Enduring motor memory of walking on a split-belt treadmill in young children and adults

Brittany Lissinna[1], Allison Smith[1], Kaylie La[1], Jaynie F Yang[1,2*]

[1] Department of Physical Therapy, [2] Neuroscience and Mental Health Institute, University of Alberta, Edmonton, AB, Canada

*Corresponding author:
Email: jaynie@ualberta.ca

Short title: Memory of split-belt walking across age groups

Keywords: motor adaptation, locomotion, human

Abstract

Adults and children modify their motor program to accommodate persistent changes in movement conditions, called motor adaptation. A memory trace is seen when reintroduction of the previously novel condition results in a smaller initial error in comparison to the first exposure. We used an adapted walking pattern on a split-belt treadmill to study the persistence of the memory trace over days, weeks and months, in young children (3-6 yr old), younger adults (20-30 yr old) and older adults (50-70 yr old). We found that retention was evident up to 2 weeks after initial exposure in all age groups, but children and younger adults retained better than older adults. Further, suggestions of a memory trace remained 4 months after the initial exposure in children and younger adults, but not in older adults. Only older adults demonstrated forgetting between trials on a single day when a short break was introduced, but this forgetting was diminished with repeated trials. The results indicate that long term memory of motor adaptation is affected by age, but it may be possible to enhance the memory in older adults by repeated exposure.

Introduction

Motor memory from skills mastered during childhood, such as riding a bicycle, using chopsticks, sports- and music-related skills seem to endure a life-time, even when the skills are rarely re-experienced in adulthood. In contrast, new motor learning is less enduring in older adults, such as with sequence learning in a serial reaction time test (1-3), and in visuomotor tracking (2). In sequence learning, the deterioration with age has been linked to a decline in cognitive function and cortico-striatal structures, reviewed in (4).

Motor adaptation, a form of motor learning that modifies a well learned motor program to persistent changes in the environment (5), is also affected by age. Children adapt at a slower rate than younger adults in some forms of motor adaptation, such as reaching in a novel force-field (6), and walking on a split-belt treadmill (7, 8). Adaptation rates in older adults are variable when compared with younger adults. For example, older adults had slower adaptation rates to visuomotor rotations (9-11), and showed similar or slower rates when reaching in a force-field (12, 13), and walking on a split-belt treadmill (14, 15).

Interestingly, the reduction in the rate of adaptation in older adults has been linked with a decline in cognitive resources. Adaptation rates within a session declined when performed simultaneously with a competing cognitive task during split-belt walking (15, 16). Further, slower adaptation to visuomotor rotation was correlated with a reduced volume in the striatum, prefrontal and sensorimotor cortical areas based on MRI imaging (17).

Retention of motor adaptation over the long term is less clear. Motor adaptation likely involves a number of memory processes, including modification of an internal model (18), and other model-free processes (19, 20). Memory of motor adaptation has mainly been quantified in two

ways – retention and savings. Retention is defined as a smaller movement error when one is exposed to a previously learned adaptation (21, 22), while savings is a faster rate of relearning the previously learned perturbation after a period of wash-out (i.e., extended removal of the previous perturbation) (23). Interestingly, retention is evident by the age of 6 years, whereas savings is not seen until 12 years old (24). Savings, but not retention, has also been linked to 'model free' mechanisms of motor memory (19, 20).

Here, we focus on age-related, longitudinal retention of split-belt adaptation over days, weeks and months between exposures, in three age groups - young children, younger adults and older adults. Long term retention of motor adaptation has scarcely been documented, and is of special interest with respect to its usefulness in motor rehabilitation (25). We expected retention to decline with the passage of time in all age groups, and the rate of decline to be age-dependent. We used the split-belt paradigm, because it is a novel task for adults and children alike, and it is not dependent on visual acuity or muscle strength, both of which may vary with age.

MATERIALS AND METHODS

Participants

Participants were a sample of convenience, consisting of volunteers with no known neurological or musculoskeletal problems that would interfere with motor learning. All participants were naïve to split-belt walking. Three age groups were targeted: children (3-6 years old), young adults (20-30 years old) and older adults (50-70 years old). We chose very young children because their slow time course of learning may give them an advantage for retention (22). Young adults were included because their adaptation and retention is robust and well known.

Older adults were studied because there is evidence for within-session forgetting (15). The age range for the children was smaller than the other age groups because their motor adaptation continues to mature with age, and we wished to minimize the variability associated with development (23).

All adult participants gave informed, written consent prior to participate; a parent gave the same consent for the young children, and verbal assent was obtained from the children at the time of the experiments. The protocols were approved by the Health Research Ethics Board – Health Panel (Pro00043622), University of Alberta.

Experimental Protocol

Experiment I

Participants were tested on 2 separate days, at intervals of either 24 hours, 1 week or 2 weeks (Figure 1). Since retention is evident after a gap of 24 hours in young adults (23) and children ≥6 years old (24), we chose to repeat this interval, and examine 2 longer intervals (1 and 2 weeks), which have not been reported for any age group. The allocation of participants to intervals was partly determined by availability of the participants, but was otherwise random.

On Day 1, two tied-belt trials (belts running at the same speed) were followed by 5 split-belt trials (one belt 2 times faster than the other) (Figure 1). Trials were 3 min long, separated by a short 1 min break. Because some adults are known to exhibit surprise when first exposed to split-belt walking, resulting in an exaggerated initial error (23), a 'surprise' trial of no more than 5 strides, 5 seconds long, with the 2:1 split-belt speeds was inserted between the two tied-belt trials, as done by others (23). In our experience (7, 8), children do not display this 'surprise', so no surprise trial was inserted for the children. On Day 2, participants started with 5 split-belt

trials at the speeds experienced on Day 1, to determine their retention of the split-belt adaptation from Day 1. This was followed by 2 tied-belt trials to quantify the size of the aftereffect, which indicated if participants of all ages learned equally well.

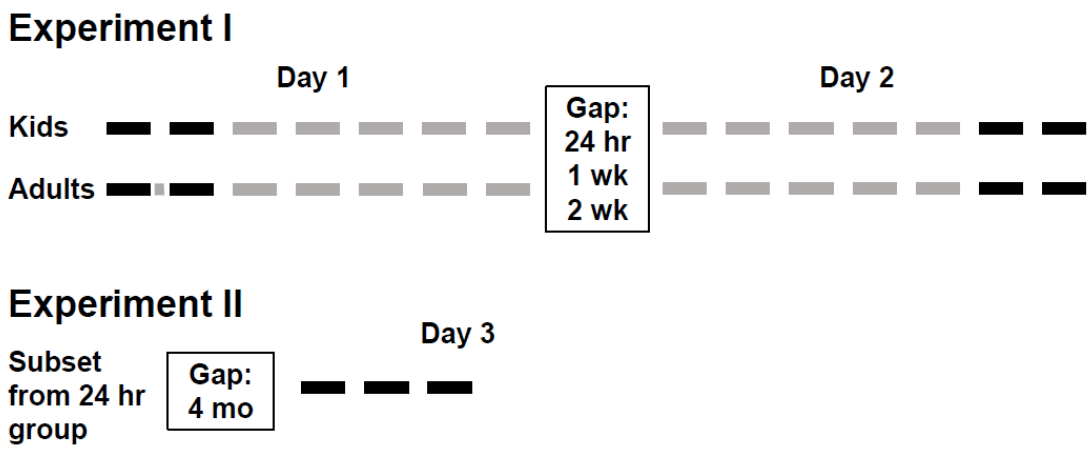

Figure 1. Experimental sequence. Each walking trial is represented by the solid horizontal bars. Black bars represent tied-belt trials and gray represent split-belt trials. All trials were 3 min long except the surprise trial of 5 strides, 5 seconds long, short gray bar between 2 tied-belt trials on Day 1. Experiment I: all participants experienced the same sequence of trials, with the exception of the surprise trial for adults only. Three gap durations between Day 1 and Day 2 were used for each of the age groups. Experiment II: a subset of participants from the group with a 24 hour gap between Day 1 and Day 2 returned for Day 3, which consisted of tied-belt walking only, to determine if any memory trace remained.

Participants walked on a Woodway split-belt treadmill (Woodway USA Inc., Waukesha, WI) with two belts driven by separate motors, controlled by a computer. The two belts were either set at the same speed (tied-belt) or at different speeds (split-belt). All adult participants walked with a tied-belt speed of 0.7m/s, and split-belt speeds of 1.4 m/s for the fast belt and 0.7 m/s for the slow belt. These speeds and the 2:1 speed ratio for the split-belt were chosen to minimize fatigue, especially for the older adults. Because children vary greatly in height, the speed for their tied-belt trials was set as a function of their leg length. The distance from the greater trochanter to the lateral malleolus was measured in meters, and the tied-belt speed (which is also the slow belt speed during split-belt walking) was set to that measure in meters per second,

as we have done in the past (8). The fast to slow belt ratio was also 2:1 for the children. For each participant, the leg on the fast belt (i.e., left or right leg) was chosen randomly on the first day, and then held constant for subsequent measures.

Each bout of walking was 3 minutes long with a short standing rest (about 1 minute) between bouts (Figure 1). We chose trials of this length to accommodate young children, who were more amenable to performing multiple 3-min bouts of walking with short breaks rather than longer stretch of continuous walking.

For safety, all participants held onto a front handrail and an adult stood behind the children to be ready to support them in case they tripped, and to ensure they walked with one foot on each belt. Children and adults were shown a movie or TV show of their choice while they walked. Adults were instructed not to look at the treadmill belts, and not to think about their walking. Children were asked questions about the movie throughout the trials to divert their attention from walking. These distractions were not meant to be difficult cognitive tasks, but simply to ensure the child was not focusing on walking.

Experiment II

A subset of the participants (n=11) from the group with a 24-hour initial gap been Day 1 and Day 2 in Experiment I returned for a final experiment (Day 3) 4 months after Day 1, to determine if any memory trace remained. These were participants who expressed interest in participating in subsequent experiments. In addition, 2 other participants who had similar Day 1 and Day 2 experiences from another study also came for a third exposure 4 months later, so a total of 13 participants were included. Day 3 consisted of 3 trials, 3 minutes each, at the tied-belt speed, to determine if there was any asymmetry remaining. The children's leg length was re-measured on Day 3 in case of growth spurts in the intervening 4 months, the treadmill belts were adjusted if

needed, but no adjustments were needed as the changes in leg length were smaller than the speed increments on the treadmill.

Instrumentation

For each experimental day, the participants were instrumented with infrared emitting markers (Northern Digital Inc., Waterloo, ON), positioned bilaterally at the top of the pelvis directly above the hip, the greater trochanter, the knee joint line, the ankle marker, placed below the lateral malleolus at the same vertical level as the marker on the head of the 5th metatarsal. Two 3-D Investigator motion sensors were positioned on each side of the treadmill to capture the positions of the infrared markers. Infrared marker positions were recorded using the NDI First Principles software (Northern Digital, Waterloo, ON). In addition, all trials were recorded with a video camera so that we could observe the participant as we analyzed the data. This was especially important for the children, who sometimes played on the treadmill, such as switching their feet on the belts or jumped unexpectedly. A custom-made timer generated a visible numeric code on the video and a TTL pulse that was simultaneously recorded with the NDI start/stop signal on a separate computer to synchronize the video image with the data.

Data Analysis

Experiment I

Video recordings of the walking were viewed prior to analyzing the data so that steps in which the participant was not stepping with one foot on each belt (usually a child being playful), could be excluded. We focused on step length symmetry in this study, because step length is a global measure of spatial and temporal adaptation on the split-belt treadmill (8). Custom written MatLab (The Mathworks Inc., Natick, MA) code was used to analyze the data. Step length was

defined as the antero-posterior distance between the ankle markers of each leg at the time of heel strike of each foot. Slow step length (SL_S) and fast step length (SL_f) refer to the step length at heel strike of the slow leg and fast leg, respectively. Step symmetry (SS) was calculated as the difference between the fast and slow step length, normalized to the sum of the fast and slow step lengths:

$$SS = \frac{SL_f - SL_S}{SL_f + SL_S} \quad \text{..... (1)}$$

A negative step symmetry indicates that the slow step was longer than the fast step. Step symmetry was calculated for all steps on the two experimental days. For each participant, the data were smoothed using epochs of 9-step averages, arbitrarily chosen as a compromise between the fast and slow time courses of learning in adults and children, respectively. The data were then averaged across participants within each of the groups to show the time course of adaptation for each experimental day.

Since children in the age range of 3 to 6 years old do not always show adaptation (8), we first determined if there was evidence of adaptation in each child. A standard t-test was used for Day 1 data from each child to determine if the average step symmetry of the last split-belt trial was significantly more positive than the first split-belt trial, using $p<0.05$ as the criterion. We used the average of all steps from the first and last trial because children are more variable in their walking, and the time course of learning is slower than adults (8). For comparison, we ran the same test on the adult trials. In addition, presence of an after-effect on Day 2, indicating the presence of learning, was confirmed by the average step symmetry of the first 10 steps on the tied-belt being more positive than the last 30 steps on the split-belt (standard t-test). If both of the above comparisons showed no differences, the child was removed from further analysis,

188 since it indicates the child showed no adaptation on both days, and the data would unfairly
189 show that the child had perfect retention (see calculation of Forgetting, below).

190 The differences in the time course of adaptation on Day 1 between the age groups were
191 determined by collapsing the data across the 3 gap durations. This was done separately for each
192 age group. This was justified since there were no significant differences in age between the
193 participants with different gap periods.

194 Step symmetry during specific time windows in the experiment days were of interest. Baseline
195 symmetry was defined as the average step symmetry from all steps in the second tied-belt trial
196 on Day 1. This represents tied-belt walking to determine if all participants were similarly
197 symmetric prior to experiencing split-belt walking. The initial error for split-belt walking on Day
198 1 (IE_1) was defined as the average of the first 9 steps on the split-belt. The nine-step average was
199 an arbitrary choice to accommodate the fast and slow time course of learning in adults and
200 children, respectively. The final error on Day 1 (FE_1) was the average of the last 30 steps on the
201 split-belt, reflecting the symmetry achieved by the end of Day 1 (23). Initial error on Day 2 (IE_2)
202 was the average of the first 9 steps on the split-belt. Forgetting between Day 1 to Day 2 was
203 defined as the difference between the initial error on Day 2 and the final error on Day 1, with
204 negative values indicating forgetting (Forgetting = IE_2 - FE_1). The aftereffect (AE_2) was the
205 average of the first 9 steps of the tied-belt on Day 2 immediately following the split-belt trials.

206 Since older adults may forget between trials within a day, we estimated forgetting between
207 trials on Day 1 by calculating the average of the 9 steps just before and just after a break, with
208 the difference between these averages representing forgetting between trials. There were 5
209 split-belt trials on Day 1, separated by 4 breaks. Only the adults were considered in this

comparison, as the children are more variable step-to-step, and their variability is likely to mask any real differences between trials.

Experiment II

The same step-symmetry calculations were performed for the participants with a 4-month gap duration. We used their asymmetry on the tied-belt trials, as an indication of their retention of split-belt walking.

Statistical Analysis

One-way ANOVAs were used to compare the ages between participant groups within the same gap interval (i.e., 24 hours, 1 week, 2 weeks) to determine if the groups were equivalent in age. Because there were no age differences within each age group, the time course of adaption on Day 1 was collapsed across different gap durations within each age group. This data were then modeled by a mixed, linear regression model to compare participants in the different age groups using the log likelihood ratio test. In addition, we used Akaike Information Criteria to determine if a linear or exponential model provided a better fit of the time course for each age group.

Two-way ANOVAs (factors: age group and gap interval) determined the differences between the groups in: 1) baseline stepping symmetry, 2) initial error when first exposed to split-belt walking (IE_1), 3) final error of Day 1 (i.e., FE_1), 4) after-effect (AE) in Day 2, and 5) forgetting between Day 1 and Day 2.

Within-day forgetting for Day 1 was compared between the younger adults and older adults using a mixed model ANOVA (between-subject factor: age group, and within-subject factor: breaks). Within-day forgetting for Day 2 was analyzed in the same way. A significant difference

was set at $p<0.1$ for all comparisons to avoid Type II errors with the small number of participants per group.

Results

Experiment I

Seventy-four volunteers (31 children, 21 younger adults, 22 older adults) participated in this part of the study. Data from one child were eliminated from analysis because they showed no signs of motor adaption, resulting in 73 sets of data. The number of participants contributing data in each group, the number of females in each group, and the average age are shown in Table 1.

Table 1. Participant numbers and ages from each experimental groups. N is the number of participants contributing data in the group with the number of female (f) participants in parentheses. Age is represented by years (yr) with mean ± 1 SD.

			Experiment I		**Experiment II**
Gap duration		24 hours	1 week	2 weeks	4 months
Kids	N (f)	12(6)	9(3)	9(3)	5(3)
	Age (yr)	4.6±0.9	4.9±0.9	4.4±0.5	4.3±0.8
Young adults	N (f)	7(4)	7(2)	7(4)	5(3)
	Age (yr)	23.5±3.7	24.0±2.0	25.6±2.3	22.4±3.2
Old adults	N (f)	7(4)	8(6)	7(4)	3(3)
	Age (yr)	62.1±8.6	59.7±5.7	59.7±7.1	56.9±5.1

All participants completed the trials without problem except for one child, who did not want to continue after 4 split-belt trials on Day 1; this same child completed all trials on Day 2. This child's data was included for analysis on both days, although the first day had somewhat fewer steps than most other children. The age of the participants within each age group were not different from each other (see Table 2, Age).

Table 2. Statistical results. The results from all statistical comparisons are listed here. The measures included: Age measured in years, Baseline step symmetry was the average of step symmetry (see equation in **MATERIALS and METHODS, Data Analysis**) from all steps in the second tied-belt trial on Day 1. SB Initial error was the average of the first 9 steps of split-belt walking on Day 1. SB Final error was the average of the last 30 steps of split-belt walking on Day 1. Aftereffect was the average of the first 9 steps of tied-belt walking on Day 2. Day 1 to Day 2 Forgetting was the difference in step symmetry between the first 9 steps of split-belt walking on Day 2 minus the SB Final error on Day 1. Within Day Forgetting was the difference in step symmetry between the first 9 steps after a break minus the last 9 steps before the break.

Measure	Statistic	Results	Post-hoc
Age	1-way ANOVA	Gap: F=0.891, p=0.422 (children) Gap: F=1.110, p=0.351 (YA) Gap: F=0.267, p=0.769 (OA)	
Baseline step symmetry	2-way ANOVA	Age: F=0.92, p=0.274 Gap: 0.462, p=0.632 Interaction: 1.314, p=0.274	
Day 1			
SB Initial error	2-way ANOVA	Age: F=0.243, p=0.785 Gap: F=0.731, p=0.485 Interaction: F=1.326, p=0.270	
SB Final error	2-way ANOVA	Age: F=4.442, p=0.016 Gap: F=1.681, p=0.194 Interaction: F=2.158, p=0.084*	1-way ANOVA age: 24 hr: F=6.235, p=0.007* 1 wk: F=1.461, p=0.256 2 wk: F=0.149, p=0.863
Day 2			
Aftereffect	2-way ANOVA	Age: F= 1.186, p=0.312 Gap: F=1.472, p=0.237 Interaction: F=1.550, p=0.198	
Forgetting			
Day 1 to Day 2	2-way ANOVA	Age: F=2.357, p=0.103 Gap: F= 0.203, p=0.817 Interaction: F=0.543, p=0.705	1-way main effect age: F=2.645, p=0.078* 1-way main effect gap: F=0.280, p=0.757
Within Day 1	Mixed model ANOVA	Age: F=2.978, p=0.092* Interaction: F=1.964, p=0.123	1-way RM ANOVA: YA: F=0.311, p=0.817 OA: F=4.524, p=0.006*

Similarities between the groups

Symmetry of walking in the tied-belt condition on Day 1 was compared between the 9 groups, using the average of all steps from the second tied-belt trial, and confirmed to be the same between the groups (Table 2, Baseline step symmetry). Initial error at the beginning of split-belt walking on Day 1, defined as the average step symmetry from the first 9 steps, was again confirmed to be the same between groups (Table 2, SB Initial error). This indicated that all groups were equally perturbed by the split-belt condition. Thus, even though the actual speeds at which they walked were not the same for the children and the adult groups, the 2:1 ratio of belt speed, and the use of leg length in the case of children to calculate a slow walking speed were successful in generating a similar perturbation for all participants.

Time course of adaptation and overall learning

The time course of adaptation during split-belt walking on Day 1 is shown in Figure 2 for each of the age groups, averaged across the gap durations, to determine how age affects the initial rate of adaptation. This was possible, because the protocol for Day 1 was identical for all gap durations, and there were no significant differences between the groups within an age range. The time course for each group represents epochs of 9-step averages, truncated at the lowest number of total steps obtained from all participants (i.e., the number of steps obtained from the child who completed 4 instead of 5 split-belt trials). All groups showed a reduction in step asymmetry through the adaptation period, with the children showing a slower time course of adaptation, and more variability between participants compared to the adults. The best fitting model for all age groups was a linear model, using the Akaike Information Criteria (26). Linear fit was best for all groups probably because of our use of the 9-step averaging to describe the time course. The age groups were significantly different from each other in the linear model, with

285 children being different from younger adults [log ratio(LR) chi2 (62)=462.65, prob=0.0000] and

286 older adults [LR chi2(62)=180.36, prob=0.0000], but younger and older adults were not different

287 from each other [LR Chi2(62)=-282.29, prob=1.000].

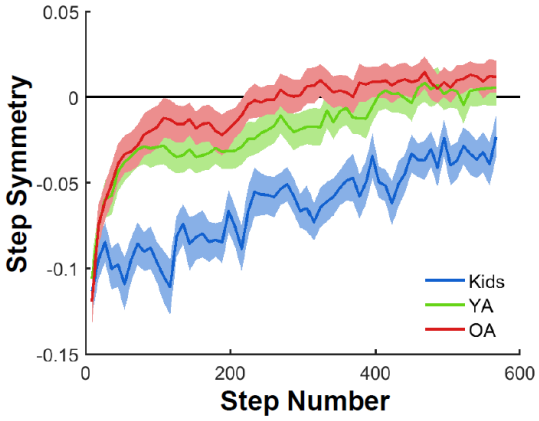

Figure 2. Time course of split-belt adaptation on Day 1. Average (9-step average) step symmetry is shown for the 3 age groups over the adaptation period of ~15 min. The age groups each contain data from n=30 for young children (Kids), n=21 for younger adults (YA), and n=22 for older adults (OA).

288 Overall learning on Day 1 was quantified as the final error on Day 1 for each participant, using

289 the average of the last 30 steps on the split-belt for each participant (i.e., not the truncated data

290 in Figure 2). The final error on Day 1 was significantly different between the groups (significant

291 interaction between Age and Gap duration; Table 2, Day 1, SB Final error). One-way ANOVAs in

292 post-hoc testing of differences between age groups at each of the gap intervals indicated that

293 only children showed more asymmetry at the end of Day 1 compared to both of the adult

294 groups, and only for the 24 hour groups (Table 2, Day 1, SB Final error, Post-hoc). Pairwise

295 comparison for the 24 hr groups indicated differences were between the children compared to

296 younger adults (p=0.022), and children compared to older adults (p=0.024). This is consistent

297 with the slower time course of learning in children shown in Figure 2.

298 The aftereffect on Day 2, a reflection of overall learning over the two days, was the same across

299 all age groups and time gaps (Table 2, Day 2, Aftereffect). Thus, the amount of learning over two

300 days of exposure to split-belt walking was the same for all age groups, even though the two days

301 of walking were separated by different intervals from 24 hours to 2 weeks.

Forgetting between Day 1 and Day 2

The time course of change in step symmetry during split-belt adaptation is superimposed for each of the groups on Day 1 and Day 2 in Figure 3. All groups showed evidence of retention on Day 2, as reflected by the starting steps on Day 2 (orange) being less asymmetric than the starting steps on Day 1 (blue). The smaller initial error on Day 2 led to all groups achieving symmetric walking sooner on Day 2 compared to Day 1. The amount of forgetting can be seen by comparing the difference between the average of the final 30 steps on Day 1 (called Final Error Day 1, FE1, shown by the blue symbols at the far right of each trace in Figure 3) to the beginning steps on Day 2 (orange). The forgetting between days is shown in bar graph form for all groups in Figure 4, which represents the difference in step symmetry between the first 9 steps on Day 2 (Initial Error Day 2, IE2) and the last 30 steps on Day 1 (FE1).

Statistical tests of forgetting were not significantly different for the age x gap interaction (Table 2, Forgetting, Day 1 to Day 2). Thus, 1-way ANOVAs were used to examine the main effect of age collapsed across gap durations, and main effect of gap duration collapsed across age. Only a significant effect for age was observed (Table 2, Forgetting, Day 1 to Day 2).

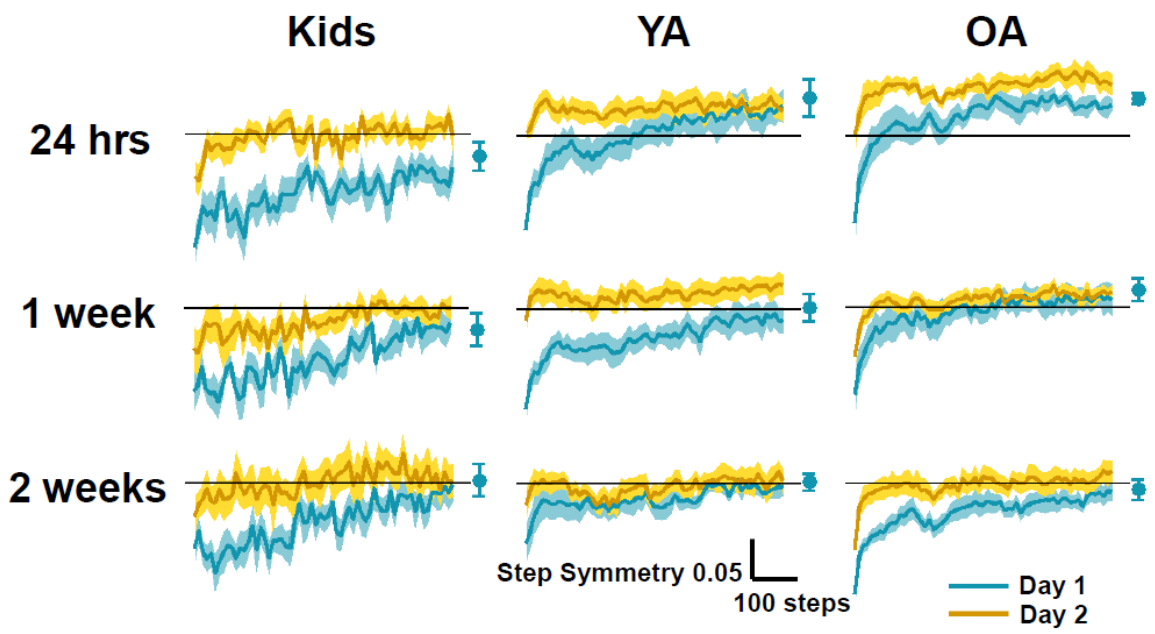

Figure 3. Average split-belt adaptation on Day 1 and Day 2 for each age group and gap durations. Adaptation is shown for 9 step averages across 567 steps, with the final step symmetry on Day 1 (average of last 30 steps, final error) shown as a blue symbol with ±1 SEM. Abbreviations: Kids - children 3-6 y.o.; YA - younger adults, OA - older adults.

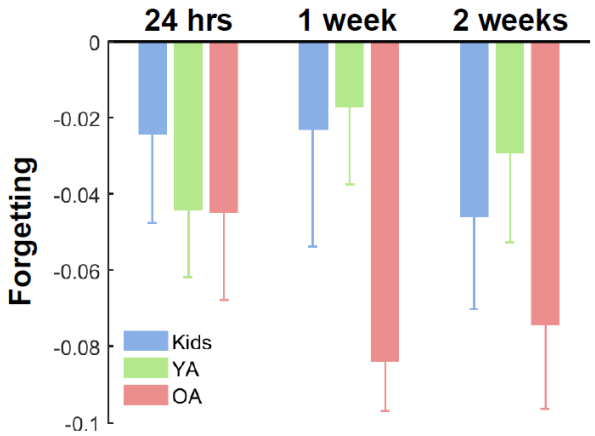

Figure 4. Forgetting from Day 1 to Day 2 for all age groups and gap intervals. Forgetting is the difference between the Initial Error on Day 2 minus the Final Error on Day 1. Means and 1 SEM are shown. Older adults (OA) showed more forgetting compared to the younger age groups (Kids and younger adults – YA).

Forgetting between trials within a day

We examined whether participants forgot between trials within a day, as has been reported for older adults (15). Children were not included in this comparison because their step-to-step variability is large, and we do not have the statistical power to examine this question. Data from younger and older adults of different gap durations on Day 1 were combined to examine the effect of age, since Day 1 was the same for everyone. Figure 5 shows the step symmetry before and after each one-minute break, together with the initial 9 steps on the split-belt, for each of the 2 age groups. Statistical comparison of the forgetting within Day 1, represented by the difference between the average of the 9 steps immediately after a break minus the average of the 9 steps immediately before the break, showed older adults forgot significantly more than the younger adults (mixed model ANOVA with repeated-measures over breaks, and between-subject factor of age). There was no interaction, but a significant main effect of age (Table 2, Forgetting, Within Day 1). Post-hoc test using one-way repeated measures ANOVA for each age group indicated the older adults showed a significant effect of breaks, whereas the younger adults did not (Table 2, Forgetting, Within Day 1, post-hoc). Further pairwise comparisons for the older adults using paired t-tests indicated the differences were between the first break and all the others ($p=0.052$, 0.023, 0.023, for Break 1 vs Breaks 2, 3 and 4, respectively), whereas Break 2 through 4 were not different from each other ($p=0.857$, 0.140, 0.171, for Breaks 2 vs 3, 2 vs 4 and 3 vs 4, respectively). Forgetting between trials was examined for Day 2, separately for each of the gap durations. No differences were found between younger and older adults on Day 2, but the statistical power was low for Day 2 compared to Day 1 because of the smaller number of participants in each group.

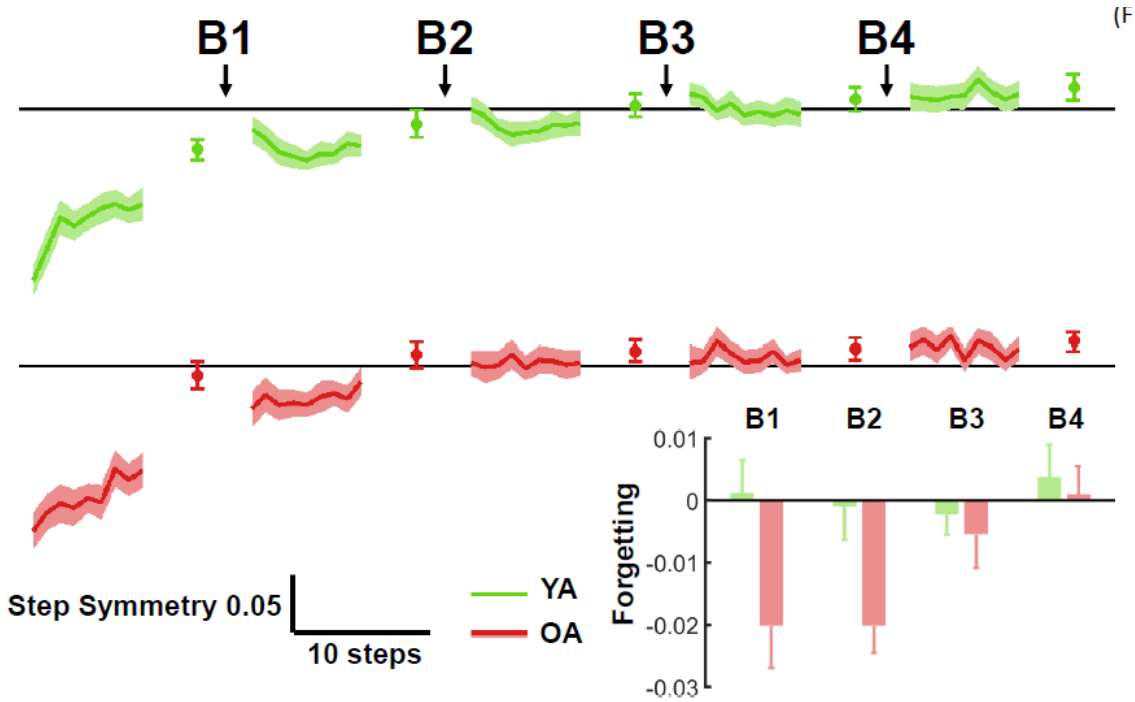

Figure 5. Forgetting between trials on Day 1. The step-by-step average step symmetry for each age group is shown for the first 9 steps on the split-belt treadmill, then the overall average of the last 9 steps before the breaks (B) and the step-by-step average of the first 9 steps right after the breaks, averaged across participants in each adult age group. The bar graph insert represents the quantified difference in forgetting between trials (forgetting = average of first 9 steps after break – average of last 9 steps before break). YA- younger adults, OA – older adults.

EXPERIMENT II

A subset of participants (n=11) from the 24 hour group in Experiment I, who had expressed continued interest in participating in our work, returned for an experiment on Day 3, four months later (Figure 1). In addition, 2 other participants with similar prior experiments identical to Experiment I also participated in Day 3. Their characteristics are shown in Table 1. The asymmetry on the tied-belt treadmill on Day 3 (4 months from Day 1) was used to reflect any residual memory of split-belt walking. Because the total number of participants with Day 3 data is very small (n=13), we did not attempt any statistical tests. Interestingly, all participants had a positive step symmetry except for one older adult. A positive step symmetry suggests the

presence of a memory trace. Further supporting evidence that this positive step symmetry was a memory trace was only seen in the children and younger adults. Both groups exhibited a return to perfect step symmetry (i.e., zero) over the 3 trials (Figure 6). In contract, no change was seen in step symmetry over the 3 trials for older adults. Hence, the positive bias of the step symmetry in older adults is more likely a result of slight differences in the placement of the ankle markers between Day 1 and Day 3, which was 4 months apart.

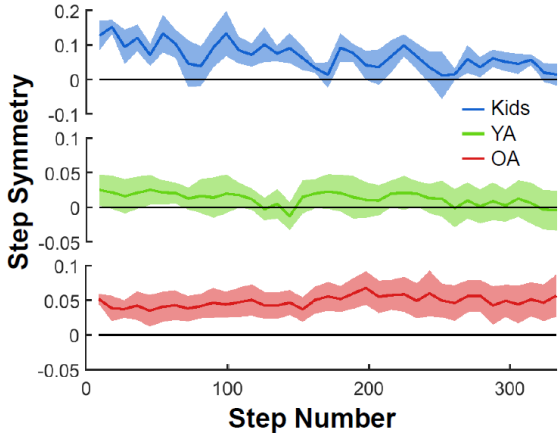

Figure 6. Step symmetry on the tied-belt treadmill on Day 3. Intial step symmetry during tied-belt walking on Day 3, which was 4 months after Day 1, suggests a positive bias in step symmetry in all groups, with a gradual return to perfect symmetry in kids and younger adults only. The small number of participants precluded statistical comparisons. N=5, 5, 3 for Kids, Younger Adults (YA) and Older Adults (OA).

DISCUSSION

This is the first report of retention in split-belt adaptation greater than 24 hours. Novel findings include: 1) retention remains strong for children and younger adults after a 1- and 2-week interval from the first exposure, whereas older adults had forgotten significantly more, and 2) there is a suggestion that a memory trace remains after a 4-month interval from initial exposure for children and younger adults only, but this will require verification with a larger study. We confirmed that the rate of adaptation within the first day of exposure is considerably slower in children than both groups of adults. Further, within the first day of split-belt walking, older adults forgot more than younger adults between trials. This within-day forgetting in older adults

369 was short-lived, and only evident between the first and second trial on Day 1. Contrary to our
370 original expectation, young children were no better than younger adults at retention, at least up
371 to a 2-week interval after initial adaptation.

372 Characteristics of adaptation in children

373 Since maturation of split-belt adaptation occurs up to 18 years of age, and in a previous study,
374 some children in the 3-6 yr old age range did not show signs of adaptation (8), we first ensured
375 that adaptation occurred in all child participants. Surprisingly, in this study, only one child
376 showed no adaptation in step symmetry, and was excluded. Movement variability is also
377 considerably larger in children, but the variability does not impede their ability to adapt to force
378 fields during upper limb reaching (6, 27), and stepping on a split-belt treadmill (7, 8). To
379 improve our ability to detect differences between age groups, we included more child
380 participants to compensate for their variability. Nevertheless, the statistical power remained
381 low, so it is possible that there were differences between children and other age groups that
382 were not detected as a result.

383 The rate of adaptation on Day 1 was considerably slower in children compared to adults, as has
384 been reported for both split-belt adaptation (7, 8) and other forms of adaptation (6). The slower
385 time course of adaptation to split-belt walking in young children could be explained by the
386 absence of a 'cognitive component' of learning, associated with the faster time course of motor
387 adaptation (19, 20). This is because the fast time course of split-belt adaptation can be
388 interfered with by a concurrent cognitive task during adaptation in adults, and especially in
389 older adults who presumably have less cognitive resources (15). Further, the fact that young
390 children did not exhibit 'savings' 24 hours later (24) is also consistent with an immature

391 cognitive component of motor learning, since savings in other forms of adaptation (i.e.,
392 visuomotor rotation) may result from a cognitive process of motor selection or retrieval (20, 28).

393 Another difference in the degree of adaptation achieved between children and adults is that
394 some adults (young and old) exhibited over learning, defined as a positive step symmetry at the
395 end of the adaptation on both Day 1 and Day 2, whereas children did not (Figure 2). This
396 phenomenon is not consistent between the different groups of adults and has occasionally been
397 observed by others (24). The reason for the over learning is unknown. Because the children take
398 longer to adapt, we do not know if they would have achieved over learning if they were given a
399 longer period of adaptation. In an extended learning period provided to children 6-8 years old in
400 a previous study, no over learning was described (24).

401 Endurance of the memory in all age groups

402 There was a trend for the greatest difference between age groups in their memory of
403 adaptation to appear at 1-week after initial exposure (Figure 4). By 2 weeks after initial
404 exposure, all groups have forgotten a considerable amount, but still far from completely
405 forgotten. We estimated the proportion of forgetting by dividing the average initial asymmetry
406 on the split-belt on Day 2 by the average initial error on the split-belt treadmill on Day 1 (step
407 symmetry of -0.11±0.07). In this way, average forgetting at 2 weeks was estimated to be 42%,
408 52% and 67% for children, younger and older adults, respectively.

409 While we do not know how long the memory trace for split-belt walking remains, it was
410 surprising that even 4 months after the initial exposure, a memory trace remained in children
411 and younger adults, as shown by their initial positive step symmetry followed by a gradual
412 return to perfect step symmetry over 3 trials (Figure 6). In contrast, older adults did not exhibit
413 this pattern, but instead showed a steady pattern of step symmetry throughout the 3 trials. The

consistent positive bias in the older adults could have resulted from other technical reasons, such as a slight difference in the placement of the markers on the participants over the 2 days that were separated by 4 months. Because the number of participants in Experiment II is very small, these results remain to be confirmed in future studies.

In the motor memory of finger-sequence tasks, children showed faster consolidation (29) and less susceptibility to interference from other tasks (30), suggesting an advantage in motor memory for children. In split-belt adaptation here, however, children were comparable but not superior to younger adults in their ability to retain the learning.

Forgetting in older adults

In motor skill learning, retention of learning over days in older adults has generally been inferior to younger adults. For example, older adults did not retain their skills as well as younger adults 24 hours after learning a ballistic task, a precision tracing task (31), a visuomotor tracking task (32), and a finger sequence task (1-3). Much less is known about retention of motor adaptation in older adults over days.

The forgetting in older adults within the first day of exposure confirms the earlier findings of Malone and Bastian (2016) that older adults forget between trials on the first day of exposure. Some procedural differences between the two studies include the duration of each split-belt trial, and the rest period between trials, both of which were considerably shorter in our study. Thus, instead of the five 3-min split-belt trials with 1 min rest between trials in this study, Malone and Bastian (2016) used three 5-min trials with 5 min rest between trials. With the short rest between trials and more repeated trials in our study, forgetting was only evident between the first and second trials in older adults, whereas it was evident between both rest

periods in the earlier study. This suggests that with repeated exposure and shorter breaks, older adults may be able to retain the learning better. Indeed, retention of split-belt adaptation in adult participants after stroke, over 12 sessions of repeated practice, has shown about half of the participants can retain the learning, resulting in their over ground walking becoming more symmetric (25). Many of the participants in that study were over 50 years old, and whether a participant responded to the intervention did not appear to be age-related, although the number of participants was small. Overall, repeated practice may be one way to improve retention in older adults.

Other factors may influence retention of motor learning in older adults. For example, in skill learning of finger sequences, sleep is important for consolidation of memory (3, 33). Whether sleep influences retention of motor adaptation is less clear, since it did not affect retention of a visuomotor rotation task (34). Limited evidence suggests acute, aerobic exercise may enhance subsequent motor learning and memory in persons with stroke (35) and Parkinson's disease (36). Finally, neuromodulation with non-invasive brain stimulation may also hold promise for improving certain types of motor adaptation in older adults (37), but its influence on motor memory remains to be seen.

Conclusions

Motor adaptation of split-belt walking is retained over days and even months in children and younger adults. Retention is less robust in older adults, but repeated trials within a day seem to allow older adults to forget less between trials. Other ways in which to improve retention in older adults would be worth exploring.

Acknowledgements

We thank Drs Kristin Musselman and Erin Vasudevan for helpful comments on earlier versions of this manuscript. We thank Dr Ming Ye for assistance with statistical modeling and testing, and Dr Jacques Bobet for programing assistance with preparing the figures. Funding for pilot work was provided in part by an NSERC Discovery Grant #2011-138181 to JFY. Funding to BL was provided by the Women and Children's Health Research Institute summer research studentship, Alberta Innovates Health Solutions summer studentship, and NSERC Undergraduate Student Research Award over several time periods.

References

1. Brown RM, Robertson EM, Press DZ. Sequence skill acquisition and off-line learning in normal aging. PLoS One. 2009;4(8):e6683.
2. Nemeth D, Janacsek K. The dynamics of implicit skill consolidation in young and elderly adults. J Gerontol B Psychol Sci Soc Sci. 2011;66(1):15-22.
3. Spencer RM, Gouw AM, Ivry RB. Age-related decline of sleep-dependent consolidation. Learn Mem. 2007;14(7):480-4.
4. King BR, Fogel SM, Albouy G, Doyon J. Neural correlates of the age-related changes in motor sequence learning and motor adaptation in older adults. Front Hum Neurosci. 2013;7:142.
5. Martin TA, Keating JG, Goodkin HP, Bastian AJ, Thach WT. Throwing while looking through prisms. II. Specificity and storage of multiple gaze-throw calibrations. Brain. 1996;119 (Pt 4):1199-211.
6. Konczak J, Jansen-Osmann P, Kalveram KT. Development of force adaptation during childhood. J Mot Behav. 2003;35(1):41-52.
7. Musselman KE, Patrick SK, Vasudevan EV, Bastian AJ, Yang JF. Unique characteristics of motor adaptation during walking in young children. J Neurophysiol. 2011;105(5):2195-203.
8. Vasudevan EV, Torres-Oviedo G, Morton SM, Yang JF, Bastian AJ. Younger is not always better: development of locomotor adaptation from childhood to adulthood. J Neurosci. 2011;31(8):3055-65.
9. Fernandez-Ruiz J, Hall C, Vergara P, Diiaz R. Prism adaptation in normal aging: slower adaptation rate and larger aftereffect. Brain Res Cogn Brain Res. 2000;9(3):223-6.
10. Buch ER, Young S, Contreras-Vidal JL. Visuomotor adaptation in normal aging. Learn Mem. 2003;10(1):55-63.

11. Vandevoorde K, Orban de Xivry JJ. Internal model recalibration does not deteriorate with age while motor adaptation does. Neurobiol Aging. 2019;80:138-53.
12. Trewartha KM, Garcia A, Wolpert DM, Flanagan JR. Fast but fleeting: adaptive motor learning processes associated with aging and cognitive decline. J Neurosci. 2014;34(40):13411-21.
13. Huang HJ, Ahmed AA. Older adults learn less, but still reduce metabolic cost, during motor adaptation. J Neurophysiol. 2014;111(1):135-44.
14. Bruijn SM, Van Impe A, Duysens J, Swinnen SP. Split-belt walking: adaptation differences between young and older adults. J Neurophysiol. 2012;108(4):1149-57.
15. Malone LA, Bastian AJ. Age-related forgetting in locomotor adaptation. Neurobiol Learn Mem. 2016;128:1-6.
16. Conradsson D, Hinton DC, Paquette C. The effects of dual-tasking on temporal gait adaptation and de-adaptation to the split-belt treadmill in older adults. Exp Gerontol. 2019;125:110655.
17. Wolpe N, Ingram JN, Tsvetanov KA, Henson RN, Wolpert DM, Cam CAN, et al. Age-related reduction in motor adaptation: brain structural correlates and the role of explicit memory. Neurobiol Aging. 2020;90:13-23.
18. Wolpert DM, Miall RC, Kawato M. Internal models in the cerebellum. Trends Cogn Sci. 1998;2(9):338-47.
19. Huang VS, Haith A, Mazzoni P, Krakauer JW. Rethinking motor learning and savings in adaptation paradigms: model-free memory for successful actions combines with internal models. Neuron. 2011;70(4):787-801.
20. Morehead JR, Qasim SE, Crossley MJ, Ivry R. Savings upon Re-Aiming in Visuomotor Adaptation. J Neurosci. 2015;35(42):14386-96.
21. Brashers-Krug T, Shadmehr R, Bizzi E. Consolidation in human motor memory. Nature. 1996;382(6588):252-5.
22. Joiner WM, Smith MA. Long-term retention explained by a model of short-term learning in the adaptive control of reaching. J Neurophysiol. 2008;100(5):2948-55.
23. Malone LA, Vasudevan EV, Bastian AJ. Motor adaptation training for faster relearning. J Neurosci. 2011;31(42):15136-43.
24. Musselman KE, Roemmich RT, Garrett B, Bastian AJ. Motor learning in childhood reveals distinct mechanisms for memory retention and re-learning. Learn Mem. 2016;23(5):229-37.
25. Reisman DS, McLean H, Keller J, Danks KA, Bastian AJ. Repeated split-belt treadmill training improves poststroke step length asymmetry. Neurorehabil Neural Repair. 2013;27(5):460-8.
26. Akaike H. New Look at Statistical-Model Identification. Ieee T Automat Contr. 1974;Ac19(6):716-23.
27. Takahashi CD, Nemet D, Rose-Gottron CM, Larson JK, Cooper DM, Reinkensmeyer DJ. Neuromotor noise limits motor performance, but not motor adaptation, in children. J Neurophysiol. 2003;90(2):703-11.
28. Huberdeau DM, Haith AM, Krakauer JW. Formation of a long-term memory for visuomotor adaptation following only a few trials of practice. J Neurophysiol. 2015;114(2):969-77.

536 29. Ashtamker L, Karni A. Motor memory in childhood: early expression of
537 consolidation phase gains. Neurobiol Learn Mem. 2013;106:26-30.
538 30. Dorfberger S, Adi-Japha E, Karni A. Reduced susceptibility to interference in the
539 consolidation of motor memory before adolescence. PLoS One. 2007;2(2):e240.
540 31. Roig M, Ritterband-Rosenbaum A, Lundbye-Jensen J, Nielsen JB. Aging increases
541 the susceptibility to motor memory interference and reduces off-line gains in motor skill
542 learning. Neurobiol Aging. 2014;35(8):1892-900.
543 32. Centeno C, Medeiros D, Beck MM, Lugassy L, Gonzalez DF, Nepveu JF, et al. The
544 effects of aging on cortico-spinal excitability and motor memory consolidation.
545 Neurobiol Aging. 2018;70:254-64.
546 33. Tucker M, McKinley S, Stickgold R. Sleep optimizes motor skill in older adults. J
547 Am Geriatr Soc. 2011;59(4):603-9.
548 34. Doyon J, Korman M, Morin A, Dostie V, Hadj Tahar A, Benali H, et al. Contribution
549 of night and day sleep vs. simple passage of time to the consolidation of motor
550 sequence and visuomotor adaptation learning. Exp Brain Res. 2009;195(1):15-26.
551 35. Siengsukon CF, Boyd LA. Sleep enhances implicit motor skill learning in
552 individuals poststroke. Top Stroke Rehabil. 2008;15(1):1-12.
553 36. Steib S, Wanner P, Adler W, Winkler J, Klucken J, Pfeifer K. A Single Bout of
554 Aerobic Exercise Improves Motor Skill Consolidation in Parkinson's Disease. Front Aging
555 Neurosci. 2018;10:328.
556 37. Hardwick RM, Celnik PA. Cerebellar direct current stimulation enhances motor
557 learning in older adults. Neurobiol Aging. 2014;35(10):2217-21.
558