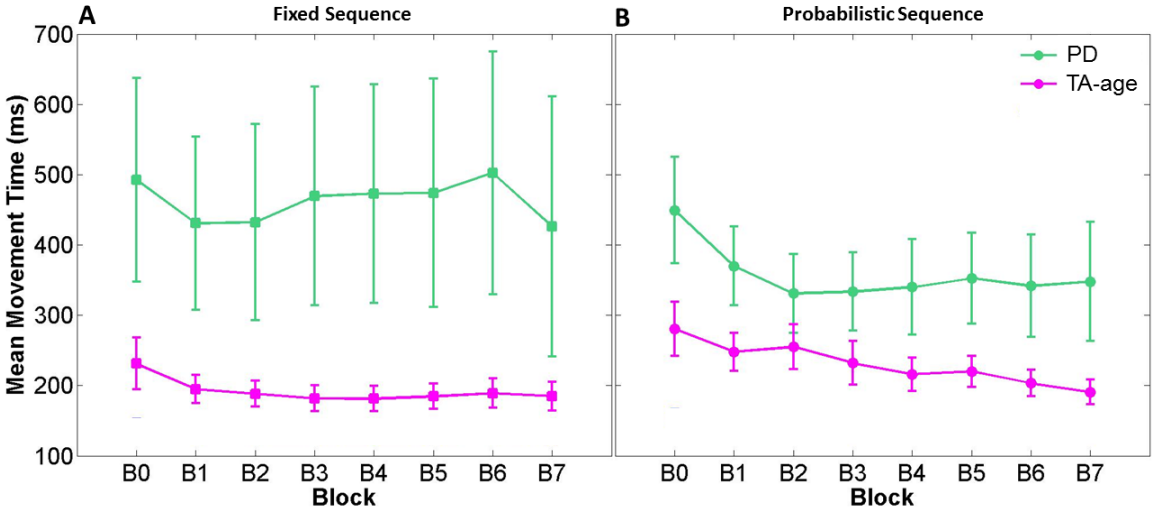

Figure 5.7: Mean movement time. There were no differences between the blocks for any of the groups. Error bars indicate standard error.

The decomposition of response time into RT and MT revealed significant findings; specifically, that the RT of typically aging adults and those with PD are not significantly different, but the MT is significantly different. The inconsistent results found in studies investigating age-related or PD-related impairment in learning may be due to the frequent interchangeable use of response and reaction times. While the results found in this study suggest that patients with PD do decrease their RT during the learning blocks for both FX and PB sequences, they only exhibit learning of the fixed sequence.

Cluster analysis reveals functional groups that can be matched with clinical populations

Cluster analysis is a technique used to structure large complex data sets into relatively homogenous groups without any predetermined criteria (Lloyd, 1982; MacQueen, 1967). In our previous study (Study 2 of this dissertation), we found that applying *k*-means cluster analysis to the RT time series of the typically aging adults separated the adults into three clusters (TA1, TA2, and TA3). We performed cluster analysis again on the typically aging adults, but included the patients with PD and interestingly, the patients with PD were interspersed with the typically aging adults, rather than being classified into their own cluster. Separate one-way ANOVAs revealed no significant differences between the groups for age, $F(3,51) = 1.29$, $p = 0.3$ (see Figure 5.8A), the Wisconsin Card Sort Task, $F(3,51) = 0.41$, $p = 0.7$, or physical activity level, $F(3,51) = 0.22$, $p = 0.9$.

Table 5.2: Demographic information of the different clusters from the typically aging group.

Cluster	Age (Mean ± S.D.)	Sex	Physical Activity Level #	2-back (% correct)	Wisconsin Card Sorting Test				MMSE
					Correct (%)	Perseverative Response (%)	Perseverative Errors (%)	Non-perseverative Errors (%)	
TA1	63.9 ± 1.45	15 female; 8 male	10 high; 6 moderate; 7 low	43.7 ± 3.64	68.9 ± 14.4	29.7 ± 15.6	13.6 ± 9.27	17.5 ± 15.4	29.5 ± 1.03
TA2	63.5 ± 1.15	9 female; 4 male	4 high; 5 moderate; 4 low	33.7 ± 5.84	73.9 ± 12.0	36.0 ± 9.24	16.0 ± 8.34	10.2 ± 6.44	29.7 ± 0.49
TA3	69.7 ± 3.19	3 female; 3 male	1 high; 4 moderate; 1 low	19.4 ± 7.46	69.8 ± 14.4	38.6 ± 11.0	18.9 ± 11.0	11.2 ± 8.42	29.5 ± 0.84

[#] Physical activity levels were determined based on the GPAQ calculated from the number of days and amount of time spent engaged in physical activity.
FX – fixed sequence group; PB – probabilistic sequence group; TA – typically aging adults.

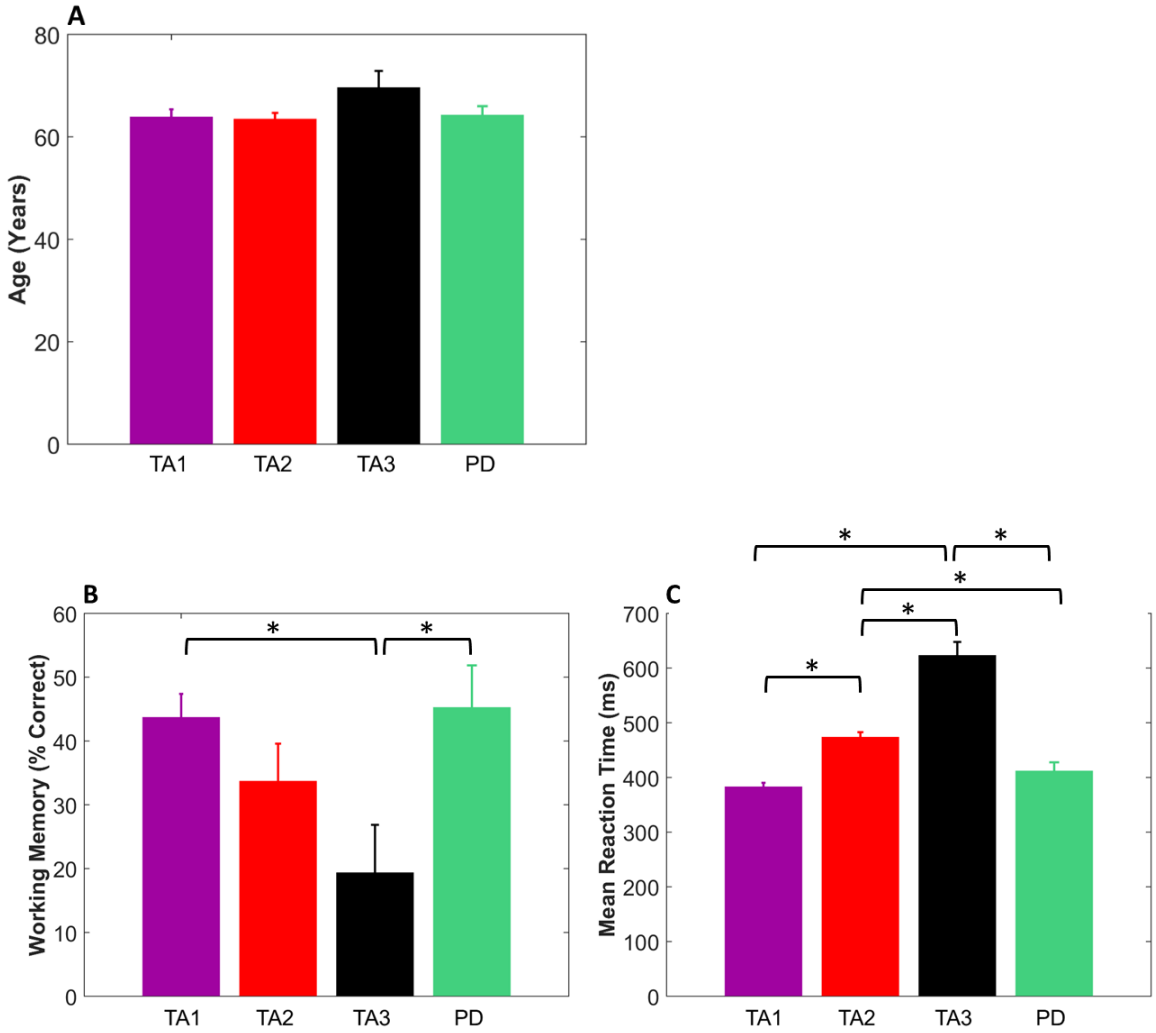

Figure 5.8: Characteristics of the three TA clusters and patients with PD. A) Age was not significantly different between the groups. B) Working memory, as assessed by percent correct in the *n*-back test, was significantly higher in the TA1 cluster and the PD group than the TA3 cluster. C) The overall mean RT of the TA1 cluster and PD group were significantly faster than the TA2 and TA3 clusters.

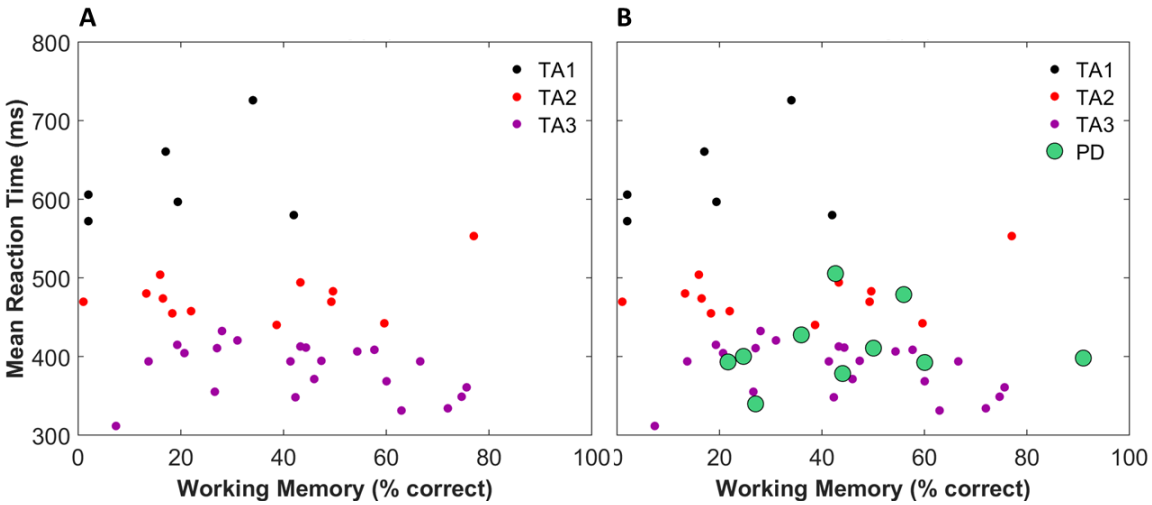

Figure 5.9: Visualization of the three typically aging adult clusters. A) *k*-means cluster analysis revealed three clusters for the typically aging group separated by overall mean reaction time and working memory (from Study 2 of this dissertation). B) The patients with PD were interspersed within the typically aging group, rather than being a cluster of their own.

Mean reaction times of the patients with PD compared to the typically aging clusters

A three-way mixed factorial (4 x 2 x 8) ANOVA on Group (TA1, TA2, TA3, PD) x Sequence Type (FX, PB) x Block (B0-7) on the RT with Block as the within subject variable indicated a main effect of Block, $F(7,308) = 57.0$, $p < 0.001$ and Group, $F(3,44) = 61.9$, $p < 0.001$ and a significant interaction between Block x Sequence x Group, $F(21,208) = 1.89$, $p = 0.01$.

Simple effects analysis indicated that in the fixed sequence group, there was no significant difference in RT between TA1 and PD for all blocks (all $p > 0.5$). Both TA1 and PD were significantly faster than TA2 (both $p < 0.05$) and TA3 (both $p < 0.001$) and TA2 was significantly faster than TA3 ($p < 0.001$) for all blocks. However, in the probabilistic sequence group, there was a different trend. In B1, TA1 was significantly faster than TA2 ($p < 0.001$), TA3 ($p < 0.001$), and PD ($p = 0.04$), but no significant difference was found between TA2 and PD ($p = 0.2$). Both TA2 and PD were faster than TA3 (both $p < 0.001$). However, in B4, B5 and B7, the same trend as the fixed sequence

groups was exhibited: no difference was found between TA1 and PD (p = 0.3), both TA1 and PD were significantly faster than TA2 (both p < 0.009) and TA3 (both p < 0.001), and TA2 was significantly faster than TA3 (p < 0.001).

Fixed sequence. As reported in study 2 of this dissertation, TA1 exhibited a significant decrease from B1 to B4 (p = 0.001), an increase from B4 to B5 (p = 0.01), and a decrease from B5 to B6 (p < 0.001) and B7 (p = 0.002). TA2 exhibited a significant decrease from B1 to B4 (p = 0.034) and TA3 exhibited no significant changes. PD exhibited a significant decrease from B1 to B4 (p = 0.001) and a marginally significant increase from B4 to B5 (p = 0.08).

Probabilistic sequence. TA1 and TA2 exhibited a significant decrease from B5 to B7 (both p < 0.04). TA3 and PD exhibited a significant decrease from B1 to B4 (both p > 0.05).

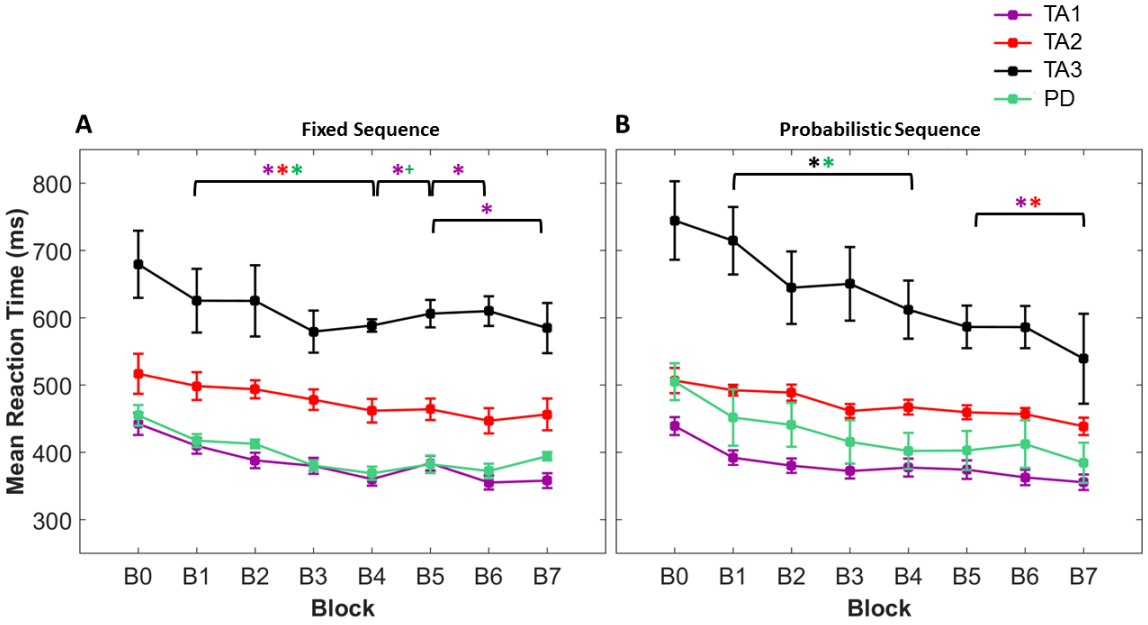

Figure 5.10: Mean RT for the clusters of typically aging adults and those with PD. A) In the fixed sequence groups, TA1 and PD exhibited a decrease in RT from B1 to B4 and B4 to B5, TA2 exhibited a decrease from B1 to B4, and TA3 exhibited no changes. (B) In the probabilistic sequence groups, TA3 and PD exhibited a decrease from B1 to B4 and TA1 and TA2 exhibited a decrease from B5 to B7.
* Indicates significance level of p < 0.05; + indicates significance level of p < 0.10. Error bars indicate standard error.

Positive correlation between baseline RT and years since diagnosis of PD

A Pearson correlation coefficient was computed to assess the relationship between the preliminary tests (age, *n*-back score, Wisconsin card sorting task score, physical activity, MMSE, MDS-UPDRS, Hoehn & Yahr score, and years since diagnosis of PD) and RT performance. A positive correlation was found between the mean baseline (B0) RT and years since diagnosis of PD, r = 0.78, $p = 0.008$ (Figure 5.12). Thus, the longer a patient has been diagnosed with PD, the slower their baseline RT. No significant correlations were found for TA, further suggesting that this reduction in baseline RT is not age-related.

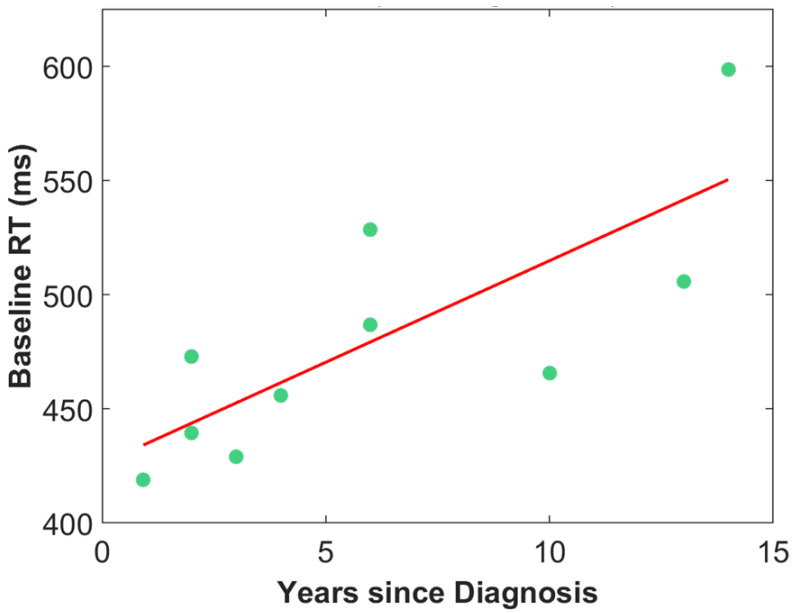

Figure 5.11: Positive correlation between baseline RT and years since diagnosis of PD.

Relative spectral power

Separate ANOVAs were conducted on the alpha and beta bands for each sequence type. The typically aging adults were compared based on their clusters with the patients with PD.

Fixed sequences

Alpha. A four-way mixed factorial (4 x 4 x 2 x 4) ANOVA on Group (TA1, TA2, TA3, PD) x Region (Frontal, Central, Parietal, Occipital) x Hemisphere (Left, Right) x Block (B1, B4, B5, B7) on the alpha band with Block as the within subject variable indicated a main effect of Block, $F(3,93) = 3.17$, $p = 0.03$ and a significant interaction between Region x Hemisphere x Block, $F(9,198) = 4.69$, $p < 0.001$. Simple effects analysis indicated that in the left hemisphere, the central region exhibited significantly greater alpha power than the frontal region ($p = 0.01$) and the occipital region ($p = 0.03$) in B1. In the right hemisphere, the central region exhibited significantly lower alpha power than the frontal region in B1 ($p = 0.007$) and marginally lower in B7 ($p = 0.08$), lower alpha than the parietal region in B1 ($p = 0.02$) and B7 ($p = 0.05$), and attenuated alpha than the occipital region in B1 ($p = 0.02$) and B7 ($p = 0.03$). There was also greater alpha power overall in the left hemisphere in the frontal region in B7 ($p = 0.03$) and in the central region in B1 ($p = 0.001$) and B7 ($p = 0.05$).

Pairwise comparisons between contrasts that were determined *a priori* revealed greater alpha power in B7 than B5 that approached significance in the left frontal region ($p = 0.08$) and left central region ($p = 0.06$) in TA1. The TA2 and TA3 clusters did not exhibit any differences in blocks in the alpha band. The PD group also exhibited a decrease in alpha power with learning, but only in the left central region ($p = 0.02$), and greater alpha

in B5 than B4 in the right frontal (p = 0.03) and right central (p = 0.03) regions and approaching significance in the left frontal and parietal (both p = 0.07) regions. In addition, the PD group also exhibited greater alpha power in B7 than B4 in the right central region (p = 0.02), right parietal region (p = 0.03), right occipital region (p = 0.02) and approaching significance in the left central region (p = 0.06) and right frontal region (p = 0.08).

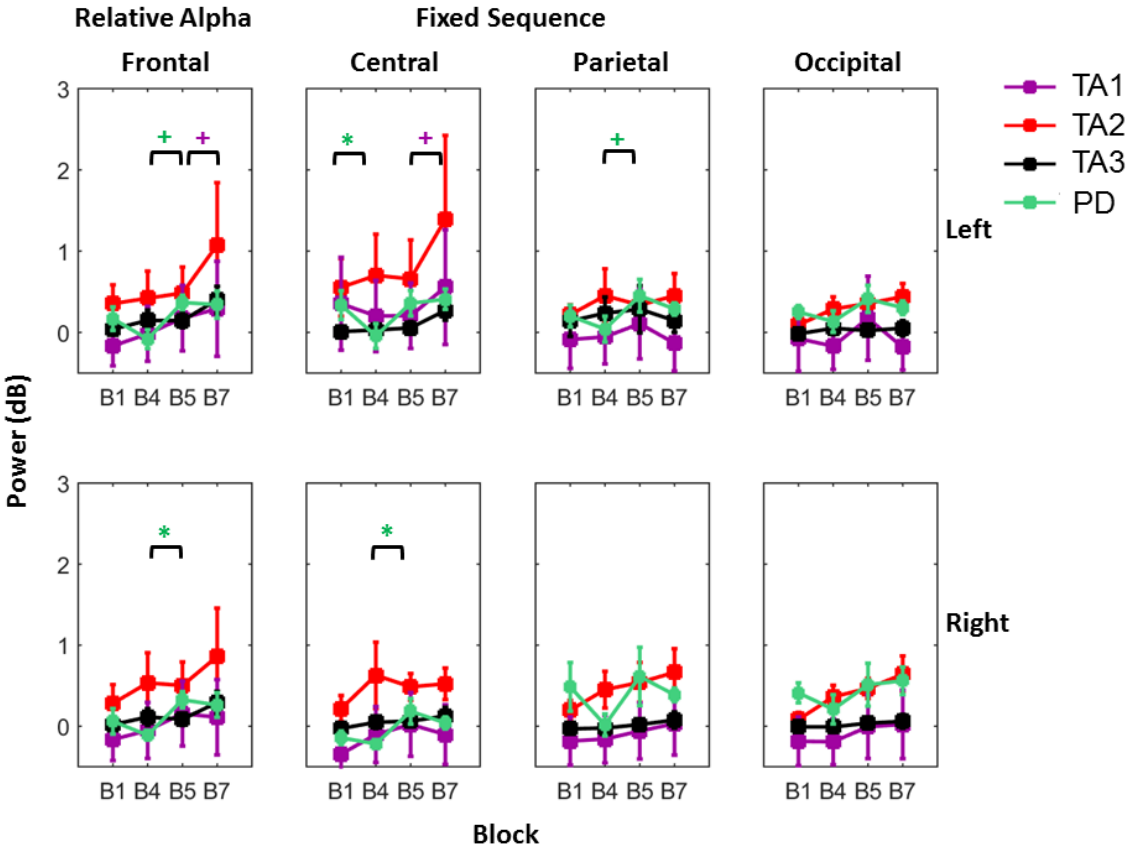

Figure 5.12: Relative alpha power for B1 (early learning), B4 (late learning), B5 (random – no sequence), and B7 (transfer of learning) for the fixed sequence groups.

Beta. In the beta band, there were no significant main effects or interactions. Pairwise comparisons revealed significantly greater beta power in B7 than B5 in the left (p = 0.003) and right (p = 0.005) frontal regions and left central region (p = 0.02). As in the

alpha band, no significant differences were found in the TA2 and TA3 clusters. In addition, no changes were exhibited the PD group.

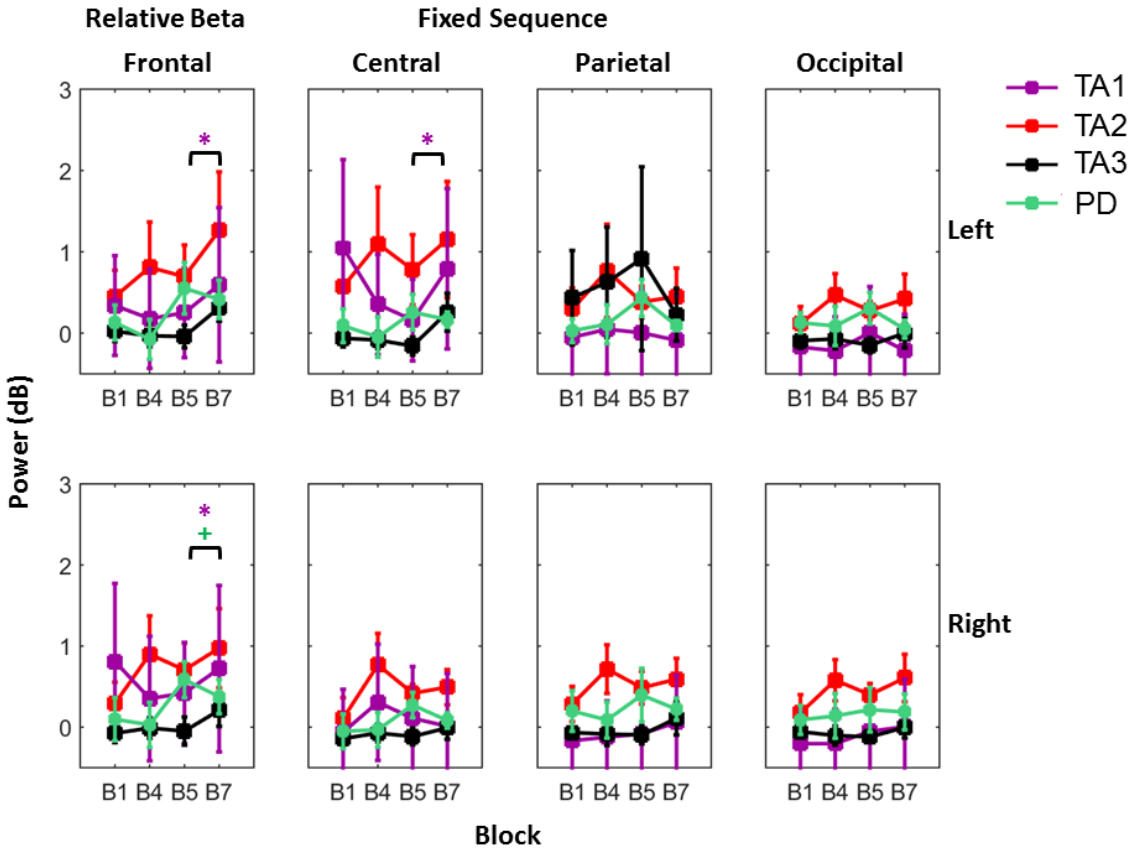

Figure 5.13: Relative beta power for B1, B4, B5, and B7 for the fixed sequence groups.

Probabilistic sequences

Alpha. A four-way mixed factorial (4 x 4 x 2 x 4) ANOVA on Group (TA1, TA2, TA3, PD) x Region (Frontal, Central, Parietal, Occipital) x Hemisphere (Left, Right) x Block (B1, B4, B5, B7) on the alpha band with Block as the within subject variable indicated a main effect of Region, $F(3,60) = 2.99$, $p = 0.04$. *Post hoc* analysis with Bonferroni correction on Region revealed significantly greater alpha power in the frontal

region than the occipital region (p = 0.03) and marginally lower alpha power in parietal region than the frontal and central regions (both p = 0.07).

Pairwise comparisons between contrasts that were determined *a priori* revealed no differences in TA1, TA2, or PD. TA3 exhibited marginally greater alpha power in B4 than B1 in the left occipital region (p = 0.07) and significantly greater power in B5 than B4 in the left central region (p = 0.05) and approaching significance in the right frontal region and parietal regions (both p = 0.07).

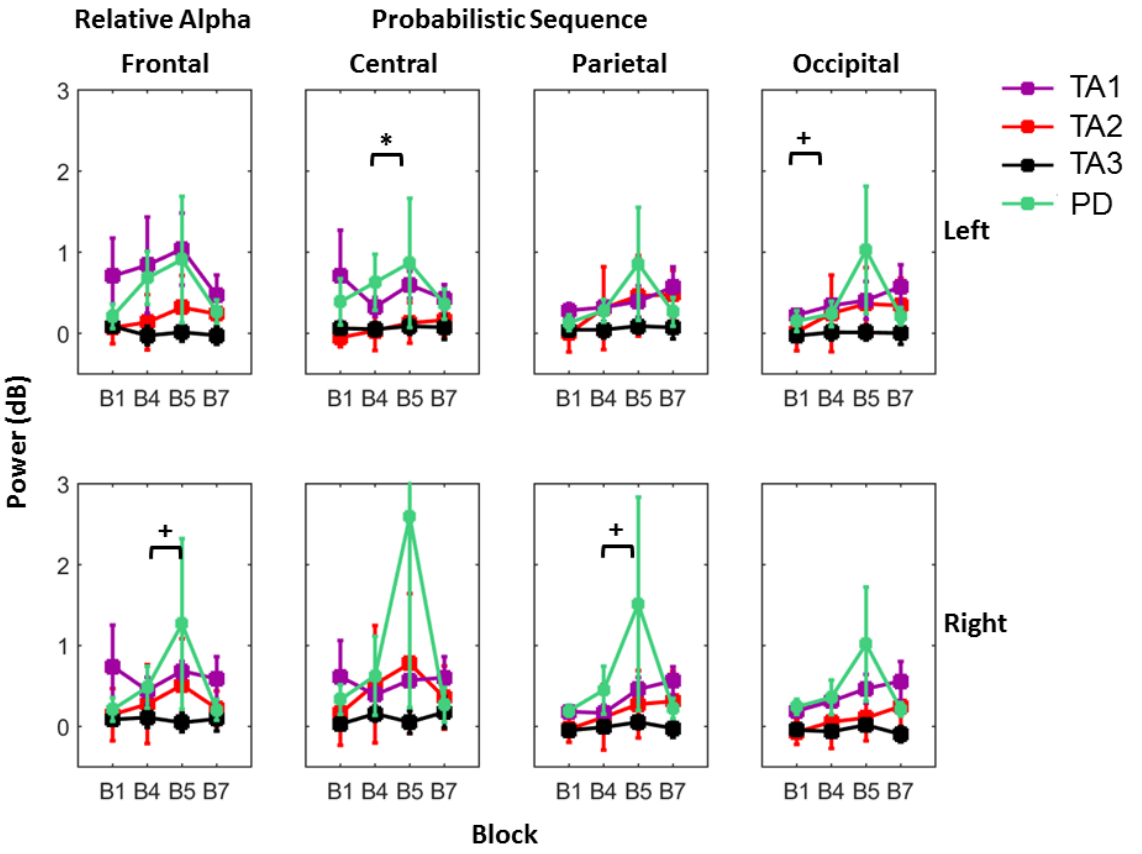

Figure 5.14: Relative alpha power for B1, B4, B5, and B7 for the probabilistic sequence groups.

Beta. In the beta band, there were significant interactions between Region x Block, F(9,180) = 2.29, p = 0.02, Region x Block x Group, F(27,180) = 1.77, p = 0.02, Region x Hemisphere x Block, F(9,180) = 2.02, p = 0.04, and Region x Hemisphere x Block x Group,

F(27,180) = 1.63, p = 0.03. Simple effects analysis revealed that in B1, B4, and B5, TA3 exhibited significantly greater beta power in the left and right frontal regions compared to TA2 (all $p < 0.05$) and marginally greater than TA1 (B1 and B4, p = 0.07; B5, p = 0.02). In addition, PD exhibited significantly greater beta power than TA2 in the right occipital region in B1 and B4 (both p = 0.05) and marginally greater power in the right parietal region (p = 0.06). In B4, PD also exhibited marginally greater beta power than TA1 (p = 0.06) in the right occipital area. Greater differences between the TA clusters and PD appeared in B5 with PD exhibiting marginally greater beta power than TA1 in the left occipital region (p = 0.06) and significantly greater power in the right occipital region compared to TA1 (p = 0.02) and TA2 (p = 0.05). Pairwise comparisons did not exhibit any differences between blocks in TA or PD.

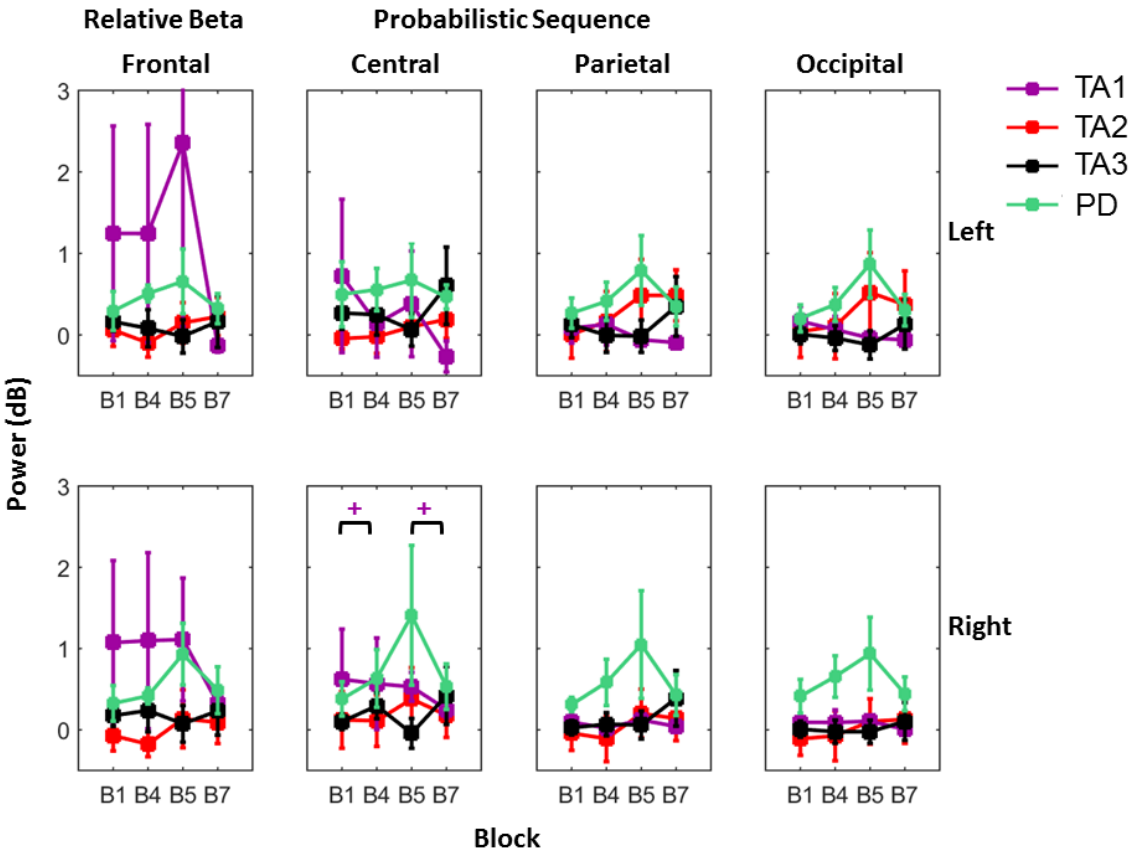

Figure 5.15: Relative beta power for B1, B4, B5, and B7 for the probabilistic sequence groups.

Coherence analysis

Fixed sequences

Alpha. A four-way mixed factorial (4 x 5 x 2 x 4) ANOVA on Group (TA1, TA2, TA3, PD) x Region (Fz pairing with each of the following: Frontal, Central, Temporal, Parietal, Occipital) x Hemisphere (Left, Right) x Block (B1, B4, B5, B7) on the alpha band with Block as the within subject variable indicated a main effect of Region, $F(4,88) = 163.3$, $p < 0.001$. *Post hoc* analysis with Bonferroni correction on Region revealed significant greater cortico-cortical connectivity in the frontal region than the other regions (all $p < 0.001$). In addition, there was greater connectivity in the central region than the temporal, parietal, and occipital regions (all $p < 0.001$); greater connectivity in the temporal and parietal regions than occipital region (both $p < 0.001$), but no difference between the temporal and parietal regions ($p = 0.9$).

Pairwise comparisons between contrasts that were determined *a priori* revealed greater cortico-cortical connectivity in the right temporal region in B4 compared to B1 ($p = 0.05$) in TA2. No differences were found in TA1, TA3, or PD.

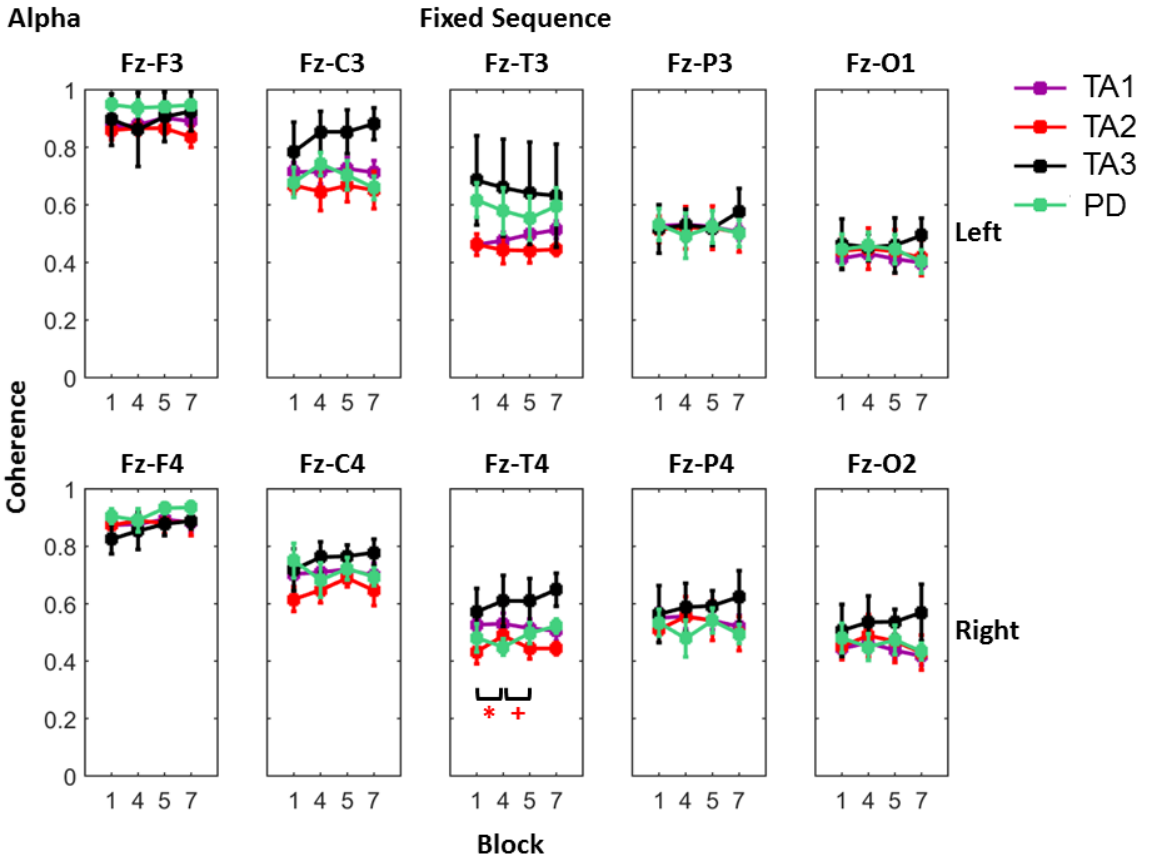

Figure 5.16: Coherence in the alpha band for B1, B4, B5, and B7 for the fixed sequence groups.

Beta. In the beta band, there was also a main effect of Region, $F(4,88) = 138.9$, $p < 0.001$ and significant interactions between Region x Hemisphere, $F(4,88) = 3.08$, $p = 0.02$ and Region x Hemisphere x Block x Group, $F(36,264) = 1.52$, $p = 0.04$. Simple effects analysis revealed that in B1, TA3 exhibited significantly greater cortical connectivity than TA2 in the left central region ($p = 0.05$) and TA1 ($p = 0.005$) and TA2 ($p = 0.008$) in the left temporal region. In B4, TA3 exhibited greater cortical connectivity than TA1 and TA2 in the left central region (both $p = 0.05$) left temporal region (both $p < 0.008$). In B5, TA3 exhibited greater cortical connectivity than TA1, TA2, and PD in the left central (all $p < 0.04$) and left temporal (TA1 and TA2, $p < 0.01$; PD, $p = 0.07$) regions. In B7, TA3 exhibited significantly greater connectivity in in the left central region than TA1, TA2

(both $p = 0.02$), and PD ($p = 0.05$), in the left temporal region than TA1 ($p = 0.05$) and TA2 ($p = 0.03$), and marginally greater in the right occipital region than TA1 ($p = 0.06$).

At the region level, TA1, TA2, and PD exhibited similar trends for all blocks: significantly greater connectivity in the frontal region compared to the other regions (all $p < 0.001$), greater connectivity in the central region than the temporal, parietal, and occipital regions (all $p < 0.001$), greater connectivity in the parietal and temporal regions than the occipital region (both $p < 0.02$), and no differences between the temporal and parietal regions ($p > 0.1$). TA3 exhibited some differences in that connectivity in the frontal region was significantly greater than the temporal, parietal, and occipital regions (all $p < 0.05$), but not different from the central region ($p = 0.2$). Additionally, the central region was not significantly different from the temporal region ($p = 0.4$), but the temporal region exhibited significantly greater connectivity than the parietal and occipital regions (both $p < 0.02$). This pattern of connectivity is different in TA3 compared to PD and the other TA clusters suggests that at least in TA3, impairments may be related to cortico-cortical connectivity in the beta band.

Pairwise comparisons found marginally greater connectivity in the right central region in B5 than B7 ($p = 0.06$) in TA1. In TA2, there was greater right temporal connectivity in B4 than B1 ($p = 0.03$) and B5 than B4 ($p = 0.005$), greater parietal connectivity in B5 than B7 in both the left ($p = 0.04$) and right ($p = 0.05$) hemispheres and marginally greater occipital connectivity in B5 than B7 in both the left ($p = 0.06$) and right ($p = 0.07$) hemispheres. In TA3, right central connectivity was greater in B5 than B7 ($p = 0.01$) and in PD, right frontal connectivity was greater in B5 than B4 ($p = 0.03$).

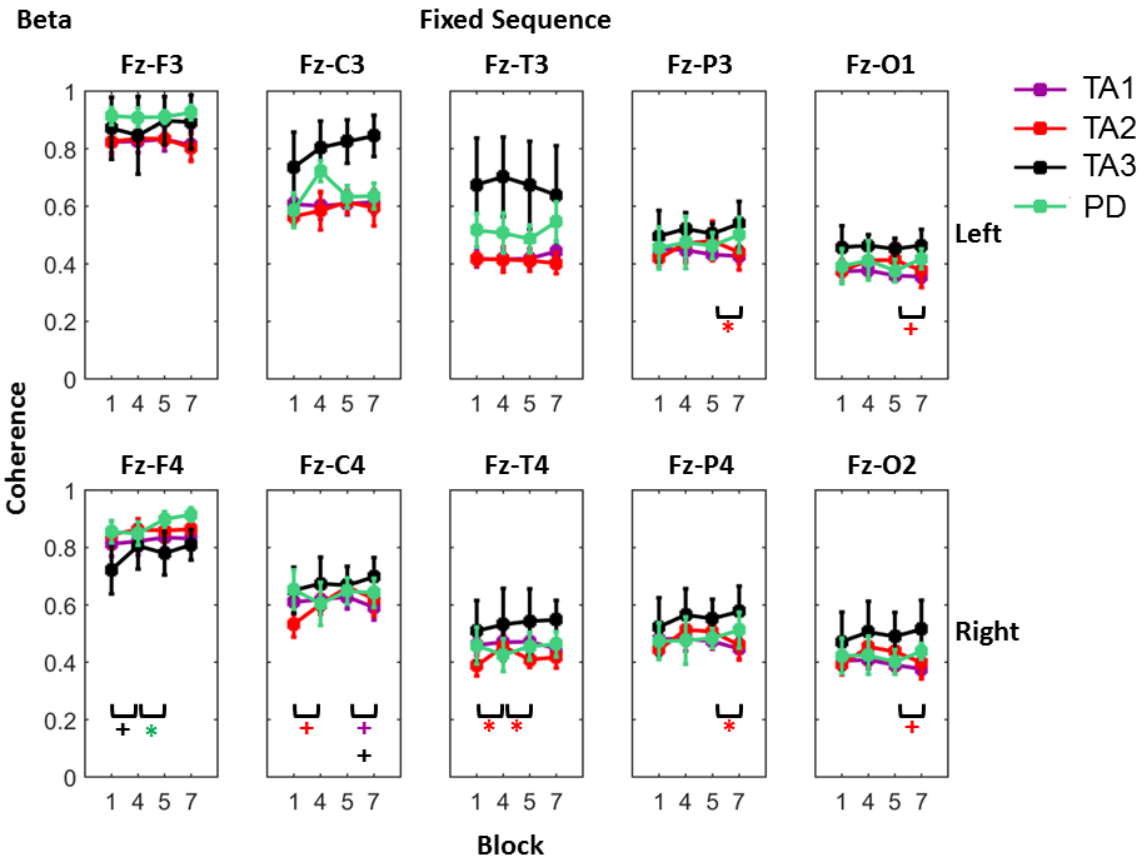

Figure 5.17: Coherence in the beta band for B1, B4, B5, and B7 for the fixed sequence groups.

Probabilistic sequences

Alpha. There was a main effect of Region, $F(4,84) = 120.2$, $p < 0.001$ and Hemisphere, $F(1,21) = 10.0$, $p = 0.005$, but no significant interactions. *Post hoc* analysis with Bonferroni corrections on Region revealed significant greater coherence in the frontal region than the other regions (all $p < 0.001$). In addition, there was greater cortical connectivity in the central region than the temporal, parietal, and occipital regions (all $p < 0.001$); greater connectivity in the temporal and parietal regions than occipital region (both $p < 0.002$), but no difference between the temporal and parietal regions ($p = 1.0$). Additionally, the right hemisphere exhibited greater connectivity than the left hemisphere.

Pairwise comparisons between contrasts of interest that were determined *a priori* revealed significantly greater connectivity in B4 than B1 in the left occipital area (p = 0.004) in TA1. There was significantly greater connectivity in B4 than B5 in the left frontal area in TA2 (p = 0.02). In TA3, there was significantly greater connectivity in B5 than B7 in the right parietal area (p = 0.03) and approached significance in the right central area (p = 0.07) and in the left occipital area for B1 (p = 0.06). In PD, B1 had significantly greater connectivity than B4 in the left frontal area (p = 0.05).

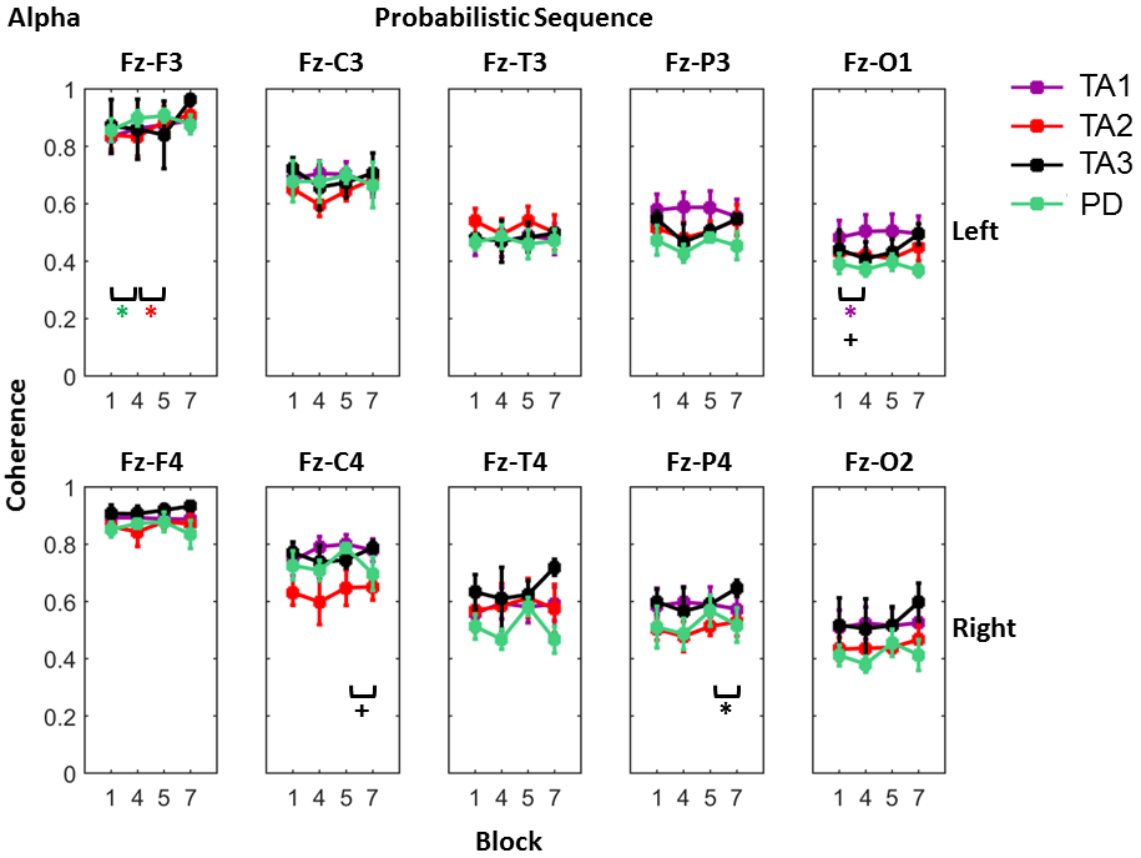

Figure 5.18: Coherence in the alpha band for B1, B4, B5, and B7 for the probabilistic sequence groups.

Beta. In the beta band, there was also a main effect of Region, $F(4,84) = 120.2$, $p < 0.001$ and Hemisphere, $F(1,21) = 9.0$, $p = 0.007$ and a significant interaction between Region x Hemisphere x Block, $F(12,252) = 3.2$, $p < 0.001$. Simple effects analysis revealed

the same trend in region for all blocks and both hemispheres as seen in the alpha band. Additionally, greater connectivity was exhibited in the right hemisphere for all blocks and regions (all $p < 0.05$).

Pairwise comparisons found significantly greater connectivity in the right frontal area in B5 than B7 in TA1 ($p = 0.01$), the left frontal in B5 than B4 in TA2 ($p = 0.004$), marginally greater connectivity in the left occipital area in B4 than B5 in TA3 ($p = 0.08$), and greater connectivity in B1 than B4 in the right central area ($p = 0.04$) and right temporal area ($p = 0.05$). In PD, significantly greater connectivity was exhibited in B1 compared to B4 in the right central ($p = 0.04$) and temporal ($p = 0.05$) regions and marginally greater connectivity in B5 than B7 in the right frontal area ($p = 0.06$).

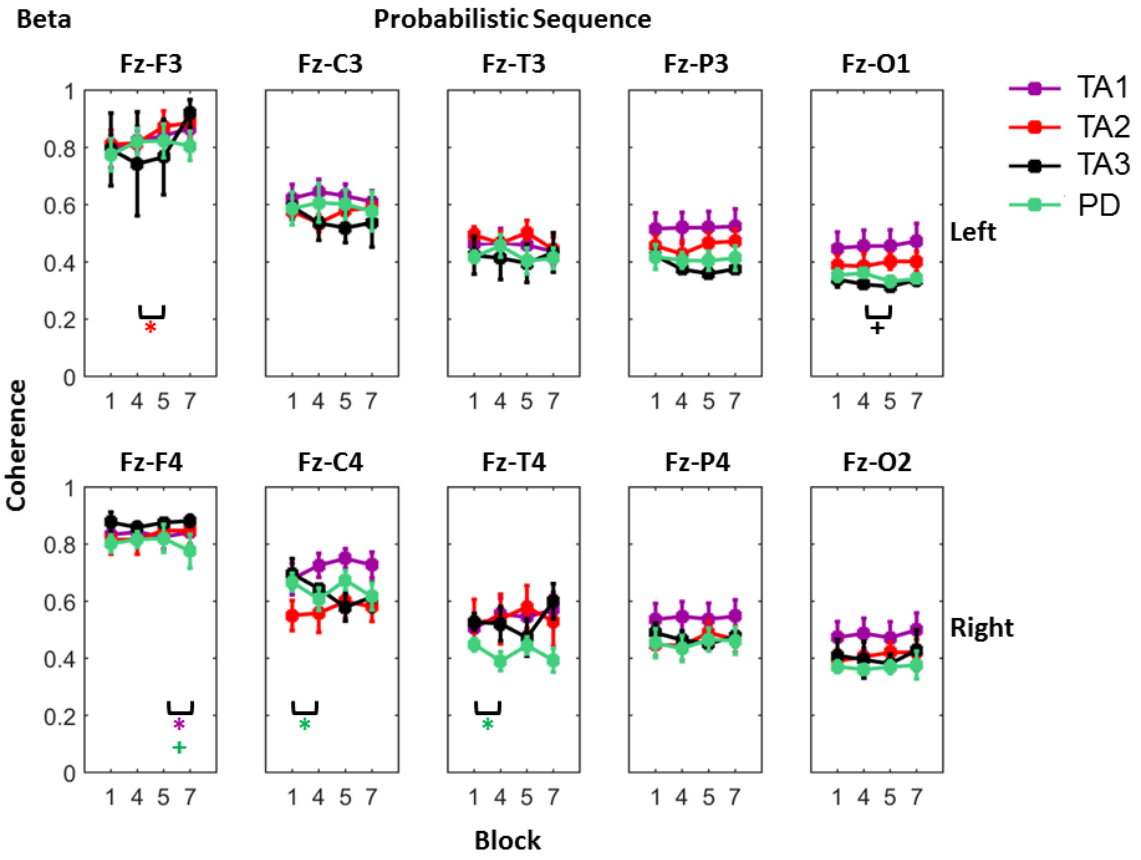

Figure 5.19: Coherence in the beta band for B1, B4, B5, and B7 for the probabilistic sequence groups.

Posttest

The posttest required participants to rate their confidence on a scale of 1-5 whether they had seen the presented chunk in any of the blocks. Some of the chunks presented to participants were from the assigned sequence and some were random chunks. Participants were also shown entire sequences, one of which was their assigned sequence.

Fixed sequence. In TA1, there was a marginally significant difference between the chunks ($p = 0.06$) and no difference between entire sequences ($p = 0.3$). Surprisingly, there was a significant difference in TA2 for chunks ($p = 0.05$), even though they did not exhibit learning of the sequence, but not for the entire sequence ($p = 0.4$). There was no difference for chunk or sequence in TA3 (both $p > 0.1$) or PD (both $p > 0.2$).

Probabilistic sequence. There was no difference between the chunks or sequences in any of the groups (all $p > 0.05$), indicating that participants in the probabilistic group were unable to recognize the chunks or their assigned sequence.

Discussion

By directly comparing fixed and probabilistic sequences in typically aging adults and those with PD, we demonstrated that motor sequence learning impairments in patients with PD are likely related to the aging process. Some typically aging adults (those with higher working memory capacity) and those with PD (also with higher working memory capacity) learned the fixed sequence, but neither were able to learn the probabilistic sequence, suggesting that reduced working memory in aging contributes to the impairment.

Disentangling cognitive and movement deficits

We found that reaction time provided a more accurate understanding of learning in PD than response time. Analysis of response time suggested that only the typically aging adults were able to learn the fixed sequence; however, decomposing response time into reaction and movement times revealed that the large response times exhibited by the patients with PD were due to their large movement times. Patients with PD exhibited a significant decrease in reaction time during the learning blocks and an increase when presented with random stimuli, thus demonstrating that were able to learn the fixed sequence. In the probabilistic sequence group, while patients with PD did exhibit a decrease in reaction time during the learning blocks, they did not exhibit any changes in subsequent blocks, suggesting that as we saw in Study 1 of this dissertation, that they improved in the motor component of the task, but did not exhibit learning of the sequence. These results suggest that motor sequence learning impairments in these patients are related to their aging and not PD. Importantly, the results highlight a potential factor contributing to the inconsistencies in the literature: namely, the interchangeable use of response and reaction time. These measurements are distinct and the difference is particularly important when assessing patients with a movement disorder. Separating these measurements may provide clarity to current literature where some studies find that patients with PD are impaired at learning motor sequences (Carbon, et al., 2010; Doyon, et al., 1997; Gamble, et al., 2014; Gobel et al., 2013; Jackson, et al., 1995; Muslimovic, et al., 2007; Seo, et al., 2010; Shin & Ivry, 2003; Westwater, et al., 1998; Wilkinson, et al., 2009), while others suggest no impairment (Agostino, Sanes, & Hallett, 1996; Mentis, et al., 2003; Pascual-Leone, et al.,

1993; Pendt, Reuter, & Muller, 2011; P. J. Reber & Squire, 1999a; Siegert, et al., 2006; Smith, et al., 2001; Wilkinson & Jahanshahi, 2007).

Most SRT paradigms used in current literature are unable to distinguish between response time (movement time and reaction time combined) and reaction time (only); however, the modified SRT task used in this study provided a method for recording reaction time and movement time separately. While reaction time was not significantly different between the typically aging adults and those with PD, movement time was significantly slower in patients with PD. This was expected as patients with PD present with motor symptoms such as bradykinesia that resulted in slower movement times. This is a critical distinction as reaction time is the measure used to infer learning in the SRT task and it is possible that studies that find impairments in learning in PD are actually reflecting movement deficits with their use of response times, rather than learning deficits.

Previous studies have suggested that impairments in PD may be a function of disease severity (Carbon & Eidelberg, 2006; Muslimovic, et al., 2007; Stephan, et al., 2011) and it is important to note that the patients in this study were all in the early stages of the disease (Hoehn & Yahr stage of I-II). Impairment in learning the probabilistic sequence is also consistent with previous studies that used different probabilistic sequence structures (Seo, et al., 2010; Wilkinson, et al., 2009), as well as other tasks that require the learning of probabilities, such as category learning (Ashby & Ell, 2001a; Ashby & Maddox, 2005, 2011; Keri, 2003; P. J. Reber, 2013; Seger, 2006a). Together, the impairments found in these studies and those found in the current study suggest an important role of the basal ganglia in learning probabilities (Knowlton, et al., 1996).

Comparing patients with PD to the developmental landscape of typically aging adults

In Study 2 of this dissertation, we demonstrated that combining all typically aging adults into one group fails to provide a clear understanding of age-related changes in performance. By using cluster analysis, we created a developmental landscape of our sample of typically aging adults that separated the adults into three clusters that were found to be distinct by mean reaction time and working memory capacity, but not age. When the patients with PD were included in this developmental landscape, they did not create a separate cluster of their own, but were interspersed within the typically aging adults, suggesting that for mean reaction time and working memory, these patients were similar to typically aging adults. Specifically, the patients with PD were not significantly different in mean reaction time or working memory capacity than TA1 (the fastest cluster with the highest *n*-back score). Thus, cluster analysis provided a unique method of matching patients with a control group that did not rely on age, but rather functional characteristics that are important for the task, elucidating deficits exhibited by patients with PD. Using such methods may prove crucial in future studies for attaining a clearer understanding of changes related to disease and age.

Patients with PD exhibit an impairment in transfer of learning

Transfer is an essential component of motor learning that provides an assessment of learning and whether performance can be maintained in a different context or variation of the skill. The TA1 cluster exhibited both learning and transfer of the fixed sequence; however, the patients with PD were unable to transfer their learning to a novel fixed sequence, suggesting a role for the basal ganglia in transfer of learning to a new context. However, some studies suggest that depending on the cognitive demands of the task,

patients with PD are able to transfer learning to a related motor task, although patients exhibited deficits in tasks related to working memory (Mendes, et al., 2012), and that levodopa medication impairs learning, but not generalization (Shohamy, et al., 2006). Transfer is not assessed in most SRT studies, thus more research is needed to determine whether patients with PD are impaired at transferring their learning within the motor sequence learning framework.

Working memory plays an important role in motor sequence learning

Given that the patients with PD had a similar *n*-back score and mean reaction time as TA1, it was not surprising that no differences were found between the reaction times of the patients with PD and the TA1 cluster in the fixed sequence group and that both were faster than the TA2 and TA3 clusters. These results further suggest that working memory plays an important role in motor sequence acquisition. This is consistent with previous studies that have found that visuospatial working memory capacity is related to both explicit (Bo, et al., 2009) and implicit (Bo, et al., 2011, 2012; Seidler, et al., 2012) motor sequence learning. In addition, studies have found that while typically aging adults exhibit reduced working memory (Li, et al., 2008), they are able to maintain their performance levels (Bo, et al., 2012). We observed a maintenance of performance in the TA1 cluster and patients with PD in the fixed sequence group, but not in the TA2 and TA3 clusters or any of the participants in the PB group, suggesting that there may be a threshold up to which performance may be maintained even with reduced working memory. Since the fixed sequence is less complex, the working memory capacity available may be adequate for sequence learning in the patients with PD and the TA1 cluster, but is not adequate for

the more complex probabilistic sequence. The reduced working memory in the TA2 and TA3 clusters may be inadequate for learning the fixed sequence.

Potential behavioral markers of Parkinson's disease

We found a significant positive correlation between the mean baseline reaction time and years since diagnosis of PD. This is not surprising as bradykinesia expresses in the symptomatology of PD and deteriorates with progression of the disease (Fahn & Jankovic, 2007). This further suggests that impairments exhibited by patients with PD in previous studies may be due to an inability to produce movement, resulting in a slower reaction time, rather than an inability to learn the sequence. This correlation suggests that there may be other behavioral variables, in addition to baseline RT, that may be potential markers to track the progression of PD or that may aid in the early detection of the disease.

Few differences between typically aging adults and those with PD in cortical activations and connectivity

In our previous study (Study 2 of this dissertation), we found distinct cortical activations between the young and typically aging adults that suggested that learning can be inferred from the cortical activations in addition to the behavioral measures. These cortical activations (or lack of them when no learning occurred) were observed across groups in the current study as well. For both fixed and probabilistic sequence groups, no significant interactions involving group were found in the alpha band for cortical activations or connectivity, suggesting few differences between the typically aging adults and those with PD and further providing evidence that the deficits exhibited by the patients with PD are related to the aging process.

In the fixed sequence group, we found greater alpha power in B5 than B4 in the left occipital and right parietal regions across groups, supporting our previous findings of cortical activations indicating learning. The patients with PD exhibited greater alpha power in B1 than B4 in the left central region, suggesting an attenuation in alpha with learning, also consistent with our previous findings. Additionally, patients also exhibited an increase in B5 compared to B4 in bilaterally in the frontal region, right central, and left parietal regions. However, patients exhibited significantly greater beta power in B5 than B7 in the right frontal area, suggesting less cortical activation in B7. Together with the behavioral data, these cortical activations further provide evidence that the patients with PD learned the fixed sequence, but were unable to transfer their learning to a novel sequence.

In the probabilistic sequence group, no differences were found between blocks in alpha power in the TA1 cluster, the TA2 cluster, or the patients with PD, but the TA3 cluster exhibited increased activation in late learning compared to the random block, suggesting that with more learning blocks, they may be able to learn the sequence. Coherence analysis demonstrated greater alpha connectivity in B4 compared to B1 in the left frontal region in patients with PD and greater beta connectivity in B1 compared to B4 in the right central and temporal regions and in B5 compared to B7 in the right frontal region was also exhibited by patients with PD, both bands indicating no learning. Thus, consistent with results from our previous study (Study 2 of this dissertation), learning (or the lack of it) can be inferred from cortical activations.

In the patients with PD, coherence analysis exhibited greater connectivity in B4 than B1 in the alpha band in the left frontal area and greater connectivity in the beta band in B1 than B4 in the right central and temporal regions. These are consistent with the

behavioral results that patients with PD were unable to learn the probabilistic sequence and the increase alpha power and decreased beta power during the learning blocks are indicative of the impairment. The lack of parietal, temporal, and occipital activation is consistent with previous studies (Carbon, et al., 2010) and greater frontal activation may reflect a potential compensatory mechanism (Catalan, et al., 1999; Nakamura, et al., 2001). In addition, the patients with PD in this study were on dopaminergic medication, which studies have found to have a negative effect on learning, as well as suppress activation of cortical regions (Argyelan, et al., 2008; Carbon & Eidelberg, 2006; Huang et al., 2007; Kwak, et al., 2010, 2012). This may reflect the impairment in learning the probabilistic sequence, but it is more likely to be age-related as the typically aging adults were also impaired at learning the probabilistic sequence.

The beta band may be more sensitive to differences and may reflect PD-related compensation

Unlike the alpha band, coherence analysis of the beta band in the fixed sequence group exhibited differences between groups. The TA3 cluster exhibited greater beta connectivity than the TA1 and TA2 clusters in the left central and temporal regions in for all the blocks. In addition, the TA1 and TA2 clusters and patients with PD exhibited the greatest connectivity in the frontal region, then the central region, then temporal and parietal regions, and the least connectivity in the occipital regions. However, the TA3 cluster exhibited a different pattern of connectivity, with similar connectivity in the frontal and central regions, the central and temporal regions, and the parietal and occipital regions. Significantly greater connectivity was found in the frontal region than the temporal, parietal, and occipital regions and in the temporal region compared to the parietal and

occipital region. These different connectivity patterns may be indicative of the impairments in learning the fixed sequence in the TA3 cluster; however, it should be noted that the TA2 cluster was also impaired at learning the sequence, but did not exhibit these different connectivity patterns.

In the probabilistic sequence groups, during B1, the TA3 cluster exhibited greater beta power than the TA1 and TA2 clusters bilaterally in the frontal region and patients with PD exhibited greater beta power than the TA2 cluster in right occipital region. However, by B4, patients with PD exhibited significantly greater beta power than the TA1 cluster in the right occipital region. This trend continued in B5, where patients with PD exhibited significantly greater beta power than the TA1 cluster in the right central region and bilaterally in the occipital region as well as in B7 in the right parietal region. This pattern is the opposite of what we observed in the behavioral data, where the patients with PD were significantly slower than the TA1 cluster in B1, but by B4 were not significantly different from the TA1 cluster and both were significantly faster than the TA2 cluster. This difference suggests that there may be compensatory mechanisms that are PD-related in the cortical activations that are not captured by behavioral measures. These differences in beta power are supported by previous studies in clinical populations that have found a reorganization of beta band connectivity in patients with focal hand dystonia (Jin, Lin, Auh, et al., 2011; Jin, Lin, & Hallett, 2011) and patients with PD (Ahn, et al., 2015).

Furthermore, coherence analysis of the beta band in the fixed sequence group indicated a similar pattern. The TA3 cluster was not significantly different from the patients with PD in B1 or B4, but in B5 and B7, the TA3 cluster exhibited significantly greater beta connectivity than the TA1 and TA2 clusters as well as the patients with PD in the left

central and temporal regions. This suggests that in the learning blocks, patients with PD exhibit similar cortical connectivity as the TA3 cluster, but in B5 and B7, patients with PD exhibit similar cortical connectivity to the TA1 and TA2 clusters. However, despite showing similar cortical connectivity as the TA3 cluster (who did not learn the fixed sequence), patients with PD exhibited learning of the sequence, suggesting that they may be employing compensatory mechanisms.

Conclusion

To our knowledge, this is the first time that probabilistic and fixed sequences have been directly compared in patients with PD. We demonstrated that deficits related to learning in patients with PD are likely to be related to the aging process, as opposed to disease-related. From the response time data, it appeared that patients with PD were unable to learn either sequence type; however, by unraveling movement deficits from cognitive deficits, we found that movement time had a greater contribution to response time, concealing the learning present in reaction time. This decomposition of response time revealed that patients with PD were able to learn the fixed sequence, but like the typically aging adults, were unable to learn the probabilistic sequence. This distinction between response and reaction time is crucial and may be partly responsible for the diverse results in current literature on PD-related impairments in motor sequence learning. Another factor that may contribute to the equivocal results in literature may be the way patients are matched with control participants based simply on age. We demonstrated that statistical methods, such as cluster analysis, could be used to create groups of controls that match better with clinical populations based on functional characteristics important for performing the task, rather than chronological age.

Few differences were found in cortical activation patterns and connectivity between the typically aging adults and those with PD, further suggesting that the observed deficits were age-related. However, differences were found in the beta band that may reflect PD-related deficits. Both spectral power and coherence analysis exhibited similar patterns during the learning blocks in which patients with PD exhibited similar cortical activations or connectivity as the TA2 or TA3 cluster, but by trial blocks B4 and B5, patients with PD exhibited similar activations or connectivity as TA1. This is an interesting finding that suggests that patients with PD may be compensating to learn the fixed sequence, but the probabilistic sequence may be too complex to benefit from the compensatory mechanisms.

Our results suggest that by more deeply understanding how motor sequence learning is affected by functional characteristics using statistical methods, tightly controlling variables for an accurate assessment of learning and movement deficits and sequence types, and studying cortical dynamics using time-sensitive methods, we can attain a clearer understanding of impairments in patients with Parkinson's disease. Together, these techniques prove effective for a deeper understanding of the dynamic motor learning process and provide evidence that patients with PD are indeed impaired at probabilistic motor sequence learning, but the impairment may be related to aging, rather than Parkinson's disease.

Chapter 6: Discussion

Summary and Implications

Motor sequence learning is a critical ability underlying all activities of daily living and it is important to study it in an adaptive learning framework, as it occurs in real life. The studies in this dissertation are the first to examine motor sequence learning from a novel perspective and advocate moving towards a more ecologically valid assessment of sequence learning in the laboratory.

Probabilistic sequences are representative of adaptive motor learning

The results from these studies suggest that probabilistic sequences are more effective than fixed sequences to assess the adaptive learning required in learning motor skills in everyday life. This is an important finding that addresses a specific methodological problem that has wide implications for future SRT studies. While previous studies have used alternate methods to generate probabilistic sequences, such as a finite-state grammar, (Jimenez & Mendez, 1999; Jimenez, et al., 1996) and the alternate serial reaction time task (Feeney, et al., 2002; D. V. Howard & Howard, 2001; J. H. Howard & Howard, 1997; Song, et al., 2007a, 2007b), no other studies have used probabilistic sequences generated by a first-order transitional probabilistic structure that results in more complex and entirely probabilistic sequences. The ability to manipulate probabilities and determine the effects of different types of sequences on motor sequence learning may be useful in more deeply understanding the learning processes. In addition, we found a differential effect of aging on the sequence type. While some typically aging adults and those with PD learned the fixed sequence, neither learned the probabilistic sequence. This finding is critical as probabilistic sequences more accurately reflect the learning acquired in daily life, since

ultimately our aim is to better understand motor skill learning that is adaptive to changes in the environment and suggests impairments in typically aging adults and those with PD.

Additional parameters may help better assess learning

We found that additional variables, such as variability, transfer, and quantifying online and offline learning provide greater insight into the learning process. A reduction in motor performance variability has been an important characteristic of motor learning (R. G. Cohen & Sternad, 2009; Wulf & Schmidt, 1997) and incorporating this variable into the SRT task may provide greater insight into the learning of different types of sequences and different populations. In addition, transfer is an essential component of assessing motor learning and whether performance can be maintained in a different context, but it has also not been used in the SRT paradigm. Here, we found that the learning of both fixed and probabilistic sequence structures can be transferred to novel sequences created using the same underlying structure, but that patients with PD may be impaired at transferring their learning to a novel sequence. Furthermore, we found that fixed sequences employ both online and offline learning, but probabilistic sequences employ only offline learning and contributed to the literature that offline learning can occur in the time interval of a few minutes (Hotermans, et al., 2006; Schmitz, et al., 2009).

Differentiating between cognitive and movement deficits

SRT studies have generally used reaction and response time measurements interchangeably. In the traditional SRT task, there is a choice between four buttons and the participants place a finger on each of the buttons and press the button that corresponds to the location of the stimulus. However, in this design, the reaction and movement times cannot be distinguished. We have demonstrated that this difference is critical and provides

insight into the learning of motor sequences, particularly in patients with movement disorders, by differentiating cognitive and movement deficits. Since PD is a movement disorder with symptoms such as bradykinesia and difficulties in initiating movement, the decomposition of response time into reaction and movement time allowed us to determine that movement time had a greater contribution to the overall response time. Thus, even though the patients with PD appeared to be unable to learn the fixed sequence according to the response time, the reaction time demonstrated that they were able to learn the sequence, but their slow and highly variable movement times were masking the learning. This is a critical finding that can provide clarity into whether patients with PD are impaired at motor sequence learning. Consistent with previous studies suggesting that patients with PD can learn simple sequences, our results suggest that patients with PD can learn fixed sequences, but not the more complex probabilistic sequences.

Statistical methods can be used to characterize diverse populations and age-related differences

Aging studies typically compare young adults that are a tightly controlled group by age (usually within a range of 18-25 years), to typically aging adults that can range from 50 to over 80 years. This is a large age range, the grouping of which implies that 50-year-old adults and 80-year-old adults are expected to perform at a similar level. However, intuition and the few studies that have compared typically aging adults suggest age-related differences in motor sequence learning between “middle-aged” and older adults (Feeney, et al., 2002). In order to characterize our diverse population of typically aging adults, we used cluster analysis to separate the participants based on their entire reaction time series. We found that the clusters were not significantly different by age, but were significantly

different by their mean reaction time and *n*-back score, which assesses working memory capacity. In addition, patients with PD were not clustered into a separate group and performed at a similar level as typically aging adults with a similar mean reaction time and *n*-back score.

Moving towards functionally-matched control groups

The cluster analysis also suggests that rather than age-matching clinical populations with control groups, it is important to characterize the clinical population and match controls according to functional characteristics. These characteristics can include factors that are important for the task in order to attain a clearer understanding of impairments related to aging or disease. The age-matched typically aging group selected initially was unable to provide a clear insight into the effects of aging on motor sequence learning. Cluster analysis and other statistical methods are critical to deeply understanding the relationships between functionally-related and age-related differences of task and individual variability and their confounding of experimental conclusions.

Insights from cortical dynamics

Power and coherence analysis of the EEG data provided a more in-depth assessment of learning, particularly for participants that did not exhibit learning in their behavioral data. Consistent with previous fMRI studies, young adults exhibited cortical activations in the frontal, central, parietal, and occipital areas during the learning blocks (Bo, et al., 2011, 2012; Braver & Barch, 2002; Braver, et al., 2001; Poldrack, et al., 2005; Seidler, et al., 2005). Interestingly, although the TA1 cluster did not exhibit learning of the probabilistic sequence, greater beta power activity suggests that participants were attempting to learn the sequence, but were unable to do so. This indicates that these

participants may need more practice blocks for the learning to be revealed in the behavioral data (Wu & Hallett, 2005). Similarly, the TA2 and TA3 clusters did not exhibit learning of either sequence type, but exhibited cortical connectivity in the temporal region, indicating visuo-spatial perception that did not translate to learning, since there were no changes during the learning blocks. There was also greater activation in the frontal regions in the patients with PD and less activation in the parietal, temporal, and occipital areas consistent with previous fMRI studies, suggesting a compensatory mechanism via the frontal regions (Carbon, et al., 2010; Catalan, et al., 1999; Nakamura, et al., 2001). The cortical activations and connectivity reflect both learning and transfer, even in the absence of behavioral markers of learning. In the beta band, patients exhibited compensatory mechanisms that are likely to be in response to deficits due to PD. Thus, cortical dynamics may contain indications of learning and compensation that are not attained through behavioral measures.

Impairments in probabilistic motor sequence learning may be related to the aging process, rather than related to Parkinson's disease

Taken together, the behavioral and EEG data suggest a critical finding: that the impairments in the learning of probabilistic sequences in the patients with PD are more likely to be age-related, rather than related to Parkinson's disease. This is indicated by various pieces of evidence conducted in these studies through the different analyses.

First, the patients with PD were not grouped in a separate cluster from the typically aging adults by the *k*-means cluster analysis, suggesting no inherent differences in the reaction time series data of patients with PD. Most patients were grouped with the TA1 cluster and both groups had no significant differences in mean RT or *n*-back score. When

the mean reaction times of each block were plotted, those of the patients with PD and the TA1 cluster were almost identical, except for one important difference: patients with PD were unable to transfer their learning to a novel fixed sequence.

Second, the TA2 and TA3 clusters were unable to learn the fixed sequence and both clusters also had significantly lower *n*-back scores indicating a critical role of working memory in motor sequence learning consistent with previous literature (Bo, et al., 2009; Bo, et al., 2011, 2012; Bo & Seidler, 2010).

Third, none of the typically aging clusters or the patients with PD were able to learn the probabilistic sequence. Probabilistic sequences are more challenging to learn, as seen in the results of the young adults, and have greater basal ganglia involvement (Aron & Poldrack, 2006; Ashby & Ell, 2001b; Ashby & Maddox, 2005, 2011; Ashby & O'Brien, 2005; Keri, 2003; Knowlton, et al., 1996; P. J. Reber, 2013; Seger, 2006a). However, probabilistic sequences reflect the adaptive learning required in real life more accurately. This is an important finding that addresses the functional quality of life with age and the ability to learn and perform activities of daily living.

Fourth, the cortical activations and connectivity did not exhibit differences between the typically aging adults and those with PD in the probabilistic sequence groups, suggesting that at least in the early stages of PD, impairments in motor sequence learning are more likely to be age-related. It is important to remember that the patients in this study were in the early stages of Parkinson’s disease and those in more advanced stages may have greater impairments that are related to PD.

Future directions

The results from these studies open greater avenues of research for future studies. The transitional probabilities used to create the probabilistic sequences used in these studies can be manipulated to create sequences with different complexities and assess whether typically aging adults and those with PD are also impaired at learning less complex probabilistic sequences. Perhaps gradually manipulating the probabilities from simple to more complex would aid in the learning of these sequences. In addition, the learning processes can be analyzed to uncover dynamics changes within and between blocks determine age- or PD-related differences in online and offline learning of motor sequences. These novel methods of analysis can provide greater insight into not only the nature of the impairments, but may also provide methods for interventions and improving learning.

Alternative statistical models can be used to model the landscape for the typically aging adults to assess differential effects of aging on motor sequence learning and to explore variability between and within individuals. This is particularly important for variables such as reaction time that have been demonstrated to be highly variable both within and between individuals. An example of an alternative approach is random coefficient modeling that provides a technique where individual performance as well as population level effects can be explored. In addition, covariates can be included in the model to account for differences between individuals and groups based on alternative variables such as preliminary assessments. The performance of the individual can be lost in general linear models and both perspectives are important in order to characterize typically aging as well as clinical populations as they can add additional variability due to diverse symptoms.

In order to achieve a more refined assessment of the spectral power and coherence analysis, frequency bands should be determined individually for each participant, rather than using fixed frequency bands. This would prevent contamination of power calculations by neighboring frequency bands, which is an important consideration as some bands change in the opposite direction during cognitive tasks, thus canceling any effects. For example, theta increases during engagement in a cognitive task, but alpha decreases, so a contamination of theta in the alpha power calculations may cancel out any changes in alpha.

To further examine cortico-cortical connectivity, functional connectivity analysis can be used to characterize large scale brain networks. Functional connectivity is the statistical association or dependency between brain regions that accounts for both linear and nonlinear associations and can be measured via synchronization likelihood calculations (Pijnenburg et al., 2008; Stam, Jones, Nolte, Breakspear, & Scheltens, 2007; Stam & van Dijk, 2002) and information theory methods such as mutual information and graph theory (Jin, Lin, Auh, et al., 2011; Jin, Lin, & Hallett, 2011). While previous research allows for the investigation of the interactions occurring between different cortical areas, they provide limited interpretations of the dynamics at the global network level (Doyon et al., 2009b; Jin, Lin, Auh, et al., 2011; Jin, Lin, & Hallett, 2011). Further research into changes in functional connectivity while performing a motor sequence learning task to provide greater insight into both behavioral differences and neural underpinnings of motor sequence learning.

Conclusion

To our knowledge, this is the first time that probabilistic and fixed sequences have been directly compared in typical young adults, typically aging adults, and patients with PD. We introduced a novel type of probabilistic sequence that more accurately reflects motor sequence learning, analyzed the underlying learning processes, and assessed transfer to a novel sequence. By unraveling movement and cognitive deficits and matching participants based on functional characteristics, we found that some typically aging adults and those with PD learned the fixed sequence, but not the probabilistic sequence, indicating age-related impairments in probabilistic motor sequence learning. We used a neuroimaging method that matches the temporal resolution of the task to assess differences in cortical dynamics between groups and across the task. By using these techniques, we provide a deeper understanding of this dynamic motor learning process and how it changes with age and Parkinson's disease.

References

Agostino, R., Sanes, J. N., & Hallett, M. (1996). Motor skill learning in Parkinson's disease. *Journal of the Neurological Sciences, 139*, 218-226.

Ahn, S., Zauber, S. E., Worth, R. M., Witt, T., & Rubchinsky, L. L. (2015). Interaction of synchronized dynamics in cortex and basal ganglia in Parkinson's disease. *European Journal of Neuroscience, 42*, 2164-2171.

Aizenstein, H. J., Butters, M. A., Clark, K. A., Figurski, J. L., Stenger, V. A., Nebes, R. D., et al. (2006). Prefrontal and striatal activation in elderly subjects during concurrent implicit and explicit sequence learning. *Neurobiology of Aging, 27*, 741-751.

Alexander, G. E., Delong, M. R., & Strick, P. L. (1986). Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annual Review of Neuroscience, 9*, 357-381.

Argyelan, M., Carbon, M., Ghilardi, M. F., Feigin, A., Mattis, P., Tang, C., et al. (2008). Dopaminergic suppression of brain deactivation responses during sequence learning. *Journal of Neuroscience, 28*, 10687-10695.

Armstrong, T., & Bull, F. (2006). Development of the World Health Organization Global Physical Activity Questionnaire (GPAQ). *Journal of Public Health, 14*, 66-70.

Aron, A. R., & Poldrack, R. A. (2006). Cortical and subcortical contributions to stop signal response inhibition: Role of the subthalamic nucleus. *Journal of Neuroscience, 26*, 2424-2433.

Aron, A. R., Poldrack, R. A., & Wise, S. P. (Eds.). (2009) Encyclopedia of Neuroscience (Vols. 2). Oxford: Academic Press.

Ashby, F. G., & Ell, S. W. (2001a). The neurobiology of human category learning. *Trends in Cognitive Sciences, 5,* 204-210.

Ashby, F. G., & Ell, S. W. (2001b). The neurobiology of human category learning. *Trends in Cognitive Sciences, 5*, 204-210.

Ashby, F. G., & Maddox, W. T. (2005). Human category learning. *Annual Review of Psychology, 56,* 149-178.

Ashby, F. G., & Maddox, W. T. (2011). Human category learning 2.0. *Annals of the New York Academy of Sciences, 1224*, 147-161.

Ashby, F. G., & O'Brien, J. B. (2005). Category learning and multiple memory systems. *Trends in Cognitive Sciences, 9,* 83-89.

Balslev, D., Nielsen, F. Å., Frutiger, S. A., Sidtis, J. J., Christiansen, T. B., Svarer, C., et al. (2002). Cluster analysis of activity-time series in motor learning. *Human Brain Mapping, 15,* 135-145.

Bennett, I. J., Howard, J. H., & Howard, D. V. (2007). Age-related differences in implicit learning of subtle third-order sequential structure. *Journal of Gerontology: Psychological Sciences, 62*, 98-103.

Bergman, H., & Deuschl, G. (2002). Pathophysiology of Parkinson's disease: From clinical neurology to basic neuroscience and back. *Movement Disorders, 17,* S28-S40.

Bhakuni, R., & Mutha, P., K. (2015). Learning of bimanual motor sequences in normal aging. *Frontiers in Aging Neuroscience, 7*, 76.

Bo, J., Borza, V., & Seidler, R. D. (2009). Age-related declines in visuospatial working memory correlate with deficits in explicit motor sequence learning. *Journal of Neurophysiology, 102*, 2744-2754.

Bo, J., Jennett, S., & Seidler, R. D. (2011). Working memory capacity correlates with implicit serial reaction time task performance. *Experimental Brain Research, 214*, 73-81.

Bo, J., Jennett, S., & Seidler, R. D. (2012). Differential working memory correlates for implicit sequence performance in young and older adults. *Experimental Brain Research, 221*, 467-477.

Bo, J., & Seidler, R. D. (2010). Spatial and symbolic implicit sequence learning in young and older adults. *Experimental Brain Research, 201*, 837-851.

Bock, O., Schneider, S., & Bloomberg, J. (2001). Conditions for interference versus facilitation during sequential sensorimotor adaptation. *Experimental Brain Research, 138*, 359-365.

Bonstrup, M., Hagemann, J., Gerloff, C., Sauseng, P., & Hummel, F. C. (2015). Alpha oscillatory correlates of motor inhibition in the aged brain. *Frontiers in Aging Neuroscience, 7*, 193.

Bostan, A. C., Dum, R. P., & Strick, P. L. (2010). The basal ganglia communicate with the cerebellum. *Proceedings of the National Academy of Sciences, 107*, 8452-8456.

Bostan, A. C., & Strick, P. L. (2010). The cerebellum and basal ganglia are interconnected. *Neuropsychology Review, 20*, 261-270.

Braver, T. S., & Barch, D. M. (2002). A thoery of cognitive control, aging cognition, and neuromodulation. *Neuroscience and Biobehavioral Reviews, 26*, 809-817.

Braver, T. S., Barch, D. M., Keys, B. A., Carter, C. S., Cohen, J. D., Kaye, J. A., et al. (2001). Cognitive processing in older adults: evidence for a theory relating cognitive control to neurobiology in healthy aging. *Journal of Experimental Psychology: General, 130*, 746-763.

Brown, P. (2003). Oscillatory nature of human basal ganglia activity: Relationship to the pathophysiology of Parkinson's disease. *Movement Disorders, 18*, 357-363.

Brown, R. M., & Robertson, E. M. (2007). Off-line processing: Reciprocal interactions between declarative and procedural memories. *Journal of Neuroscience, 27*, 10468-10475.

Carbon, M., & Eidelberg, D. (2006). Functional imaging of sequence learning in Parkinson's disease. *Journal of the Neurological Sciences, 248*, 72-77.

Carbon, M., Reetz, K., Ghilardi, M. F., Dhawan, V., & Eidelberg, D. (2010). Early Parkinson's disease: Longitudinal changes in brain activity during sequence learning. *Neurobiology of Disease, 37*, 455-460.

Cassidy, M., Mazzone, P., Oliviero, A., Insola, A., Tonali, P., Di Lazzaro, V., et al. (2002). Movement-related changes in synchronization in the human basal ganglia. *Brain, 125*, 1235-1246.

Catalan, M. J., Ishii, K., Honda, M., Samii, A., & Hallett, M. (1999). A PET study of sequential finger movements of varying length in patients with Parkinson's disease. *Brain, 122*, 483-495.

Cleeremans, A., & McClelland, J. L. (1991). Learning the structure of event sequences. *Journal of Experimental Psychology: General, 120,* 235-253.

Cleeremans, A., Servan-Schreiber, D., & McClelland, J. L. (1989). Finite state automata and simple recurrent networks. *Neural Computation, 1,* 372-381.

Cohen, M. X. (2014). *Analzying Neural Time Series Data*. Cambridge, MA: The MIT Press.

Cohen, R. G., & Sternad, D. (2009). Variability in motor learning: relocating, channeling and reducing noise. *Experimental Brain Research, 193,* 69-83.

Cools, R. (2011). Dopaminergic control of the striatum for high-level cognition. *Current Opinion in Neurobiology, 21,* 402-407.

Cools, R., & D'Esposito, M. (2006). Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biological Psychiatry, 69,* e113-e125.

Cudeck, R., & Harring, J. R. (2007). Analysis of nonlinear patterns of change with random coefficient models. *Annual Review of Psychology, 58,* 615-637.

Cudeck, R., & Harring, J. R. (2010). Developing a random coefficient model for nonlinear repeated measures data. In S.-M. Chow, E. Ferrer & F. Hsieh (Eds.), *Statistical methods for modeling human dynamics: An interdisciplinary dialogue*. New York: Routledge.

Curran, T. (1997). Effects of aging on implicit sequence learning: Accounting for sequence structure and explicit knowledge. *Psychological Research, 60,* 24-41.

D'Esposito, M., Zarahn, E., Aguirre, G. K., & Rypma, B. (1999). The effect of normal aging on the coupling of neural activity to the bold hemodynamic response. *NeuroImage, 10,* 6-14.

Daselaar, S. M., Rombouts, S. A. R. B., Veltman, D. J., Raaijmakers, J. G. W., & Jonker, C. (2003). Similar network activated by young and old adults during the acquisition of a motor sequence. *Neurobiology of Aging, 24*, 1013-1019.

DeCoster, J., & O'Mally, J. (2011a). Specific sequence effects in the serial reaction time task. *Journal of Motor Behavior, 43*, 263-273.

DeCoster, J., & O'Mally, J. (2011b). Specific Sequence Effects in the Serial Reaction Time Task. [Article]. *Journal of Motor Behavior, 43*(3), 263-273.

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics. *Journal of Neuroscience Methods, 134*, 9-21.

Dennis, N. A., Howard, J. H., & Howard, D. V. (2006). Implicit sequence learning without motor sequencing in young and old adults. *Experimental Brain Research, 175*, 153-164.

Destrebecqz, A., & Cleeremans, A. (2001). Can sequence learning be implicit? New evidence with the process dissociation procedure. *Psychonomic Bulletin & Review, 8*, 343-350.

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

Doya, K. (2000). Complementary roles of basal ganglia and cerebellum in learning and motor control. *Current Opinion in Neurobiology, 10*, 732-739.

Doyon, J. (2008). Motor sequence learning and movement disorders. *Current Opinion in Neurology, 21*, 478-483.

Doyon, J., Bellec, P., Amsel, R., Penhune, V., Monchi, O., Carrier, J., et al. (2009a). Contributions of the basal ganglia and functionally related brain structures to motor learning. *Behavioural Brain Research, 199*, 61-75.

Doyon, J., Bellec, P., Amsel, R., Penhune, V. B., Monchi, O., Carrier, J., et al. (2009b). Contributions of the basal ganglia and functionally related brain structures to motor learning. *Behavioural Brain Research, 199*, 61-75.

Doyon, J., & Benali, H. (2005). Reorganization and plasticity in the adult brain during learning of motor skills. *Current Opinion in Neurobiology, 15*, 161-167.

Doyon, J., Gaudreau, D., Laforce, R., Castonguay, M., Bedard, P. J., Bedard, F., et al. (1997). Role of the striatum, cerebellum, and frontal lobes in the learning of a visuomotor sequence. *Brain and Cognition, 34*, 218-245.

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

Doyon, J., & Ungerleider, L. G. (2002). Functional anatomy of motor skill learning. In L. R. Squire & D. L. Schacter (Eds.), *Neuropsychology of Memory, 3rd edition* (pp. 225-238): The Guilford Press.

Dum, R. P., & Strick, P. L. (2009). Basal ganglia and cerebellar circuits with the cerebral cortex. In M. S. Gazzaniga (Ed.), *The Cognitive Neurosciences* (4th ed., pp. 553-563). Cambridge, MA: The MIT Press.

Fahn, S., & Jankovic, J. (2007). *Principles and Practice of Movement Disorders.* Philadelphia: Churchill Livingstone Elsevier.

Fallon, S. J., Smulders, K., Esselink, R. A., van de Warrenburg, B. P., Bloem, B. R., & Cools, R. (2015). Differential optimal dopamine levels for set-shifting and working memory in Parkinson's disease. *Neuropsychologia, 77*, 42-51.

Feeney, J. J., Howard, J. H., & Howard, D. V. (2002). Implicit learning of higher order sequences in middle age. *Psychology and Aging, 17*, 351-355.

Feigin, A., Ghildardi, M. F., Carbon, M., Edwards, C., Fukuda, M., Dhawan, V., et al. (2003). Effects of levodopa on motor sequence learning in Parkinson's disease. *Neurology, 60*, 1744-1749.

Filoteo, J. V., & Maddoz, W. T. (2007). Category learning in Parkinson's disease. In M. Sun (Ed.), *Research Progress in Alzheimer's Disease and Dementia* (Vol. 3): Nova Science Publishers, Inc.

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research, 12*, 189-198.

Fraser, S. A., Li, K. Z. H., & Penhune, V. B. (2009). A comparison of motor skill learning and retention in younger and older adults. *Experimental Brain Research, 195*, 419-427.

Fries, P. (2005). A mechanism for cognitive dynamics: neuronal communication through neuronal coherence. *Trends in Cognitive Sciences, 9*, 474-480.

Fuhrer, H., Kupsch, A., Halbig, T. D., Kopp, U. A., Scherer, P., & Gruber, D. (2014). Levodopa inhibits habit-learning and Parkinson's disease. *Journal of Neural Transmission, 121*, 147-151.

Fukuda, M., Edwards, C., & Eidelberg, D. (2001). Functional brain networks in Parkinson's disease. *Parkinsonism and Related Disorders, 8,* 91-94.

Gabrieli, J. D. E., Singh, J., Stebbins, G. T., & Goetz, C. G. (1996). Reduced working memory span in Parkinson's disease: Evidence for the role of a frontostriatal system in working and strategic memory. *Neuropsychology, 10,* 322-332.

Gaillard, V., Destrebecqz, A., Michiels, S., & Cleeremans, A. (2009). Effects of age and practice in sequence learning: A graded account of ageing, learning, and control. *European Journal of Cognitive Psychology, 21,* 255-282.

Gamble, K. R., Cummings, T. J., Lo, S. E., Ghosh, P. T., Howard, J. H., & Howard, D. V. (2014). Implicit sequence learning in people with Parkinson's disease. *Frontiers in Human Neuroscience, 8,* 563.

Ghilardi, M. F., Eidelberg, D., Silvestri, G., & Ghez, C. (2003). The differential effect of PD and normal aging on early explicit sequence learning. *Neurology, 60,* 1313-1319.

Gobel, E. W., Blomeke, K., Zadikoff, C., Simuni, T., Weintraub, W., & Reber, P. J. (2013). Implicit perceptual-motor skill skill learning in Mild Cognitive Impairment and Parkinson's disease. *Neuropsychology, 27,* 314-321.

Goetz, C. G., Fahn, S., Martinez-Martin, P., Poewe, W., Sampaio, C., Stebbins, G. T., et al. (2007). Movement Disorder Society-sponsored revision of of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Process, format, and clinimetric testing plan. *Movement Disorders, 22,* 41-47.

Goetz, C. G., Tilley, B. C., Shaftman, S. R., Stebbins, G. T., Fahn, S., Martinez-Martin, P., et al. (2008). Movement Disorder Society-sponsored revision of the Unified

Parkinson's Disease Rating Scale (MDS-UPDRS): Scale presentation and clinimetric testing results. *Movement Disorders, 23,* 2129-2170.

Grant, D. A., & Berg, E. A. (1948). A behavioural analysis of degree of reinforcement and ease of shifting to new responses in a Weigl-type card sorting problem. *Journal of Experimental Psychology, 38,* 404-411.

Hayes, H. A., Hunsaker, N., Schaefer, S. Y., Shultz, B., Schenkenberg, T., Boyd, L., et al. (in press). Does dopamine replacement medication affect postural sequence learning in Parkinson's disease? *Motor Control*.

Hedden, T., & Gabrieli, J. D. E. (2004). Insights into the ageing mind: A view from cognitive neuroscience. *Nature Reviews Neuroscience, 5,* 87-97.

Hellwig, B., Haubler, S., Lauk, M., Guschlbauer, B., Koster, B., Kristeva-Feige, R., et al. (2000). Tremor-correlated cortical activity detected by electroencephalogram. *Clinical Neurophysiology, 111,* 806-809.

Helmuth, L. L., Mayr, U., & Daum, I. (2000). Sequence learning in Parkinson's disease: A comparison of spatial-attention and number-response sequences. *Neuropsychologia, 38,* 1443-1451.

Hikosaka, O., Nakamura, K., Sakai, K., & Nakahara, H. (2002). Central mechanisms of motor skill learning. *Current Opinion in Neurobiology, 12*, 217-222.

Hoehn, M. M., & Yahr, M. D. (1967). Parkinsonism: onset, progression and mortality. *Neurology, 17,* 427-442.

Hotermans, C., Peigneux, P., Maertens de Noordhout, A., Moonen, G., & Maquet, P. (2006). Early boost and slow consolidation in motor skill learning. *Learning & Memory, 13,* 580-583.

Houk, J. C., & Wise, S. P. (1995). Distributed modular architectures linking basal ganglia, cerebellum, and cerebral cortex: Their role in planning and controlling action. *Cerebral Cortex, 5*, 95-110.

Howard, D. V., & Howard, J. H. (2001). When it does hurt to try: Adult age differences in the effects of instructions on implicit pattern learning. *Psychonomic Bulletin & Review, 8,* 798-805.

Howard, D. V., Howard, J. H., Japikse, K., DiYanni, C., Thompson, A., & Somberg, R. (2004). Implicit sequence learning: Effects of level of structure, adult age, and extended practice. *Psychology and Aging, 19,* 79-92.

Howard, J. H., & Howard, D. V. (1997). Age differences in implicit learning of higher order dependencies in serial patterns. *Psychology and Aging, 12*, 634-656.

Howard, J. H., & Howard, D. V. (2013). Aging mind and brain: is implicit learning spared in healthy aging? *Frontiers in Psychology, 4*, 817.

Howard, J. H., Howard, D. V., Dennis, N. A., & Kelly, A. J. (2008). Implicit learning of predictive relationships in three-element visual sequences by young and old adults. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 34*, 1139-1157.

Huang, C., Tang, C., Feigin, A., Lesser, M., Ma, Y., Pourfar, M., et al. (2007). Changes in network activity with the progression of Parkinson's disease. *Brain, 130*, 1834-1846.

Hutchison, W. D., Dostrovsky, J. O., Walters, J. R., Courtemanche, R., Boraud, T., Goldberg, J., et al. (2004). Neuronal oscillations in the basal ganglia and

movement disorders: Evidence from whole animal and human recordings. *Journal of Neuroscience, 24*, 9240-9243.

Jackson, G. M., Jackson, S. R., Harrison, J., Henderson, L., & Kennard, C. (1995). Serial reaction time learning and Parkinson's disease: Evidence for a procedural learning deficit. *Neuropsychologia, 33*, 577-593.

Jaeggi, S. M., Buschkuehl, M., Jonides, J., & Perrig, W. J. (2008). Improving fluid intelligence with training on working memory. *Proceedings of the National Academy of Sciences, 105*, 6829-6833.

Jenkins, I. H., Brooks, D. J., Nixon, P. D., Frackowiak, R. S. J., & Passingham, R. E. (1994). Motor sequence learning: A study with positron emission tomography. *Journal of Neuroscience, 14*, 3775-3790.

Jimenez, L., & Mendez, C. (1999). Which attention is needed for implicit sequence learning? *Journal of Experimental Psychology: Learning, Memory, and Cognition, 25*, 236-259.

Jimenez, L., Mendez, C., & Cleeremans, A. (1996). Comparing direct and indirect measures of sequence learning. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 22*, 948-969.

Jin, S. H., Lin, P., Auh, S., & Hallett, M. (2011). Abnormal functional connectivity in focal hand dystonia: Mutual information analysis in EEG. *Movement Disorders, 26*, 1274-1281.

Jin, S. H., Lin, P., & Hallett, M. (2011). Abnormal reorganization of functional cortical small-world networks in focal hand dystonia. *PLoS One, 6*, e28682.

Jueptner, M., Frith, C. D., Brooks, D. J., Frackowiak, R. S. J., & Passingham, R. E. (1997). Anatomy of motor learning. II. Subcortical structures and learning by trial and error. *Journal of Neurophysiology, 77*, 1325-1337.

Kelly, R. M., & Strick, P. L. (2000). Rabies as a transneuronal tracer of circuits in the central nervous system. *Journal of Neuroscience Methods, 103*, 63-71.

Keri, S. (2003). The cognitive neuroscience of category learning. *Brain Research Reviews, 43*, 85-109.

King, B. R., Fogel, S. M., Albouy, G., & Doyon, J. (2013). Neural correlates of the age-related changes in motor sequence learning and motor adaptation in older adults. *Frontiers in Human Neuroscience, 7*, 1-13.

King, B. R., Harring, J. R., Oliveira, M. A., & Clark, J. E. (2011). Statistically characterizing intra- and inter-individual variability in children with Development Coordination Disorder. *Research in Developmental Disabilities, 32*, 1388-1398.

Klimesch, W. (1999). EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Research Reviews, 29*, 169-195.

Knowlton, B. J., Mangels, J. A., & Squire, L. R. (1996). A neostriatal habit learning system in humans. *Science, 273*, 1399-1402.

Kwak, Y., Mueller, M. L. T. M., Bohnen, N. I., Dayalu, P., & Seidler, R. D. (2010). Effect of dopaminergic medications on the time course of explicit motor sequence learning in Parkinson's disease. *Journal of Neurophysiology, 103*, 942-949.

Kwak, Y., Mueller, M. L. T. M., Bohnen, N. I., Dayalu, P., & Seidler, R. D. (2012). L-dopa changes ventral striatum recruitment during motor sequence learning in Parkinson's disease. *Behavioural Brain Research, 230*, 116-124.

Laming, D. R. J. (1968). *Information theory of choice-reaction times*: Academic Press Inc.

Laming, D. R. J. (1969). Subjective probability in choice-reaction experiments. *Journal of Mathematical Psychology, 6*, 81-120.

Levy, R., Hutchison, W. D., Lozano, A. M., & Dostrovsky, J. O. (2000). High-frequency synchronization of neuronal acvtivity in subthalamic nucleus of Parkinsonian patients with limb tremor. *Journal of Neuroscience, 20*, 7766-7775.

Li, S., Huxhold, O., Smith, J., Schmiedek, F., Rocke, C., & Lindenberger, U. (2008). Working memory plasticity in old age: Practice gain, transfer, and maintenance. *Psychology and Aging, 23,* 731-742.

Lin, C. J., Wu, A. D., Udompholkul, P., & Knowlton, B. J. (2010). Contextual interference effects in sequence learning for young and older adults. *Psychology and Aging, 25*, 929-939.

Lloyd, S. P. (1982). Least squares optimization in PCM. *IEEE Transactions of Information Theory, 28*, 129-137.

Lu, X. F., Hikosaka, O., & Miyachi, S. (1998). Role of monkey cerebellar nuclei in skill for sequential movement. *Journal of Neurophysiology, 79*, 2245-2254.

MacQueen, J. (1967). *Some methods for classification and analysis of multivariate observations.* Paper presented at the Fifth Berkeley Symposium on Mathematical Statistics and Probability, Berkeley.

McDonald, R. J., & White, N. M. (1993). A triple dissociation of memory systems: hippocampus, amygdala, and dorsal striatum. *Behavioral Neuroscience, 107*, 3-22.

Mendes, F. A., Pompeu, J. E., Lobo, A. M., da Silva, K. G., Oliveira, T. P., Zomignani, A. P., et al. (2012). Motor learning, retention and transfer after virtual-reality-based training in Parkinson's disease - effect of motor and cognitive demands of games: a longitudinal, controlled clinical study. *Physiotherapy, 217-223*.

Mentis, M. J., Dhawan, V., Nakamura, T., Ghilardi, M. F., Feigin, A., Edwards, C., et al. (2003). Enhancement of brain activation during trial-and-error sequence learning in early PD. *Neurology, 60*, 612-619.

Middleton, F. A., & Strick, P. L. (2000a). Basal ganglia and cerebellar loops: motor and cognitive circuits. *Brain Research Reviews, 31*, 236-250.

Middleton, F. A., & Strick, P. L. (2000b). Basal ganglia output and cognition: Evidence from anatomical, behavioral, and clinical studies. *Brain and Cognition, 42*, 183-200.

Milak, M. S., Shimansky, Y., Bracha, V., & Bloedel, J. R. (1997). Effects of inactivating individual cerebellar nuclei on the performance and retention of an operantly conditioned forelimb movement. *Journal of Neurophysiology, 78*, 939-959.

Moisello, C., Crupi, D., Tunik, E., Quartarone, A., Bove, M., Tononi, G., et al. (2009). The serial reaction time task revisited: A study on motor sequence learning with an arm-reaching task. *Experimental Brain Research, 194*, 143-155.

Muellbacher, W., Ziemann, U., Wissel, J., Dang, N., Kofler, M., Facchini, S., et al. (2002). Early consolidation in human primary motor cortex. *Nature, 415*, 640-644.

Mueller, S. T. (2010). A partial implementation of the BICA cognitive decathlon using the Psychology Experiment Building Language (PEBL). *International Journal of Machine Consciousness, 2*, 273-288.

Mure, H., Tang, C. C., Argyelan, M., Ghilardi, M. F., Kaplitt, M. G., Dhawan, V., et al. (2012). Improved sequence learning with subthalamic nucleus deep brain stimulation: Evidence for treatment-specific network modulation. *Journal of Neuroscience, 32*, 2804-2813.

Muslimovic, D., Post, B., Speelman, J. D., & Schmand, B. (2007). Motor procedural learning in Parkinson's disease. *Brain, 130*, 2887-2897.

Nagy, H., Keri, S., Myers, C. E., Benedek, G., Shohamy, D., & Gluck, M. A. (2007). Cognitive sequence learning in Parkinson's disease and amnestic mild cognitive impairment: Dissociation between sequential and non-sequential learning of associations. *Neuropsychologia, 45*, 1386-1392.

Nakamura, T., Ghilardi, M. F., Mentis, M., Dhawan, V., Fukuda, M., Hacking, A., et al. (2001). Functional networks in motor sequence learning: Abnormal topographies in Parkinson's disease. *Human Brain Mapping, 12*, 42-60.

National Institute of Neurological Disorders and Stroke. (2015). Parkinson's Disease: Hope Through Research. Retrieved October 12, 2015, 2015

Nemeth, D., & Janacsek, K. (2010). The dynamics of implicit skill consolidation in young and elderly adults. *Journal of Gerontology: Psychological Sciences, 66B*, 15-22.

Newell, K. M. (1991). Motor skill acquisition. *Annual Reviews of Psychology, 42*, 213-237.

Newell, K. M., & Shapiro, D. C. (1976). Variability of practice and transfer of training: Some evidence toward a schema view of motor learning. *Journal of Motor Behavior, 8*, 233-243.

Nissen, M. J., & Bullemer, P. (1987). Attentional requirements of learning: Evidence from performance measures. *Cognitive Psychology, 19*, 1-32.

Nunez, P. L. (2000). Toward a quantitative description of large-scale neocortical dynamic function and EEG. *Behavioral and Brain Sciences, 23*, 371-437.

Owen, A. M., Doyon, J., Dagher, A., Sadikot, A., & Evans, A. C. (1998). Abnormal basal ganglia outflow in Parkinson's disease identified with PET: Implications for higher cortical functions. *Brain, 121*, 949-965.

Packard, M. G., & Knowlton, B. J. (2002). Learning and memory functions of the basal ganglia. *Annual Review of Neuroscience, 25*, 563-593.

Parkinson's Disease Foundation. (2015). Statistics on Parkinson's. Retrieved October 2, 2015, 2015, from http://www.pdf.org/en/parkinson_statistics

Pascual-Leone, A., Grafman, J., Clark, K., Stewart, M., Massaquoi, S., Lou, J. S., et al. (1993). Procedural learning in Parkinson's disease and cerebellar degeneration. *Annals of Neurology, 34*, 594-602.

Peigneux, P., Maquet, P., Meulemans, T., Destrebecqz, A., Laureys, S., Degueldre, C., et al. (2000). Striatum forever, despite sequence learning variability: A random effect analysis of PET data. *Human Brain Mapping, 10*, 179-194.

Pendt, L. K., Reuter, I., & Muller, H. (2011). Motor skill learning, retention, and control deficits in Parkinson's disease. *PLoS One, 6*, e21669.

Penhune, V., & Doyon, J. (2002). Dynamic cortical and subcortical networks in learning and delayed recall of timed motor sequences. *Journal of Neuroscience, 22,* 1397-1406.

Pijnenburg, Y. A. L., Strijers, R. L. M., vd Made, Y., van der Flier, W. M., Scheltens, P., & Stam, C. J. (2008). Investigation of resting-state EEG functional connectivity in frontotemporal lobar degeneration. *Clinical Neurophysiology, 119,* 1732-1738.

Poldrack, R. A., Sabb, F. W., Foerde, K., Tom, S. M., Asarnow, R. F., Bookheimer, S. Y., et al. (2005). The neural correlates of motor skill automaticity. *Journal of Neuroscience, 25,* 5356-5364.

Polich, J. (1997). EEG and ERP assessment of normal aging. *Electroencephalography and Clinical Neurophysiology, 104,* 244-256.

Prashad, S., Du, Y., & Clark, J. E. Probabilistic sequences offer a unique window on motor sequence learning.

Ratcliff, R. (1993). Methods for dealing with reaction time outliers. *Psychological Bulletin, 114,* 510-532.

Reber, A. S. (1967a). Implicit learning of artificial grammars. *Journal of Verbal Learning and Verbal Behavior, 6,* 855-863.

Reber, A. S. (1967b). Implicit learning of artificial grammars. *Journal of Verbal Learning and Verbal Behavior, 6,* 317-327.

Reber, A. S. (1989a). Implicit learning and tacit knowledge. *Journal of Experimental Psychology: General, 118,* 219-235.

Reber, A. S. (1989b). Implicit learning and tacit knowledge. *Journal of Experimental Psychology, 118,* 219-235.

Reber, P. J. (2013). The neural basis of implicit learning and memory: A review of neuropsychological and neuroimaging research. *Neuropsychologia, 51*, 2026-2042.

Reber, P. J., & Squire, L. R. (1994). Parallel brain systems for learning with and without awareness. *Learning & Memory*, *1*, 217-229.

Reber, P. J., & Squire, L. R. (1999a). Intact learning of artificial grammars and intact category learning by patients with Parkinson's disease. *Behavioral Neuroscience, 113*, 235-242.

Reber, P. J., & Squire, L. R. (1999b). Intact learning of artificial grammars and intact category learning by patients with Parkinson's disease *Behavioral Neuroscience, 113*, 235-242.

Reed, J., & Johnson, P. (1994). Assessing implicit learning with direct tests: Determining what is learned about sequence structure. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 20*, 585-594.

Rieckmann, A., & Backman, L. (2009). Implicit learning in aging: Extant patterns and new directions. *Neuropsychology Review, 19*, 490-503.

Rieckmann, A., Fischer, H., & Backman, L. (2010). Activation in striatum and medial temporal lobe during sequence learning in younger and older adults: Relations to performance. *NeuroImage, 50*, 1303-1312.

Robertson, E. M. (2007). The serial reaction time task: Implicit motor skill learning? *Journal of Neuroscience, 27*, 10073-10075.

Robertson, E. M., Pascual-Leone, A., & Miall, R. C. (2004). Current concepts in procedural consolidation. *Nature Reviews Neuroscience, 5*, 576-582.

Rossini, P. M., Rossi, S., Babiloni, C., & Polich, J. (2007). Clinical neurophysiology of aging brain: From normal aging to neurodegeneration. *Progress in Neurobiology, 83*, 375-400.

Ruitenberg, M. F. L., Duthoo, W., Santens, P., Notebaert, W., & Abrahamse, E. L. (2015). Sequential movement skill in Parkinson's disease: A state-of-the-art. *Cortex, 65*, 102-112.

Sakai, K., Hikosaka, O., Miyauchi, S., Takino, R., Sasaki, Y., & Putz, B. (1998). Transition of brain activation from frontal to parietal areas in visuomotor sequence learning. *Journal of Neuroscience, 18*, 1827-1840.

Salthouse, T. A. (1996). The processing-sped theory of adult age differences in cognition. *Psychological Review, 103*, 403-428.

Sanchez, D. J., Yarnik, E. N., & Reber, P. J. (2014). Quantifying transfer after perceptual-motor sequence learning: how inflexible is implicit learning? *Psychological Research, 79*, 327-343.

Schmitz, R., Schabus, M., Perrin, F., Luxen, A., Maquet, P., & Peigneux, P. (2009). Recurrent boosting effects of short inactivity delays on performance: an ERPs study. *BMC Research Notes, 2*, 170.

Schnitzler, A., & Gross, J. (2005). Normal and pathological oscillatory communication in the brain. *Nature Reviews Neuroscience, 6*, 285-296.

Schultz, W., Tremblay, L., & Hollerman, J. R. (2000). Reward processing in primate orbitofrontal cortex and basal ganglia. *Cerebral Cortex, 10*, 272-283.

Schvaneveldt, R. W., & Gomez, R. L. (1998). Attention and probabilistic sequence learning. *Psychological Research, 61*, 175-190.

Seger, C. A. (1994). Implicit learning. *Psychological Bulletin, 115*, 163-196.

Seger, C. A. (2006a). The basal ganglia in human learning. *Neuroscientist, 12*, 285-290.

Seger, C. A. (2006b). The basal ganglia in human learning. *Neuroscientist, 12*(4), 285-290.

Seidler, R. D. (2007). Older adults can learn to learn new motor skills. *Behavioural Brain Research, 183*, 118-122.

Seidler, R. D. (2010). Neural correlates of motor learning, transfer of learning, and learning to learn. *Exercise and Sport Sciences Reviews, 38*, 3-9.

Seidler, R. D., Bernard, J. A., Burutolu, T. B., Fling, B. W., Gordon, M. T., Gwin, J. T., et al. (2010). Motor control and aging: Links to age-related brain structural, functional, and biochemical effects. *Neuroscience and Biobehavioral Reviews, 34*, 721-733.

Seidler, R. D., Bo, J., & Anguera, J. A. (2012). Neurocognitive contributions to motor skill learning: The role of working memory. *Journal of Motor Behavior, 44*, 445-453.

Seidler, R. D., Purushotham, A., Kim, S. G., Ugurbil, K., Willingham, D., & Ashe, J. (2005). Neural correlates of encoding and expression in implicit sequence learning. *Experimental Brain Research, 165*, 114-124.

Seo, M., Beigi, M., Jahanshahi, M., & Averbeck, B. B. (2010). Effects of dopamine medication on sequence learning with stochastic feedback in Parkinson's disease. *Frontiers in Systems Neuroscience, 4*, 1-9.

Shin, J. C., & Ivry, R. B. (2003). Spatial and temporal sequence learning in patients with Parkinson's disease or cerebellar lesions. *Journal of Cognitive Neuroscience, 15*, 1232-1243.

Shohamy, D., Myers, C. E., Geghman, K. D., Sage, J., & Gluck, M. A. (2006). L-dopa impairs learning, but spares generalization in Parkinson's disease. *Neuropsychologia, 44*, 774-784.

Siegert, R. J., Taylor, K. D., Weatherall, M., & Abernethy, D. A. (2006). Is implicit sequence learning impaired in Parkinson's disease? A meta-analysis. *Neuropyschology, 20*, 490-495.

Simon, J. R., Howard, J. H., & Howard, D. V. (2010). Age differences in implicit learning of probabilistic unstructured sequences. *Journal of Gerontology: Psychological Sciences, 66B*, 32-38.

Smith, J. G., & McDowall, J. (2006). The implicit sequence learning deficit in patients with Parkinson's disease. *Neuropsychologia, 44*, 275-288.

Smith, J. G., Siegert, R. J., & McDowall, J. (2001). Preserved implicit learning on both the serial reaction time task and artificial grammar in patients with Parkinson's disease. *Brain and Cognition, 45*, 378-391.

Song, S., Howard, J. H., & Howard, D. V. (2007a). Implicit probabilistic sequence learning is independent of explicit awareness. *Learning & Memory, 14*, 167-176.

Song, S., Howard, J. H., & Howard, D. V. (2007b). Sleep Does Not Benefit Probabilistic Motor Sequence Learning *Journal of Neuroscience, 27*, 12475-12483.

Song, S., Howard, J. H., & Howard, D. V. (2008). Perceptual sequence learning in a serial reaction time task. *Experimental Brain Research, 189*, 145-158.

Squire, L. R., & Zola, S. M. (1996). Structure and function of declarative and nondeclarative memory systems. *Proceedings of the National Academy of Sciences, 93*, 13515-13522.

Srinivasan, R., Nunez, P. L., & Silberstein, R. B. (1998). Spatial filtering and neocortical dynamics: Estimates of EEG coherence. *IEEE Transactions on Biomedical Engineering, 45*, 814-826.

Stadler, M. A. (1992). Statistical structure and implicit serial learning. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 18*, 318-327.

Stadler, M. A., & Frensch, P. A. (1998). *Handbook of implicit learning*. Thousand Oaks, CA: Sage Publications, Inc.

Stam, C. J., Jones, B. F., Nolte, G., Breakspear, M., & Scheltens, P. (2007). Small-world networks and functional connectivity in Alzheimer's disease. *Cerebral Cortex, 17*, 92-99.

Stam, C. J., & van Dijk, B. W. (2002). Synchronization likelihood: an unbiased measure of generalized synchronization in multivariate data sets. *Physica D, 163*, 236-251.

Stephan, M. A., Meier, B., Zaugg, S. W., & Kaelin-Lang, A. (2011). Motor sequence learning performance in Parkinson's disease depends on the stage of disease. *Brain and Cognition, 75*, 135-140.

Strick, P. L., & Card, J. P. (1992). Transneuronal mapping of neural circuits with alpha herpesviruses. In J. P. Bolam (Ed.), *Experimental Neuroanatomy: A practical appraoch* (pp. 81-101). Oxford, UK: Oxford University Press.

Timmermann, L., Goss, J., Dirks, M., Volkmann, J., Freund, H., & Schnitzler, A. (2003). The cerebral oscillatory network of parkinsonian resting tremor. *Brain, 126,* 199-212.

Tremblay, P., Bedard, M., Langlois, D., Blanchet, P. J., Lemay, M., & Parent, M. (2010). Movement chunking during sequence learning is a dopamine-dependent process: a study conducted in Parkinson's disease. *Experimental Brain Research, 205,* 375-385.

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

Weiermann, B., & Meier, B. (2012). Incidental sequence learning across the lifespan. *Cognition, 123,* 380-391.

Westwater, H., McDowall, J., Siegert, R., Mossman, S., & Abernethy, D. (1998). Implicit learning in Parkinson's disease: Evidence from a verbal version of the serial reaction time task. *Journal of Clinical and Experimental Neuropsychology, 20,* 413-418.

Whelan, R. (2008). Effective analysis of reaction time data. *Psychological Record, 58,* 475-482.

White, N. M. (1997). Mnemonic functions of the basal ganglia. *Current Opinion in Neurobiology, 7,* 164-169.

Wichmann, T., Bergman, H., & DeLong, M. R. (1994). The primate subthalamic nucleus. III. Changes in motor behavior and neuronal activity in the internal pallidum induced by subthalamic inactivation in the MPTP model of Parkinsonism. *Journal of Neurophysiology, 72,* 521-531.

Wilkinson, L., & Jahanshahi, M. (2007). The striatum and probabilistic implicit sequence learning. *Brain Research, 1137*, 117-130.

Wilkinson, L., Khan, Z., & Jahanshahi, M. (2009). The role of the basal ganglia and its cortical connections in sequence learning: Evidence from implicit and explicit sequence learning in Parkinson's disease. *Neuropsychologia, 47*, 2564-2573.

Williams, D., Tijssen, M., van Bruggen, G., Bosch, A., Insola, A., Di Lazzaro, V., et al. (2002). Dopamine-dependent changes in the functional connectivity between basal ganglia and cerebral cortex in humans. *Brain, 125*, 1558-1569.

Willingham, D. B. (1998). A neuropsychological theory of motor skill learning. *Psychological Review, 105*, 558-584.

Willingham, D. B. (1999). Implicit motor sequence learning is not purely perceptual. *Memory & Cognition, 27*, 561-572.

Willingham, D. B., & Goedert-Eschmann, K. (1999). The relation between implicit and explicit learning: Evidence for parallel development. *Psychological Science, 10*, 531-534.

Willingham, D. B., Greenberg, A. R., & Thomas, R. C. (1997). Response-to-stimulus interval does not affect implicit motor sequence learning, but does affect performance. *Memory & Cognition, 25*, 534-542.

Willingham, D. B., Nissen, M. J., & Bullemer, P. (1989). On the development of procedural knowledge. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 15*, 1047-1060.

Willingham, D. B., Wells, L. A., Farrell, J. M., & Stemwedel, M. E. (2000). Implicit motor sequence learning is represented in response locations. *Memory & Cognition, 28*, 366-375.

Wu, T., & Hallett, M. (2005). The influence of normal human ageing on automatic movements. *Journal of Physiology, 562*, 605-615.

Wulf, G., & Schmidt, R. A. (1997). Variability of practice and implicit motor learning. *Learning, Memory, and Cognition, 23*, 987-1006