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RESEARCH ARTICLE

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Anticipatory eye movements and long-term memory in early infancy

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Abstract

Advances in our understanding of long-term memory in early infancy have been made possible by studies that have used the Rovee-Collier's mobile conjugate reinforcement paradigm and its variants. One function that has been attributed to long-term memory is the formation of expectations (Rovee-Collier & Hayne, 1987); consequently, a long-term memory representation should be established during expectation formation. To examine this prediction and potentially open the door on a new paradigm for exploring infants' long-term memory, using the Visual Expectation Paradigm (Haith, Hazan, & Goodman, 1988), 3-month-old infants were trained to form an expectation for predictable color and spatial information of picture events and emit anticipatory eye movements to those events. One day later, infants' anticipatory eye movements decreased in number relative to the end of training when the predictable colors were changed but not when the spatial location of the predictable color events was changed. These findings confirm that information encoded during expectation formation are stored in long-term memory, as hypothesized by Rovee-Collier and colleagues. Further, this research suggests that eye movements are potentially viable measures of long-term memory in infancy, providing confirmatory evidence for early mnemonic processes.

KEYWORDS

infancy, long-term memory, visual expectations, anticipatory eye movements

1 | INTRODUCTION

For the longest time, infants were thought to be incapable of establishing enduring long term memories. As recently as 2004, Neisser suggested that our inability to remember events from before we were approximately 2 years of age, also known as infantile amnesia, stems from an inability to establish early memory traces in the first place (<u>Neisser</u>, 2004). Yet, with the advent of the Mobile Conjugate Reinforcement Paradigm over four decades ago (<u>Rovee & Rovee</u>, 1969), as well as the deferred imitation paradigm (e.g., Barr & Hayne, 2000; <u>Barr, Rovee-Collier, & Campanella, 2005; Meltzoff, 1988, 1995</u>), a steady stream of research has highlighted the sophisticated capacity that exists in early infancy for establishing longterm memories, the specificity of those memories, and the similarity of early memory mechanisms to those seen with adult memory (<u>Hayne, 2004;</u> <u>Rovee-Collier, 1997, 2001</u>).

Many studies of memory in infants have used visual recognition paradigms and have generally found that infants can only remember for a few seconds or minutes at most (Kagan & Hamburg, 1981; Werner & Perlmutter, 1979). In one habituation study, for example, 4-month-old infants were found to remember information for a maximum of 15 s (Kagan & Hamburg, 1981). More recently, Pascalis, de Haan, Nelson, and de Schonen (1998), demonstrated with a visual pairedcomparison task that some infants exhibit long-term memory for faces after an interval of 1 day. Conditioning paradigms (as well as deferred imitation tasks), in contrast, have suggested the maintenance of a response over much longer intervals; for example, a conditioned eye blink response, an indicator of memory, was exhibited by 10-, 20-, and 30-day olds, after a delay of 10 days (Little, Lipstitt, & Rovee-Collier, 1984) and 2 weeks in 5-month olds (Invkovich, Collins, Eckerman, Krasnegor, & Staton, 1999). The Mobile Conjugate Reinforcement Paradigm, an operant conditioning paradigm, has been particularly influential in furthering our understanding of infant memory development. In the mobile paradigm, infants learn to kick to produce movement in an overhead mobile placed on their crib. A retention interval of hours, days, or weeks is then inserted between the

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training sessions and the testing session, during which memory is assessed (for a more detailed description of the Mobile Conjugate Reinforcement Paradigm, see Rovee-Collier, 1997; <u>Rovee-Collier & Hayne, 1987; Rovee-</u> <u>Collier, Hayne, & Colombo, 2001</u>).

One of the first mobile studies showed that infants exhibit retention of the conditioned kicking response over a delay of 24 hr (Rovee & Fagen, 1976). Subsequent studies have demonstrated that infants as young as 3 months of age exhibit retention after delays of up to 7 days (Sullivan, Rovee-Collier, & Tynes, 1979). The intervals across which infants exhibit retention are not fixed, though, but are affected by conditions present during learning (Adler, Gerhardstein, & Rovee-Collier, 1998; Hayne, 1990; Ohr, Fagen, Rovee-Collier, Hayne, & Vander Linde, 1989). Additional mobile studies have demonstrated that infants' long-term memories are specific to the content available during encoding (Adler & Rovee-Collier, 1994; Rovee-Collier, 1997) and seem to obey many of the same principles as found in adults (Rovee-Collier, 1997; Rovee-Collier et al., 2001). These findings, as well as others (e.g., Adler, Inslicht, Rovee-Collier, & Gerhardstein, 1998), strongly suggest that the memory tapped by this operant conditioning task, as well as by deferred imitation tasks (e.g., Barr & Hayne, 2000; Barr, Rovee-Collier, & Campanella, 2005; Meltzoff, 1988, 1995), is sophisticated and shares many of the same properties as adult memory.

Taken together, previous studies have shown that infants are capable of remembering information over a significant period of time. But, what is the function of forming long-term memories in the first place? Rovee-Collier and Hayne (1987) (also see Grossberg, 1995) posited that one function of memory formation is to enable individuals to prepare prospectively to act based on expectations for future events. By forming long-term memories for experienced events, expectations can be constructed for forthcoming events. With these expectations as a basis, cognitive resources are freed up for information processing to proceed more efficiently (Haith, Benson, Roberts, & Pennington, 1994).

Studies examining infants' capacity to form expectations have primarily used the Visual Expectation Paradigm (e.g., Haith et al., 1988) in which the timing of infants' eye movements relative to the onset of predictable visual events is measured. In the prototypical VExP, infants view images that appear either in a predictable leftright alternating sequence or in an unpredictable random and irregular sequence. The primary index of an expectation is anticipatory behavior in which eye movements are initiated to locations where visual events will occur prior to their onset. Haith et al. (1988) demonstrated that infants' anticipatory eye movements were more numerous and the latency of the infants' reactive eye movements after picture onsets was faster when images appeared in a predictable spatiotemporal sequence than in an unpredictable sequence (Haith et al., 1988). These results provide evidence that infants form expectations for events that are not perceptually available and that rudimentary future-oriented processes are evident early in life (Haith, 1994). Subsequent studies have revealed that infants form expectations for more complex, asymmetric (e.g., left-left-right) spatial sequences (Canfield & Haith, 1991), for events' temporal parameters (Adler, Haith, Arehart, & Lanthier, 2008; Wass,

Lewis, & Haith, 1998), as well as the content of those events (Adler & Haith, 2003).

When forming expectations, information currently perceptually available needs to be merged with information remembered about past events to generate predictive representations of those upcoming events and plan appropriate behavior (Haith, 1994). In this way, behaviors in anticipation of future events are guided by expectation representations that have been formed from memories for similar previous events. Thus, formation of future-oriented expectations relies on memory (Haith et al., 1994). From this perspective, the function of long-term memories is not for the memories themselves, but rather to enable predictions about future events and guide behaviors (Grossberg, 1995; <u>Rovee-Collier & Hayne, 1987; Rovee-Collier & Sullivan, 1980</u>).

The VExP is a paradigm that has been used to study expectations, but might also be used to study long-term retention of information that infants' have experienced during the formation of their expectations in the first place. This could be accomplished by placing a delay between the infants' first encounter with a predictable sequence of events and a subsequent encounter. In the present study, we use the VExP to study the long term retention of visual expectations. To do this, infants were exposed to a sequence of visual events consisting of colorful and geometric-patterned stimuli in which the stimuli on one side (e.g., left) always contained the same invariant color combination whereas the stimuli on the other side (e.g., right) contained varied color combinations.

2 | EXPERIMENT 1-MEMORY AFTER 24 HR

The first step in this research was to determine whether infants formed a long-term memory trace during expectation formation. To do this, infants were initially exposed to a spatially predictable stimulus sequence (left-right-left-right) in which all the stimuli on one side (e.g., right) consisted of the same invariant color combination (e.g., red/ green) and the stimuli on the other side (e.g., left) consisted of varied color combinations (e.g., blue/yellow, red/yellow, blue/green, etc). Twenty-four hours later, infants were tested with an identical stimulus sequence in which the specific invariant color combination and the spatial location on which the invariant color combination was presented was the same as it had been the previous day. We reasoned that, if infants establish a long-term memory during expectation formation, then they would exhibit anticipatory eye movements at the outset of the test day at a rate similar to that at the end of expectation training 24 hr earlier.

3 | METHODS

3.1 | Participants

Data were collected from eight 3-month-old infants (four males and four females), ranging in age from 88 days to 124 days (M = 100.25 days, SD = 11.60). Infants in the sample were Caucasian (n = 4), Asian

(n = 2), African (n = 1), and mixed ethnicity (n = 1), and were primarily drawn from middle- to upper-socioeconomic status (SES) families. All infants were full term at birth with no reported complications, and appeared in good health. An additional six infants participated, but insufficient data were collected from them because they cried or were inattentive (i.e., disinterested or did not look at the stimuli on a minimum of 60% of the trials; n = 4), or due to experimenter error (n = 2). The high dropout rate in this experiment is consistent with the dropout rates reported in other visual expectation studies (e.g., <u>Adler et al., 2008; Adler & Haith, 2003; Haith et al., 1988</u>).

The participants and their caregivers were recruited through a monthly list purchased from a local marketing company (Z Retail Marketing Inc., Toronto, Ontario). The purchased lists provided the name and addresses of expecting families as well as new parents. An invitation letter was sent to all the families on the list outlining the general goals of the studies conducted at the Visual and Cognitive Development Project at York University. Enclosed with the letter was a self-addressed postcard that could be returned by families who were interested in participating. Parents also had the option to respond to the invitation letter via phone, email, or an online form. Families who returned postcards, or contacted us via phone, email or who filled out the online form, were contacted and provided with more specific information about the current study. Families who were willing to participate were then asked to provide a day and time best suited to their schedule and to the infant being most attentive.

3.2 | Stimuli and apparatus

The stimuli were computer-generated graphic images of checkerboards, vertical stripes, concentric circles, and diamond shapes in four possible color combinations: red/green, red/blue, yellow/green, and blue/yellow (see Figure 1). The infants viewed the images on a Sony LCD colour monitor (model 1302) that was 20.3 cm in height and 25.4 cm in width, with a 1024 × 768 pixel resolution. The LCD monitor was mounted above a specialized crib in which the infants laid during their participation. The stimuli were 4.5° squared in size and appear at 5.7° to the left or right of the infant's visual center.

During the experimental session, babies laid supine in the specialized crib that had the LCD monitor, displaying the stimuli, mounted 48 cm above (see Figure 2). Between the infant and the monitor, there was a 30×30 cm infrared-reflecting, visible-transmitting mirror that allowed the infant an unobstructed view of the stimuli on the monitor. A remote pan-tilt infrared eye tracking camera (Model 504, Applied Science Laboratories [www.a-s-l.com], Bedford, MA) was also placed overhead. Black felt curtains were hung around the crib to limit light entry and reduce distraction.

Using bright pupil technology, the eye tracker recorded the participants' eye movements via reflection in the infrared mirror at a



FIGURE 1 Examples of graphic images used as stimuli

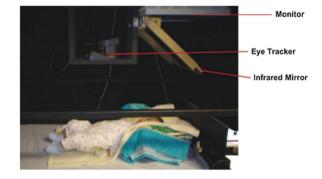


FIGURE 2 Infant viewing stimuli overhead while lying in specialized crib apparatus

temporal resolution of 60 Hz. Diodes on the camera emitted infrared light that reflected off the infrared mirror on to the infant and then back off the infant's retina through the pupil to produce a backlit white pupil. The infrared light also produced a point reflection on the corneal surface of the eye. Through proprietary software (Applied Science Laboratories), the eye fixation position was calculated from the relation between the corneal reflection and the centroid of the backlit pupil.

Two Dell computers were used to control the experimental session, one generated and presented the stimuli using DirectRT software (Empirisoft Inc., New York, NY, Available online at: http://www.empirisoft.com/DirectRT.aspx), while the other controlled the eye tracker camera and collected the eye movement data. The experimenter viewed the infant's eye movements and stimulus presentation on the data collection computer as a picture-in-picture video, via video capture software. The picture-in-picture video and the digital data were written to the data-collecting computer and synchronized in real time with a unique, stimulus-dependent, numerical code received from the stimulusgenerating computer. More specifically, the stimulus-generating computer sent a unique, time-stamped numerical code through a parallel port to the data-collecting computer, indicating the onset of the stimulus array and indicating the type of stimulus array. This synchronization in the data file allowed coordination of the eye movement sequences with specific stimuli.

3.3 | Procedure

On day 1 of participation, families were provided with an overview of the procedure of the experiment and shown the experimental setup. Parents were then asked to provide informed consent and to complete a form requesting demographic information. Subsequently, parents placed their infant in the specialized crib and, if necessary, a pacifier was offered to the infant during calibration. Minor adjustments to the position of the infant's head were also made as necessary. Before the start of the experimental trials, the eye tracker was calibrated by recording the infant's fixations to two known locations on the LCD monitor at which a calibration stimulus (concentric squares) was presented. All subsequently recorded eye tracker values were filtered through the calibration file to produce measures of eye position. The 2-point calibration procedure was repeated at the outset of day 2, 24 hr later, to account for any changes in the positioning of the infant's head relative to the monitor and eye tracker.

During the experimental phase, infants viewed a series of pictures appearing on the left and right sides of a computer screen. A total of 70 pictures were presented on each of two consecutive days in all conditions, of which the first 10 on the first day served as the baseline phase during which pictures appeared randomly 5.7° to the left or right of visual center. These initial 10 baseline trials were included to expose the infants to the locations and type of stimuli that were to be used in the experiment. The remaining 60 pictures on day 1 and all 70 pictures on day 2 appeared on the left or the right of the screen in a predictable alternating left-right sequence, each with a picture duration of 700 ms and an ISI of 1000 ms. The checkerboard, vertical stripes, concentric circles, and diamond shapes stimuli were used in four possible color combinations (red/green, red/blue, yellow green, and blue/yellow). The color combination that constituted the invariant content and the side on which this color combination appeared were counterbalanced across infants. Infants' level of anticipatory responding and reactive latencies were assessed.

On day 1, infants viewed stimuli where the pictures presented on one side (e.g., right) consisted of the same color combination (invariant side), whereas the pictures presented on the other side (e.g., left) consisted of different color combinations (varied side). In Experiment 1, on day 2, infants viewed the same invariant color combination on the same side as they had viewed the pictures on day 1.

3.4 | Data reduction

The raw digital data recorded by the eye tracker was imported into a MATLAB toolbox called ILAB for analysis (Gitelman, 2002). ILAB allows for the analysis of eye movements, by parsing out and individually displaying horizontal and vertical components of the eye movements on a trial-by trial basis. Scan paths of the eye for each trial was also displayed by ILAB, thereby allowing for the analysis of the nature of the eye movements (timing, direction and distance) relative to the stimuli. With the use of ILAB, an experimenter identified which of infants' eye movements were anticipatory or reactive in nature based on their timing relative to picture onset (Haith et al., 1988). Anticipations were the primary measure of the underlying cognitive construct of expectations and were defined as eye movements that began prior to the onset of a visual event. Anticipations were calculated separately for each side (invariant and varied) as a percentage of all valid eye movement trials by the following formula: number of anticipation trials/(number of anticipation trials + number of reactive trials), where the denominator reflects the total number of trials on which the scorer judged the infant as having made a valid eye movement. On the trials where infants did not exhibit anticipations, the latency of the infants' reactive eye movement to the stimulus was recorded.

In order for an eye movement to be included in the final data sample, it needed to meet a set of criteria. Only data from infants who attended (looked at the stimulus at some point during the trial) on a minimum of 60% of the experimental trials on day 1 were included (e.g., Adler & Haith, 2003; Adler & Orprecio, 2006). In Experiment 1, the mean proportion of trials attended was 61.5%, in Experiment 2, the mean proportion was 63.3%, and in Experiment 3, the mean proportion was 63.1%. Eye movements were considered to be anticipatory if they occurred after the offset of the previous stimulus and within the first 167 ms after the onset of the next stimulus. This latency value is designated as the anticipatory cut-off because previous studies have determined that 3-month-old infants cannot make reactive eye movements to the onset of a stimulus faster than 167 ms (Adler & Haith, 2003; Canfield, Smith, Brezsnyak, & Snow, 1997). If an eye movement occurred 167 ms after stimulus onset but before 167 ms after stimulus offset, then that movement was classified as a reactive eye movement (see Figure 3). Finally, the eye movement to a stimulus had to trace a path that was more than 50% of the distance to the intended stimulus from that eye movement's starting location. This was assessed through the infants' scan path in conjunction with the location of the stimulus presented. The 50% criterion has been used in previous studies using infants' eye movements (e.g., Adler & Haith, 2003; Adler & Orprecio, 2006) and is typically taken as an indication that the eve movement was intentional and not random.

The primary measure of memory for expectation information was a savings ratio, which has historical precedence for use in the study of the development of retention (Cornell, 1980; Parsons, Fagan, & Spear, 1973; Richardson-Klavehn & Bjork, 1988). The savings ratio provides a relative measure that expresses the degree of an infant's responding on a long-term retention test relative to that infant's responding on an immediate retention test. Use of a savings ratio index indicates how much of the information available immediately at the end of training is retained after a delay. Here savings was calculated by dividing the infant's percent of anticipations during the first 20 trials on day 2 by the infant's percent of anticipatory eye movements on the last 20 trials on day 1. By comparing the first 20 trials¹ on day 2 to the last 20 trials on day 1, the degree of retention on day 2 can be compared to the highest level of performance on day 1. A group's mean savings measure equal to or greater than a theoretical ratio of 1.00 indicates that the group of infants anticipated after a 24 hr retention interval at or above the level they anticipated at the end of the learning phase 24 hr earlier, thereby exhibiting retention. A group's mean savings ratio significantly less than 1.00 indicates that the group of infants anticipated at a level below what they had anticipated at the end of the learning phase 24 hr earlier, thereby exhibiting forgetting or discrimination. All results were analyzed with valid statistical

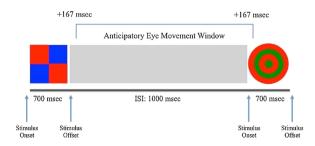


FIGURE 3 Cut-off time for anticipatory eye movements

methodology using the statistical software program STATA, with all tests conducted at an alpha level of p < .05.

4 | RESULTS

4.1 | Anticipatory eye movements

A repeated-measures, two-way analysis of variance (ANOVA) was performed to compare the infants' exhibited proportion of anticipatory eye movement with Day (day 1 vs. day 2) as the repeated measure and Content Side (invariant and varied) as a within-subjects factor. The main effect of Day was not significant, F(1, 28) = 2.67, p = .11, indicating that the proportion of anticipations made by infants on day 2 (M = 45.29, 95%CI [36.63, 53.96]) did not differ from day 1 (M = 36.31, 95%CI [27.65, 44.98]). A significant main effect was observed for Content Side, F(1, 28) = 11.53, p = .002, $\eta^2 = .27$. Infants made significantly more anticipations to the invariant side (M = 50.12.) 95%CI [42.57, 57.88]) in comparison to the varied side (M = 31.48, 95%CI [23.84, 39.12]), irrespective of day (see Figure 4). There was also no significant interaction between Content Side and Day, F(1, 28) < 1, indicating that the difference in anticipatory eye movements between the invariant and varied content sides did differ from days 1 to 2.

In order to investigate infants' memory for the content information encoded during expectation formation on day 1, the mean savings measure for each infant was computed. Two separate related sample *t* tests were conducted to compare the mean savings ratio of infants to the invariant (M = 1.36, 95%CI [1.04, 1.67]) and varied (M = 1.43, 95% CI [1.14, 1.73]) content side to the theoretical ratio of 1.00. Infants' mean savings ratio was not significantly different from 1.00 on either the invariant side, t(7) = 1.55, p = .17, or on the varied side, t(2) = 1.25, p = .34. These results suggest that infants exhibited retention on day 2 of the event information encoded during expectation formation 24 hr

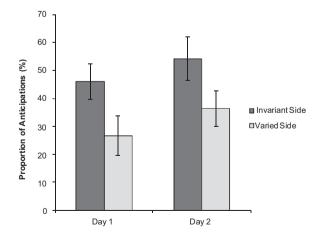


FIGURE 4 Mean proportion of anticipatory eye movements in Experiment 1 in which the invariant color combination and its side of presentation were not changed after the 24 hr retention interval. Results show infants' mean proportion of anticipations as a function of invariant color combination side versus varied color combination side for day 1 and day 2. Error bars indicate ± *SE*

earlier and recognized this event information after a 24 hr retention interval for both the invariant and varied sides (see Figure 5).

4.2 | Reactive eye movements

On the trials during which infants did not execute an anticipatory eye movement, the median reactive latencies to the target after the image onset for each infant were calculated. A two-way repeated ANOVA was performed to compare whether there were differences in the reactive latencies across day 1 and day 2, as well as between content sides (varied, invariant). The main effect of Day was not significant, F(1, 28) < 1, indicating that there was no difference in infants' median reactive latencies on day 1 (M = 389.50, 95%CI [358.20, 420.80]) relative to day 2 (M = 380.78, 95%CI [349.48, 412.08]). Consistent with Adler and Haith's (2003) previous finding that the latency of infants' reactive eye movements were unaffected by invariant color content versus varied color content, the main effect of Content Side was not significant, F(1, 28) < 1, (invariant side; M = 348.38, 95%CI [351.11, 417.64]; varied content side: M = 385.91, 95%CI [354.34, 417.67]), nor was the interaction between Day and Content Side, F(1, 28) = 2.39, p = .13.

Savings ratio data suggest that during expectation formation on day 1, infants encoded the event information and remembered this information when encountering the same sequence of events 24 hr later. Alternatively, infants may have not encoded the specific event information (e.g., red/blue color combination) in long-term memory, but may instead have encoded general information such as "constant information on left side." To distinguish between these two possibilities, the specific content information was changed in Experiment 2.

5 | EXPERIMENT 2-DISCRIMINATION OF COLOR CHANGE AFTER 24 HR

To determine whether infants encoded the specific invariant color combination in long-term memory, infants in the current experiment were trained to form a content expectation as in Experiment 1.

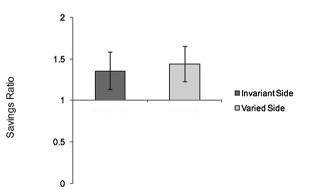


FIGURE 5 Mean savings ratio of anticipatory eye movements in Experiment 1 in which the invariant color combination and its side of presentation were not changed after the 24 hr retention interval. Results show infants' mean savings ratio relative to theoretical retention ratio of 1.00 as a function of invariant color combination side

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Twenty-four hours later, infants were exposed the same event sequence as on day 1, but the specific colors on the invariant side were novel and did not match what were presented on day 1.

6 | METHODS

6.1 | Participants

Participants and their caregivers were recruited in the same manner as in Experiment 1. Data were collected from eight 3-month-old infants (four males and four females), ranging in age from 88 days to 124 days (M = 108 days, SD = 12.32). Infants in the sample were Caucasian (n = 3), Asian (n = 2), Hispanic (n = 1), and mixed ethnicity (n = 1), and were primarily drawn from middle-to upper-socioeconomic status (SES) families. All infants were full term at birth with no reported complications, and appeared in good health. An additional seven infants participated, but insufficient data were collected from them because they cried or were inattentive (i.e., disinterested, or looked at their hands or other parts of the visual field; n = 4), or due to experimenter error (n = 3).

6.2 | Stimulus and apparatus

The equipment set-up and stimuli for this experiment were identical to those of Experiment 1. During the experimental session, infants were placed in the same specialized crib with the LCD monitor above, as in Experiment 1.

6.3 | Procedure

The procedure for Experiment 2 was exactly the same as the procedure of Experiment 1, with one exception. The stimulus sequence on day 1 was identical to that used in Experiment 1. In the current experiment, however, on day 2, infants received a different invariant color combination than they had seen on day 1, but the side on which the invariant color combination was presented was the same as day 1. If infants, for example, were presented with an invariant color combination of blue/yellow on the right side on day 1, then they might be presented with an invariant color combination of red/green on the right side on day 2.

6.4 Data reduction

Eye movement data were coded and then analyzed in the same manner as in Experiment 1.

7 | RESULTS

7.1 | Anticipatory eye movements

A repeated-measures, two-way analysis of variance (ANOVA) was performed to compare infants' proportion of anticipations with Day (day 1 vs. day 2) as the repeated measure and Content Side (invariant and varied) as a within-subjects factor. The main effect of Day was not significant, *F*(1, 28) < 1, indicating that the proportion of anticipations made by infants on day 2 (*M* = 35.81, 95%CI [29.79, 41.83]) did not differ from day 1 (*M* = 37.69, 95%CI [31.67, 43.71]), irrespective of Content Side. There was a significant main effect of Content Side, *F*(1, 28) = 32.26, *p* = .00, η^2 = .52, indicating that infants made significantly more anticipations (*M* = 46.19, 95%CI [42.21, 50.16]) to the invariant content side in comparison to the varied content side (*M* = 27.31, 95% CI [22.20, 32.41]), irrespective of day. The interaction between Day and Content Side was also not significant, *F*(1,28) = 1.27, *p* = .27 indicating that the difference in anticipatory eye movements between the invariant and varied content sides did differ from days 1 to 2 (see Figure 6).

In order to determine whether infants encoded the specific event content information in memory during expectation formation on day 1, and whether they discriminated a change in the invariant colors that at the outset of day 2 before re-learning occurred, the mean savings ratio was computed as in Experiment 1. Two separate sample t tests were conducted to compare the mean savings ratio of infants for the invariant (M = .72, 95%CI [.46, .99]) and varied (M = 1.39, 95%CI [.99, 1.79]) content sides, relative to the theoretical ratio of 1.00. Results revealed that the mean savings ratio for the invariant side was significantly less than 1.00, t(6) = 3.79, p = .00, d = .79, indicating that infants exhibited fewer anticipations during the first 20 trials of day 2 relative to the last 20 trials of day 1 (see Figure 7). This finding suggests that infants discriminated the change in the invariant color combination. No significant difference in the mean savings ratio was found for the varied side t(6) = .88, p = .41, indicating that infants' proportion of anticipations during the first 20 trials of day 2 relative to the last 20 trials of day 1 did not differ.

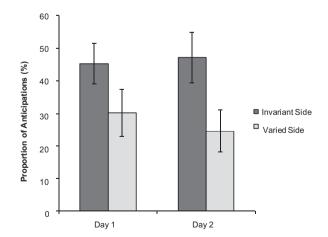


FIGURE 6 Mean proportion of anticipatory eye movements in Experiment 2 in which the invariant color combination was changed but not its side of presentation after the 24 hr retention interval. Results show infants' mean proportion of anticipations as a function of invariant color combination side versus varied color combination side for day 1 and day 2. Error bars indicate ± *SE*

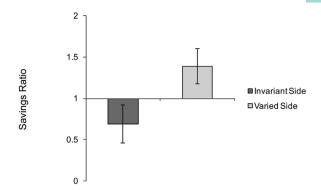


FIGURE 7 Mean savings ratio of anticipatory eye movements in Experiment 2 in which the invariant color combination was changed but not its side of presentation after the 24 hr retention interval. Results show infants' mean savings ratio relative to theoretical retention ratio of 1.00 as a function of invariant color combination side

7.2 | Reactive eye movements

As in Experiment 1, a two-way repeated ANOVA revealed that there was no significant difference in the main effects of Day, F(1, 28) = 1.39, *n.s.*, or Content Side, F(1,28) < 1. Further, the interaction of Day and Content Side was not significant, F(1, 28) < 1, indicating that infants' median reactive latencies to the invariant relative to the varied content side was the same on day 2 as on day 1.

The anticipatory savings ratio in Experiment 2 supports the conclusion that infants discriminated the novel event color information on the invariant side on day 2 and, as a consequence, decreased their anticipatory responding only to the invariant side on which specific content information had changed. The decrease in anticipatory eye movements is likely due to the novel invariant color information not matching the specific color information encoded in long-term memory during expectation formation on day 1. That infants discriminated the novel event content information into long-term memory during expectation formation into long-term memory during expectation formation—a finding that is consistent with Rovee-Collier's studies of infants' long-term memory using the mobile paradigm (Adler & Rovee-Collier, 1994; Adler et al., 1998b; Rovee-Collier, 1997).

8 | EXPERIMENT 3-DISCRIMINATION OF SPATIAL CHANGE AFTER 24 HR

The invariant content events have other predictable event parameters in addition to their color information and that is their spatial location. That is, the invariant content events might always occur on a particular side, for example, the left (or the right). Do infants encode the specific predictable spatial information of the invariant events in long-term memory as well? In a study of infants' formation of content expectations, however, Adler and Haith (2003) found that anticipatory eye movements were sensitive to the predictability of event content, but not to an event's spatial information. In Experiment 3, therefore, infants were trained to form a content expectation as in the previous two experiments, but 24 hr later, infants' memory for the spatial location of the invariant events was tested by changing the side on which the invariant events occurred.

9 | METHODS

9.1 | Participants

The participants and their caregivers were recruited in the same manner as in Experiments 1 and 2. Data were collected from eight 3-month-old infants (four males and four females), ranging in age from 88 days to 124 days (M = 112.50 days, SD = 15.12). Infants in the sample were Caucasian (n = 4), Asian (n = 1), African (n = 1), Hispanic (n = 1), and mixed ethnicity (n = 1), and were primarily drawn from middle- to upper-socioeconomic status (SES) families. All infants were full term at birth with no reported complications, and appeared in good health. An additional seven infants participated, but insufficient data were collected from them because they cried or were inattentive (i.e., disinterested, or looked at their hands or other parts of the visual field; n = 5), or due to experimenter error (n = 2).

9.2 | Stimulus and apparatus

The stimuli, apparatus, equipment set-up, and data collection for this experiment were identical to those outlined for Experiments 1 and 2.

9.3 | Procedure

The procedure for Experiment 3 was identical to that used in both Experiments 1 and 2 on day 1. On day 2, however, infants viewed the same invariant color combination as day 1, but on the opposite side. That is, for example, if they viewed the invariant color combination of red/green on the left side of the screen on day 1, they then viewed the same red/green invariant combination on day 2 but on the right side.

9.4 | Data reduction

Eye movement data were coded and then analyzed in the same manner as in Experiments 1 and 2. Note that the invariant side on each day is physically on different sides of the screen. That is, if the invariant side was on the left on day 1, then the invariant side was situated on the right on day 2, and vice versa. Analyses across days was based on being the invariant side.

10 | RESULTS

10.1 | Anticipatory eye movements

As in Experiments 1 and 2, a repeated-measures, two-way ANOVA was performed to compare the proportion of infants' anticipations with Day (day 1 vs. day 2) as the repeated measure and Content Side

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(invariant and varied) as a within-subjects factor. The main effect of Day was not significant, F(1, 28) = 2.44, p = .13, indicating that proportion of anticipations made by infants on day 1 relative to day 2 did not differ, irrespective of Content Side. There was a significant main effect of Content Side, F(1, 28) = 4.51, p = .04. Infants made significantly more anticipations (M = 38.01, 95%CI [31.89, 44.14]) to the invariant side in comparison to the varied side (M = 28.00, 95%CI [20.31, 35.70]) (see Figure 8). The interaction between Day and Content Side was not significant, F(2, 30) = 3.62, p = .07, indicating that there were no differences in anticipatory eye movements between the invariant and varied sides from days 1 to 2. This analysis approached significance, however, as can be seen in Figure 8, infants made many more anticipatory eye movements to the invariant than the varied side on day 1 (but not day 2), consistent with the previous two experiments and Adler and Haith (2003).

Once again to determine whether infants actually remembered the information encountered on day 1 and then discriminated a change in that information on day 2, a mean savings ratio was computed. Two separate sample t tests were conducted to compare the mean savings ratio to a theoretical ratio of 1.00, which indicates recognition, for the invariant (M = .69, 95%CI [.46, .92]) and varied (M = 1.09, 95%CI [.50, 1.69]) content sides. The mean savings ratio for the invariant side was not significantly less than 1.00, t(6) = 1.78, p = .17, indicating that infants exhibited the same level of anticipatory eye movements during the first 20 trials of day 2 relative to the last 20 trials of day 1 to the invariant side (see Figure 9). This finding suggests that infants did not discriminate the change in the spatial location of the invariant color events. No significant difference in the mean savings ratio was exhibited for the varied side t(4) = .39, p = .71, indicating that infants' proportion of anticipations during the first 20 trials of day 2 relative to the last 20 trials of day 1 did not differ (see Figure 9).

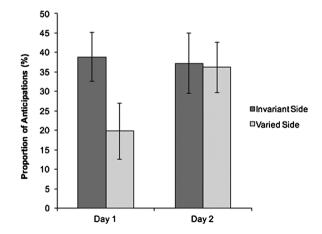


FIGURE 8 Mean proportion of anticipatory eye movements in Experiment 3 in which the spatial location of the invariant color combination was changed but not the particular color content after the 24 hr retention interval. Results show infants' mean proportion of anticipations as a function of invariant color combination side versus varied color combination side for day 1 and day 2. Error bars indicate $\pm SE$

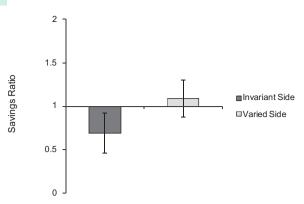


FIGURE 9 Mean savings ratio of anticipatory eye movements in Experiment 3 in which the spatial location of the invariant color combination was changed but not the particular color content after the 24 hr retention interval. Results show infants' mean savings ratio relative to theoretical retention ratio of 1.00 as a function of invariant color combination side. Error bars indicate ± *SE*

10.2 | Reactive eye movements

Median reactive eye movement latencies were subjected to a two-way repeated ANOVA with Day (day 1 vs. day 2) as the repeated measure and Content Side (invariant and varied) as a within factor. Neither of the main effects of Day, F(1, 28) < 1, *n.s.*, Content Side, F(1, 28) < 1, *n.s.*, nor the interaction of Day and Content Side, F(1, 28) < 1, *n.s.*, were significant. These outcomes indicate that the infants' median reactive latencies did not differ from day 1 irrespective of side and between the invariant or varied sides irrespective of day, and that latencies to the invariant relative to the varied content side was the same on day 2 as on day 1.

Consistent with the prediction generated from the finding of a dissociation between the processing of color content versus spatial information in infants' expectation representations by Adler and Haith (2003) and from levels of processing (Adler et al., 1998a, 1998b; Adler & Rovee-Collier, 1994; Craik & Lockhart, 1972), the findings from this experiment suggest that the spatial location of invariant content events was either not encoded or not encoded particularly deeply in long-term memory. As a consequence, infants exhibited an equivalent level of anticipatory responding to the invariant content events even when the spatial location of those events had changed.

11 | GENERAL DISCUSSION

Numerous studies have previously demonstrated that young infants can retain event information in memory over an extended period of time (e.g., Little et al., 1984; Rovee & Fagen, 1976; Watson & Rayner, 1920). Many other studies have demonstrated that infants are capable of forming expectations and exhibiting anticipatory eye movements for spatial (Canfield & Haith, 1991), content (Adler & Haith, 2003), and temporal parameters (Adler et al., 2008; Wass et al., 1998) of future events. These two domains, memory and expectations, have been theoretically linked by the proposal that the function of memory is to provide a foundation upon which

expectations for future events are formed (Grossberg, 1995; Haith, 1994; <u>Rovee-Collier & Hayne, 1987; Rovee-Collier & Sullivan, 1980</u>). That is, a function of long-term memories is not simply for the memories themselves but to inform future decisions and behaviors. The current study is the first to demonstrate this link in infants. Across three experiments, 3-month-old infants were shown to encode events' content parameters, but not events' spatial parameters that they had processed during the formation of an expectation representation. As a consequence, infants then discriminated a change in the events' content parameters.

11.1 | The relation between memory and expectations

As previous expectation studies (e.g., Adler & Haith, 2003; Wentworth & Haith, 1992) have shown, across all three experiments in the current study, infants initially formed an expectation for the predictable event content (invariant color combination). As a result, infants made a greater percentage of anticipatory eye movements to the invariant color side than to the equally spatially predictable varied color side. If an integral characteristic of memory is the formation of expectations, as theorized (Grossberg, 1995; Haith, 1994; Rovee-Collier & Hayne, 1987; Rovee-Collier & Sullivan, 1980), then the information, that is, invariant color combination and spatial location, that is encountered during initial expectation formation should be remembered over the long term. The findings from Experiment 1 indicated that infants continued to exhibit a greater percentage of anticipations to the invariant side as evidenced by a mean savings ratio greater than 1.00. This finding suggested that the information to which they were initially exposed was retained even after 24 hr and that the encoded information guided their behavior to future events after the retention interval. Moreover, the findings from Experiment 1 indicate that infants encoded the information that defined the sequence of events, thereby forming an expectation for the events with predictable content. Subsequently, this information was retrieved to facilitate anticipatory eye movements to the same events after the retention interval.

Although the results of Experiment 1 demonstrate that infants' anticipatory eye movements were facilitated by exposure to the same event sequence and event parameters 24 hr earlier, the possibility exists that all that was facilitated was the entrainment of a behavioral rhythm. In any examination of memory, in order to demonstrate that the originally encountered information has actually been encoded in memory, not only must retention for that original memory be exhibited, but also discrimination of novel information must be exhibited as well (Atucha & Roozendaal, 2015; Hutchinson & Turk-Browne, 2012; Poirer et al., 2012; Sannino et al., 2012). As studies with Rovee-Collier's mobile conjugate reinforcement paradigm (Adler, 1997: Adler & Rovee-Collier, 1994; Rovee-Collier, Adler, & Borza, 1994) have demonstrated, discrimination of information that was not originally encoded in a memory has been an integral part of any infant memory study. To this end, in Experiments 2 and 3, infants viewed novel invariant color information and a novel spatial location of the invariant events that did not match the event information that they

had encountered 24 hr earlier. Infants in Experiment 2 discriminated a novel content parameter after a 24 hr retention interval, as evidenced by mean savings ratios less than 1.00. This result demonstrates that infants not only show retention of the originally encoded event information, but also discriminate novel information. This finding further demonstrates that the memory that is established during expectation formation is not just a general memory of invariant versus varied, but is specific to the nature of the originally encountered event parameters.

Together, the experiments of this study showing that information encoded during expectation formation is stored in long-term memory, confirm the link, theorized by Rovee-Collier and others, that a function of memory is to inform future behavioral decisions through expectations. This capacity potentially provides a foundation in how experiences early in life can inform cognitive processing and behavioral decisions to relatively immediate future events, as well as to events much later in development.

11.2 | Differential processing of content and spatial information

Adler and Haith (2003), in their study of infants' capacity to form an expectation for the content of events, found a dissociation in the sensitivity of different categories of eye movements to different event parameters (also see Adler et al., 2008). Anticipatory eve movements. for example, were found to be sensitive to the predictability of content information but not spatial information. Because anticipatory eye movements are generated by higher cortical levels (Hanes, Patterson, & Schall, 1998) and reactive eye movements by lower subcortical levels (Krauzlis, Basso, &Wurtz, 2000; Krauzlis & Dill, 2002), as a consequence of the dissociation, Adler and Haith proposed that the different event parameters are processed at a different cognitive level. Content information, such as color, which influences anticipatory eye movements was proposed to be processed to deeper level than spatial information which seems to influence reactive eye movements (Adler & Haith, 2003). If Adler and Haith (2003) were correct that the different event parameters were processed to different levels, then the memorability of those parameters would be predicted to be different as well, as suggested by level-of processing models of memory (Craik & Lockhart, 1972). That is, if infants encode content information to a deeper level than spatial information then infants should remember the content information better as well.

The results of Experiments 2 and 3 suggest that the hypothesis of Adler and Haith (2003) may be correct. Infants discriminated a change in content information, but not a change in spatial information. Thus, as predicted, content information was processed to a deeper level than spatial information during expectation formation. Whether the spatial information was encoded at all in long-term memory cannot be determined from this study. The possibility exists that a shorter retention interval would be sufficient for the effect of encoding the spatial information to manifest itself. Previous mobile studies have shown that different stimulus features, even if differentially encoded, seem to be equally memorable at shorter retention intervals, when at longer intervals those features are remembered differentially (Adler et al., 1998a,b; Adler & Rovee-Collier, 1994). Perhaps, therefore, the effect of differential levels of processing and memory encoding of event parameters during expectation formation is manifested over the course of longer retention intervals, but not shorter ones. Future studies will have to examine this possibility.

11.3 | Validity of using VExP to study memory

Theoretically, long-term memory has been related to expectations (Adler et al., 2008; Adler & Haith, 2003; Haith et al., 1988), but this has never been directly explored before at any age. The findings of the current study demonstrate that the Visual Expectation Paradigm (VExP) and anticipatory eye movements are valid means for assessing the characteristics of long-term memory in early infancy. Furthermore, because the characteristics of saccadic eye movements, including anticipatory eye movements, are relatively mature and stable very early in infancy (Canfield et al., 1997; Shea, 1992), anticipations may provide a means by which memory can be studied continuously and in an operationally similar manner over the development from infancy into childhood. With such a tool in hand, a much more thorough and complete picture of the developmental course of memory mechanisms could be attained.

In summary, this study has demonstrated for the first time that there is a link between expectation formation and the functioning of long-term memory. The current study also demonstrates that the VExP and anticipatory eye movements can be used to study infant long-term memory. Not only can infants' long-term memory be assessed with the expectation paradigm, but also the specificity of those memory traces can be analyzed. Future studies will need to confirm the current findings and build upon them by documenting the specific intervals at which infants can remember the different types of information encoded during expectation formation. In addition, other phenomena that have been shown to occur with infant memory, for example, the effect of context, memory reactivation and memory modification, need to be assessed with the expectation paradigm in order to confirm the link between the establishment of long-term memories and expectation formation. Further, though the effect sizes were strong demonstrating robust effects, any future studies should increase the number of participants to increase the statistical power. Nonetheless, this study has extended our knowledge of infants' cognitive expectations and shown that expectations are in fact linked to memory. Finally, this study represents and builds upon the legacy left by Carolyn Rovee-Collier and her contributions to the understanding of memory development. There can be no better tribute.

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ENDNOTES

¹ Because whether or not an infant exhibits a valid eye movement on any given trial could not be controlled, a window of 20 trials was used both post- and pre-retention interval to insure that there were enough valid trials to provide a reliable savings measure. Use of 10 trials as the window, for example, could result in some infants having only 1 or 2 trials in either the numerator (post-retention interval) or denominator (pre-) of the savings ratio, which would not construct a reliable measure. Further, any infant that failed to provide any trials in either the post- or pre-retention interval window, was not included in the savings analysis. For this reason, the df's for the savings analyses do not match within or across experiments or seem consistent with the number of infants who participated in the study.

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