Procedural memory in infancy: Evidence from implicit sequence learning in an eye-tracking paradigm

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ABSTRACT

Procedural memory underpins the learning of skills and habits. It is often tested in children and adults with sequence learning on the serial reaction time (SRT) task, which involves manual motor control. However, due to infants' slowly developing control of motor actions, most procedures that require motor control cannot be examined in infancy. Here, we investigated procedural memory using an SRT task adapted for infants. During the task, images appeared at one of three locations on a screen, with the location order following a five-item recurring sequence. Three blocks of recurring sequences were followed by a random-order fourth block and finally another block of recurring sequences. Eye movement data were collected for infants ($n = 35$) and adults ($n = 31$). Reaction time was indexed by calculating the saccade latencies for orienting to each image as it appeared. The entire protocol took less than 3 min. Sequence learning in the SRT task can be operationalized as an increase in latencies in the random block as compared with the preceding and following sequence blocks. This

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pattern was observed in both the infants and adults. This study is the first to report learning in an SRT task in infants as young as 9 months. This SRT protocol is a promising procedure for measuring procedural memory in infants.

Introduction

Multiple systems appear to underlie learning and memory abilities (Eichenbaum, 2010; Schacter, Wagner, & Buckner, 2000; Squire, 2004). However, the development of procedural memory, the memory system that underlies the learning of habits and skills, has not been fully tracked from infancy to adulthood. Procedural memory involves the acquisition of particular motor or cognitive skills or habits and is implicated in the skills and habits that underlie language learning (Ullman, 2016) and social interaction (Lieberman, 2000). Skills and habits emerge in everyday life as the repeated execution of a task leads to improvements in the speed and/or accuracy with which the task is completed (Magallón, Narbona, & Crespo-Eguílaz, 2016; Squire, 2009; Zwart, Vissers, Kessels, & Maes, 2019). Using the same measure for infants and adults has important benefits for tracking the development of procedural memory because performance can be directly compared. For example, different looking paradigms have been used to test infants and adults. The visual paired comparison test is a looking paradigm, now understood to tap declarative memory (Manns, Stark, & Squire, 2000), which is well established as a measure in infancy that can also be used with adults (Richmond, Sowerby, Colombo, & Hayne, 2004). Statistical learning has also been examined in infants, children, and adults using a looking paradigm (Amso & Davidow, 2012), and the authors suggested that statistical learning showed developmental invariance based on observation of few or no differences in response patterns between age groups. However, procedural memory is frequently studied by investigating changes in motor behavior during sequence learning and, as such, has not been tracked from infancy to adulthood.

The serial reaction time (SRT) task and related tasks that measure sequence learning have frequently been used to examine the development of procedural memory over the life span beginning at 4 years of age (Adi-Japha, Badir, Dorfberger, & Karni, 2014; Howard, Howard, Dennis, & Kelly, 2008; Janacsek, Fiser, & Nemeth, 2012; Zwart et al., 2019). Nissen and Bullemer (1987) developed the original SRT procedure to investigate sequence learning. Participants pressed one of four keys in response to corresponding locations where a visual cue could appear on a screen. The spatiotemporal order was either random or determined by repeating a 10-item sequence 10 times in each block for eight blocks in total without indication of when the sequence or the blocks started or ended. In the random condition, participants performed the same number of trials and blocks. Results indicated that participants in the sequence condition improved in performance, with mean reaction time decreasing over blocks, whereas participants in the random condition did not. Even though the original study lacked a within-participant comparison, this SRT procedure became very influential in testing procedural memory. Recent studies (e.g., Karatekin, Marcus, & White, 2007; Lum, Kidd, Davis, & Conti-Ramsden, 2010) have added one or more random blocks after repeating blocks to confirm within-participant effects. When a random block is introduced, reaction time typically slows. This is because the predictions the participant has learned based on the repeated sequence are no longer valid. If a final sequence block is reintroduced, participants once again exhibit the learned behavior and reaction time decreases (Karatekin et al., 2007). This systematic decrease in reaction time during sequence blocks and increase in reaction time during random block trials is the hallmark of the SRT task (Robertson, 2007). The final sequence block is a manipulation check to ensure that any observed response time increase between the sequence block prior to the random block and the random block itself was not simply due to fatigue.

The kind of sequence that is used also varies among studies. In first-order sequences, such as those used by Nissen and Bullemer (1987) and Lum et al. (2010), the spatiotemporal order of visual stimuli can be predicted from the preceding item with varying degrees of probability. Second-order sequences, such as those used by Karatekin et al. (2007), are also common. In these sequences, the location of an upcoming visual stimulus can be predicted by combining the two preceding items, but the preceding item is not predictive in itself. Howard and Howard (1997) introduced the alternating SRT task, another frequently used variation (e.g., Howard & Howard, 2013; Nemeth, Janacsek, & Fiser, 2013) of the target sequence that focuses on second-order sequences. In the alternating SRT task, the position of every second appearance of the visual stimulus follows a given spatiotemporal sequence, whereas locations of intermittent items are chosen randomly.

The development of procedural memory might be invariant from childhood to adulthood. For example, Karatekin et al. (2007) examined four cross-sectional age groups from 8-year-olds to young adults. Furthermore, Lum et al. (2010) also found little evidence of change in performance in a longitudinal study from 5.5 to 6.5 years of age. However, some studies found evidence for change in procedural memory. Examining sequence learning over the life span from 4 years of age, Janacsek et al. (2012) found evidence for sequence learning to be most efficient until 11 years, with a decline in performance from that age onward. Lukács and Kemény (2015) also found evidence for change and argued for an inverted U-shaped pattern of performance on the SRT task across the life span with a peak at 30 years of age. However, data analysis method seems to influence the nature of the change observed in procedural memory. Janacsek et al. (2012) pointed out that if their data were corrected for baseline performance by normalizing the reaction times of the participants, the data indicated that children increase their efficiency for sequence learning between 4 and 11 years of age. Zwart et al. (2019) concluded in their meta-analysis that studies finding increases in sequence learning ability from school age to young adulthood are consistent if data were normalized. But if one considers raw data, children are consistent in their ability to learn from school age onward. This pattern of results underscores the need for a single task that can be used over the life span starting from infancy. In the current study, normalized data are reported because normalization of the data in some studies indicated an increase in the sequence learning ability in childhood. If the ability to learn sequences increases in childhood, showing effects of sequence learning ability in infants for normalized data would be a more robust test of sequence learning in infancy because effects would be smaller in normalized data.

Due to the lack of appropriate measures applicable across the life span, the developmental trajectory of procedural memory has not been fully documented from infancy to adulthood. A key problem in designing studies to examine procedural learning and memory in infants is connected with infants' slowly developing control of the motor actions often involved in tests of procedural learning. This has excluded them from most procedural tasks that involve motor control, including the SRT (Nissen & Bullemer, 1987) and related tasks. One way to circumvent the infant motor control problem is to instead use the ocular-motor system, which matures quickly during the first year of life (Braddick & Atkinson, 2011; Scerif et al., 2005). Vakil, Bloch, and Cohen (2017) compared reaction times based on manual responses to ocular-motor responses and found similar sequence learning in both measures in a study with adults. In the current study, therefore, we capitalized on infants' mature eye movements by developing an infant-friendly ocular-motor adaptation of the SRT task that can be used to examine procedural memory in both infants and adults. Instead of button presses, saccades (i.e., rapid eye movement from one visually fixated location to another) are measured.

Previous work based on the timing of saccades suggests that infants can learn visual sequences. Using the visual expectation paradigm (VExP), Haith and colleagues demonstrated that infants learn to expect the next item to appear in a particular location and can make an eye movement in anticipation of the item appearing there (Canfield & Haith, 1991; Canfield, Smith, Brezsnynak, & Snow, 1997; Haith, Hazan, & Goodman, 1988). In this procedure, visual stimuli are presented in the left or right visual field followed by a 1-s interval when no stimulus is presented. During this interval, infants perform anticipatory saccades to the anticipated visuospatial location of the upcoming stimulus, indicating expectation-based learning of the visuospatial sequence (e.g., Canfield & Haith, 1991). Note that although infants do perform anticipatory saccades significantly more often to upcoming locations of stimuli, most saccades actually occur after the item appears. However, these reactive saccades are also

taken to index learning because even reactive saccades to a predictable sequence item location are executed earlier than baseline (Canfield & Haith, 1991, Haith et al., 1988). The complexity of the visual sequences that can be learned as measured with the VExP increases developmentally. Canfield and Haith (1991) reported that 2-month-olds could learn an alternating left–right pattern, whereas 3-month-olds learned more complex asymmetric left–left–right patterns and 6- to 12-month-olds learned an even more complex visuospatial sequence, with anticipatory saccades increasing and reaction time decreasing from 6 to 9 months of age but no additional changes occurring between 9 and 12 months (Reznick, Chawarska, & Betts, 2000). The VExP differs from the SRT procedure in that the VExP paradigm lacks a comparison between a learned sequence and a random sequence. Instead, the VExP measures saccades toward the correct (upcoming) position compared with saccades to the wrong position.

Using a habituation procedure, Kirkham, Slemmer, Richardson, and Johnson (2007) presented evidence for learning of even more complex sequences. In their study, 11-month-old infants watched objects appear one at a time in one of six different locations in a 2 × 3 grid. The location of each item was either unpredictable or perfectly predictable based on the location of the previous item. The study revealed that the infants looked longer at stimuli with novel and unpredictable sequences compared with predictable sequences that included the learned spatiotemporal order, indicating sequence learning. Although these findings indicate a capacity for learning visuospatial sequences, analogous to findings from SRT, it is difficult to directly compare performance on these infant tasks with data collected with older children and adults on SRT tasks. It is for this reason that we developed an ocular-motor SRT task for infants.

The current study protocol used a first-order sequence (Lum et al., 2010; Nissen & Bullemer, 1987; Vicari, Marotta, Menghini, Molinari, & Petrosini, 2003). First-order sequence learning has been linked to activity of the basal ganglia and, thus, has been implicated in procedural memory processes in adults (Clark, Lum, & Ullman, 2014). Procedural memory, as opposed to declarative memory, is usually defined as inaccessible to conscious recollection (Squire, 2009), and scientific attention has been aimed at designing studies where participants are unaware of the learned sequences (Song, Howard, & Howard, 2007; Stark-Inbar, Raza, Taylor, & Ivry, 2017). For example, to reduce awareness, Song et al. (2007) embedded the repeating sequence within an alternating pattern using the alternating SRT. Awareness of a learned sequence might affect how flexibly the learned representation can be used (Esser & Haider, 2017) and might increase variability in performance (Yordanova, Kirov, & Kolev, 2015). Structural features of the target sequence (e.g., its length) influence how easy it is for participants to become aware of the target sequence, and perceptually salient features of the stimuli can emphasize but also distract from the structural features of the target sequence. Thus, in the current study, we aimed to strike a balance between a sequence that infants are able to learn and that adults cannot verbally describe. One adaptation introduced in the current study is the use of infant-friendly images, a novel one for each trial, instead of using the same visual stimulus, an asterisk or a dot, throughout (Robertson, 2007). This changes the perceptually salient features of the stimuli used and adds the possibility of verbal processing for each image, but the spatiotemporal structure is unchanged. It is important to note that although the spatial pattern repeats, the images change across trials, meaning that it is more difficult to detect the repeating spatial sequence and that even if items are labeled by participants, verbal processing will not aid sequence learning.

Changes in eye movements toward predictable stimuli have also been used to index procedural learning in adults. Specifically, saccade latency has been used to measure procedural learning on the SRT task with adult participants (Marcus, Karatekin, & Markiewicz, 2006; Vakil et al., 2017) and children aged 8 years and older (Karatekin et al., 2007). Saccade latency is comparable to motor response time. Instead of measuring time taken to press the button, reaction time is measured from the appearance of a stimulus at a new location until the participant starts to saccade to the new location. This indexes how quickly a person shifts his or her attention to a new visuospatial location. The assumption is the same as with manual SRT tasks; that is, a reaction to a predictable location should be faster than a reaction to a random location. Thus, both manual and ocular-motor reaction times tap procedural memory (Marcus et al., 2006; Vakil et al., 2017). The SRT task has been widely used in older children with productive language and adults (Lum, Ullman, & Conti-Ramsden, 2013; Magallón et al., 2016; Robertson, 2007). But to the best of our knowledge it has not been used in infancy.

In the current study, we tested a new ocular-motor adaptation of the SRT task that is suitable for infants but that could be used with adults as well. Participants were presented with 125 trials divided into five blocks. Each trial presented a novel infant-friendly image in one of three locations on a screen, with the new location always being different from the previous trial's location. In the first three blocks and the final block, the spatiotemporal order of items was defined by a five-item spatiotemporal sequence. In the fourth block, the spatiotemporal order was random.

The critical index of SRT sequence learning in this ocular-motor adaptation of the SRT task, as in SRT tasks in general, is a significantly longer mean reaction time in the random block compared with both the immediately preceding and immediately following recurring sequence blocks (Robertson, 2007). Therefore, we examined mean reaction times within these three blocks and predicted that saccade latencies would be significantly longer in the random block compared with the preceding and following sequence blocks for all participants, infants and adults alike. Furthermore, for the disruption during the critical phase to be plausibly linked to procedural memory, some kind of learning would need to occur during a learning phase, which in the current study is represented by the first three blocks. A significant increase in reaction time across the learning phase would invalidate the ocular-motor SRT task. However, we expected the participants to demonstrate a decrease in mean saccade reaction times across the learning phase. Given prior studies of infant learning (for a review, see Rovee-Collier & Barr, 2010), we predicted that mean reaction times would decrease more in infants than in adults. In addition, to check whether the adults had any explicit knowledge of the recurring sequences after performing the ocular-motor SRT task, we asked them to report any sequences that they noted.

Method

Participants

The current study was approved by the regional ethical review board, Linköping Sweden (2017/386-32).

Infants

Parents were invited by mail to participate with their infants in developmental psychology research. Those who expressed an interest were contacted by phone and informed about the study. In the current study, 35 monolingual Swedish infants (17 female) took part. All were full-term (gestational age 38 weeks or later; $M = 40.51$ weeks, $SD = 0.92$). At time of testing, their mean age was 9.38 months ($M = 285.2$ days, $SD = 10.04$, range = 268–309). The parents of the participating infants reported that their infants were healthy and typically developing. These parents were highly educated (83% of mothers and 63% of fathers had a university degree). An additional 28 infants began the protocol but were not included in the final sample. Of these 28 infants, 15 failed to complete the protocol due to fussiness (i.e., actively trying to get away from watching the screen) and 13 infants did not meet inclusion criteria of the test protocol (i.e., saccade latencies identified for less than half of all trials in at least one block [see below]). This rate of attrition is common in infant eye-tracking studies that use parameters similar to those of the current experiment (e.g., Kirkham et al., 2007, Experiment 2; Wang et al., 2012, Experiment 2).

Adult comparison group

As a comparison group, 31 adults (18 female) with a mean age of 24 years ($SD = 3.18$, range = 20–37) working or studying at Linköping University completed testing. All were healthy and had normal or corrected-to-normal vision. A further 5 adults began the experiment, but their data could not be included due to technical failure ($n = 3$), experimenter error ($n = 1$), or failure of the system to detect the eyes during eye tracking ($n = 1$).

Ocular-motor SRT task

The SRT task (Nissen & Bullemer, 1987) was adapted for use in an eye-tracking paradigm, and the same protocol was run with infants and adults. For infants and adults, reaction times were measured by saccade latencies.

Apparatus and task materials

Gaze data were collected with a Tobii T120 eye tracker (Tobii, Stockholm, Sweden). The screen size of the monitor was 1280 × 1024 pixels. The firmware version of the T120 was 2.0.7. We used a 64-bit Windows computer with operating system Windows 7 Enterprise (Service Pack 1). The processor was a 3.60-GHz Intel CPU with 12 GB of RAM. Tobii Studio 3.4.8 was used to calibrate the eye-tracking system to the participants' eyes, and the experiment was run on MATLAB Version 2016b (The Math-Works, Natick, MA, USA). The custom script used for running the experiment used Psychtoolbox Version 3.0.13 (Brainard, 1997; Kleiner et al., 2007) and TobiiPro SDK Version 1.1.0.21 (Tobii) for MATLAB.

All images for the ocular-motor SRT task were infant-friendly drawings of animals and cartoon characters (see Fig. 1). They were presented with a size of 400 × 400 pixels, corresponding to 9.9 visual degrees for a viewing distance of about 60 cm, the approximate distance between the participant's eyes and the screen. The three on-screen positions of the images (lower left corner, lower right corner, and top center) were defined by an equidistant triangle with a distance of 14.9 visual degrees between the images' centers.

This implementation of the ocular-motor SRT is based on gaze contingency. This means that trials were started based on gaze positions. Once a fixation on the target image was registered, a new trial was triggered automatically by the system and a new image was displayed at a new spatial location. The procedure always started with an image that was presented at the center of the screen. After the participant had fixated on this image for at least 200 ms, the image disappeared and simultaneously the first trial of the SRT task started. In each trial, a new image appeared at the spatial location immediately after the previous image disappeared. The spatial location of the item (Location A, B, or C; see Fig. 1) was determined either by the visual spatial sequence (during sequence block trials [see details below]) or following a pseudorandom order (during random block trials [see below]). Although the spatial location sequence repeated in the sequence trial block, different images were used on each trial. To ensure that the participant oriented to the screen, each trial onset was accompanied by
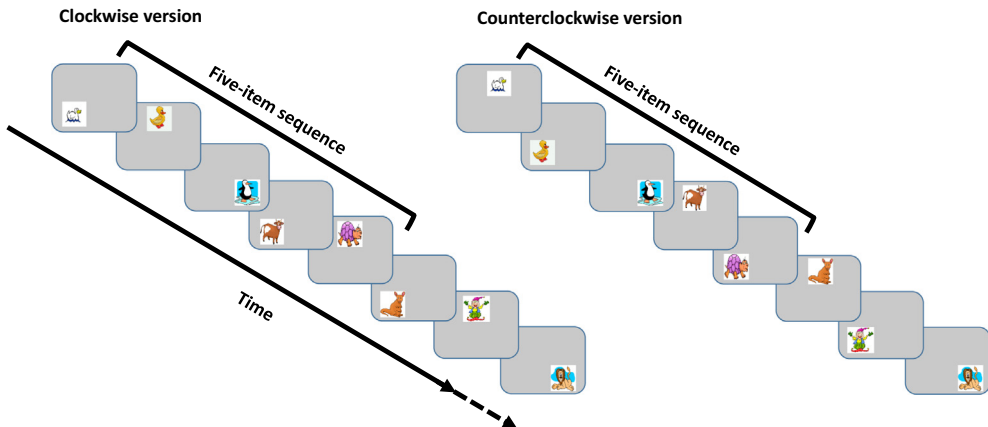

Fig. 1. Infant-friendly ocular-motor serial reaction time task. Images are shown in one of three different locations (A, B, or C) one at a time. After the infant fixated on an image for at least 200 ms, it disappeared and the next image immediately appeared at a new spatial location. Order of spatial locations was determined by a five-item sequence (A-B-C-A-B) that was repeated five times during each sequence block. Each infant saw either a clockwise version, where positions A, B, and C corresponded to lower left corner, top center, and lower right corner, respectively, or a counterclockwise version, where positions A, B, and C corresponded to top center, lower left corner, and lower right corner, respectively.

one of 85 short unrelated infant-friendly sounds that lasted less than a second. These sounds were not related to either the image or the location. Each new trial began only after the participant fixated for at least 200 ms on the area where the current image was being shown.

In this implementation, participants were presented with five blocks of 25 trials each. Blocks 1, 2, 3, and 5 were "sequence blocks," whereas Block 4 was a "random block." In each sequence block, a five-item sequence was presented five times. The visuospatial sequence was A-B-C-A-B (following Vicari et al., 2003), with each letter corresponding to one of three spatial locations on-screen. Infants were tested with one of two versions to control for possible directional biases in visual spatial sequence learning (see Bulf, de Hevia, Gariboldi, & Macci Cassia, 2017, who found a directional bias for sequence learning in infancy). In the clockwise version, A corresponded to lower left corner, B to top center, and C to lower right corner for on-screen position, with most transitions being in the clockwise direction. In the counterclockwise version, A corresponded to top center, B to lower left corner, and C to lower right corner (see Fig. 1). All trials followed directly after each other, with no indication of when the sequence or blocks ended or began. In the random block (Block 4), a pseudorandom order of 25 visuospatial locations was used, which was C-B-A-C-B-A-C-B-C-B-A-B-A-C-A-C-B-A-B-A-B-A-B-A-C for the clockwise version; the reversed order was used in the counterclockwise version. It is important to note that reversals (Vaquero, Jiménez, & Lupiáñez, 2006) were frequent in both the sequence and random blocks: 10 in the sequence blocks and 12 in the random block. Note that reversal occurred when sequences were repeated (... A-B-C-A-B-A-B-C-A-B ...).

If necessary, the experimenter could attract the infant's attention to the screen by activating an attention-getter. This changed the background color and replayed the infant-friendly sound that was played at that trial's onset. The attention-getter was activated on average 5.11 times ($SD = 6.52$) per infant. On these trials, valid saccade latencies could still be obtained as the attention-getter attracted the infant to the screen, and the next trial would be triggered automatically after the infant fixated on the relevant area for 200 ms (see below for details). Furthermore, the experimenter could manually trigger the next trial; this occurred on average in 0.23 trials ($SD = 0.69$) per infant. These trials could not yield valid saccade latencies and were automatically dropped from analyses.

On-line data processing

While participants were being tested, gaze data were analyzed automatically to identify fixations on target images that would trigger the following trial contingent on participants' gaze. At any one time during the experiment, the system would analyze the 26 most recent gaze data samples. Each gaze data sample consisted of x and y coordinates that define the position of the gaze on-screen, and these were collected with a frequency of 120 Hz (every 8.33 ms). Thus, the 26 most recent gaze data samples assessed an infant's gaze behavior for at least the last 200 ms. These data were "smoothed" by replacing each value by a mean using a "moving average" with a window of 5 data points. A fixation was then identified if during this time period no eye movement that was faster than the threshold of 35° per second was detected. In this case, the position of the fixation was estimated by calculating the mean for all x and y coordinates during the last 200 ms. If the position of the fixation matched the position of the current image, the next trial was triggered by displaying a new image at a new location and removing the current image. However, if no fixation was identified or the identified fixation was not on target, the system would analyze the 26 most recent data samples again. If a new data sample had been collected by the system during the interim, the data sample would be updated before the analysis was repeated. This ensured that the time lag of the system was no more than a few milliseconds and that the participant had fixated for at least 200 ms on the current image before the next trial was triggered.

Procedure

When an infant and parent arrived at the lab, the procedure was explained to the parent, who then signed a consent form. Once the infant showed signs of comfort, such as smiles and positive vocalizations, the experimental procedure was started. The infant was seated in his or her parent's lap in front of the Tobii T120 monitor. A 36-s infant-friendly video clip was used to attract attention to the screen.

While the infant watched the video, the distance from the monitor to the infant was adjusted to approximately 60 cm, with the monitor centered in front of the infant's face.

Parents were instructed not to comment on what was shown on-screen. They were also informed that it did not matter if their infants looked away for a short while, but if infants did not attend to the monitor for a longer period, parents could try to direct infants' attention to the monitor by saying "Look at that" in Swedish.

After the monitor's position was adjusted, the experimenter started the Tobii Studio infant calibration procedure using five calibration points. A curtain that separated the experimenter from the parent and infant was closed before starting the calibration procedure. After successful calibration, several stimulus videos (total duration of 244 s) were shown. These were unrelated to the SRT task and were part of a larger study. The SRT task started once the other videos were finished. Infants completed the SRT task on average in 104.3 s (SD = 22.5), with the longest duration being 169 s.

Adults were tested using the same setup and stimuli as were used with infants. Because adults were recruited to participate in a BabyLab study, they were informed that they were part of a comparison group in an experiment designed to test a newly adapted task for infants. After adult participants signed a consent form, they were asked to sit down in front of the Tobii T120 monitor and the calibration procedure was started. For adults, the SRT task started immediately after calibration. After adults finished the SRT task, they were asked, "Did you notice anything in particular during the test situation?" If they said yes, they were asked to expand. Participants could, for instance, refer to sounds, pictures, or patterns. If they mentioned a pattern, they were asked to describe the pattern.

Statistical analysis

In the current study, the analyzed responses were saccade latencies. Saccade latencies were included only for trials in which a saccade from the location of the previous image to the location of the current image occurred between 0.1 and 2 s from trial onset. Criteria for identification of saccade latencies followed Kenward et al. (2017). Saccade latencies were extracted for raw eye gaze data by a custom-made script using MATLAB Version 2016b. Gaze data were processed by first removing all invalid data points, as defined by Tobii Studio. If possible, gaze data position was based on an average for both eyes' gaze positions. If data for only one eye were available, those data were used. As with the on-line analysis, data were smoothed by applying a moving average over 5 samples to x and y coordinates separately, and then eye movement velocity was calculated and saccades were defined as periods of eye movement above 35° per second. To avoid data misclassification, response saccades are classified as those saccades whose origin matched the position of the previous image and whose destination matched the position of the current image. All periods of eye movement above 35° per second occurring between 100 ms and either 2000 ms or the beginning of the next trial (whichever occurred first) were analyzed for origin and destination. If origin and destination matched, the response saccade was identified and latency was calculated from trial onset to the beginning of the saccade.

Participants were included in the analyses if they completed all five blocks. Due to infants being more easily distracted than adults, an additional inclusion criterion for infants was used. For infants, saccade latencies needed to be identified for at least half of all trials per block (for a minimum of 13 of the 25 trials per block). Data for 35 infants met this criterion, but 13 additionally tested infants were excluded for not meeting this criterion (see "Participants" section). This ensured that the mean for a given block is based on at least 13 valid saccade latencies from that block and that infants were following the procedure as intended.

First, to test whether infants and adults exhibited sequence learning, we analyzed performance on the critical phase Sequence Block 3, random block, and final sequence block. Following Lum, Conti-Ramsden, Page, and Ullman (2012), each individual's saccade latencies were transformed to z scores. Transformation to a z score for a given trial was calculated based on an individual's median and standard deviation for all valid trials in these three blocks. The transformation ensures that all participants' shortest and longest saccade latencies had similar values. Because the data were positively skewed, the median rather than the mean was more representative of the central tendency for the transformation. For this reason, the median was used as an anchor when transforming each individual's data to z scores (Fig. 2). The mean of the transformed data for each block was then calculated based on z values
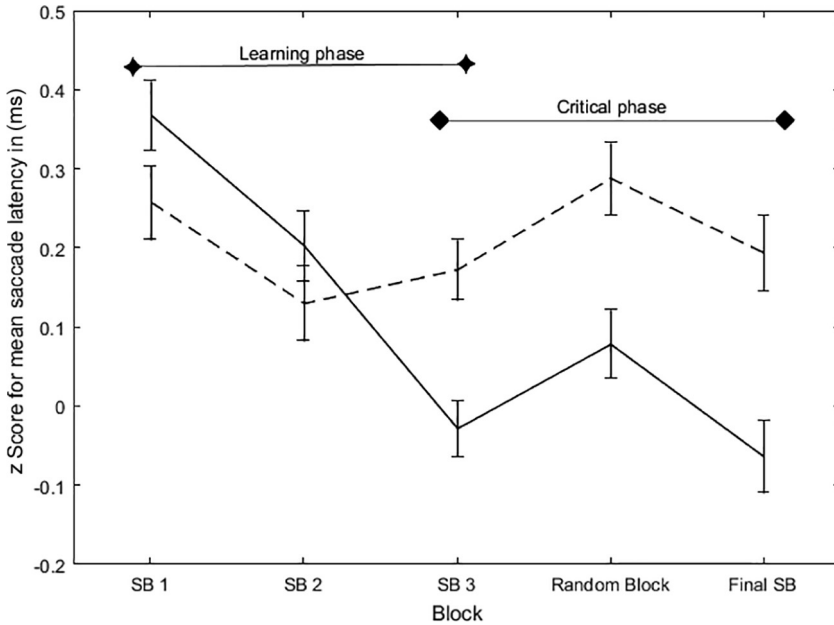
Fig. 2. Infants' (solid line) and adults' (dashed line) means for z scores per block. The median is used as a reference for the z transformation. This is why most values are higher than 0. SB, sequence block.

for all valid trials for a given block per participant. This resulted in three mean z values per participant for saccade latencies. Changes between blocks were compared by a mixed-design analysis of variance (ANOVA) with repeated measures across blocks and factors such as age group (infant or adult) and gender as between-group measures.

Furthermore, the learning phase was also analyzed based on z scores. Here, saccade latencies were transformed using the median and standard deviation for all valid saccade latencies in Sequence Blocks 1, 2, and 3 to calculate z scores for each valid trial. As above, for each block (Block 1, 2, or 3), a z score mean was then calculated, which was used as the dependent variable in the repeated-measures ANOVA. Mauchly's test of sphericity was not significant for any of the presented analyses. For post hoc comparison of differences between groups, Bonferroni corrections were used throughout. Statistical analyses were performed in MATLAB Version 2016b. Partial eta-square is reported following the definition of Richardson (2011). Files for gaze data and the MATLAB script that extracts the saccade latencies and performs the analyses are published and available for download (Koch et al., 2019).

Results

To illustrate the raw data pattern of results, descriptive statistics for raw saccade latencies in milliseconds can be found in Table 1. As this table shows, saccade latencies of infants are nearly twice

Table 1
Means and standard deviations for saccade latencies (ms) for infants and adults per block.

		Block 1 sequence		Block 2 sequence		Block 3 sequence		Block 4 random		Block 5 sequence	
Group	n	M	SD	M	SD	M	SD	M	SD	M	SD
Infants	35	347.7	50.5	334.7	44.8	319.9	46.0	328.3	52.0	318.5	48.9
Adults	31	186.6	22.8	183.3	25.8	180.9	16.8	190.5	26.8	183.5	18.3

those of adults and also the variability in saccade latencies of infants is nearly twice that of adults. All the subsequent analyses are based on z-transformed values (see Method for details). Due to the debate about whether data should be normalized (Janacsek et al., 2012; Lukács & Kemény, 2015; Zwart et al., 2019), all analyses were performed with raw scores as well. These results can be found in the online supplementary material, showing that the findings and conclusion are the same irrespective of whether data were normalized. First, the critical phase (Sequence Block 3, the random block, and the final sequence block) of the ocular-motor SRT was examined. Next, we conducted an analysis to examine the learning phase (Sequence Blocks 1–3).

Preliminary analyses of the critical phase

We first assessed whether gender or sequence order was associated with task performance. Gender did not enter into any significant main effects, $F(1, 62) = 0.18$, $p = .67$, $\eta_p^2 < .01$, or any two-way or three-way interaction terms (all ps > .10). For infants, order (clockwise vs. counterclockwise) was tested, but this factor did not enter any main effects, $F(1, 31) = 1.65$, $p = .21$, $\eta_p^2 = .05$, or any two-way or three-way interactions (all ps > .10). The factors gender and order were not considered in any subsequent analyses.

Performance during the critical phase

A mixed-design ANOVA was run with repeated measures across blocks (3–5) and between-group factor age (infant vs. adult). This analysis indicated that there was a main effect of block, $F(2, 128) = 5.71$, $p < .01$, $\eta_p^2 = .08$, and a main effect of age, $F(1, 64) = 5.39$, $p = .02$, $\eta_p^2 = .08$, but no interaction between block and age, $F(2, 128) = 0.37$, $p = .69$, $\eta_p^2 < .01$. For both infants and adults, there was a significantly longer mean reaction time in the random block compared with both the immediately preceding sequence block ($p = .02$) and the immediately following recurring sequence block ($p < .01$).

Learning phase

Furthermore, it is of interest to analyze how saccade latencies changed during the learning phase; this concerns Sequence Blocks 1 to 3. Again, a mixed-design ANOVA was run with repeated measures across blocks (here, Sequence Blocks 1–3) and between-group factor age (infant vs. adult). This analysis yielded an interaction between age and block, $F(2, 128) = 8.65$, $p < .001$, $\eta_p^2 = .12$, as well as a main effect of age, $F(1, 64) = 8.64$, $p < .01$, $\eta_p^2 = .12$, and block, $F(2, 128) = 18.76$, $p < .001$, $\eta_p^2 = .23$. For infants, differences in mean reaction time were not significant between Block 1 and Block 2 ($p = .15$), but mean reaction time decreased significantly from Block 2 to Block 3 ($p = .02$). For adults, differences were not significant between blocks (both ps > .10). It is important to note that saccade latency did not significantly increase during the learning phase for either infants or adults because such an increase during the learning phase might indicate that the task was not working as intended (e.g., due to fatigue of participants). Thus, an increase in saccade latency was observed only for the random sequence during the critical phase (see above).

Adult explicit sequence knowledge

Three participants reported that they noticed a sequence that was three items long (A-B-C), and one participant reported a sequence that was four items long (A-B-A-C), but no one reported a five-item or longer sequence; thus, no one identified a whole correct sequence. Eight participants said that they were looking for a pattern but did not find one, seven participants commented that the images and sounds used were infant friendly or funny, and six participants commented that images changed very quickly. Two participants said that they were trying to match the sounds to the images but did not find a pattern there.

Discussion

Here, we tested a novel infant-friendly ocular-motor version of the SRT task adapted for infants using saccade latencies as the main measure. Participants were presented with three sequence blocks, followed by a random block and then a final sequence block. Performance on the ocular-motor SRT task indicates sequence learning when mean reaction time on the random block is significantly slower compared with both the preceding and following recurring sequence blocks. This pattern was significant for all participants, infants and adults alike. For the learning phase, no significant deceleration in reaction times was observed for either adults or infants. This indicates that a deceleration in reaction time was unique to the random block. For infants, faster saccade latencies were apparent for Block 3 compared with Block 2. This decrease in saccade latencies is in line with previous research on sequence learning showing better performance across the initial blocks (Magallón et al., 2016). The change in saccade latencies for infants may reflect general task learning effects. However, because deceleration in response time was observed only for the random block, the finding here shows specific sequence learning for infants and adults.

As can be expected based on previous research (Kenward et al., 2017), adults' overall saccade latencies were nearly twice as fast as those of infants and the infants showed a much higher variability in latencies compared with adults (see Table 1). Despite accounting for differences in absolute reaction time by normalizing the data, specific sequence learning effects were found in both infants and adults. As mentioned in the Introduction, whether the data were normalized or not influenced the interpretation of developmental trends in some studies (Janacsek et al., 2012; Lukács & Kemény, 2015; Zwart et al., 2019), and for this reason we chose the more stringent normalization approach in our analysis. Importantly, in the current study, the pattern of results did not differ between normalized data (see Results) and raw data (see supplementary material); infants and adults showed similar changes in performance in the final three blocks. The mean reaction times of both infants and adults were slower in the random block compared with the preceding sequence block as well as the subsequent recurring sequence block. This suggests that participants in both age groups were able to use their knowledge of the sequence to restore their speed of response in the final sequence block. These findings suggest that developmental changes can be tracked from infancy to adulthood using the same measure. Whether development of procedural memory is best described as developmentally invariant or entails developmental changes in performance is beyond the scope of the current study.

The development of an infant SRT task provides a number of possibilities for future research avenues to test theoretical models of procedural learning. Specifically, researchers have suggested that procedural memory consists of two subprocesses, namely sequence learning and statistical learning (Kóbor et al., 2018, Simor et al., 2019). Whereas sequence learning refers to the ability to learn the serial order of longer sets of items (5–12) (Simor et al., 2019), statistical learning refers to the ability to learn frequency or probability relations between items (Saffran & Kirkham, 2018). Nemeth et al. (2013) suggested that there are different developmental trajectories for these two subprocesses of procedural memory from 11 years of age onward. They argued that although statistical learning decreases, higher-order sequence learning increases in efficiency from 11 years of age onward. Amso and Davidow (2012) suggested that there is developmental invariance from infancy in statistical learning, suggested by the same pattern of responses across age groups. But until now there has not been an SRT task suitable for use with infants to directly compare performance on statistical learning and sequence learning. The results presented here provide evidence of specific sequence learning effects (Robertson, 2007) in infants and adults. By assessing sequence learning (using the ocular-motor SRT task) and statistical learning (using, e.g., Amso & Davidow's (2012) task) in various age groups, future research could document and compare the developmental trajectory of both subprocesses of procedural memory.

Methodological modifications of the ocular-motor SRT task need further discussion. The visuospatial sequence used in the current study is shorter (five items) than sequences that are usually presented to adult participants (10 items) (Nissen & Bullemer, 1987; Robertson, 2007). In addition, three locations instead of four locations were used. Despite these simplifications, of the adult participants, only four of them reported noticing any visuospatial sequence, and none of them identified the correct one. This

may have been partly due to the fact that new images were presented on each trial, which is a deviation from previous SRT tasks for which dots or asterisks were used as stimuli. The images were primarily used to motivate attention for the infants, but perhaps an unintended consequence was that adults were not aware of the regularity in the spatiotemporal locations of the sequence. That is, one effect could be that adults' attention focused more on the content of the images, making it harder to detect the visuospatial sequence. Despite the added complexity due to novel images on each trial, we speculate that this does not change the procedural aspect of the task but assume that the visual attractiveness of the images kept infants engaged with the task. All participants were given about 200 ms to attend to each image, and thereafter the image automatically disappeared and the next trial was triggered. This reduced individual variation for how much visual attention a participant devoted to images and also controlled for visual attention between infants and adults. We did not examine the effect of stimulus material, but overall saccade latencies did not seem to be slower for 9-month-olds or adults in the current study (cf. Kenward et al., 2017). Furthermore, each new image appeared simultaneously with the offset of the old image without any gap or delay between trials, which is contrary to common SRT tasks that use a time delay of 200–500 ms (Robertson, 2007). So, participants could not "linger" on an image before responding because it disappeared after 200 ms of visual attention, just as the next image appeared. We think that the combination of gaze-contingent trial onset and lack of delays between images made it difficult to detect and verbally describe this simplified sequence. More sophisticated screening methods for detecting awareness about the sequence (e.g., Stark-Inbar et al., 2017) would better describe to what degree adults are aware of the target sequence. Future research should cross-validate the current method with a more traditional SRT method with adults to confirm that similar patterns of behavior emerge. Given the fact that the findings of this study point to implicit sequence learning by adults, it seems likely that the infants also learned the task implicitly. Thus, we propose that the newly adapted ocular-motor SRT task introduced in the current study provides a measure of procedural memory that can be used from infancy to adulthood.

Limitations

In total, 63 infants started the test in the current study. Of these, 15 infants did not finish it due to fussiness and a further 13 were excluded due to not passing the inclusion criterion, which was that more than half of the trials per block needed to provide valid saccade latencies. Lack of valid saccade latencies could be due to improperly tracked eye gaze even though infants were looking at the screen. To improve the eye gaze data, more sophisticated gaze data imputation techniques could be used, which might identify more valid saccade latencies. Another reason for missing saccade latencies could be that infants did not saccade toward the upcoming image but rather looked away from the screen after a new trial was triggered. In these cases, infants disengaging from the task would, if it happened too often, jeopardize the validity of this task as a procedural task. Triggering trials without attending to the next trial would not lead to procedural learning. This was the reason for implementing the inclusion criterion for infants. Attrition is common in infancy studies, and attrition in the current study was not different from what might be expected when studying infant cognition (Kirkham et al., 2007; Rovee-Collier & Barr, 2002; Wang et al., 2012). Our 9-month-old participants had been watching 4 min of television before starting the SRT procedure. Had we started with the SRT procedure earlier, fewer infants might have been lost due to fussiness. Furthermore, inclusion criteria need to be balanced between ensuring that infants actually perform the intended task and including as many infants as possible (Oakes, 2010). Future studies could examine improvements of inclusion criteria for infants.

For adults, the increase in saccade latencies in the random condition was much lower than what Vakil et al. (2017) observed in a sequence learning study. Several differences might contribute to our finding. As mentioned above, we used meaningful images that were novel for each trial, but we also used three positions instead of four positions. The main difference when it comes to motor behavior is that Vakil et al. changed the sequence after participants viewed 648 trials, as opposed to 75 trials in the current study. Because procedural memory depends on practice, a longer learning phase is likely to lead to a stronger disruption by the random sequence. The much lower number of trials in our study compared with other prior studies could also be a reason for adults not noticing the correct visuospatial sequence. This could be verified empirically.

Vaquero et al. (2006) pointed to the problems of reversals during sequence learning. Reversals are found when the target appears in the same area in the first and third trials of three consecutive trials. Vaquero et al. argued that reversals occur more often in random sequences than in structured sequences. With only three positions to choose from, reversals were rather common in the current study in both conditions (10 in sequence blocks and 12 in the random block). Vaquero et al. recommended using a different structured sequence as the control comparison. In the current experiment, we used two versions—a clockwise version and a counterclockwise version—during sequence learning and compared these with when the sequence was random. It might be possible to use the counterclockwise version as the control sequence when infants learned the clockwise version (and vice versa). We have not tested this. Another methodological change could be to use four positions instead of three. With more positions to choose from, this could also reduce the problem with reversals, but whether infants can learn the structure in a sequence that is based on four locations remains to be tested. Establishing the SRT protocol for infants means that it will be possible to test these variations.

Recently, issues about reliability of SRT and related tasks have been discussed (Conway, Arciuli, Lum, & Ullman, 2019; West, Vadillo, Shanks, & Hulme, 2018). A limitation of the ocular-motor SRT presented here is the lack of test–retest data. Infants have been tested only once. Infants could be tested several times to assess consistency in procedural learning.

Future directions

Theoretically, it is important to develop several memory measures that can track the development of different memory systems over the life course. The development of such measures would allow researchers to track the emergence of abilities that depend on specific memory systems but also to examine whether the systems emerge independently of each other or whether they are dependent on each other. With respect to procedural memory, the adapted ocular-motor SRT task could be used to track encoding, consolidation, and recall processes. Of particular interest would be the issue of memory consolidation during sleep (Fischer, Drosopoulos, Tsen, & Born, 2006).

Future research should more closely examine the development of procedural memory in infants. By using the same SRT task from infancy through childhood and into adulthood, the development trajectory of sequence learning can be examined. A possible developmental invariance or a decline in saccade latencies might be possible outcomes. It is not clear whether the adapted SRT task works for the elderly population, and this will be an interesting future examination.

Whether or not infants can also learn sequences that reflect second-order dependencies (and not just first-order dependencies as used here) remains to be seen. First- and second-order sequence learning might rely on distinct neural networks in adults (Clark et al., 2019; Lum et al., 2018). If infants are not able to learn second-order dependencies, this might be an aspect of the procedural memory system that develops with age. Future research could investigate whether this possible change is due to better integration of the procedural memory system with other systems or changes in the procedural memory system itself. It may be possible to develop an alternating SRT variant of this task.

The ocular-motor SRT protocol outlined in this study takes less than 3 min to complete, which makes it usable for infant research. This also means that the task can be integrated into a protocol that includes other cognitive measures, allowing procedural memory to be compared with other cognitive abilities and their development. In adults, it has been demonstrated that implicit sequence learning, which the SRT task entails, relies on a network of brain structures that is rooted in the basal ganglia, in particular portions of the striatum (Clark et al., 2014; Hardwick, Rottschy, Miall, & Eickhoff, 2013). Neuroimaging studies have demonstrated dissociations between procedural and declarative memory systems in adults (Squire, 2009). The current task provides a promising tool to examine the developmental origin of the dissociation between memory systems.

Conclusion

The novel infant-friendly ocular-motor adaptation of the SRT task introduced here enables rapid assessment of implicit sequence learning and is a promising procedure for measuring procedural memory in infants. The results indicate that infants show procedural memory similar to that of adults.

Acknowledgments

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jecp.2019.104733.

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