

# Differences in implicit motor learning between adults who do and do not stutter

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## ABSTRACT

Implicit learning allows us to acquire complex motor skills through repeated exposure to sensory cues and repetition of motor behaviours, without awareness or effort. Implicit learning is also critical to the incremental fine-tuning of the perceptual-motor system. To understand how implicit learning and associated domain-general learning processes may contribute to motor learning differences in people who stutter, we investigated implicit finger-sequencing skills in adults who do (AWS) and do not stutter (ANS) on an Alternating Serial Reaction Time task. Our results demonstrated that, while all participants showed evidence of significant sequence-specific learning in their speed of performance, male AWS were slower and made fewer sequence-specific learning gains than their ANS counterparts. Although there were no learning gains evident in accuracy of performance, AWS performed the implicit learning task more accurately than ANS, overall. These findings may have implications for sex-based differences in the experience of developmental stuttering, for the successful acquisition of complex motor skills during development by individuals who stutter, and for the updating and automatization of speech motor plans during the therapeutic process.

## 1. Introduction

Speech production is a highly specialised and complex human behaviour, relying on the smooth and efficient coordination, integration, and automaticity of several neural and physiological processes. Although directed by the explicit goal of communication, the typical course of speech and language development can be seen as an intuitive adaptation to our linguistic environment, without conscious effort or awareness (Perruchet and Pacton, 2006; Shanks, 2005). During infancy, early recognition of statistical regularities or high-probability auditory patterns in our environment, such as the sound and syllable strings that form words and sentence structures, are thought to call upon domain-general mechanisms of implicit learning which shape the development of speech motor skill (Saffran et al., 1996; Slone and Johnson, 2018; Kirkham et al., 2002). Implicit mechanisms continue to facilitate speech motor control through updating of motor plans and allowing for the adaptability of our responses to the demands and

dynamics of verbal communication (Houde and Jordan, 1998, 2002; Gracco and Abbs, 1985). Speech motor development draws upon our linguistic, auditory, cognitive, as well as motor capacities, all of which have been implicated in the experience of developmental stuttering. This less typical course of speech development and the precise role of implicit learning therein, particularly for those who persist to experience speech dysfluency throughout their lives, is not well understood, however.

Overtly characterised by involuntary dysfluencies in speech production that present as sound repetitions, prolongations or blocks on the initiation of speech segments, the behavioural symptoms of developmental stuttering typically emerge between the ages of 18 months and 6 years (Bloodstein et al., 2021; Yairi and Ambrose, 2005). At this stage of development, the demands of coordinated movement and learning increase to enable longer, more complex linguistic and articulatory structures to be produced. Moments of dysfluency are marked by disruptions of the respiratory, phonatory, and/or articulatory movements,

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as well as disruption of their coordination (Max et al., 2004). Those who stutter report physical, emotional, and cognitive impacts of dysfluency, with feelings of “losing control” (Tichenor and Yaruss, 2018), and being unable to consciously progress with speech production (Bloodstein and Shogan, 1972; Moore and Perkins, 1990). Developmental stuttering has a strong neurobiological basis, with genetic inheritance, neural development and sex differences influencing the diverging trajectories of children who initially develop stuttering but gain fluency in their early years from those who persist to experience stuttering into adulthood (Ambrose et al., 1997; Benito-Aragón et al., 2020; Briley et al., 2021; Chang et al., 2018; Chow et al., 2020; Garnett et al., 2019). Behavioural indicators that interact with these risk factors of persistence include higher stuttering frequency, differences in expressive and receptive language abilities, and reduced accuracy of verbal sequencing skills (Singer et al., 2020, 2022; Walsh et al., 2021; Walsh et al., 2018).

For adults who persist to experience stuttering, the most commonly practised treatment approaches aim to reduce its frequency by targeting speech motor and cognitive behaviours, as well as managing the potential of relapse that can challenge the long-term maintenance of treatment gains (Andrews and Craig, 1988; Bloodstein et al., 2021; Craig and Hancock, 1995). Behavioural interventions often use speech retraining approaches for the establishment and retention of fluency-enhancing behaviours, such as the use of prolonged speech (Goldiamond, 1965), or by modifying the initiation of speech sound production, using techniques such as gentle voice onsets (Webster, 1974). Successful treatment outcomes rely in part on motor skill learning and maintenance, so that these skills can be automatically and effortlessly applied to everyday communicative contexts, with individuals no longer feeling the need to consciously monitor their speech (Bloodstein et al., 2021).

### 1.1. Implicit learning of motor skill

Throughout development and in everyday life, much of our behaviour comprises of a set of movements or responses that are organized into sequences. Without intention or awareness, complex skills can emerge as a consequence of repeated exposure to sensory cues and the repetition of a motor act, or set of responsive actions. This process of implicit learning does not involve or depend on declarative memory systems, but is assumed to rely on a procedural learning system, whereby learning is evidenced through gradual improvement in performance, and without awareness or the ability to describe what is learned (Cohen and Squire, 1980; Nissen and Bullemer, 1987; Willingham et al., 2002). Where explicit instruction and conscious imitation are involved from the outset, explicitly acquired skills can also become proceduralized, more implicit and automatic with extensive practice (Ashby and Crossley, 2012).

Whether under implicit or explicit conditions, the successful acquisition of complex motor skills follows a process of learning movement responses, of how to control and adjust these movements, and of improving the efficiency of their execution with increased speed and accuracy (Doyon et al., 2009; Luft and Buitrago, 2005). More complex motor skills, which involve successive gestures in an appropriate, finely tuned, temporal order, such as the movement of the articulators to produce speech or of the fingers to play a musical instrument, bind together to form motor chunks or hierarchical associative structures that facilitate their memory representation, as well as their increasingly automatic and flexible execution (Doyon et al., 2018; Krakauer et al., 2019; Sakai et al., 2004). Evidence of learning can be observed not only in improved performance capacities and adaptability, but also in the reduced attentional demands of motor performance (Magill and Anderson, 2017; Schmidt and Lee, 2005).

From early practice, within-session performance improvements can emerge rapidly across initial trials, also inducing experience-dependent changes at a neural level (Doyon and Benali, 2005; Doyon et al., 2018; Fogel et al., 2017; Karni et al., 1998). This fast learning phase is followed

by slower learning, which sees performance improve even without additional practice (Karni et al., 1998). These “off-line” improvements of motor skill and the stabilization of their memory representation requires the passing of time after practice. Whereas, explicitly learned skill and movement goals are also enhanced by sleep, skills that are acquired unintentionally can show offline gains regardless of sleep (Cohen et al., 2005; Fogel et al., 2017; Nemeth et al., 2010; Robertson et al., 2004). Motor memory consolidation between sessions, along with further practice opportunities, can lead to well-learned, less attention-demanding, and more robust motor skill performance and chunk formation that are resistant to interference or decay (Krakauer and Shadmehr, 2006; Song, 2009; Wymbs et al., 2012).

Implicit or incidental learning is of particular importance to the acquisition of complex skills that require incremental fine-tuning of the perceptual-motor system and updating of internal models through repeated practice (Fitts, 1964; Tourville and Guenther, 2011). From early development, we rely on repeated practice of discrete motor actions in the correct sequential order, as well as the repeated exposure to positive instances or patterns in our environment that enable prediction-feedback learning of associations, to improve the accuracy and efficiency of movement responses (Doyon et al., 2009; Hikosaka et al., 2002; Perruchet and Pacton, 2006). In addition, already established motor programs may require updating of their internal states (i.e., neural representations) through implicit recalibration of the existing motor controller, when new or divergent contextual and sensorimotor information is available (Krakauer et al., 2019). These motor adaptation processes can also take place without explicit strategizing, conscious effort or awareness (Taylor et al., 2014). In speech motor production, feedback and feedforward processes rely on relevant auditory and somatosensory information to guide and adapt speech sound production and termination in a timely and fluent manner (Bohland et al., 2010; Chang and Guenther, 2020) – processes that have been found to be disrupted in developmental stuttering (Cai et al., 2014).

### 1.2. Motor learning in persons who stutter

To date, the majority of behavioural research has employed either motor sequencing or adaptation paradigms to investigate whether persons who do and do not stutter differ in their performance of explicitly instructed oral or manual motor skills. Investigations of motor sequence learning, involving male participants, have shown the acquisition of 10-element syllable or finger sequences to be slower in male adults who stutter (AWS) than in age- and sex-matched fluent speakers, particularly in early practice trials (Bauerly and De Nil, 2011, 2015; Smits-Bandstra et al., 2006a). Practice under single and dual-task conditions has further demonstrated slower, more variable, and attention-demanding performance of finger sequencing in male AWS than male adults who do not stutter (ANS) (Bauerly and De Nil, 2015; Smits-Bandstra and De Nil, 2007, 2009), with their sequences also differentiated by greater within-chunk intervals in male AWS (Smits-Bandstra and De Nil, 2013).

More recent studies, involving both male and female participants, have found that, although AWS can be less accurate or slower compared to ANS at early stages of learning, AWS can improve their verbal or finger sequencing performance with extended practice and retain their learning gains following a latent period of 24 hours, similarly to ANS (Korzeczek et al., 2020; Masapollo et al., 2021). The processing and articulatory demands of a syllable sequencing task, evidenced in neural activation of regions supporting verbal working memory and motor planning, were also found to decrease for AWS and ANS alike (Masapollo et al., 2021). In these recent studies, the potential effects of sex-based differences in motor learning were not explored.

Two studies of implicit motor learning in AWS have reported reduced sensorimotor learning on an auditory-motor adaptation task (Kim and Max, 2021) and reduced sequence learning on a verbal serial reaction time task (SRTT), when compared to the performance gains of ANS (Smits-Bandstra and Gracco, 2013). Although implicit sequence-specific

learning was significantly reduced compared to that of ANS, performance in AWS was found to not significantly differ from that of individuals with Parkinson’s disease (Smits-Bandstra and Gracco, 2013), for whom SRTT studies have demonstrated clear impairments in procedural memory and implicit learning (Clark et al., 2014). These observed similarities may point towards shared impairments in the underlying functional networks required for successful learning, retention, and automatization of motor sequencing skills, involving the cortico-striato-thalamo-cortical circuit (Chang and Guenther, 2020; Smits-Bandstra and Gracco, 2013, 2015; Smits-Bandstra and De Nil, 2007), and in the basal ganglia, specifically (Alm, 2004, 2021; Cler et al., 2021; Craig-McQuaide et al., 2014; Giraud et al., 2008).

1.3. The current study

With limited evidence of implicit motor learning abilities in individuals with persistent developmental stuttering, it remains unclear whether AWS experience difficulties in learning more complex motor skills, consolidating these skills, and whether modality-independent learning processes are also implicated. With the aim of understanding how implicit motor learning and its supporting procedural processes may contribute to hypothesized motor learning difficulties in people who stutter, we investigated implicit motor sequencing skills in adults who do and do not stutter on the Alternating Serial Reaction Time (ASRT) task. The ASRT is an implicit finger-sequencing paradigm, which previous research has shown to tap into implicit sequence learning and procedural memory processes, without participants developing explicit awareness of the hidden sequence pattern (Howard et al., 2004; Song et al., 2007).

A non-verbal experimental paradigm was used with the purpose of investigating the modality-independent procedural processes that support complex motor skill acquisition, while also eliminating the possible confound of the speech demands placed by a verbal sequencing task. Previous research has demonstrated comparable differences in finger-sequencing and syllable-sequencing performance between AWS and ANS (Bauerly and De Nil, 2015; Smits-Bandstra et al., 2006a).

Explicitly instructed sequences may be stored in their component elements instead of consolidating as a functional representation of the whole or chunked sequence (Beukema et al., 2019). By adapting the

standard deterministic sequence of stimulus driven SRTTs to a probabilistic sequence, which is dependent on the statistical regularities of how visual or auditory stimuli are presented, the acquisition of complex sequences can be probed more closely (Krakauer et al., 2019; Nissen and Bullemer, 1987). Thus, instead of following a first-order sequence (e.g., 1-2-3-4), the ASRT task involves probabilistic sequence learning. Within a probabilistic sequence, random (R) elements alternately intersperse the sequenced pattern (e.g., 1-R-2-R-3-R-4-R), creating a second-order conditional paradigm (Fletcher et al., 2005; Howard and Howard, 1997; Remillard, 2008). Because the fixed elements of the sequence are followed by random elements, the extension of learning to a three element-dependency within the sequence results in second-order conditional probabilities having to be learned (Remillard, 2008), whilst also rendering a complexity to the sequence that makes it more difficult to reach the explicit awareness of participants completing the task.

Adapting the original 10-trial sequence paradigm of the SRTT (Nissen and Bullemer, 1987), a 10-element alternating sequence was used in the current study, in which five elements followed a sequence-specific pattern and, between these, every second element was random (see Fig. 1). Unlike previous adaptations (Fletcher et al., 2005), there were no conditions placed on the alternating elements, other than these being random. Within a given sequence, e.g., 1-R-2-R-3-R-4-R-3-R in which the numbers represent the spatial locations of the visual stimulus, some three-element chunks or “triplets” would occur more frequently than others. In the current example, 1\_2, 2\_3, 3\_4, 4\_3, and 3\_1 would occur in higher frequency than 1\_3, 1\_4, 2\_1, 2\_4 etc., because two second-order sequenced elements as well as two consecutive random elements could contribute to the formation of higher-frequency triplets (e.g., 132 could be formed by 1-R-2 or R-3-R), while only consecutive random elements could form low-frequency triplets (e.g., 123 could only be formed by R-2-R) (Janacsek et al., 2012). This triplet-level processing involves frequency learning, as well as probability learning, with high-frequency triplets being more predictable than low-frequency triplets (Szegedi-Hallgató et al., 2019). Previous studies have demonstrated that, in addition to general motor skill learning that is measured by improvements in speed of performance across blocks, sequence-specific learning can be measured by comparing the speed and accuracy of participants’ responses to the final elements of high-frequency triplets to that of their response to the final elements of low-frequency triplets

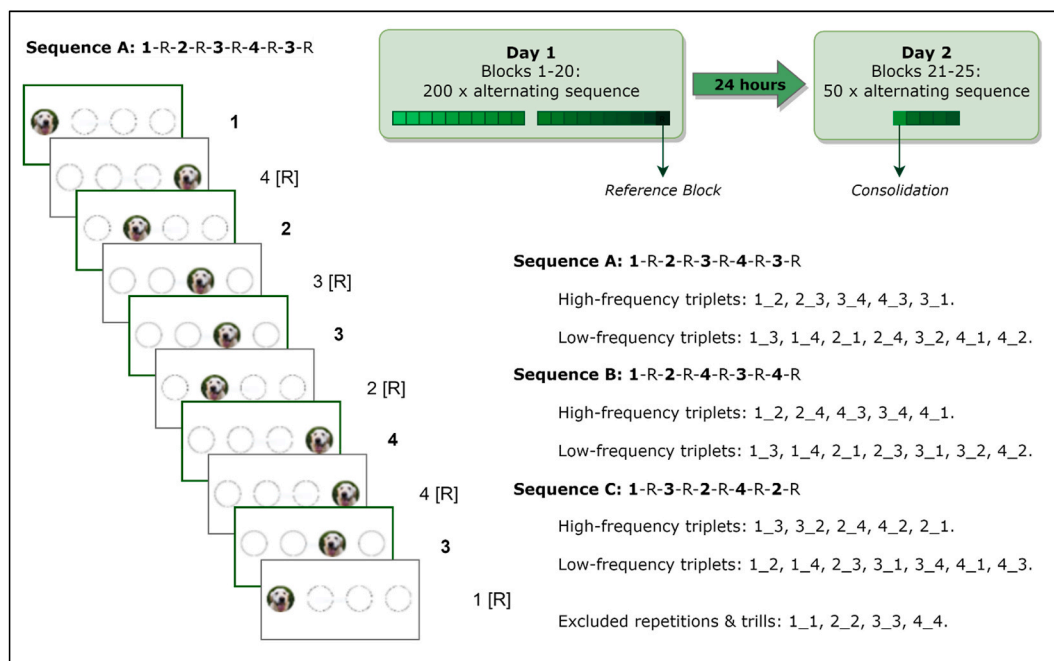


Fig. 1. Alternating Serial Reaction Time (ASRT) task design and experimental schedule.

(Howard and Howard, 1997; Janacek et al., 2012; Savic and Meier, 2016; Song et al., 2007).

On the ASRT, participants are presented with a visual stimulus or spatial marker, to which they are instructed to provide a motor response, thereby implicating perceptual motor skills or “spatio-motor sequencing” (Dennis et al., 2006; Nemeth et al., 2009). Although perceptual cues have been found to enhance sequence learning (Robertson and Pascual-Leone, 2001), perceptual sequence representations have also been found to develop more slowly (Gheysen et al., 2009) and to consolidate differentially to motor representations (Albouy et al., 2013, 2015). Thus, to investigate memory consolidation, the ASRT task was repeated in a second session, after an intervening period of approximately 24 hours, during which participants had no further exposure to the task.

Employing a complex sequence and omitting any explicit instruction of what was to be learned, the task was used to evaluate the ability of participants to acquire a new skill in an implicit manner. We hypothesized that, when compared to sex- and age-matched ANS, implicit sequence-specific learning would be reduced in AWS on a nonverbal motor sequencing task. These reductions would be evidenced by slower reaction times, decreased accuracy, and reduced consolidation, when comparing group performance in response to high- and low-frequency triplets.

## 2. Material and methods

### 2.1. Participants

Fifteen AWS (4 female, mean age = 31.9 years  $\pm$ 7.3) and 15 ANS (5 female, mean age = 31.6 years  $\pm$ 6.5) performed the ASRT task across two sessions, which were separated by approximately 24 hours. The groups were matched by age and sex, as previous research has implicated the influence of each factor on motor skill and reaction time performance (Bennett et al., 2007; Der and Deary, 2006; Dorfberger et al., 2009; Lissek et al., 2007; Nemeth and Janacek, 2011), with implicit motor learning abilities being similarly stable in adults between 18 and 44 years of age (Janacek et al., 2012). All participants self-identified as either male or female. The sex of participants was not balanced within each group, as the male to female ratios reflect the unequal prevalence ratio observed among AWS in the general population (Yairi and Ambrose, 2013). Three additional male AWS participants and one male ANS were not included due to incomplete data, failure to follow task instructions, or delays in follow-up testing. Based on previous research using the ASRT paradigm, two samples of 15 participants were originally deemed sufficient for between-group comparison on the task (Fletcher et al., 2005; Howard and Howard, 1997; Howard et al., 2006; Song et al., 2007).

All participants were right-handed as tested by the Edinburgh Handedness Inventory (Oldfield, 1971), with no significant difference in handedness quotient between the AWS and ANS groups. Self-reporting indicated that none of the participants had any diagnosed health, neurological, physical or sensory concerns, other than a diagnosis of developmental stuttering for the AWS. Additional information regarding participants' experience of practising musical instruments or of video gaming was also collected by intake questionnaire at study enrollment. The groups did not differ significantly in their reported years of music practice ( $t(28) = -1.14, p = .26, d = -0.42$ ) or of experience in video gaming ( $t(28) = 1.56, p = .13, d = 0.57$ ).

Written informed consent was obtained from all participants before participation. The research protocol was approved by the Research Ethics Board at the University of Toronto.

### 2.2. Assessment measures

As part of the investigation, participants in both groups completed tests of Short-Term Memory (STM) and Working Memory (WM), which

were measured by verbally presented Digit Span Forward and Backward tasks, respectively (Gignac and Weiss, 2015; Oberauer et al., 2000; Wechsler, 1981). Using number strings that were generated in line with the methods of the Wechsler Adult Intelligence Scale-Revised (Wechsler, 1981), participants were asked to repeat a series of single digits, which were presented at a rate of one digit per second and which increased in length by one digit on every second trial. Repetition was required firstly in the same order as the digits were presented, with the longest span recalled correctly noted as Longest Digit Span Forward (LDSF). On the second task, repetition was required in the reverse order of how the digits were presented, with this longest span recalled correctly noted as Longest Digit Span Backward (LDSB). All participants completed the task verbally and were provided with two trials of each digit length, starting with three digits for the Digit Span Forward and two digits for the Digit Span Backward, until two trials of the same digit length could not be repeated correctly, up to a maximum of 10 digits.

Although the ASRT task does not require rehearsal or transformation of information for implicit sequence learning, the effect of WM capacity on the stabilization of sequence memory traces during consolidation (Janacek and Nemeth, 2013), as well as the effect of possible group differences in WM (Eichorn et al., 2016; Yang et al., 2019) on sequence learning, were of interest to the current investigation. Independent samples t-tests did not reveal a significant difference between the groups on the measure of STM, but did indicate a significantly lower mean WM score on Digit Span Backward for the AWS group (see Table 1).

Sustained attention was also measured across participant groups by use of the Conners' Continuous Performance Test (Conners CPT 3<sup>TM</sup>; Conners, 2014; Conners et al., 2018). The CPT 3<sup>TM</sup> test provides measures of Hit Reaction Time (HRT) in milliseconds across six blocks of continuous performance. The CPT 3<sup>TM</sup> also provides a proportional percentage of errors by omission (missed targets), commission (incorrect responses), and perseveration (responses made in less than 100 ms and deemed to be anticipatory, repetitive, or impulsive). The data from the CPT 3<sup>TM</sup> were transformed to norm-referenced t-scores for HRT and error type by the test software (Multi-Health Systems Inc.). The CPT 3<sup>TM</sup> was used as a standardised measure of sustained attention to rule out any problems with inattention among participants, as well as performance differences between the groups. Independent t-tests indicated no significant differences between the groups across any of these measures (see Table 1).

Stuttering severity of the participants in the AWS group was scored using the Stuttering Severity Instrument (SSI-4; Riley, 2009) by the first author. To test for reliability, 30% of participants' reading and conversational speech samples were rescored independently by the final author. Intraclass correlation coefficients, ICC (3,1) (Shrout and Fleiss, 1979), were calculated by two-way mixed-effect ANOVA models (Vallat, 2018), indicating good absolute agreement on reading scores of percent syllables stuttered (0.86) and excellent absolute agreement on conversation scores of percent syllables stuttered (0.95). AWS participants' SSI-4 outcomes ranged from very mild ( $n = 8$ ), mild ( $n = 4$ ), moderate ( $n = 2$ ), to severe ( $n = 1$ ).

The potential correlation, as well as covariation, between participants' scores on STM, WM and sustained attention measures, along with data of participants' experience of practising music and of video gaming, and the variables of Speed and Accuracy on the ASRT task, were also explored.

### 2.3. Experimental procedure

To measure implicit motor sequence learning, the Alternating Serial Reaction Time (ASRT) task, as adapted by (Janacek et al., 2012; Janacek and Nemeth, 2012; Nemeth et al., 2010), was used. This version of the ASRT task involved the visual stimulus of a dog's head, which appeared in one of four circular placeholders topographically aligned in the middle of a computer screen. Participants were not informed prior to the task about the presence of a sequence or the nature

**Table 1**  
Participant characteristics and assessment scores.

	AWS			ANS			Difference		
	Mean	SD	Median	Mean	SD	Median	t	P	d
Age	31.93	7.32	34	31.6	6.47	31	0.13	.90	0.05
LDSF	7.33	1.29	8	7.47	1.3	7	0.28	.78	-0.10
LDSB	4.93	0.96	5	6.2	1.57	6	-2.67	.01	-0.97
EHI Handedness	86.67	16.76	90	92	10.14	100	-1.05	.30	-0.39
CPT 3 <sup>TM</sup> HRT	52.2	10.1	51	49.27	6.93	47	0.93	.36	0.34
CPT 3 <sup>TM</sup> Omissions	46.8	5.29	45	46.87	4.82	45	0.04	.97	-0.01
CPT 3 <sup>TM</sup> Commissions	48.6	5.9	49	52.67	11.93	50	1.18	.25	-0.43
CPT 3 <sup>TM</sup> Perseverations	45.8	1.37	45	46.2	2.01	45	0.64	.53	-0.23
%SS	4	4.19	3						

Abbreviations: LDSF = Longest Digit Span Forward, raw scores of Short-term Memory (Gignac and Weiss, 2015; Wechsler, 1981); LDSB = Longest Digit Span Backward, raw scores of Working Memory (Gignac and Weiss, 2015; Wechsler, 1981); EHI = Edinburgh Handedness Inventory Handedness Quotient by percent (Oldfield, 1971); CPT 3<sup>TM</sup> = Conners' Continuous Performance Test Third Ed. (Conners, 2014); HRT = Hit Reaction Time on measure of sustained attention, in T-score conversion of mean reaction time is based on the sample mean and sample standard deviation of normative sample of 1,400 cases (Conners CPT 3<sup>TM</sup>); Omissions = T-score of errors by missed targets on measure of sustained attention (Conners CPT 3<sup>TM</sup>); Commissions = T-score of errors by incorrect response on measure of sustained attention (Conners CPT 3<sup>TM</sup>); Perseverations = T-score of responses made in less than 100 ms following the presentation of a stimulus; %SS = percent syllables stuttered, averaged across reading and conversation samples from the Stuttering Severity Instrument Fourth Edition (SSI-4; Riley, 2009).

of the task, and were only instructed to respond as quickly and as accurately as possible in order to "catch the dog". Responses involved pressing one of four buttons on a response box (Cedrus Corporation, San Pedro, CA, USA) with the right hand. The box was connected to a laptop computer (Dell Latitude with Intel® Core™ i5-6200U processor at 2.30 GHz), running the experimental program (designed using SuperLab 5.0 software, Cedrus Corporation, San Pedro, CA, USA), and was projected to an external 19-inch monitor (Dell Model P2213t with input rating of 100–240V–50/60Hz 1.5A) via a high-definition multimedia interface (HDMI) connection. The task did not involve any auditory information. Participants were seated approximately 30 cm from the monitor, with the response box comfortably placed below their right hand on the table before them. All participants were instructed to lightly place the four fingers of their right hand, from index to little finger, on the response box's four colour-coded buttons.

As outlined in Fig. 1, the participants' first sessions consisted of 20 blocks, within each of which a 10-element alternating sequence was repeated 10 times. In this study, one of three different sequence variations was assigned to each participant: A) 1-R-2-R-3-R-4-R-3-R, B) 1-R-2-R-4-R-3-R-4-R, and C) 1-R-3-R-2-R-4-R-2-R. The sequences were assigned in equal distribution across both groups, with six AWS and six ANS participants performing Sequence A, six participants in each group performing Sequence B, and three participants in each group performing Sequence C. Five randomly presented stimuli were included at the beginning of each block, which resulted in the dog stimulus being presented 105 times per block. Participants were informed that the first five responses were included for warm-up purposes only. Thus, only the 100 button presses corresponding to the repeating sequence were included in the final analyses. Within each block, each consecutive stimulus was presented 120 ms after the participant responded by button press to the presentation of the previous stimulus, whether correct or incorrect. After each block, participants were provided with feedback which informed them of their accuracy (i.e., how many dogs they had caught), as well as their relative speed in responding (whether this was faster or slower than the previous block). The inclusion of feedback can provide participants with the motivation to persist, as well as guidance in learning that would normally take the form of extrinsic factors or sensory information in non-experimental complex motor skill acquisition (Salmoni et al., 1984). Feedback on response time was calculated by comparing a participant's mean reaction time (RT) on the block they had just completed with that of the previous block. If their mean RT had increased (responses slowed), they were encouraged to respond more quickly. If their mean RT had decreased (responses sped up), they were encouraged to maintain their improved speed. Task instructions, narration, stimuli and feedback were presented visually, in written format, and did not include any sound throughout. All participants took

a break of approximately 2–3 min after completing 10 blocks, and were encouraged to stand up and leave the task during this time.

Following the completion of 20 blocks in the first session, all participants returned for a second session on the next day, scheduled approximately 24 hours after their first. During this second session, participants completed another five blocks, the first of which was used for consolidation testing (Block 21) and four for additional practice (Blocks 22–25). The five blocks on day two were identical to those completed the previous day, with the same 10-element alternating sequence repeated 10 times within each of the five blocks, and with the inclusion of feedback. During the practice session on day one, improvements in performance were classed as on-line learning gains. Any additional improvements on Block 21, the first ASRT block on the second day, relative to the last practice block on day one (Block 20) were deemed to reflect off-line gains and consolidation of implicit sequencing skills.

#### 2.4. Tests of explicit awareness

After completing the five additional blocks of practice on day two, explicit awareness of the task was assessed by tests of recollection, reproduction, and recognition of the sequence. The test of *recollection*, a questionnaire administered verbally by the researcher, asked participants if they had observed anything significant or special about the task. Participants were asked to rate how sure they were of having observed anything significant on a five-point Likert scale, with the lowest rating indicative of not having noticed anything and the highest affirming that they had noticed a pattern. The questions increased in specificity surrounding any reported knowledge of sequenced events, with participants ultimately being asked to guess what the sequence involved in terms of length and spatial locations, buttons, or finger movements (Janacek et al., 2012; Song et al., 2007). As a test of *reproduction*, participants completed a free-generation button-press task, during which they could reproduce the previously performed sequence manually. For this test of explicit awareness, participants used the same response buttons to make the stimulus appear on the computer screen, as was used during the practice sessions. Each participant had five trials to generate a 10-element sequence they remembered completing during the experimental sessions and five trials to generate a 10-element sequence they would not have completed (Song et al., 2007). Finally, to test for *recognition*, participants were presented with 20 sequences of 10 elements each. Among these 20 sequences, 10 followed the same pattern as those completed during practice and 10 interspersed sequences followed a pattern that was different to those previously experienced (e.g., reversed-order or alternate-order sequences). Participants were simply asked to provide a yes or no verbal response, when

asked if they recognised the sequence from previous exposure. The tests of sequence recollection, reproduction and recognition were completed in the same order by all participants. Group averages of participant results were analysed by independent samples *t*-tests.

## 2.5. Statistical analysis and modelling

Motor sequence learning was inferred from two measures of motor performance across two practice sessions (Magill and Anderson, 2017; Schmidt and Lee, 2005). Specifically, performance was measured by calculating the accuracy of trials (i.e., correct vs. incorrect button presses), and the latency of participants' responses for the last element of high- and low-frequency triplets on each of the 25 blocks (20 blocks on day one and 5 blocks on day 2).

As illustrated in Fig. 1, the three sequences used in the current study provided for different high- and low-frequency triplet structures, depending on the assigned sequence. For participants completing Sequence A, triplets beginning and ending with sequenced elements 1\_2, 2\_3, 3\_4, 4\_3, and 3\_1 were more frequent, because not only did the sequenced elements contribute to their formation but random elements could also follow the same pattern. Within Sequence A, 1-1-2 or 1-2-2 could be formed by second-order sequenced elements (i.e., 1-R-2) or alternating random elements (i.e., R-1-R and R-2-R). The low-frequency triplets were not dependent upon the sequenced elements and ended with a random element (e.g., 1-1-3 could only be formed by R-1-R). Each participant's response on the ASRT was thereby classified as high- or low-frequency, depending on the two previous elements and whether these formed triplets that were more or less frequent and predictable (Janacek et al., 2012). However, triplets that started and ended with the same element (e.g., 1\_1 or 2\_2), which comprised of two random elements and occurred in lower frequency, formed either repetitions (111, 222, 333, and 444) or trills (121, 212, 343, 434, etc.). In accordance with previous research (Howard et al., 2004; Janacek et al., 2012), these triplets were excluded from the analysis, as it was not possible to balance their presentation across participants (Song et al., 2007), and studies have shown that repetitions and trills can tap into prepotent response tendencies (Howard et al., 2004; Remillard and Clark, 2001). Thus, across participants and two practice sessions, an average of 65.99% of triplets ( $SD = 3.58$ ) were classified as high-frequency and 24.68% ( $SD = 4.02$ ) as low-frequency.

In addition to repetitions and trills, RTs of 100 ms or less were also removed from the analysis, as these were deemed to be the result of participants' error of perseveration or potential instrumentation issues (Connors, 2014; Luce, 1986; Whelan, 2008). On average, 1.14% of trials were excluded from AWS' (mean = 28.53 trials  $\pm$  28.44) and 1.81% of trials from ANS' performance data (mean = 45.27 trials  $\pm$  35.90). Levene's test indicated comparable variance between the mean numbers of trials removed ( $p = .22$ ). An independent samples *t*-test revealed no significant differences between the groups ( $t(28) = -1.41, p = .17, d = -0.52$ ).

In the current adaptation of the ASRT task, consideration was also given to the repeated element of each 10-element sequence. That is, in each sequence, one element occurred twice as a sequenced element. In Sequence A (1-2-3-4-3-), element "3" was repeated, in Sequence B (1-2-4-3-4-), element "4", and in Sequence C (1-3-2-4-2-), element "2". Post-hoc analyses were carried out, in order to ensure that sequence-specific learning was not confounded by a frequency effect of these individual elements. Because the analyses of performance Speed and Accuracy involved only response data for the final element of high- and low-frequency triplets, post-hoc analyses were conducted on a subset of the performance data by exclusion of triplets ending with the repeated sequence element, and examined for the same predictive effects.

For the investigation of performance Speed, the use of mean or median RT was not deemed appropriate for analysing data that was inherently variable, skewed, and gathered under frequency conditions that involved different numbers of trials (Kliegl et al., 2010; Whelan,

2008). RT data were reciprocally transformed to a measure of "response speed", which was equal to response/seconds and calculated as 1000/RT, with higher values indicating higher "response speeds" (i.e., derived from shorter RTs). This transformation was applied in order to reduce the skew of RT distribution and mitigate the effect of outlying RTs when these were uncharacteristically short, while still maintaining sufficient variability of the data and satisfactory power for analysis (Baron, 1985; Kliegl et al., 2010; Whelan, 2008).

A mixed-effects modelling approach was used to analyse the effects of predictor variables, which included Frequency, Block, Group, and Sex, on the performance outcome variables of Accuracy and performance Speed. The application of hierarchical mixed-effects models that allow for analysis of all individual observations across repeated measures along with stimulus-specific characteristics, such as frequency, has been found to support modelling of smaller sample sizes (Wiley and Rapp, 2019). In addition, linear mixed-effects models can appropriately account for the ex-Gaussian distribution of repeated RT measures and the inherent within-subject random effects on each participant's performance (Koerner and Zhang, 2017; Lo and Andrews, 2015; Sternberg and Backus, 2015; Whelan, 2008).

Performance Speed was analysed by generalized linear mixed-effects models (GLMMs), in accordance with the gamma distribution of the dependent variable, and with already transformed RT data connected by the identity link function (Bates et al., 2021). The best fitting model to the distribution of transformed or untransformed RT was selected by comparison of each model's Akaike Information Criterion, AIC (Lo and Andrews, 2015). Model predictors were first compared in linear mixed-effects models by ANOVA and Chi-squared test in R (Bates, 2005). Along with fixed variables of interest that sought to decipher sequence learning outcomes (i.e., Block and Frequency), Sex was also included in the modelling owing to its proclivity to influence motor skill and RT (Der and Deary, 2006; Lissek et al., 2007). Modelling including Block (1–25), Group (AWS vs. ANS), Frequency (High-vs Low-Frequency triplets), and Sex (Male vs Female) as predictor variables was found to be the most sensitive in predicting performance Speed on the ASRT ( $X^2(100, N = 30) = 215.99, p < .001$ ). Accuracy data were dichotomous (correct vs. incorrect) and analysed by logistic GLMM, in accordance with binomial distribution of the dependent variable. The best fitting GLMM also included Block, Group, Frequency, and Sex as fixed effects in the analysis of participants' Accuracy ( $X^2(100, N = 30) = 146.90, p = .002$ ). All models incorporated the random-effects of Participant factor as intercept only. In order to control for the effects of participants' speed-accuracy trade-off on task performance, the GLMM for performance Speed included the additive factor of Accuracy, while the GLMM analysing performance Accuracy included the additive factor of Speed.

Although an analysis by sex of participants was not planned as part of the original research question, considering its predictive power had not been supported in previous research (Trofimova et al., 2020), it was found to contribute to the best fitting models for the analysis of Speed and Accuracy data. The significant effects of Sex and Group were also further explored through stratified analyses, each of which involved smaller sample sizes than originally planned for between-group comparisons. However, by using a repeated measures approach on the ASRT task, the large number of measured observations could also be taken into account (Brybaert and Stevens, 2018). According to calculations in G\*power 3.1.9.6 (Faul et al., 2009), a repeated measures ANOVA for between-factors comparison, with alpha at .05, would have 80% power to detect an effect size of  $f = 0.237$  with a sample of 30 participants,  $f = 0.288$  with 21 male participants, and  $f = 0.488$  with nine female participants, across two participant groups, with an average of 2,267 repeated measures. Therefore, stratified analyses would not be sufficiently powered to detect effects smaller than  $f = 0.288$  between the male participant groups and  $f = 0.488$  between the female participant groups. Hence, the use of GLMMs, to estimate differences in predictive effects across the groups, was considered to be a more sensitive and powerful approach to the analysis of this highly variable time-series

data, as compared to repeated measures ANOVAs (Brybaert and Stevens, 2018; Lo and Andrews, 2015).

Within each GLMM of performance Speed and Accuracy, a main effect of Block would indicate general skill learning, while a main effect of Frequency would indicate sequence-specific learning in accordance to triplet type. A significant interaction effect between Block and Frequency would then indicate different rates of learning for each triplet type, whereby response performance for high-frequency triplets is expected to improve at a greater rate than for low-frequency triplets (Janacsek et al., 2012; Nemeth et al., 2010). In all analyses of implicit sequence learning, Block 20 (the last block on the first day), was used as the reference criterion to compare within-session performance on earlier blocks, as well as to evaluate off-line consolidation between the last block on day one (Block 20) and the first block on day two (Block 21), and further changes in performance on the second day (comparing Block 20 with Blocks 22 through to 25). Interactions between fixed effects were further analysed through data stratification and remodelling by Sex and Group. Statistical analyses were carried out using *statsmodels* in Python (Seabold and Perktold, 2010) and the *lme4* package in R Studio (Bates et al., 2015).

Additional measures of memory (STM and WM) and sustained attention (CPT 3<sup>TM</sup>; Conners, 2014) were analysed by independent *t*-test and linear mixed-effects models (LMM) to investigate group differences in mean scores and across task performance, respectively. Pearson's correlation coefficients were also calculated to investigate the correlation between the participants' scores on the additional measures of STM and WM, and the motor performance outcomes of participants' overall mean performance Accuracy and mean performance Speed, as well as those during Block 21 of consolidation testing. The false discovery rate (FDR) of multiple comparisons was controlled for through application of the Benjamini-Hochberg procedure (Benjamini and Hochberg, 1995). Significant relationships between these cognitive measures and ASRT performance were then further explored by including the measure as a fixed effect in subsequent GLM modelling of performance Speed and Accuracy.

### 3. Results

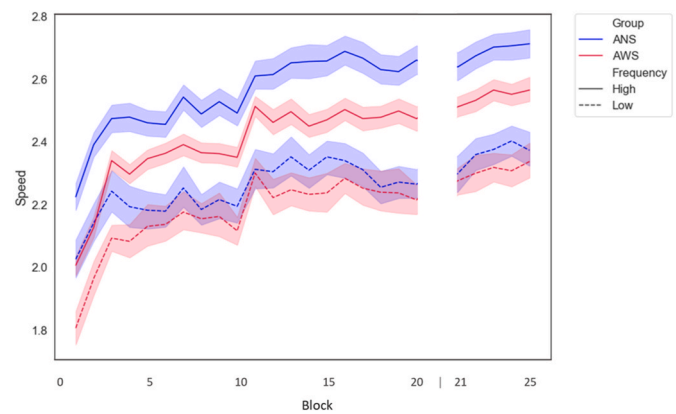
Accuracy was overall high for participants throughout the ASRT task, while the participants' performance Speed was more sensitive to the effects of learning. Therefore, we first report the results of performance Speed, followed by a brief report of performance Accuracy below.

#### 3.1. Performance Speed

With the reference criterion of performance set for Block 20, participants' overall performance Speed was analysed across practice, with individual blocks as GLMM factor. Accuracy was included as additive factor to control for the potential effects of a speed-accuracy trade-off on participants' Speed of performance. By the end of their first session, the groups' general performance Speed increased significantly when compared to that in earlier blocks (Blocks 1–6: all  $p$ 's < .05; Block 8:  $b = -0.139$ ,  $SE = 0.052$ ,  $z = -2.67$ ,  $p = .008$ , 95% CI [-0.24, -0.04]), reflecting significant improvements in general skill performance throughout early practice (see Fig. 2).

The main effect of Group on performance on Block 20 was not significant, revealing no significant difference between AWS' and ANS' general performance Speed at the end of practice on day one (Group AWS:  $b = -0.072$ ,  $SE = -0.056$ ,  $z = -1.28$ ,  $p = .20$ , 95% CI [-0.18, -0.04]). The main effect of Frequency indicated that participants responded to high-frequency triplets with shorter latencies than to low-frequency triplets (Frequency Low:  $b = -0.392$ ,  $SE = 0.066$ ,  $z = -5.96$ ,  $p < .001$ , 95% CI [-0.52, -0.26]).

When looking at effects of consolidation, the main effect of Block 21 on participants' overall performance Speed was found to be significant ( $b = 0.120$ ,  $SE = 0.053$ ,  $z = -2.28$ ,  $p = .023$ , 95% CI [-0.22, -0.02]),



**Fig. 2.** Average performance Speed (calculated as 1000/RT) for correct responses on the ASRT task by AWS (red) and ANS (blue) participant groups to High (solid lines) and Low (dashed lines) Frequency triplets, across practice on day one (Blocks 1–20) and day two (Blocks 21–25), with 95% confidence interval bands. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

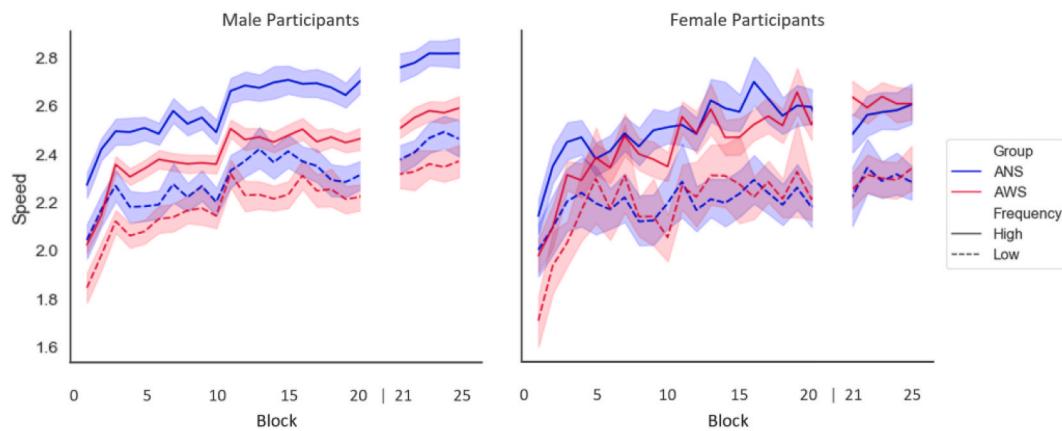
but was mediated by further interaction effects between Group and Block 21 ( $b = 0.240$ ,  $SE = 0.08$ ,  $z = 3.01$ ,  $p = .003$ , 95% CI [0.08, 0.40]). Overall, Speed of performance was found to be significantly influenced by the main effect of Sex ( $b = 0.114$ ,  $SE = 0.047$ ,  $z = 2.42$ ,  $p = .015$ , 95% CI [0.02, 0.21]). A significant three-way interaction effect was also found between Group, Block 21, and Sex ( $b = -0.249$ ,  $SE = 0.095$ ,  $z = -2.62$ ,  $p = .009$ , 95% CI [-0.44, -0.06]). Therefore, interaction effects were further investigated by sex-stratified analyses for male and female participant groups separately.

**Male participants:** The male-stratified GLMM revealed differential performance between male AWS and ANS that reached significance at the end of the first practice session, with the main effect of Group on Block 20 indicating a tendency among male ANS to exhibit faster general performance speeds (Group AWS:  $b = -0.238$ ,  $SE = 0.035$ ,  $z = -6.72$ ,  $p < .001$ , 95% CI [-0.31, -0.17]). A significant interaction effect between Group and Frequency Low was also found for the male participant groups (Group AWS x Frequency Low:  $b = 0.147$ ,  $SE = 0.062$ ,  $z = 2.37$ ,  $p = .018$ , 95% CI [0.03, 0.27]), showing differences in sequence-specific learning between male AWS and male ANS (see Fig. 3).

When data were analysed separately for the male AWS and ANS groups, the male ANS GLMM revealed a significant main effect of Frequency for male ANS participants (Frequency Low:  $b = -0.393$ ,  $SE = 0.050$ ,  $z = -7.91$ ,  $p < .001$ , 95% CI [-0.49, -0.30]), and further yielded significant Frequency by Block interactions, early in the first practice session (Frequency x Blocks 1–3,  $p$ 's < .05) as well as on Blocks 10 and 13 ( $p$ 's < .05). The male AWS GLMM also revealed a significant main effect of Frequency for male AWS participants (Frequency Low:  $b = -0.247$ ,  $SE = 0.038$ ,  $z = -6.50$ ,  $p < .001$ , 95% CI [-0.32, -0.17]), but no significant changes in their sequence-specific learning throughout practice (Frequency x Block interactions: all  $p$ 's > .05) (see Fig. 3).

Comparing group performances at the end of day one (Block 20) with that at the beginning of day two (Block 21) in separate GLMMs, no significant effect of consolidation on general performance Speed was found among male AWS (Block 21:  $b = 0.022$ ,  $SE = 0.031$ ,  $z = 0.70$ ,  $p = .49$ , 95% CI [-0.04, 0.08]) nor among male ANS (Block 21:  $b = 0.030$ ,  $SE = 0.041$ ,  $z = 0.74$ ,  $p = .46$ , 95% CI [-0.05, 0.11]). Both male AWS and ANS groups made their most significant gains in general skill learning in early practice blocks, with significant effects seen across Blocks 1–10 (all  $p$ 's < .01), and additional gains seen in male ANS on Blocks 19 and 23 ( $p$ 's < .05) and for male AWS on Blocks 23–25 of day two ( $p$ 's < .05).

**Female participants:** In a female-stratified GLMM, analyses of performance Speed among female participants revealed no significant differences between female AWS and female ANS (Group AWS:  $b =$



**Fig. 3.** Average performance Speed (calculated as 1000/RT) for correct responses on the ASRT task by male and female AWS (red) and ANS (blue) participants to High (solid lines) and Low (dashed lines) Frequency triplets, across practice on day one (Blocks 1–20) and day two (Blocks 21–25), with 95% confidence interval bands. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$-0.072$ ,  $SE = 0.06$ ,  $z = -1.19$ ,  $p = .23$ , 95% CI  $[-0.19, 0.05]$ ). No significant interaction effect was found between female Group and Frequency factors, indicating that sequence-specific learning did not differ between female AWS and ANS by the end of the first practice session, on Block 20 (Group AWS  $\times$  Frequency Low:  $b = 0.113$ ,  $SE = 0.105$ ,  $z = 1.07$ ,  $p = .28$ , 95% CI  $[-0.09, 0.32]$ ). Among female participants overall, the analysis of consolidation indicated a significant main effect of Block 21 ( $b = -0.130$ ,  $SE = 0.057$ ,  $z = -2.82$ ,  $p = .023$ , 95% CI  $[-0.24, -0.02]$ ); however, this was mediated by a significant interaction between Group and Block 21 (Group AWS  $\times$  Block 21:  $b = 0.241$ ,  $SE = 0.086$ ,  $z = 2.81$ ,  $p = .005$ , 95% CI  $[0.07, 0.41]$ ) (see Fig. 3).

Separate analyses for the female AWS and ANS groups indicated early changes in sequence-specific performance for female ANS on day one (Frequency  $\times$  Block 1, Block 4, and Block 5:  $p$ 's  $< .05$ ) as well as on day two (Frequency Low  $\times$  Block 22:  $b = 0.220$ ,  $SE = 0.104$ ,  $z = 2.11$ ,  $p = .035$ , 95% CI  $[0.16, 0.43]$ ), which was only seen among female AWS participants on Block 5 (Frequency Low  $\times$  Block 5:  $b = 0.216$ ,  $SE = 0.104$ ,  $z = 2.08$ ,  $p = .038$ , 95% CI  $[0.01, 0.42]$ ).

When investigating the consolidation effect mediated by the interaction between Group factor and Block 21, female ANS were found to increase their response latencies (i.e. with slower performance Speed) in response to high-frequency triplets, at the start of the second practice session (Block 21:  $b = -0.125$ ,  $SE = 0.060$ ,  $z = -2.08$ ,  $p = .038$ , 95% CI  $[-0.24, 0.01]$ ). For female AWS, there was no significant change in performance Speed found at the start of the second practice session (Block 21:  $b = 0.110$ ,  $SE = 0.061$ ,  $z = 1.81$ ,  $p = .07$ , 95% CI  $[-0.01, 0.23]$ ).

### 3.2. Performance Accuracy

Although variable, accuracy of response was high across participants, with the AWS group averaging at 97.7% (SD = 2.6) accuracy in response to high-frequency triplets and 97.3% (SD = 3.6) in response to low-frequency triplets; while the ANS group had an average response accuracy of 95.8% (SD = 4.5) and 94.8% (SD = 5.8) for high- and low-frequency triplets, respectively. The test for equality of variances (Brown and Forsythe, 1974) indicated no significant difference between variances of performance Accuracy of AWS and ANS at the start of practice (Block 1:  $F_{BF}(1, 2558) = 3.46$ ,  $p = .06$ ); however, ANS were found to have significantly higher variance of Accuracy than AWS at the end of the first (Block 20:  $F_{BF}(1, 2467) = 9.87$ ,  $p = .002$ ) and the second practice sessions (Block 25:  $F_{BF}(1, 2562) = 7.17$ ,  $p = .007$ ).

Accuracy of performance was analysed by logistic GLMM, which was simplified in the first model with Block as a continuous variable. The main effect of Group on overall Accuracy of performance was found to be significant (Group AWS:  $b = 0.670$ ,  $SE = 0.192$ ,  $z = 3.49$ ,  $p < .001$ ,

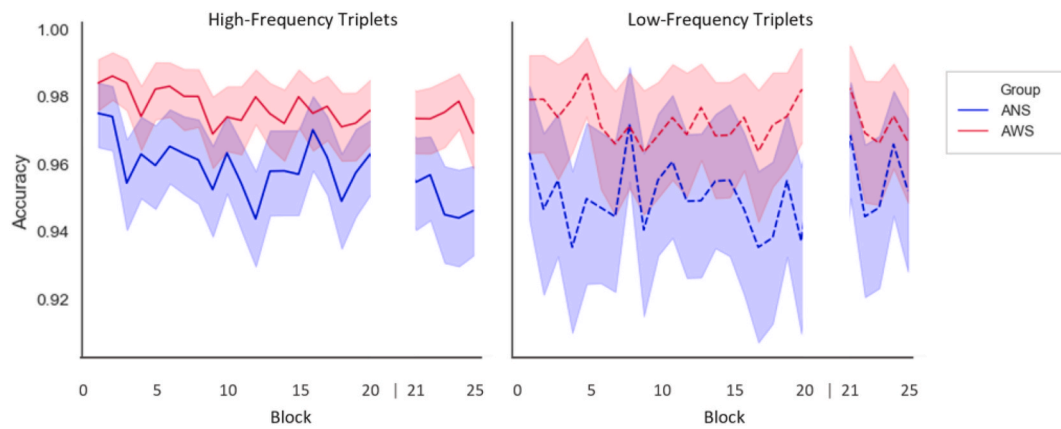
95% CI  $[0.29, 1.05]$ ), showing that AWS performed significantly more accurately than the ANS group in terms of general motor skill. The main effect of Frequency on overall Accuracy was also significant (Frequency Low:  $b = -0.551$ ,  $SE = 0.194$ ,  $z = -2.84$ ,  $p = .004$ , 95% CI  $[-0.93, -0.17]$ ), with participants generally responding to high-frequency triplets more accurately than to low-frequency triplets. However, this effect of Frequency on Accuracy was not consistent and did not reflect sequence-specific learning (see Fig. 4).

The main effect of Block on Accuracy of performance was significant ( $b = 0.024$ ,  $SE = 0.008$ ,  $z = 3.03$ ,  $p = .002$ , 95% CI  $[0.01, 0.04]$ ). Although the interaction between factors of Group and Block was not significant (Group AWS  $\times$  Block:  $b = -0.025$ ,  $SE = 0.013$ ,  $z = -1.90$ ,  $p = .06$ , 95% CI  $[-0.05, 0.00]$ ), significant interaction effects did indicate that changes in Accuracy differed between male and female participants (Sex [Male]  $\times$  Block:  $b = -0.045$ ,  $SE = 0.010$ ,  $z = -4.63$ ,  $p < .001$ , 95% CI  $[-0.06, -0.03]$ ), as well as between male and female AWS and ANS groups (Group AWS  $\times$  Sex [Male]  $\times$  Block:  $b = 0.046$ ,  $SE = 0.016$ ,  $z = 2.88$ ,  $p = .004$ , 95% CI  $[0.02, 0.08]$ ).

Thus, interaction effects were further analysed by additional stratified modelling by Sex. The stratified GLMMs, with the reference criterion of performance Accuracy set for Block 20, revealed no significant effect of Group on either male participants' (Group AWS:  $b = 0.338$ ,  $SE = 0.324$ ,  $z = 1.04$ ,  $p = .30$ , 95% CI  $[-0.30, 0.98]$ ) or female participants' Accuracy (Group AWS:  $b = -0.212$ ,  $SE = 0.530$ ,  $z = -0.40$ ,  $p = .69$ , 95% CI  $[-1.25, 0.83]$ ), indicating no significant difference between the groups in their Accuracy of performance by the end of the first day of practice (on Block 20). Only the female participants demonstrated any significant changes in general Accuracy during their first day of practice (Blocks 3, 5, 6, and 12:  $p$ 's  $< .05$ ), as well as on the first block of day two (Block 21:  $b = -0.930$ ,  $SE = 0.438$ ,  $z = -2.12$ ,  $p = .034$ , 95% CI  $[-1.79, -0.07]$ ); however, this latter effect reflected a decrease in general performance Accuracy between sessions.

In the male-stratified GLMM, neither main effect of Frequency (Frequency Low:  $b = -0.698$ ,  $SE = 0.360$ ,  $z = -1.94$ ,  $p = .052$ , 95% CI  $[-1.40, 0.01]$ ) nor of consolidation (Block 21:  $b = 0.189$ ,  $SE = 0.298$ ,  $z = 0.63$ ,  $p = .53$ , 95% CI  $[-0.40, 0.77]$ ) was significant, thereby not demonstrating evidence of sequence-specific learning in the performance Accuracy of male participants. In the female-stratified GLMM, a significant main effect of Frequency was found (Frequency Low:  $b = -1.654$ ,  $SE = 0.505$ ,  $z = -3.28$ ,  $p = .001$ , 95% CI  $[-2.64, 0.67]$ ), which mediated the consolidation effect at the start of day two (Frequency Low  $\times$  Block 21:  $b = 2.829$ ,  $SE = 0.871$ ,  $z = 3.25$ ,  $p = .001$ , 95% CI  $[1.12, 4.54]$ ) and reflected differential changes in Accuracy of performance between Blocks 20 and 21. Female ANS improved their Accuracy in response to low-frequency triplets ( $b = 2.512$ ,  $SE = 0.891$ ,  $z = 2.82$ ,  $p = .005$ , 95% CI  $[0.77, 4.26]$ ), while significantly decreasing their Accuracy





**Fig. 4.** ASRT performance Accuracy of AWS (red) and ANS (blue) participant groups by High (solid lines on the left) and Low (dashed lines on the right) Frequency triplets, across practice on day one (Blocks 1–20) and day two (Blocks 21–25), with 95% confidence interval bands. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

in response to high-frequency triplets ( $b = -0.915$ ,  $SE = 0.430$ ,  $z = -2.13$ ,  $p = .033$ , 95% CI  $[-1.76, -0.07]$ ). Female AWS did not demonstrate effects of sequence-specific learning (Frequency Low:  $b = 0.704$ ,  $SE = 1.101$ ,  $z = 0.64$ ,  $p = .52$ , 95% CI  $[-1.45, 2.86]$ ) or significant changes in their performance Accuracy across practice Blocks or in consolidation (all  $p$ 's  $> .05$ ).

### 3.3. Post-hoc analyses of frequency effects

The current version of the ASRT task was adapted to include a 10-element alternating sequence. The three sequences used in this study, (A) 1-2-3-4-3-, (B) 1-2-4-3-4-, and (C) 1-3-2-4-2-, each included one sequence element that was repeated (i.e., elements 3, 4, and 2 in Sequences A, B, and C, respectively). To ensure that participants' sequence-specific performance was not influenced solely by the frequency of the repeated element, we carried out post-hoc analyses of performance Speed and Accuracy on the high- and low-frequency triplets which did not involve a repeated element in final position. Each GLMM included fixed effects of Group, Frequency, Sex, and Block, along with random intercept for Participant, and additive factor of either Accuracy or Speed, respectively.

With triplets ending in a repeated sequence element omitted from the analyses, GLM modelling of participants' performance Speed showed a significant main effect of Frequency on overall performance, with participants responding more quickly to high-frequency triplets (Frequency Low:  $b = -0.337$ ,  $SE = 0.073$ ,  $z = -4.60$ ,  $p < .001$ , 95% CI  $[-0.48, -0.19]$ ). As in the previous GLMM of performance Speed, the main effect of Group was not found to be significant (Group AWS:  $b = -0.068$ ,  $SE = 0.069$ ,  $z = -0.99$ ,  $p = .32$ , 95% CI  $[-0.20, 0.07]$ ). The main effect of Sex was not significant (Sex [Male]:  $b = 0.082$ ,  $SE = 0.058$ ,  $z = 1.40$ ,  $p = .16$ , 95% CI  $[-0.03, 0.20]$ ) nor was its interaction with the factor of Group (Group AWS x Sex [Male]:  $b = -0.133$ ,  $SE = 0.082$ ,  $z = -1.62$ ,  $p = .11$ , 95% CI  $[-0.30, 0.03]$ ). The factors of Group and Sex did still interact with the Block factor on day one (Group AWS x Sex [Male] x Block 10:  $b = 0.240$ ,  $SE = 0.113$ ,  $z = 2.13$ ,  $p = .034$ , 95% CI  $[0.02, 0.46]$ ) and on day two (Group AWS x Sex [Male] x Block 21:  $b = -0.266$ ,  $SE = 0.117$ ,  $z = -2.26$ ,  $p = .024$ , 95% CI  $[-0.50, -0.04]$ ).

When stratified by Sex, the main effect of Group was found to significantly predict the performance Speed of male participants (Group AWS:  $b = -0.205$ ,  $SE = 0.044$ ,  $z = -4.67$ ,  $p < .001$ , 95% CI  $[-0.29, -0.12]$ ), whereas, the main effect of Group was not found to be significant in predicting the female participants' Speed of responding (Group AWS:  $b = -0.066$ ,  $SE = 0.073$ ,  $z = -0.91$ ,  $p = .37$ , 95% CI  $[-0.21, 0.08]$ ). The main effect of Frequency for triplet type remained significant across all GLMMs of Speed; however, the interaction between Frequency and Group that indicated differences in sequence-specific

learning among male participants was no longer significant (Frequency Low x Group AWS:  $b = 0.073$ ,  $SE = 0.070$ ,  $z = 1.04$ ,  $p = .30$ , 95% CI  $[-0.06, 0.21]$ ).

Logistic GLMMs of Accuracy in response to triplets without a repeated sequence element in final position revealed a significant main effects of Frequency (Frequency Low:  $b = -0.715$ ,  $SE = 0.221$ ,  $z = -3.24$ ,  $p = .001$ , 95% CI  $[-1.15, -0.28]$ ), Group (Group AWS:  $b = 0.563$ ,  $SE = 0.254$ ,  $z = 2.22$ ,  $p = .027$ , 95% CI  $[0.07, 1.06]$ ), and Sex (Male:  $b = 0.496$ ,  $SE = 0.179$ ,  $z = 2.77$ ,  $p = .006$ , 95% CI  $[0.15, 0.85]$ ) on overall performance, as in the original analysis.

Thus, the main effects of sequence-specific learning on the current version of the ASRT task, and the differences in performance Speed found between the male participant groups, specifically, were found to relate to distinctions between high- and low-frequency triplets, whether these contained a repeated sequence element or not. The rate of sequence-specific learning was not found to differ between the groups, however, when examined within this subset of performance data.

### 3.4. Tests of explicit awareness

On the Questionnaire of Explicit Awareness, the majority of participants did not report noticing a repeating sequence while completing the ASRT task. One AWS reported "the same repeating pattern every time" but could not suggest a possible length or structure for the sequence. One ANS reported noticing a pattern after 10 blocks of practice and was able to provide eight of the 10 sequence elements correctly. Four AWS and three ANS suggested three-element structures that would have been part of the sequence they practiced. None of the participant were able to describe the sequence in full. On the questionnaire's scale from one to five, an independent  $t$ -test revealed that the participant groups did not significantly differ in their ratings of explicit awareness ( $t(28) = 0.68$ ,  $p = .50$ ,  $d = 0.25$ ) (see Fig. 5).

When asked to generate a sequence they would have completed during the ASRT task, two AWS and one ANS produced the correct fixed elements of the alternating sequence on one attempt, but not on their four other attempts. One AWS produced the fixed elements of the sequence they had practiced on two trials, but did so when asked to generate a sequence they would *not* have encountered during the ASRT task. Otherwise, all participants correctly generated five untrained sequences on the second part of the sequence generation task (see Fig. 5). AWS and ANS groups did not differ significantly in their generation of trained ( $t(28) = 0.59$ ,  $p = .56$ ,  $d = 0.22$ ) or untrained sequences ( $t(28) = -1.00$ ,  $p = .33$ ,  $d = -0.37$ ).

On the final test of explicit awareness, participants were asked to indicate by verbal response whether they did or did not recognize a set of 20 sequences from the ASRT task. Ten presented examples followed

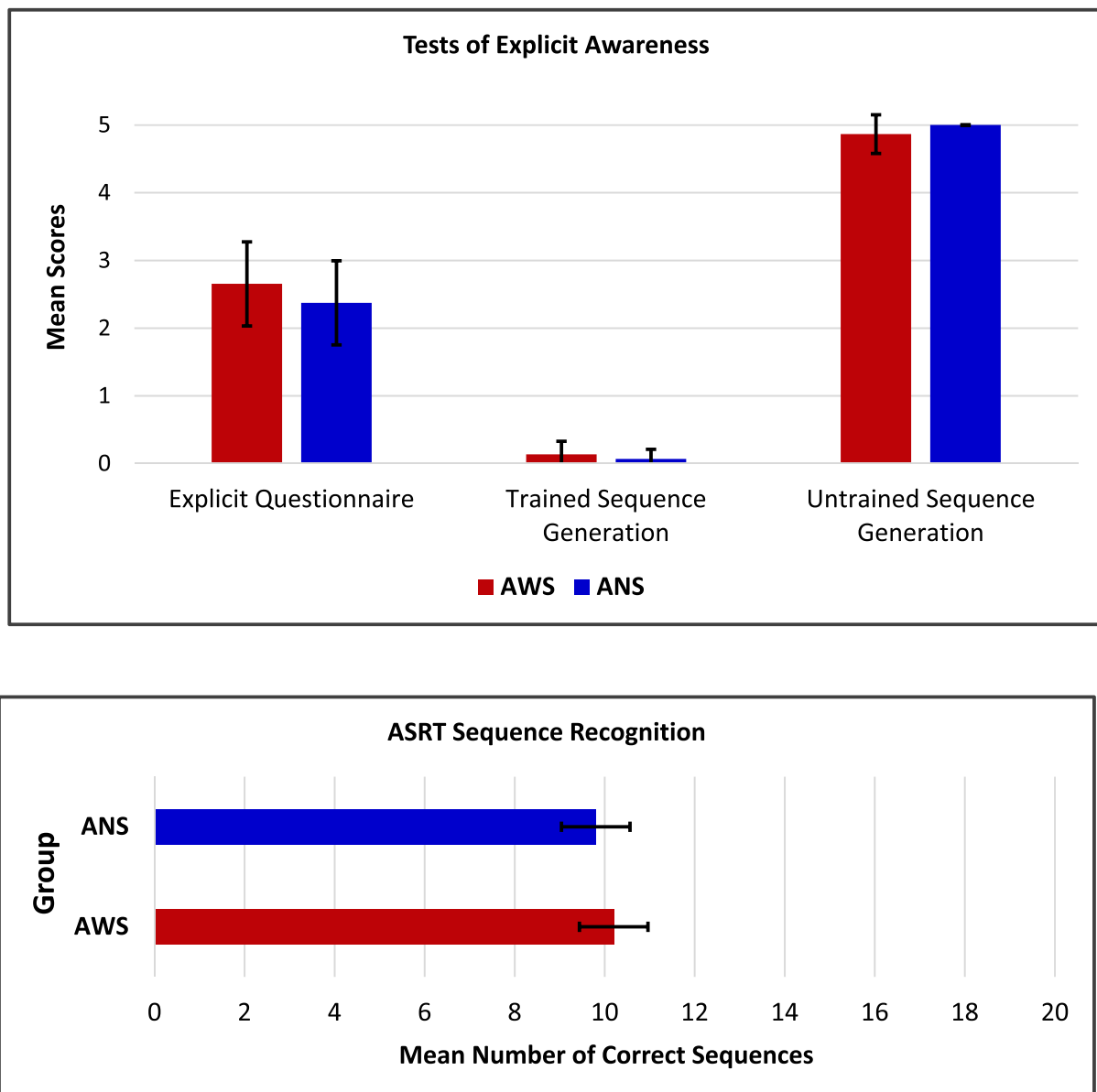


Fig. 5. Mean Scores of Explicit Awareness for AWS (red bars) and ANS (blue bars) groups. Upper bar graphs: Mean *recollection* scores on the Explicit Awareness Questionnaire (5-Point Likert Scale) and mean *reproduction* scores on tasks of Trained and Untrained Sequence Generation (5 trials per task). Lower bar graphs: Mean *recognition* scores of ASRT Sequences (10 trained and 10 untrained sequences). Bars indicate 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the sequence pattern from their practice sessions, while the remaining 10 interspersed sequences followed a reverse or alternative sequencing order. Overall, AWS and ANS did not differ significantly in their sequence recognition scores ( $t(28) = 0.80, p = .43, d = 0.29$ ) (see Fig. 5).

### 3.5. Additional measures

Pearson’s correlation coefficients were calculated separately within each group, to investigate the relationship between measures of implicit learning (mean overall ASRT Speed [transformed RT] and mean overall ASRT Accuracy [correct responses] across all 25 blocks), and measures of sustained attention on the CPT 3™ (ATT Speed [transformed RT averaged across six blocks], HRT, Omissions, Commissions), Short-term Memory (LDSF) and Working Memory (LDSB), as well as Kendall’s tau-b to investigate the relationship between ASRT performance and participants’ music and gaming experience (reported in years).

Although there was a moderate negative correlation between overall

mean ASRT Accuracy and the LDSB scores of AWS ( $r(13) = -0.64, p = .63$ ), as well as between mean Accuracy of AWS in responding to high-frequency triplets on Block 21 and their LDSB scores ( $r(13) = -0.68, p = .57$ ), these were not significant following FDR correction. Similarly, the performance Speed of ANS in response to high-frequency triplets during their first practice session was initially revealed to moderately correlate with their Gaming experience ( $r_b(13) = 0.41, p = .54$ ), but was also no longer significant following FDR correction. No additional correlations were found to be significant.

On measures of sustained attention (CPT 3™; 2014), there was no significant difference found between the groups on standardized scores of Hit Reaction Time (HRT), Omissions, Commissions, or Preservations. Analysis by LMMs also indicated no significant main effect of Group on sustained attention, as measured by average Speed of performance across 6 blocks of the CPT 3™ ( $b = -0.094, SE = 0.120, t = -0.78, p = .43, 95\% \text{ CI } [-0.33, 0.14]$ ), and no significant differences between the AWS and ANS groups across blocks of the sustained attention test (Group

x Blocks 1–5; all  $p$ 's > .05).

### 3.5.1. Effects of working memory

The AWS and ANS groups were found to significantly differ on the verbal measure of Working Memory (WM). To further investigate the nature of the relationship between WM and ASRT performance, WM scores were incorporated as a continuous fixed effect in the previously described GLMMs of performance Speed and Accuracy.

**WM and Speed:** The GLMM of performance Speed, including fixed effects of Group, Frequency, Sex, WM and factorized Block, revealed a significant main effect of WM on performance at the end of practice on day one (WM:  $b = -0.122$ ,  $SE = 0.033$ ,  $z = -3.74$ ,  $p < .001$ , 95% CI [-0.19, -0.06]), as well as significant interactions between the factors of WM and Group (Group AWS x WM:  $b = 0.127$ ,  $SE = 0.050$ ,  $z = 2.56$ ,  $p = .011$ , 95% CI [0.03, 0.22]), and between WM and Sex (Sex [Male] x WM:  $b = 0.190$ ,  $SE = 0.037$ ,  $z = 5.12$ ,  $p < .001$ , 95% CI [0.12, 0.26]). Additional interactions were found among the factors of WM, Group, Sex, Block, and Frequency and their effect on Speed, as well as a five-way interaction between all factors on Block 21 (Frequency Low x Group AWS x Sex [Male] x Block 21 x WM:  $b = 0.310$ ,  $SE = 0.148$ ,  $z = 2.10$ ,  $p = .036$ , 95% CI [0.02, 0.60]).

In a stratified GLMM of male participants, a main effect of WM was found on performance Speed at the end of practice, on day one ( $b = 0.071$ ,  $SE = 0.017$ ,  $z = 4.06$ ,  $p < .001$ , 95% CI [0.04, 0.11]). Although this effect did not differ significantly between the male AWS and ANS groups (Group AWS x WM:  $b = 0.014$ ,  $SE = 0.033$ ,  $z = 0.43$ ,  $p = .67$ , 95% CI [-0.05, 0.08]), WM was found to interact with effects of Group and Blocks 6, 11 and 12 ( $p$ 's < .05). Further stratification by group revealed a main effect of WM on the performance Speed of male AWS (WM:  $b = 0.085$ ,  $SE = 0.026$ ,  $z = 3.22$ ,  $p = .001$ , 95% CI [0.03, 0.14]), along with additional interactions between factors of WM and Blocks 1, 6, 10–12, 22 and 25 ( $p$ 's < .05) and between factors of WM, Frequency and Blocks 1, and 22 each ( $p$ 's < .05). A main effect of WM was also found to influence the performance Speed of ANS (WM:  $b = 0.070$ ,  $SE = 0.019$ ,  $z = 3.77$ ,  $p < .001$ , 95% CI [0.03, 0.11]), with interactions found between factors of WM and early practice blocks only (WM x Blocks 1, 2, 4, and 5:  $p$ 's < .05).

In a stratified GLMM of female participants, a main effect of WM was found on Speed of performance at the end of practice, on day one (WM:  $b = -0.122$ ,  $SE = 0.034$ ,  $z = -3.56$ ,  $p < .001$ , 95% CI [-0.19, -0.06]), which was mediated by a significant interaction effect with Group (Group AWS x WM:  $b = 0.126$ ,  $SE = 0.052$ ,  $z = 2.44$ ,  $p = .015$ , 95% CI [0.03, 0.23]). Additional interactions were found between WM and Group and several blocks throughout both practice sessions (Group AWS x WM x Blocks 1, 2, 4, 7, 8, 17, 21, 24, and 25:  $p$ 's < .05). Further stratified modelling by group found that there was no main effect of WM on Speed of performance for female AWS at the end of day one (WM:  $b = 0.005$ ,  $SE = 0.037$ ,  $z = 0.12$ ,  $p = .90$ , 95% CI [-0.07, 0.08]). However, WM was found to interact with performance of female AWS throughout practice (WM x Blocks 1–5, 7–9, 12, 21, 24, 25:  $p$ 's < .05). Among female ANS, the main effect WM was significant, implicating end of practice on day one (WM:  $b = -0.122$ ,  $SE = 0.036$ ,  $z = -3.42$ ,  $p = .001$ , 95% CI [-0.19, -0.05]), but WM did not interact with any other fixed effects.

**WM and Accuracy:** The continuous factor of WM was introduced to the logistic GLMM of Accuracy, which included fixed effects of Group, Frequency, Sex, and factorized Block, along with random intercept for Participant and additive factor of Speed. The main effect of WM on performance Accuracy was not found to be significant (WM:  $b = -0.064$ ,  $SE = 0.115$ ,  $z = -0.56$ ,  $p = .58$ , 95% CI [-0.29, 0.16]) nor did WM interact with Group effects (Group AWS x WM:  $b = 0.078$ ,  $SE = 0.256$ ,  $z = 0.31$ ,  $p = .76$ , 95% CI [-0.42, 0.58]). However, a significant interaction was revealed between factors of WM, Group, and the consolidation Block (Group AWS x Block 21 x WM:  $b = -0.805$ ,  $SE = 0.351$ ,  $z = -2.30$ ,  $p = .022$ , 95% CI [-1.49, -0.12]).

Stratification by Sex indicated no WM effect on performance Accuracy for male participants overall (WM:  $b = -0.046$ ,  $SE = 0.125$ ,  $z =$

$-0.37$ ,  $p = .71$ , 95% CI [-0.29, 0.20]), but WM did interact with Frequency effects and Block 2 for male ANS only (Frequency Low X Block 2 X WM:  $b = -0.753$ ,  $SE = 0.353$ ,  $z = -2.13$ ,  $p = .033$ , 95% CI [-1.45, -0.06]). There was no main effect of WM found for female participants either (WM:  $b = -0.339$ ,  $SE = 0.280$ ,  $z = -1.21$ ,  $p = .23$ , 95% CI [-0.89, 0.21]) but an interactive effect between Frequency and WM was found at the end of practice, i.e. Block 20 (Frequency Low x WM:  $b = 1.126$ ,  $SE = 0.471$ ,  $z = 2.39$ ,  $p = .017$ , 95% CI [0.20, 2.05]). This effect was seen in the performance Accuracy of female ANS only, as indicated by a significant interaction between WM and Frequency at the end of practice (Frequency Low x WM:  $b = 1.135$ ,  $SE = 0.472$ ,  $z = 2.40$ ,  $p = .016$ , 95% CI [0.21, 2.06]), as well as on early practice blocks (Frequency Low x WM x Blocks 1, 11, 14, and 15:  $p$ 's < .05).

### 3.5.2. Effects of sex on additional measures

Following the analyses of performance data on the ASRT task, which revealed a significant effect of Sex that differentiated the implicit motor learning outcomes of AWS and ANS groups, possible differences between male and female participants were further explored across the additional measures of WM, years of video gaming as well as of musical experience.

Across participants, the Mann-Whitney test showed that the WM scores of female participants (Mdn = 6) and those of male participants (Mdn = 5) were not significantly different ( $U = 125$ ,  $p = .16$  two-tailed). Although years of music and gaming experience varied considerably, female participants were not found to differ significantly from male participants in reported years of musical experience ( $U = 87$ ,  $p = .73$  two-tailed) or in years of video gaming experience ( $U = 70$ ,  $p = .21$  two-tailed).

Although the SSI-4 scores also varied between male and female AWS, results from the Mann-Whitney test of AWS participants' average percent syllables stuttered (%SS) across two speech samples indicated that there was insufficient evidence of a difference between female AWS (Mdn = 4) and male AWS (Mdn = 2) on the measure of %SS scores ( $U = 32$ ,  $p = .21$  two-tailed).

## 4. Discussion

This study aimed to investigate the nature of an hypothesized deficit in implicit motor learning among individuals with persistent developmental stuttering. Using an ASRT paradigm, we compared the motor sequencing performance of adults who do and do not stutter across two separate practice sessions, as well as investigating consolidation of implicit finger-sequencing skills by comparing their performance improvements between sessions. We hypothesized that AWS would perform the ASRT task more slowly and with lower accuracy when compared to an age and sex-matched control group of ANS. In addition, we hypothesized that AWS would show smaller gains in within-session learning as well as in consolidation of sequence-specific information between sessions.

On the ASRT task, participants across both groups demonstrated significant sequence-specific learning on measures of performance speed, but not in their accuracy of response. Overall, participants showed significant changes in their general skill performance at the early stages of learning, from blocks one to ten. Although a significant change in performance was detected on day two (on Block 21), this reflected depreciation among female ANS in general speed of response. Among male participants, AWS were found to perform significantly more slowly than their ANS counterparts, as well as differing significantly in sequence-specific learning. Neither male participant group demonstrated a significant consolidation effect of additional off-line performance gains between their two practice sessions.

As previously found in studies investigating explicit finger sequencing (Smits-Bandstra et al., 2006b) and syllable sequencing skills (Smits-Bandstra and De Nil, 2009), performance differences between male AWS and ANS were most evident during the early stages of

learning. However, unlike previous reports of explicit motor sequence learning in AWS (Korzeczek et al., 2020), the AWS participants in this study did not demonstrate a consolidation effect of performance gains following a latent period of 24 hours. A change in the performance of female ANS was found between sessions, but did not reflect consolidation of sequence-specific learning. Performance curves show that, overall, the AWS group did not catch up with their ANS counterparts by the end of practice on days one or two.

#### 4.1. Differences in sequence-specific performance

Although the groups were matched by age and sex, significant differences were found between participant groups in their efficiency of motor sequence performance based on the sex of participants. The most significant group differences were found between male AWS and ANS. This was true for their general skill performance, as well as for sequence-specific performance when measuring the differential effects of high- and low-frequencies on speed of response.

Previous research into motor sequence learning in adults with developmental stuttering has either focused solely on male participant groups (Bauerly and De Nil, 2011, 2015; Forster and Webster, 1991; Namasivayam and Van Lieshout, 2008; Smits-Bandstra et al., 2006a; Smits-Bandstra et al., 2006b; Smits-Bandstra and De Nil, 2009; Webster, 1986, 1989) or has not explored possible sex-differences in performance (Kim and Max, 2021; Korzeczek et al., 2020; Smits-Bandstra and Gracco, 2013, 2015). The number of female participants in this study was very limited and, thus, the data can only be interpreted with a high degree of caution. Although it was not part of our original research question, the analysis of participants' performance across sex-stratification does provide preliminary support of sex-related differences in motor learning performance of AWS. The female AWS and ANS participants did not demonstrate any group differences in sequence-specific performance speed, but did exhibit significant improvements in general performance speed across practice blocks. Notably, the male participants in this study demonstrated significant group differences on performance speed, as well as in sequence-specific performance (high-vs. low frequency effects) at the end of their first day of practice – differences that were evident from the very early stages of implicit sequence learning. These sex-based differences in performance measures, along with findings from previous studies that focused on male participants, may offer preliminary suggestions of difficulties in motor skill acquisition and consolidation, as well as efficiency of motor performance, which are experienced to a greater degree by males who stutter than females who stutter.

A study with a large sample of adults in the United Kingdom used simple and choice finger-tapping tasks to find sex differences across all investigated RT measures (Der and Deary, 2006), with females showing a more variable speed of performance across trials (Reimers and Maylor, 2006). The use of a finger-to-thumb opposition sequencing (FOS) paradigm, which also requires participants to complete a sequence of finger movements as quickly and accurately as possible, similarly revealed a significant male advantage in speed of sequencing performance at the end of practice, as well as in consolidation and follow-up, which was seen to emerge in late adolescence (Dorfberger et al., 2009). In contrast to these investigations of explicit motor learning, recent research using an *implicit* SRT task, in which sequence trials were compared to subsequent random trials, found that neither sex nor age predicted speed of performance within training or on post-training tests among young adult participants (Trofimova et al., 2020).

With particular relevance to the participants in the current investigation, sex differences do play a role in the incidence and persistence of developmental stuttering, with young males being at higher risk of both (Briley et al., 2021; Craig and Tran, 2005; Yairi and Ambrose, 2005). Differences have also been found between male children who do and do not stutter (CWS) in functional connectivity from an early age (Chang et al., 2008), as well as specifically between male AWS and ANS in their

functional activation of regions critical to sensorimotor integration and movement planning (Chang et al., 2009), such as the pre-supplementary motor area (pre-SMA).

The findings of the current study emphasize the need for further research into possible sex differences in motor learning among individuals who stutter. Especially if confirmed in children, our findings may point towards the influence of implicit learning abilities on the developmental trajectories of individuals who stutter from an early stage, when the acquisition of complex motor skills and integration of relevant sensorimotor information is more dependent on these capacities (Janacek et al., 2012; Kirkham et al., 2002; Slone and Johnson, 2018). Additionally, differences between male and female persons who stutter may yield their relevance to treatment planning at various stages of the lifespan.

#### 4.2. Differences in performance strategies

In this study, while AWS demonstrated superior performance accuracy, when compared to ANS, performance accuracy did not reflect motor sequence learning gains across participants. Closer analyses also revealed that, across practice, male AWS improved their speed of response to low-frequency triplets to a greater degree than their performance of high-frequency triplets. In the test of explicit awareness, some AWS participants reported focusing on their accuracy over speed of response. Although the association of group did not predict sequence-specific learning in terms of accuracy (as seen in effect of triplet frequency), the significantly fewer response errors made by AWS may indicate differing strengths and weaknesses that were influenced by their approach to performing the task, with a focus on accuracy over speed in AWS, as opposed to speed over accuracy in ANS, for example.

The implications of using performance strategies that focus on accuracy over speed of performance on a sequence-specific learning task, in which patterns or predictability are consciously sought, may point towards performance approaches that are less beneficial to implicit and statistical learning outcomes. The more functionally complex the task to be learned and the higher the cognitive processing demands for the learner, the more likely that focusing on explicit information can have a deleterious effect on procedural learning. Previous studies of motor sequence learning have found that when provided with instructions that a complex sequence is to be learned, those searching for the sequence improved their response times significantly less than those who were left unaware of the presence of the sequence (Fletcher et al., 2005). Although the segmentation of a motor sequence into chunks can occur during both explicit and implicit learning (Song and Cohen, 2014), explicit segmentation can be more reliant on WM capacities (Bo et al., 2009; Howard and Howard, 2001). WM scores were found to be significantly lower among the AWS than ANS participants in this study. Further investigation of their potential effects on implicit sequence learning found that both male and female AWS showed a significant effect of WM during motor practice, specifically on speed of performance across several practise blocks and influencing sequence-specific learning in male AWS both at an early and later stage of the ASRT task. Although general WM effects were found across both participant groups, their differential influence across practice blocks was only found in the performance speed of AWS in this study.

Conversely, investigations into the sequence learning abilities of individuals with musical and video gaming experience have found an advantage of music practice and gaming experience for implicit sequence-specific learning on the ASRT task (Romano Bergstrom, Howard and Howard, 2012), as well as for other forms of visual-spatial and auditory sequence learning (Dye et al., 2009; Green and Bavelier, 2006). A positive correlation between gaming experience and implicit sequence learning was initially found for ANS in the current study and, although this relationship did not hold statistical significance, performance speed and sequence-specific learning were also found to improve significantly more for ANS than for the AWS group. The participant

groups did not differ significantly in their reported gaming experience.

Differences in learning strengths may implicate different dependencies related to WM capacity. Previous research using the ASRT paradigm found a significant effect of reduced WM capacity on implicit sequence learning, in terms of both speed and accuracy improvements, in older adults, as compared to the performance improvements of younger participants who had higher WM capacity (Howard and Howard, 2001). It has been argued that the learning processes that support the detection of covariation in complex sequence patterns may be another unintentional mechanism of implicit learning (Howard and Howard, 2001; Krakauer et al., 2019). When the covariation of sensory events are integral to learning of the second order conditional pattern in our alternating sequence, such as those induced by movements or visual stimuli on a spatio-motor sequencing task like the ASRT (Albouy et al., 2006; Nemeth et al., 2009), this can depend on the capacity of the performer to activate a number of events simultaneously in memory (Bo and Seidler, 2009; Krakauer et al., 2019). In turn, the processing of sensory events and patterns could place demands on WM capacity, as well as on the chunking process for faster execution of the sequence. Studies have found that the perceptual representations of motor sequencing tasks can take longer to consolidate and can be more susceptible to interference during the learning process (Albouy et al., 2013, 2015; Gheysen et al., 2009). The role of WM in sequence learning and segmentation, in particular, may relate to the evidence of reduced sequence-specific learning found in male AWS in this study.

The use of digit span tasks, along with arithmetic and letter-number sequencing, have previously provided evidence of lower WM performance in AWS when compared to ANS, but was not found to significantly influence participants' response to temporal changes in syllable sequencing (Schwartz and Kotz, 2020). The more commonly used non-word repetition task, to measure phonological WM, has typically been found to differentiate AWS and ANS in their accuracy of repeating complex segmental structures (Coalson et al., 2019), their repetition of non-words with increasing syllabic length (Byrd et al., 2015), as well as in their improved performance and retention of syllable sequencing (Sasisekaran and Weisberg, 2014). Notably, these measures of WM, as well as the digit-span task used in the current study, are specific to the verbal domain. Adults who do and do not stutter have not been found to differ on measures of non-verbal WM performance (Gkalitsiou and Byrd, 2021). Although evidence of reduced non-verbal WM in children who stutter has been found to vary with age (Ntouriou et al., 2018), children's non-word repetition strengths were found to be a predictive factor of stuttering recovery over persistence at the preschool age (Spencer and Weber-Fox, 2014). In light of the current findings, the developmental significance of WM capacities in early life, along with that of the underlying neural networks that support WM and cognitive control (Engle et al., 1999; Furman et al., 2021; Mazoyer et al., 2001), warrant further consideration, as does the potential domain-specificity of both WM and sequence learning across tasks and development (Janacek and Nemeth, 2013). This could help us better understand their role in motor sequence learning and consolidation, as well as their influence in developmental stuttering.

#### 4.3. Neurological Underpinnings of implicit motor learning

There are potentially important parallels between the neural substrates of implicit motor learning and developmental stuttering. Research using motor learning paradigms with participants who do not stutter has found that speech as well as non-speech motor learning relies on the activity of the basal ganglia and pre-SMA, with the left frontal operculum and white matter connectivity to sensory brain regions implicated in successful feedback-based learning (Segawa et al., 2015). Involvement of the pars opercularis in the inferior frontal gyrus has also been found to be critical in general motor skill performance, as well as implicit sequence-specific learning (Clerget et al., 2012; Lungu et al., 2014). Whereas cortico-cerebellar connectivity is critical to adaptive,

sensorimotor learning (Doyon et al., 2009; Lametti et al., 2018), as well as to early stages of motor sequence learning (Doyon et al., 2009); the basal ganglia, and in particular the caudate and putamen, have been found to play an integral role in sequence-specific and implicit learning, even from the early stages of motor practice (Janacek et al., 2020). Dependency on these striatal regions continues into later stages of slower learning, automatization, and retention of sequences or chunks (Albouy et al., 2008; Doyon et al., 2009; Lohse et al., 2014; Lungu et al., 2014), with modulation of motor cortical regions having a decreased effect (Clerget et al., 2012; Wiltshire and Watkins, 2020).

The pars opercularis and basal ganglia also play an essential role during speech development, supporting the sequential articulation of phonemic gestures to form well-learned and automatized speech motor programs (Bohland et al., 2010; Dick et al., 2019). The role of the basal ganglia involves monitoring the cognitive, motor, and sensory contexts, to detect and signal when phoneme production should be terminated and the next unit in a speech sequence should be initiated (Chang and Guenther, 2020). It is hypothesized that impaired functioning of the basal ganglia can lead to prolongations instead of the timely termination of phonemes, to blocks instead of timely phoneme initiation, or to phoneme repetitions through signaling issues that cycle between initiation and premature termination (Chang and Guenther, 2020; Alm, 2004, 2021). A recent investigation into the motor sequence learning abilities of children diagnosed with Tourette syndrome (TS), using the ASRT, found impaired sequence learning but heightened probabilistic learning in children with TS as compared to that of their neurotypical peers – differences that were associated with basal ganglia functioning (Tóth-Fáber et al., 2021).

Further links between the supporting neural architecture of implicit sequence learning, speech motor development and developmental stuttering are supported by evidenced differences in individuals who stutter in grey matter volume in regions such as the left inferior frontal gyrus (Beal et al., 2007, 2013, 2015; Kell et al., 2009; Lu et al., 2012), cerebellum (Lu et al., 2012), and subcortically in the left putamen (Beal et al., 2013; Lu et al., 2010) and right caudate (Foundas et al., 2013). These differences have been shown to play a role in stuttering persistence and recovery in children and adults who stutter, as well as being significantly associated with measured stuttering severity (Kell et al., 2009; Giraud et al., 2008). Notably, when compared to individuals who do not stutter, differences in the functional connectivity of white matter tracts have also been found in these regions, specifically in the right inferior frontal gyrus in AWS (Beal et al., 2007; Lu et al., 2010), as well as between the left putamen and the SMA in CWS (Chang and Zhu, 2013).

Combined, these findings in developmental stuttering implicate the neural functioning of cortical and subcortical regions that form the cortico-basal ganglia-thalamocortical circuit (Chang and Guenther, 2020), in which stroke-related lesions have also been found to be specifically associated with the incidence of neurogenic stuttering (Theys et al., 2013). The finding that areas of atypical neural development and processing in individuals who stutter correspond to regions and processes implicated in implicit motor learning (Furman et al., 2021; Janacek et al., 2020; Mazoyer et al., 2001) provides indirect support for the role of implicit learning in the development and persistence of stuttering, and is deserving of future investigation.

#### 4.4. Implications for treatment and clinical research

The relevance of implicit learning conditions and differences in the learning processes relied upon by those who stutter can also be extended to the appropriateness of treatment approaches used with adults who experience persistent developmental stuttering. Traditional approaches in speech therapy tend to be dominantly explicit in their instruction, but rely on successful retention and automaticity, and thereby decreased attention, for their long-term efficacy. Stuttering treatment approaches may benefit from being tailored to the learning abilities that are

strongest in AWS, but with the provision of additional support in areas where extended training may facilitate further learning gains (e.g., working memory training; Howell et al., 2020).

Motor learning under implicit conditions is especially important early in life for the development of speech and language, as well as for other cognitive and social skills, with children demonstrating that learning implicitly is significantly more effective for them until they reach adolescence (Janacek et al., 2012). Further development of the attentional circuits in the brain sees a gradual shift away from implicit learning to greater performance gains on measures of explicit learning into adulthood (Dennis and Cabeza, 2011; Nemeth et al., 2013). Connectivity between the neural networks that support attentional control and motor task performance, along with the detection of less salient information, such as the visual perception of subtle orofacial movements during motor speech development (Uddin, 2015; Venezia et al., 2016), have also been found to differentiate children who persist to stutter from those who recover during childhood (Chang et al., 2018). At an early age, CWS demonstrate significantly lower efficiency in their abilities to orient attention (Eggers et al., 2012), with half of CWS exhibiting elevated symptoms of Attention Deficit Hyperactivity Disorder (ADHD) (Donaher and Richels, 2012; Druker et al., 2019). According to reports of individuals who persist to stutter, these attentional difficulties are also experienced in adulthood and may play a role in successful treatment outcomes (Tichenor et al., 2021).

With the aim of better informing clinical management and providing the most suitable intervention measures to the needs of the client, differences in motor learning and cognitive abilities of individuals who stutter across the lifespan must be afforded additional research effort and attention, as the results of the current investigation offer further insight into the implicated dependencies and critical processes that support complex motor skills acquisition.

#### 4.5. Limitations and future research

The current investigation has noteworthy limitations that highlight important considerations for future research. Firstly, interpretation of the study findings is limited by a relatively small sample size of participants. The focus of the current analyses was not intended to decipher sex-based differences in motor learning between individuals who do and do not stutter. Indeed, much of the previous research into the motor learning abilities of persons who stutter has mainly involved male participants. Therefore, the findings from this study are preliminary and underscore the need for motor learning research to carefully consider sex-based differences and to strive for larger and more balanced samples of male and female participants, most importantly when the condition of interest is also found to differentially affect individuals by sex and gender.

Additionally, the differential effects of WM across the participant groups and their potential influence on implicit sequence learning is worthy of further exploration. However, it is important to note that the domains of WM and of motor sequence learning that were investigated in the current study were different, with WM assessed on a verbal task and sequencing skills on a visuomotor task. The specific contribution of visuospatial WM to implicit sequence learning is also deserving of increased consideration. Furthermore, in order to better understand how the implicit motor learning abilities of individuals experiencing developmental stuttering may be implicated in the perceptual processing of speech, future research may include verbal or aurally presented stimuli in a variation of this implicit learning paradigm.

As indicated by the results of the current research, the participants' focus of attention, cognitive processing, and adopted strategies during implicit learning, as well as the potential influence of specific video gaming experience, also provide for additional avenues of future investigation. It might also be of interest to explore the relationship between stuttering behaviours and processes of implicit sequencing skills and perceptual learning in future studies. In the current

investigation, the one-time measurement of AWS' speech samples, and the limited representation of severity scores on the SSI-4, rendered the further analysis of severity effects or stuttering behaviours inappropriate, when considering the variable nature and diverse experience of stuttering.

## 5. Conclusion

In summary, we found significant differences in implicit motor sequence learning between AWS and ANS that were mediated by effects of sex across the groups. Specifically, implicit sequence learning was significantly reduced in male AWS when compared to their ANS counterparts. Significant differences on measures of WM were also found to correlate with implicit sequence learning for AWS, a group for whom accuracy was a performance strength. These findings provide further support of potential sex differences in motor learning and highlight their relevance to complex skill acquisition in individuals who experience persistent developmental stuttering. Further research into the effects of implicit motor learning abilities at distinct stages of development, their influence on proceduralization and automaticity of speech motor skills, as well as on successful treatment outcomes and maintenance in developmental stuttering is also needed.

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## Author contribution

Fiona Höbler: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft preparation, review & editing. Tali Bitan: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing. Luc Tremblay: Methodology, Supervision, Writing – review & editing. Luc De Nil: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft preparation, review & editing.

## Data availability

The authors do not have permission to share data.

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