RELATION OF INFANTS' MEMORY AND EXPECTATION FORMATION: EVIDENCE FROM ANTICIPATORY EYE MOVEMENTS AND PUPIL DIAMETER CHANGES

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Abstract

Studies have demonstrated that infants are capable of forming expectations and exhibiting anticipatory eye movements for specific parameters of future events. Theories have suggested that these abilities highlight our memory's function of providing a foundation upon which expectations for future events are formed. This study aimed to assess this hypothesis by investigating the relation between long-term memory and expectation formation in 3-month-old infants.

Infants underwent the Visual Expectation Paradigm, wherein they viewed spatially predictably alternating (left-right) sequences of shapes in which the color content remained invariant on one side but varied on the other. After a delay of 24 hours, infants were tested with either a change in the stimuli or the same stimuli. Infants' level of anticipatory eye movements were measured on both test days. This study also aimed to study the relation of working memory and long-term memory by investigating if temporal decay (a limitation of working memory), would affect infants' long-term memory for expectation information. To this end, this study assessed the effect of two different interstimulus intervals (ISIs), 1000 and 3000 msec on infants' long-term memory performance. Finally, changes in pupil diameter during encoding on Day 1 and retrieval on Day 2 were also measured.

Results indicated that infants discriminated any change in the stimuli on Day 2 relative to Day 1, indicating that they encoded and remembered this information from Day 1. However, when the ISI was increased to 3000 msec, infants no longer discriminated changes in the stimuli. These findings support the theory that visual expectation processes are related to mechanisms of long-term memory in infancy and that working memory is related to long-term memory. In addition, this study reveals the potential for assesssing eye movements and pupil size to examine long-term memory mechanisms in infants.

Keywords: infancy, long-term memory, visual expectations, temporal decay, pupil diameter

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Introduction

Whether it is non-associative learning, exhibited by single-cell organisms, or more complex and conceptual learning found in humans, memory is an important capacity found in some form in all living organisms (Rovee-Collier & Barr, 2004). Memory provides the means for retrieving information that is pertinent for survival (Rovee-Collier & Barr, 2004), and in higher order organisms, such as humans, provides a foundation for higher cognitive skills such as planning, problem solving and, more importantly, the comprehension of language. Since the first attempts to study memory, there has been much progress in our understanding of it, as well as the processes and brain areas that modulate its functioning.

Most memory researchers today, whether they have a cognitive, neuroscience or physiological orientation, agree that memory is distinguished in two different general types: short-term memory and long-term memory. Short-term memory is considered to be the ability to hold information in the mind and is of limited capacity and temporal period. This form of memory, according to some, is further dissociated into working memory (Pribram, Miller & Galanter, 1960) and sensory memory (Coltheart, 1980). Working memory consists of the capacity to actively hold information in the mind for the purpose of executing complex tasks such as learning, comprehension and reasoning (e.g. Engle, 2002). Sensory memory, on the other hand, is a form of short-term memory in which information is captured and held by our sensory organs or receptors (Coltheart, 1980).

In contrast to short-term memory, wherein the information stored has a temporary temporal dynamic, the storage of information in long-term memory is thought to be temporally permanent. This form of memory is thought to consist of different components in which the distinction is made by the type of information stored. One division casts long-term memory into explicit and implicit categories. Explicit memories are consciously accessible, whereas implicit memories are not (McDougall, 1923; Wood, Baxer & Belpaeme, 2011). Explicit memory is additionally segregated into episodic memory, which refers to memories for specifically experienced events, and semantic memory, which refers to memories about general knowledge and facts. Implicit memory is also thought to be dissociable into different types including memory that encompasses non-associative learning and classical conditioning, as well as procedural memory. This multi-system view is currently the dominant framework for researchers investigating any aspect of memory and cognition (Johnson, 2011). Many memory studies focus on further understanding the different memory types, as well as diseases, disorders or injuries that may affect their functioning (e.g Unsworth & Engle, 2007; Belleville, Chertkow & Gauthier, 2007; Piolino, Desgranges, Manning, North, Jokic & Eustache, 2007). Although these topics are important for many reasons, an understanding of the development of memory could provide us with even more insight into the functioning of memory.

Although much is known about memory in adulthood (Milner, Squire & Kandel, 1998), not as much is known about how it develops from infancy into adulthood. Adults, for example, are unable to recall memories from infancy and even have difficulty recalling those from early childhood. This phenomenon, known as “infantile amnesia” and first coined by Freud (1990, 1914), is characterized by an inability to recall autobiographical events from the early years of life. Since Freud, anecdotal evidence for infantile amnesia has been validated by numerous retrospective studies (Henri & Henri, 1895; Howe & Courage 1993; Nelson, 1993; Peterson 2002; Wang 2003). Seeing that both learning and memory share many similarities at the neuro-structural and molecular level (Josselyn & Frankland, 2012), and infants of both human and other species have been shown to have great capacity for learning (Josselyn & Frankland, 2012), the phenomenon of infantile amnesia is rather perplexing. Is it possible then that infants learn and encode information but they cannot access it as they age? Or, perhaps the encoded information is lost over time? These questions are still open for debate.

Over the past decades, there has been a continuing debate over whether infants can actually form memories and remember them for any length of time. Infantile amnesia has led some researchers to suggest that infants possess only a primitive memory system, granting any form of event encoding impossible (Mandler, 1998), and that children are unable to remember events until they are able to rehearse them verbally (Nelson, 1990). Some of the studies investigating memory abilities in infants, however, have demonstrated that they are in fact capable of remembering information over a significant period of time (Hayne, 2004).

As will be discussed more extensively later, one aim of this study was to assess the viability of a new methodology to study memory in infants that will extend the theoretical and methodological framework of our current knowledge of memory development. To this end, the visual expectation paradigm (VExP) (Haith, Hazan & Goodman, 1988) was used and anticipatory eye movement and pupil diameter changes, both of which have empirical and theoretical connections to memory, were measured over a 24-hour delay. This methodology was assessed to investigate whether it could provide a means by which our confused understanding of infant memory can be disambiguated. Although, we did not attempt to distinguish between implicit and explicit long-term memory types in terms of what young infants can exhibit, we investigated the relation between infants' memory and expectation formation which are theoretically linked. Furthermore, we examined the nature of infants' retention for particular information encoded during expectation formation.

Early Infant Memory Studies

Habituation, Paired Comparision, and Classical Conditioning. Early infant studies investigating perceptual as well as cognitive abilities including memory did so by means of habituation or classical conditioning paradigms. Habituation tasks that have been used typically involved repeatedly presenting a stimulus to an infant until their attention to the stimulus decreases to some absolute (e.g. McCall, Kennedy, & Dodds, 1977) or relative level (e.g. Cohen, Deloache, & Pearl, 1977). Infants are subsequently presented with a novel stimulus during which their attentional behaviour is recorded. The behaviour to the initial familiar stimulus at the end of the habituation period and to the novel stimulus during the test period are compared. If infants distinguish a difference between the familiar stimulus and the novel stimulus, their relative attentional behaviour to each will be different. In particular, their attention to the novel stimulus will increase relative to their attention to the familiar stimulus at the end of habituation. When using this paradigm to assess memory, a delay is simply inserted between the habituation and test phases.

In the late 1950's Robert Fantz was the first to develop the habituation paradigm to study simple visual preference in infant animals, such as chicks (Fantz, 1957; Fantz, 1958a) and infant chimpanzees (Fantz, 1958b). Fantz (1964) later demonstrated habituation in 2-month-old and 6-month-old human infants using an extension of his habituation paradigm, which has been termed the visual paired comparison task. During all exposures of the visual paired comparison task, two stimuli are presented simultaneously to infants for visual inspection. One of the stimuli is consistently unfamiliar or "novel", while the other remains unchanged. Results of the visual paired comparison task (Fantz, 1964) found that while initially infants looked equally long to both stimuli, over trials, infants progressively looked less at the familiar stimulus and attended more to the novel ones. In this seminal study, Fantz demonstrated that infants' looking to the familiar stimulus "habituated" over time, which suggested the possibility that infants were encoding the unchanging stimulus in memory, thereby enabling its familiarity (Colombo & Mitchell, 2009). As a result, the paradigm could possibly be useful in studying different aspects of infant cognition. These results were subsequently replicated in similar studies (e.g., Lewis & Goldgerg, 1969).

Using a different form of habituation, Stinson (1971, cited in Werner & Perlmutter,

1979) obtained the first evidence of infant memory in 4-month-olds. In his study, each high-amplitude suck on a nonnutritive nipple resulted in a briefly illuminated visual stimulus on a screen. After repeated exposure to the stimulus upon sucking, the infant's sucking fell to a fraction of its original level, i.e., habituation. Subsequently, a brief delay on the order of tens of seconds was inserted before infants were allowed to suck on the nipple again. Findings indicated that as the delay increased, the greater the sucking response as the infants would return to their original, pre-habituation levels of sucking. Presumably, the increased sucking after longer delays was due to forgetting the original stimulus and indicated that the infants were treating the stimulus exposure after the delay as if it were a new stimulus. Stinson concluded from the findings of his study that infants could not remember stimulus information for longer than a maximum of 15 seconds.

A more likely explanation for the relatively short delay across which infants exhibit the capacity to remember, however, is that the habituation method could only measure short-term memory abilities in infants (Rovee-Collier & Barr, 2004). In Stinson's habituation study, for example, the learned habituation decayed over seconds and not hours or even minutes, characteristic of working memory, a form of short-term memory. Working memory is known to be subject to temporal decay, the likelihood that information will be lost from the memory store as the time between encoding of information and its recall increases (Brown, 1958; Cowan, Saults, & Nugent, 1997; Peterson & Peterson, 1959). More specifically, as time progresses, the information that is held in working memory decays, unless strategies (i.e rehearsal) are used to maintain the information in an active state. In Stinson's study, as the delay between encoding and recall increased, infants' behaviour increasingly returned to pre-habituation levels. It seems likely that the information was only encoded in working memory and was being lost because of temporal decay. Temporal decay of information from working memory also has implications for long-term memory. Memory models posit that information that we pick up from the environment is initially stored in working memory and then, with the help of various memory strategies, the information is transferred to long-term memory if need be (Atkinson & Shiffrin, 1968). If information decays over time from working memory, then it may not be available to be transferred to long-term memory for later retrieval. That Stinson's and other habituation studies yield such relatively rapid decay of information suggests that these tasks are not suitable for the encoding of information in long-term memory in the first place.

Infant classical conditioning paradigms, the other method featured in early infant memory studies, were derived from versions that had been used for decades to study memory in both human and non-human adults (for review, see Woodruff-Pak & Steinmetz, 2000a, 2000b). These experiments typically involve pairing a neutral conditioned stimulus such as a tone or a light with an unconditioned stimulus, such as a loud noise or a shock. The earliest infant classical conditioning study was conducted with only one participant, during which conditioning performance was traced over a period of time. In this well-known study by Watson and Rayner's (1920), the effects of classical conditioning on an 11-month-old named Albert, was investigated. Little Albert was presented with a loud gong (US) that made him cry and withdraw (UR). Each time the gong was being sounded, little Albert would touch a white rat (CS) with his hand. After a delay of one week, Albert would withdraw his hand (CR) at the sight of the rat. After an additional delay of 5 days, Albert was brought in again and demonstrated the same reaction that was seen at the one-week mark. These results indicated that infants can be classically conditioned during their first year, and that it can be provide evidence of memory by the exhibition of the CR even after a fairly lengthy period of time (Rovee-Collier & Barr, 2004).

Studies using classical conditioning to demonstrate memory have even been extended to very young infants. For instance, Blass, Ganchrow, and Steiner (1984) conducted a study in which they demonstrated that newborns can be classically conditioned as well. Newborns were conditioned to orient their head and to pucker suck to a stroke of their head (US), after repeated exposure to the pairing of the head stroke (US) and the delivery of sucrose through a pipette (CS). In yet another study, conducted by Little, Lipstitt, and Rovee-Collier (1984), a conditioned response of an eye blink was shown to be exhibited by 10-, 20-, and 30-day-olds. In this study, Little and colleagues (1984) presented infants with a tone-air puff (CS-US) pairing, and long-term retention of the conditioned response after a delay of 10 days, was exhibited in all of the age groups aforementioned. More recent infant classical conditioning studies continue to feature eye blinks as the conditioned response in infants and have shown maintenance of the CR over an even longer period. For instance, in a study conducted by Invkovich and colleagues (1999), 4-month-olds and 5-month-olds were presented with repeated pairings of a tone (CS) and an airpuff (US) (Inkovich, Collins, Eckerman, Krasnegor & Staton, 1999). Interestingly, although infants of both age groups initially did not show any CR, they exhibited a high percentage of CR at the outset of the second session two weeks later.

Although taken altogether, the findings of the studies using both the visual paired comparison paradigm and classical conditioning suggest evidence of memory in infants, there are still several drawbacks from these paradigms. Although classical conditioning studies have demonstrated information retention in infants, a major drawback of the paradigm is the nature of the CR they are measuring. Even if the maintenance of these conditioned responses provide some evidence of memory in infants, it is likely that higher order processes involved in more complex long-term memory are not at play. The CRs that are measured in the infants are reflexive behaviours, which are considered to be implicit and not under conscious control (Rovee-Collier & Barr, 2004). As a result, the retention of these CRs is likely not involving higher order processes that modulate more complex long-term memory (Rovee-Collier & Barr, 2004). It has been suggested that responses that are emitted, rather than reflexive, could perhaps be more appropriate to study memory processes that modulate explicit memory (Rovee-Collier & Barr, 2004). Classical conditioning tasks, therefore, may underestimate infants' abilities by assessing only implicit memory.

Operant Conditioning. Unlike classical conditioning, wherein the CRs are elicited reflexive behaviours, in operant conditioning the CRs are emitted. In operant conditioning, the responses are spontaneously performed by research participants and are behaviours that are exhibited at a low to moderate rate prior to any reinforcement implementation (Rovee-Collier & Barr, 2004). Moreover, in contrast to classical conditioning, in operant conditioning, there is also no need for a biological or physical relation between the type of reinforcer and the response it influences, (Rovee-Collier & Barr, 2004). One particular operant conditioning procedure termed the mobile conjugate reinforcement paradigm, pioneered and developed by Carolyn Rovee-Collier in the late 1960's (Rovee & Rovee, 1969; for reviews, see Rovee-Collier & Hayne, 1987; Rovee-Collier, 1997) has been quite influential in furthering our understanding of infant memory development. In the mobile conjugate paradigm, infants learn to kick to produce movement in an overhead mobile placed on their crib. During this learning session, one of the infant's feet is tied to the mobile by means of a ribbon, which makes possible for their kicks to move the mobile. When the mobile conjugate paradigm is used to assess memory, a delay is inserted between the conclusion of training session and the testing session, or the stimulus is changed at test relative to training.

Studies using the mobile paradigm have shown that infants exhibit retention of the conditioned kicking over a delay of 24 hours (Rovee & Fagen, 1976). Moreover, Sullivan, Rovee-Collier, and Tynes (1979), subsequently demonstrated that infants as young as 3-months of age, when trained for 2 consecutive days, exhibit retention after delays of 2, 3, 4, 5, 6 and 8 days, and it was not until 13 days later that the forgetting occurred. These retention lengths have also been lengthened when infants have undergone longer acquisition sessions or additional training sessions (Orh, Fagen, Rovee-Collier, Hayne & Vander Linde, 1989).

Since infants older than 6 months are not captivated by the mobile task, due to changes in interest, motivation and motor skills (Hayne, 2004), a modified version of the conjugate reinforcement task was created by Rovee-Collier and Hatshorn (1997). In this task, infants learn to press a lever to make a miniature train move around a track. Six-month-olds tested with both the mobile conjugate paradigm and the train task yielded the same retention intervals for remembering, despite the differences in methodology. Testing infants of different age groups and combining the data from these two tasks has revealed that there is a linear increase in retention from the ages of 2 to 18 months of age (Hartshorn, Rovee-Collier, Gerhardstein, Bhatt, Klein, Aaron, Wondoloski & Wurtzel, 1998). Figure 1 below depicts these results.

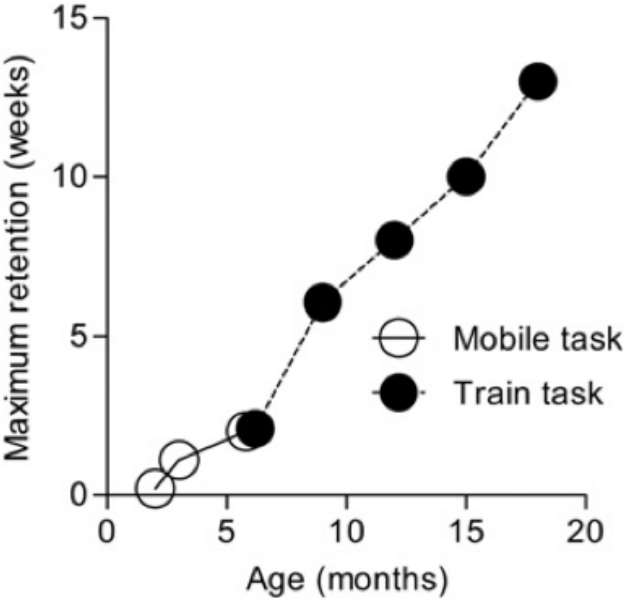

Figure 1. Based on data from Rovee-Collier (1999). Memory retention increases gradually with age in children. Rovee-Collier and colleagues used the mobile conjugate paradigm in young infants (2- to 6-month-olds) and a variant of this task, the train paradigm, in older infants (6- to 18-month-olds).

Particularly important, additional studies using the mobile conjugate paradigm have demonstrated that infants' long-term memories are amazingly specific to the content available during encoding (Rovee-Collier, 1999) and seem to obey many of the same principles as found in adults (Rovee-Collier, 1999). These findings, as well as others (Gerhardstein, Adler, Inslicht, & Rovee-Collier, 1997), strongly suggest that the memory tapped by these operant conditioning tasks is explicit in nature. Thus, these operant conditioning studies with 3-month-olds and older infants demonstrate much more extensive and enduring memories than either habituation or classical conditioning tasks[1]. Finally, the aforementioned operant conditioning studies[1], unlike any other paradigm, were able to demonstrate a continuous developmental trend in memory capabilities throughout infancy, with the length of memory retention increasing gradually with age.

Extending VExP to a Memory Paradigm

Taken all together, previous infant memory studies have shown that infants are capable of remembering information over a significant period of time, much longer than once believed. There are inconsistencies in the findings, however, wherein different studies using different paradigms have yielded evidence of memory at different age groups and over different retention delays. This has lead to varying conclusions about the nature of early memory capabilities and their development in infancy. The mobile paradigm is the only paradigm to date that has provided systematic evidence of memory

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[1] Other studies have used deferred and elicited imitation to investigate the development of memory (Barnat, Klein & Meltzoff, 1996; Hayne, Boniface, & Barr, 2000; Meltzoff, 1988, 1995). These studies have generally focused on later infancy and so are not directly relevant to the current study and for the sake of brevity a full accounting of these studies have been omitted.

in 3-month-olds that lasts for more than a few days. As a consequence, there have been long standing debates over the validity of the paradigm. For instance, some have suggested that the mobile conjugate paradigm measures only procedural memory, a form of implicit memory for particular types of actions, and as such, does not represent an accurate measure of memory for specific events that is comparable to what is typically referred to as long-term memory found in adults (Nelson, 1995). More specifically, researchers have claimed that the mobile task measures only a motor skill and as such serves as an implicit memory task and does not assess explicit memory (Nelson, 1995). These conclusions, however, are often derived from a misunderstanding of the methodology (see Hayne, 2004, for a complete discussion of these issues). Moreover, as previously mentioned, additional studies using the mobile conjugate paradigm have demonstrated that infants show memory specificity, whereby for infants between 2 and 6 months of age, only the original mobile (or train) is an effective retrieval cue when testing occurs 1 day after training, whereas a novel one is not (Rovee-Collier, 1999). This renders it very unlikely that the mobile paradigm measures only procedural memory in infants. Instead, memory for the explicit characteristics of the cue experienced during training and encoding is likely required in order to discriminate a novel cue.

Nevertheless, in the hopes of better understanding infant memory, and provide converging evidence for infants' long-term memory capacities, in the current study, we introduce and evaluate the validity of using the Visual Expectation Paradigm (Haith, Hazan, & Goodman, 1988) and pupil diameter analysis, to study infant memory. The difference between the encoding and retrieval of different types of information, namely content versus spatial information, and the effects of temporal decay will also be investigated.

When forming expectations for future events, we combine information remembered about the past with information that is currently perceptually available in order to generate representations of the conditions surrounding upcoming events (Adler et al., 2008, Adler & Haith, 2003; Haith et al., 1988). Consequently, decisions and behaviours in anticipation of future events are guided by the expectation representations. As can be seen, long-term memory for previous events likely serves an integral role in expectation formation and for planning and anticipatory behavior. Another way to view this relation is that the function of long-term memories is not for the memories themselves but to inform decisions and behaviors to future events in the form of expectation representations (Grossberg, 1995; Rovee-Collier & Sullivan, 1980). Such expectations guide our everyday behaviours, such as planning for future events and guiding our social interactions (Adler & Haith, 2003). Presumably, since little of the environment is under an infant's direct physical control and the relevant cognitive and brain functioning is thought to be relatively immature (Johnson, 1995), future-oriented behaviour was initially hypothesized to be beyond their cognitive capabilities (see Haith, Benson, Roberts & Pennington, 1994 discussion on the issue). However, Wentworth and Haith (1992, 1998), demonstrated that infants form expectations for future events, as early as eight weeks of age.

Haith, Hazan and Goodman (1988) provided the first evidence of expectations in infants for events that were not perceptually available, with a paradigm called the Visual

Expectation Paradigm (VExP). In the prototypic VExP, infants view images that appear either in a predictable left-right alternating sequence or in an unpredictable random and irregular sequence. The primary index of the expectation construct is anticipatory behavior where eye movements are programmed during the inter-stimulus interval (ISI) to locations where pictures will appear before their actual onset. Haith and colleagues (1988) demonstrated that infants' anticipatory eye movements were more numerous when images appeared in a predictable spatiotemporal sequence than in an unpredictable sequence. Moreover, the latency of the infants' reactive eye movements after picture onsets was faster when the pictures appeared in a predictable spatiotemporal sequence (Haith et al., 1988). Such 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 using the VExP have revealed that infants encode and form expectations for more complex, asymmetric (e.g., left–left–right) spatial sequences (Canfield & Haith, 1991), for the temporal parameters of visual events (Adler, Arehart, Lanthier, & Haith, 2006; Wass, Lewis, & Haith, 1998), as well as content of events (Adler & Haith, 2003).

Given that a function of long-term memory is the formation of expectations to anticipate future events (Grossberg, 1995; Haith, 1995; Rovee-Collier & Hayne, 2004), and infants have demonstrated the capacity of forming expectations, the VExP is perhaps an optimal paradigm for studying memory in infancy. By placing a delay between the infants' first encounter with the paradigm and a subsequent encounter, the VExP can be extended to allow for the study of long-term retention of information that infants' have experienced during the formation of their expectations. If memory is an integral component in the formation of expectations, as theorized (Adler et al., 2008, Adler & Haith, 2003; Haith et al., 1988), then the information that is encountered during expectation formation should be remembered over the long term. Because expectations have also been thought to play an important role in the development of different cognitive skills, such as object permanence (Harris, 1983) and contingency learning (Fagen, Morrongiello, Rovee-Collier & Gekoski, 1984), extending the VExP into a memory task might allow for the opportunity to encompass elements of previous tasks and provide a more comprehensive view of memory in infants.

We suggest that VExP is also an ideal paradigm for the study of memory because of its use of eye movements to assess cognitive abilities. Previously mentioned paradigms such as habituation and the visual paired comparison task have used visual attention and looking methodologies (Colombo & Mitchell, 2009). As a consequence, while differences in looking time seem to reflect the capacity to discriminate, when there are no differences in looking time, there is ambiguity as to whether there is the capacity to discriminate at any level, whether perceptually or cognitively (Aslin, 2007). For this reason, looking paradigms may not represent the optimal methodology for investigating memory development. Conversely, it has been suggested that perhaps eye movements may be more appropriate to study cognition in infants (Aslin, 2007).

Eye movements show rapid maturation in infants from a young age (Aslin, 1985; Shea, 1992), as demonstrated, for example, by the finding that infants from 12 down to as early as 2 months all exhibit anticipatory eye movements, during the VExP (Canfield, Smith, Brezsnyak, & Snow, 1997). Since eye movements are well developed early they provide a means for measuring any cognitive ability (including memory) in the same manner, with the same stimuli and measures, across a wide range of different age groups -- characteristics that have been missing in most previous developmental methodologies. As the VExP relies on measures of eye movements, particularly anticipatory ones, to assess cognitive abilities, it acquires this advantage, and renders the paradigm developmentally stable and appropriate for different age groups. Moreover, the VExP paradigm also allows for the assessment of pupil diameter. Pupil diameter analysis is a measure that has been used recently to explore various cognitive abilities in adults, including memory (Goldinger & Papesh, 2012). Thus, as will be further explained later, using the VExP to study infant memory will also allow for yet another measure of long-term memory across development and, consequently, a better understanding of the nature of memory development across a wide range of ages.

In this study, the VExP will be used, wherein infants will see an alternating sequence of visual events of colourful and patterned pictures, and their capacity to remember the event parameters, such as the spatial and content information, over the long term, and the effects of temporal parameters of the stimuli on their memory, will subsequently be assessed. By training infants with a particular stimulus sequence on one day and subsequently testing them on the next day with a sequence in which perceptual information has changed, their long-term memory for the original perceptual information and the relation between memory and expectations can be explored. This study, consequently, will also allow the determination of whether infants encode into long-term memory information that supports their expectations and provide a new methodology for exploring the nature of memory processes in infancy.

Differences in Information Processing for Content and Spatial Information

In the original study of infants' expectation for event content, Wenthworth and Haith (1992) showed that infants could form expectations for the content of a single repeating event. Since the content did not change, it was therefore possible that infants were just detecting the constancy of the picture event, without necessarily encoding the specific content information that defined that event. Thus, the processing load for detecting the commonality across these events was low, as infants could have just noticed that it was the same even without having to extract and compare shared content across differing events. People, however, rarely form expectations for a single repeating event, and instead form expectations for a set or category of events that share particular feature content (Adler & Haith, 2003). Previous studies have demonstrated that infants have the perceptual capacity for detecting invariance of features, such as colour, across distinct stimuli (Bornstein & Kordan, 1984; Catherwood, Crassini & Freiberg, 1989). Whether the detection of invariance of attributes of stimuli could be used in forming expectations was not examined in the original expectation study for event content (Wentworth & Haith, 1992).

To address this issue, Adler and Haith (2003) examined whether infants could form expectations for a set of events that shared common colour content as they varied along dimensions of shape and pattern. In their study, 3-month-olds viewed images of checker-boards, vertical stripes, concentric circles, and diamond-in-square shapes that appeared randomly in a spatially predictable left-right alternating sequence (Adler & Haith, 2003). Stimuli on the invariant side consisted of picture events with the same colour combination (e.g. blue/yellow), whereas on the varied side the picture events could be any of four possible colour combinations on any given trial. The results showed that infants made more anticipations toward the colour invariant side relative to the varied side. This indicated that infants do encode the invariant content from a set of events in their expectation representations, such that the predictable invariant colour content guided their expectation behaviour. In a follow-up experiment to assess the nature of the information encoded into the expectation representation, the presentation of a stimulus of a novel colour combination was occasionally included on either side. Anticipatory behaviour on the invariant side was disrupted, whereas anticipations to the varied side was not. These findings further indicate that infants, in forming expectations for related events that differ along some dimensions, encode specific shared content or event features, as evidenced by the discrimination of novel stimulus only on the invariant content side (Adler & Haith, 2003).

In the current study, infants' detection and encoding of information, the retention of that information, and the relation of these processes to expectation formation will be further explored. Of particular interest is the investigation of the apparent differences in encoding of content and spatial information during expectation formation. In the Adler and Haith (2003) study infants' anticipatory eye movements seemed to be guided more by the predictability of the color information on the invariant side than it was by the spatial information that was equally predictable for both the invariant and varied sides. In contrast, infants' reactive eye movements (after picture onset) was unaffected by the color predictability of the pictures. That is, infants' made more anticipations before picture onset to the predictable color (invariant) side than to the unpredictable color (varied) side, yet the latencies of reactive saccades made after the picture onsets showed no such difference. This finding is contrary to the theoretical framework of Haith (1993), in which anticipatory and reactive saccades are both measures of the same underlying cognitive construct of expectations. As such, any expectation that results in the increase in anticipatory behaviour is suppose to also produce a concordant facilitation of reactive behaviour. Adler and Haith (2003) therefore hypothesized that these two measures are differentially sensitive to expected event content.

Adler and Haith (2003) explained that reactive eye movements are just that, reactive to sensory input and, therefore, may be sensitive to the relatively primitive property of spatial location, and as a result be less sensitive to the content of events. This possibility could account for why reactive latencies did not differentiate performance, since spatial information did not vary between the two content sides (Adler & Haith, 2003). In contrast, anticipations occur prior to sensory input and involve top-down cognitive forecasting of events that are not perceptually available (Adler & Haith, 2003). Because the two content sides differed in their content predictability, Adler and Haith (2003) further suggested that differences in anticipations to the two sides may reflect the relative amount of cognitive processing of event's color (and pattern) content. The proposal that content information is processed separately, and to different levels than spatial information, has also been suggested by many adult behavioral studies (e.g., Biederman & Cooper, 1992; Craik & Lockhart, 1972; Treisman & Gelade, 1980) Further, other studies of infants' information processing, such as their immediate perceptual discrimination (Colombo, Mitchell, Coldren, & Freeseman, 1991) and long-term memory (Adler, Gerhardstein, & Rovee-Collier, 1998), have similarly indicated that informational parameters of events are processed to different cognitive levels.

In order to understand the differences in processing of content versus spatial information in the VExP task, the current study will include different conditions which we hypothesize will be processed and encoded differently. If the Adler and Haith (2003) hypothesis that content information is processed at a higher cognitive level than spatial information is correct, then according to the level-of-processing theory of memory (Craik & Lockhart, 1972), spatial information will not be as memorable as content information. To this end, conditions in which the content or the spatial information is changed after a delay will allow us to determine whether infants encode content and spatial information differently and whether both types of information are equally memorable.

Effects of Temporal Decay on Memory

Working memory is generally defined as a capacity-limited, short-term store that holds information one is actively attending to (Hunter, 1964; Kyllonen & Christal, 1990). Information is maintained in working memory for the purpose of performing complex cognitive tasks such as problem-solving, reasoning (Hunter, 1964; Kyllonen & Christal, 1990), mathematical skills (Bull & Scerif, 2001; Lee, Ng, & Ng, 2009), and learning (Atkinson & Shiffrin, 1968; Baddeley, 1986, 1992; Camos, 2008). In addition, any information held in working memory can be transferred from both working memory to long-term memory, which is as previously described, is a more durable and essentially limitless and permanent store of information (Atkinson & Shiffrin, 1968; Bahrick, Hall, & Berger, 1996; Shallice & Warrington, 1970).

A major limitation of working memory is temporal decay, the likelihood that information will be lost from the memory store as the time between the encoding of information and its recall or use increases (Brown, 1958; Cowan, Saults, & Nugent, 1997; Peterson & Peterson, 1959). More specifically, with the passage of a relatively short amount of time, information in working memory decays, unless strategies (e.g., rehearsal) are used to maintain the information in an active state. The possibility exists that the limitations of working memory may also negatively impact the ability to form expectations and store the information encoded during expectation formation in long-term memory. In fact, infant studies have shown that increases in the interstimulus interval (ISI) affects the ability of infants to keep information online throughout a task. For instance, Gilmore and Johnson (1995) showed that when ISIs increased to more than 3000 msec, 6-month-olds' performance on an ocularmotor delayed response task significantly decreased. This finding that increases in ISI affect an infant's ability on a task, indicates that delays that are too long for infants to keep information online in working memory (Gilmore & Johnson, 1995).

Since working memory and long-term memory are linked (Atkinson & Shiffrin, 1968; Bahrick, Hall, & Berger, 1996; Shallice & Warrington, 1970; Ranganath, Johnson, D'Esposito, 2003; Axmacher, Schmitz, Weinreich, Elger & Fell, 2008), if information decays from infants' working memory because the delay is too long then that information will not be available for transfer to long-term memory. In this study we investigated if temporal decay would affect infants' long-term memory for expectation information. To this end, this study assessed the effect of two different ISIs, 1000 and 3000 msec on infants' long-term memory performance. By including a longer 3000 msec ISI, we predict that temporal decay will occur and consequently result in poorer long-term memory encoding and performance. An ISI of 3000 msec was chosen as the appropriate delay to elicit temporal decay and study its effects on long-term memory because Gilmore and Johnson (1995) had previously shown decay at this delay in infants.

Pupil Diameter As an Indicator of Memory

Recent research with adults has suggested that analyses of pupil diameter might be a relevant measure with which to explore cognition (Goldinger & Papesh, 2012). Pupil diameter change (dilation and constriction), in fact, has previously been shown to be associated with various cognitive abilities (Stanners, Coulter, Sweet & Murphy, 1979), while at the same time being independent to changes in stimulus luminosity or changes in emotional state. Of particular interest is the finding that pupil diameter changes are related to memory. For instance, nonsense syllables made familiar through pre-experimental exposure, relative to unfamiliar nonsense syllables, yielded greater pupil dilations during the second exposure (Gadner, Mo & Borrego, 1974). More recent pupillometry studies have also demonstrated that the strength of memory is observable from different patterns of pupil diameter changes during both encoding and retrieval (Cansino & Trejo-Morales, 2008). Additionally, in yet another study by Heaver and Hutton (2011), pupil dilations were exhibited more when participants viewed images they had previously seen in comparison to novel images. Pupil dilations were also exhibited even if participants were instructed to report a previously viewed item as a novel one, which suggested that increases in pupil diameter are not under conscious control (Heaver and Hutton, 2011).

Although it seems as though memory strength is linked to pupil dilation, other studies have found contrasting results. For instance, in a recent study by Kafkas and Montaldi (2011), participants were given a surprise memory task and it was found that during incidental encoding (as opposed to previously mentioned tasks, where participants know they will be tested), as memory strength increased, pupil diameter decreased. In yet another study, Van Rijn and colleagues (2012), demonstrated that after participants learned paired associates, pupil dilations decreased with increased memory strength (Van Rijn, Dalenberg, Borst &Sprenger, 2012). Van rijn and colleagues (2012) suggested that these decreases were a result of participants requiring less retrieval effort for pairs they strongly remembered. Although the findings of these two studies are not completely concordant with the previously mentioned studies, this is most likely due to the differences in the tasks and methodology used across all the studies. Regardless, the findings from all these studies suggest that differences in pupil diameters reflect differences in memory (Goldinger & Papesh, 2012).

Other studies have demonstrated that pupil diameter analysis can even be used to study developmental populations, albeit for different cognitive and perceptual tasks. These early infant studies have observed, for instance, greater pupillary dilation to pictures of faces than to geometric shapes (Fitzgerald, 1968). In addition, more recent studies have demonstrated that 8-month-old infants react with larger pupil dilations after having observed a physically impossible event (i.e. one train entering a tunnel with another coming out) in comparison to a possible event (i.e. the same train entered and exited the tunnel) (Jackson & Sirois, 2009). To date, however, no study has yet attempted to investigate pupil diameter changes in relation to memory in infants.

Given that pupillometry methods have been used to assess memory in adults, and have successfully allowed for the study perception and cognition in infants, in this study we conducted exploratory analyses of pupillary responses of infants as they experience the VExP. Particularly, we assessed possible differences between the different days and our different conditions. Since changes in the level of anticipations were the primary measure of memory in the study, to assess the connection of pupil size changes to long-term memory in infants we looked at pupillary responses in temporal conjunction with anticipations throughout the VExP. This represented a first step in determining the viability of pupil size changes as a measure of infants' memory and allowed for a better understanding of possible cognitive changes related to memory retrieval throughout the task.

This study represents the first attempt to use such a measure with infants while assessing memory. In conjunction with the VExP, the pupil diameter analysis may represent an even more robust and developmentally stable measure for assessing long-term memory, because changes in pupils have been shown exist in all age groups and are not under conscious control (Heaver & Hutton, 2011).

Current Study

This proposed study had 5 aims: 1) to investigate the relation between long-term memory and expectations in infancy, 2) assess the possible differences in the processing and encoding of content versus spatial information, 3) assess the effects of working memory limitations, namely temporal decay on long-term memory, 4) demonstrate the validity of the VExP as a paradigm to assess long-term memory, and 5) investigate the feasibility of using the measurement of pupil diameter changes as a means to assess memory in infants.

By adding a delay between the training and test phase of the VExP, the theoretical relation between long-term memory and expectations was assessed in infants. Furthermore, the relative strengths and depth of encoding of the original perceptual information in long-term memory for was explored by training infants with a particular stimulus sequence and subsequently testing after the delay with a sequence in which either the content or spatial information had changed. As a consequence, the validity of whether anticipatory behaviours are sensitive to and guided by different event information was assessed. We hypothesized that content information is processed at a higher cognitive level than spatial information, and therefore, we predicted that content information would be more memorable than spatial information, as suggested by level-of-processing models of memory (Craik & Lockhart, 1972).

By also manipulating the temporal parameters of the stimuli, the effects of temporal decay on memory were investigated. Although temporal decay is mainly considered a limitation of working memory, we hypothesized that it would also have an effect on long-term memory. Since working memory and long-term memory are linked (Atkinson & Shiffrin, 1968; Bahrick, Hall, & Berger, 1996; Shallice & Warrington, 1970), and a previous study indicated rapid decay of information from infants' working memory (Gilmore & Johnson, 1995), we hypothesized that an increase in the ISI would compromise infants' ability to form expectations and, as a result, disrupt their long-term memory for the information encoded during expectation formation.

By allowing us to determine whether infants encode into long-term memory information that supports their expectations, this proposed study will therefore begin to bridge the gap between the development of infants' perceptual and memory capacities and help to begin to identify how these two processes are linked. Without an understanding of this link through development, a true understanding of how early experiences affect cognitive development and future behaviour cannot be formed. Finally, since some of the neural network responsible for pupillary changes has been documented, the ability to use pupil diameter as a measure of memory could provide an easy and inexpensive means for assessing relevant neural development in a population for whom fMRI is not currently a viable option.

Experiment 1: VExP and Long-Term Memory

Methods

Participants

Data were collected from 15 3-month-old infants (7 males and 8 females), ranging in age from 86 days to 125 days (*M* = 108.07 days, *SD* = 12.86). All three conditions of the experiment included 5 infant participants. Infants in the sample were Caucasian (*n* = 12) and Asian (*n* = 3) 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 13 infants participated, but insufficient data were collected from them because they cried (*n* = 5), were inattentive (i.e., disinterested, or looked at their hands or other parts of the visual field; *n* = 6), or due to experimenter error (*n* = 2). The high dropout rate in this study reflected a strict criterion for inclusion of an infants' data in the analyses. More on this criterion will be mentioned below in the data reduction section. Nevertheless, the dropout rates in this experiment are consistent with the dropout rates reported in other visual expectation studies (e.g., Adler & Haith, 2003; Haith et al., 1988; Haith & McCarty, 1990; Wentworth & Haith, 1992).

The participants and their caregivers were recruited through a monthly list purchased from Z Retail Marketing Inc., a local marketing company. 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 families who were interested in participating could return. Parents also had the option to respond to the invitation letter via phone, email, or an online form. Families who returned postcards were contacted by phone and were provided with more information about a specific study. Families who were still willing to participate were then asked to provide a day and time best suited to their schedule at which time they were able come to the Project for their participation.

For those families who did not immediately respond to the invitation letter, as many of their phone numbers as possible were found. A few weeks after families were expected to have received the invitation letter, they were contacted over the phone as a follow-up. During the phone conversation, questions about the Visual and Cognitive Development Project and its studies were answered and if families were interested in participating, an appointment was set up at a time that was convenient for their schedule.

Stimuli and Apparatus

The stimuli were computer-generated graphic images of checkerboards, vertical stripes, concentric circles and diamond shapes in four possible colour combinations: red/green, red/blue, yellow/green and blue/yellow. 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 x 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. Figure 2 depicts the graphic images used as stimuli.

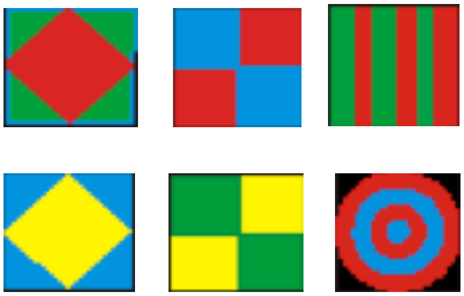

Figure 2. Examples of graphic images used as stimuli.

During the experimental session, babies laid supine in the specialized crib that had the LCD monitor, displaying the stimuli, mounted 48 cm above. Between the infant and the monitor, there was a 30 x 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. Figure 3 depicts this set up.

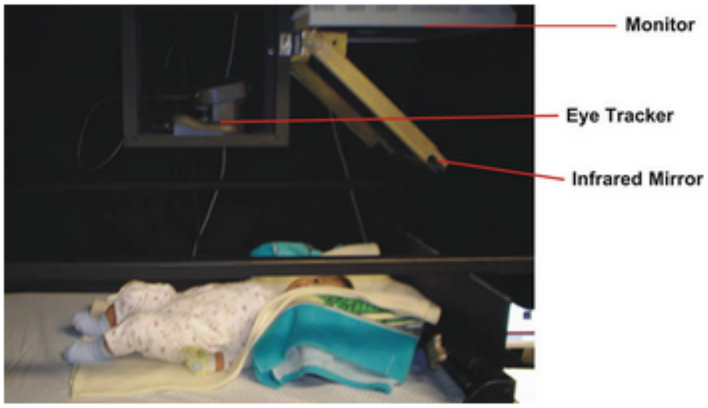

Figure 3. Infant viewing stimuli overhead while lying in specialized crib apparatus.

Using bright pupil technology, the eye tracker recorded the participants' eye movements via reflection in the infrared mirror at a 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. The eye tracker was initially calibrated by having the infant look at a stimulus (concentric squares) that was presented at known locations on the LCD screen. All subsequently recorded eye tracker values were filtered through the calibration file to produce measures of eye position data.

Two Dell computers were used to control the experimental session, one generated and presented the stimuli using DirectRT software (Empirisoft Inc., New York: NY, 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 stimulus-generating 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.

Procedure

On the first day of participation, families were provided with a quick overview of the procedure of the experiment that their baby would to be participating in and shown the experimental setup. Afterwards, the parents were asked to fill out an informed consent form, as well as a form requesting their demographic information (See Appendix A for consent form).

Parents placed their infant in the specialized crib and, if available, a pacifier was offered to the infant as the eye tracker was focused to the infant's eye by the use of a remote control. Minor adjustments to the position of the infant's head were also be made when necessary. Before the start of the experimental trials, the eye tracker was calibrated by recording the infant's fixations to known locations on the monitor at which a stimulus were presented. The stimuli that were presented were concentric squares. All subsequent eye data were filtered through these calibration values. This procedure was repeated at the outset of the second visit to account for any changes in that infant's head position relative to the monitor and eye tracker.

This experiment included three conditions: no-change, colour-change and spatial-change condition. In all three conditions, infants viewed a series of pictures appearing on the left and right sides of the screen. A total of 70 pictures were presented on both days in all conditions, of which the first 10 on the first day served as the baseline phase during which pictures appeared randomly to the left or right with a duration of 700 msec and an inter-stimulus interval (ISI) of 1000 msec. 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 served as the experimental phase during which the pictures appeared on the left or the right of the screen in a predictable alternating left-right sequence, also with a duration of 700 msec and an ISI of 1000 msec. In all conditions, the same checkerboard, vertical stripes, concentric circles, and diamond shapes stimuli were used in four possible colour combinations (red/green, red/blue, yellow green, and blue/yellow). The colour combination that constituted the invariant content and the side on which this colour combination appeared were counterbalanced across infant. In all conditions, infants' level of anticipatory responding and pupil diameter changes were assessed.

On Day 1, infants in all three conditions viewed stimuli wherein pictures on one side were always presented with the same colour combination (invariant side), whereas the other side consisted of pictures with different colour combinations (varied side). However, the stimuli infants viewed on Day 2, 24 hours later, were different across conditions. For the no change, infants received the same invariant color combination on the same side on Day 2 as Day 1. For the color change, infants received a different invariant color combination on Day 2 but on the same side as Day 1. For example, if infants were presented with an invariant color combination of blue/yellow on the right side on Day 1, then they might have been presented with an invariant color combination of red/green on the right side on Day 2. Finally, for the spatial change, infants received the same invariant color combination as Day 1 but on the opposite side. That is, for instance, if they were presented with the invariant color combination of red/green on the left side on Day 1, then they were presented with the same red/green invariant combination on Day 2 but on the right side.

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 movement data 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, a scorer identified which of infants' eye movements were anticipatory or reactive in timing (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. Although previous VExP studies demonstrated that the latency of the infants’ reactive eye movements after picture onsets were also faster for pictures that appeared in a predictable spatiotemporal sequence (e.g. Haith et al., 1988; Haith, 1994), this study excluded the analysis eye movement latencies. The rationale for this is predictability of the stimuli included in this study was based on colour content, and previous VExP studies that have included content invariability did not show any effect on reactive latencies (Adler & Haith, 2003). 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 will judge the infant as having made a valid eye movement.

In order for an eye movement to be included in the final data sample, it needed to meet a set of criteria. Only data of infants who attended (looking at the stimuli) on a minimum of 60% of the experimental trials were included (e.g. Adler & Haith, 2003; Adler & Orprecio, 2006). Eye movements were considered to be anticipatory if it occurred after the offset of the previous stimulus and within the first 167 msec 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 msec (Adler & Haith, 2003; Canfield, Smith, Brezsnyak & Snow, 1997). If an eye movement occurred 167 msec after the onset of stimulus but before 167 msec after to the stimulus offset, then it was classified as a reactive eye movement. Figure 4 depicts the cut-off time for anticipatory eye movements. 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 eye movement was intentional and not random.

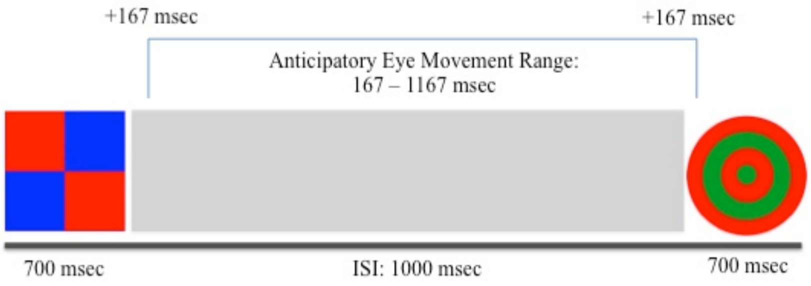

Figure 4. Cut-off time for anticipatory eye movements.

Our primary measure of memory recognition for expectation information was a savings measure. The savings measure provides a relative measure that indicates the degree of an infant’s long-term retention compared to that infant’s immediate retention or learning. That is, savings indexes indicate how much of the information available immediately at the end of training is retained after a delay. Savings was calculated by dividing the infant’s percent of anticipations during the first 30 trials on Day 2 by the infant’s percent of anticipatory eye movements on the last 30 trials on Day 1. By comparing the first 30 trials on Day 2 to the last 30 trials on Day 1, the degree of retention on Day 2 can be compared to the highest level of performance and learning on Day 1. Moreover, looking at the first 30 trials on Day 2 also controls for new learning that may occur throughout the mid to later trials on Day 2. The savings measure formula is illustrated in in Table 1. The savings measure is also an all or none measure, in that a group’s mean savings measure equal to or greater than a theoretical ratio of 1.00, indicates memory retention. A group’s mean savings measure significantly less than 1.00, suggests an impairment in or lack of retention.

$$\textbf{Savings Measure} = \frac{\text{\% Anticipatory Eye Movements in first 30 Trials (of DAY 2)}}{\text{\% Anticipatory Eye Movements in last 30 Trials (of DAY 1)}}$$

Table 1. Formula for calculating the savings measure.

Until now, no study has aimed to study pupil diameter changes in relation to memory in infants. As such, in this study our aim was to explore a preliminary analysis procedure to study this relation. Because of the nature of the questions and other performance measures we were using, the analysis we conducted in this study was to compare the pupil diameter at the initiation of the anticipations on Day 1 versus Day 2. We believed this method would likely capture the physiological and neurological changes, and as a result, pupillary changes that would occur in relation to the retrieval of expectation information from memory needed during the VExP task.

Pupil diameter was recorded at the initiation of all anticipations to both the varied side and invariant side. Three pupil recordings surrounding the anticipations were collected to account for the eye tracker's sampling error. Pupil diameter was sampled at the initiation of an anticipation, as well as a time frame (1/60 of a second) before and a time frame (1/60 of a second) after each anticipation. The mean of the 3 samplings served as the measure of pupil diameter on a given trial. Means of all pupillary diameter recordings were subsequently compared across the different days and groups of conditions.

Finally, all results were analyzed with valid statistical methodology using the statistical software program SPSS, with all tests conducted at an alpha level of $p < .05$.

Results

Preliminary Analyses

Since the invariant colour content for this experiment was drawn from four possible combinations, and it has been previously shown that 3-month-olds have differential sensitivity to various colours (Adams, Maurer & Davis, 1986; Peeples & Teller, 1975; Teller, Peeples & Sekel, 1978), it was first necessary to determine whether each of the colour combinations yielded equivalent expectancy performance across infants. For these analyses, Day 1 data from all infants in all were examined. Day 2 was not included since the infants viewed different stimuli with distinctive parameters across the different conditions and this could have additionally affected their anticipatory behaviour. Due to the small sample size of the study, our resulting data for the conditions in this experiment ISI violated assumption of parametric statistical tests when additionally separated into the different colour combination groups. The Levene's test for equality of variance indicated unequal variances in the anticipation data for the varied side (F = 5.09, p = .019). As such, non-parametric tests for independent samples were conducted on the percent anticipations across each possible invariant colour combination (red/green, red/blue, yellow/green and blue/yellow).

Two separate Krushall-Wallis tests were conducted for each content side (invariant and varied). Results indicated that there was no significant effect of colour combination on the median anticipations to the invariant side, $\chi^2(3, N = 15) = 6.11$, $p = .107$, or to the varied side, $\chi^2(3, N = 15) = 6.66$, $p = .083$. These preliminary analyses indicate that the specific colour combination that was featured on the invariant side did not have an effect on the infants' anticipatory performance. As such, for subsequent analyses, data were collapsed across infants who viewed different colour combinations as the invariant content.

Anticipations

Expectation Formation on Day 1. Before conducting analyses on memory performance on Day 2, it was essential to first assess the percent anticipations on Day 1, in order to see if the infants' anticipatory behaviours for this study were comparable to the findings of other studies of expectations for event content. Since the previous content expectation studies have demonstrated that infants form expectations for the stimuli as evidenced by a greater proportion of anticipations to the invariant side (Adler & Haith, 2003; Wentworth & Haith, 1992), it was important to first assess if this effect was also found in this study, on Day 1. A 3x2 split-plot analysis of variance was conducted comparing infants' anticipatory behaviour across the three experimental conditions (between-subjects variable – no-change, colour-change and spatial-change conditions) for the invariant color combination side and the varied color combination side (within-subjects variable). There was a significant main effect of color content side, *F* (1, 12) = 5.72, *p* = .034, indicating infants made more anticipations to the invariant color combination side (*M* = 72.89%, *SD* = 13.70) across all conditions than to the varied side (*M* = 64.21%, *SD* = 10.80). No significant interaction effect between color side and condition was found, *F* (2, 12) = .702, *p* > .05. More specifically, a greater percentage of anticipations were made to the invariant side in comparison to the varied side (see Figure 4). These results replicate and are consistent with previous VExP studies of expectations for event content (e.g., Adler & Haith, 2003; Haith et al., 1988; Haith & McCarty, 1990; Wentworth & Haith, 1992) and indicate that infants in this study also formed expectations for the content of the events as evidenced by more anticipations to content predictable events than to content unpredictable events on Day 1. Thus, any differences found in infants' anticipatory performance on Day 2 was likely due to the effects on their memory of the different conditions than to differences in the initial encoding of the expectation information.

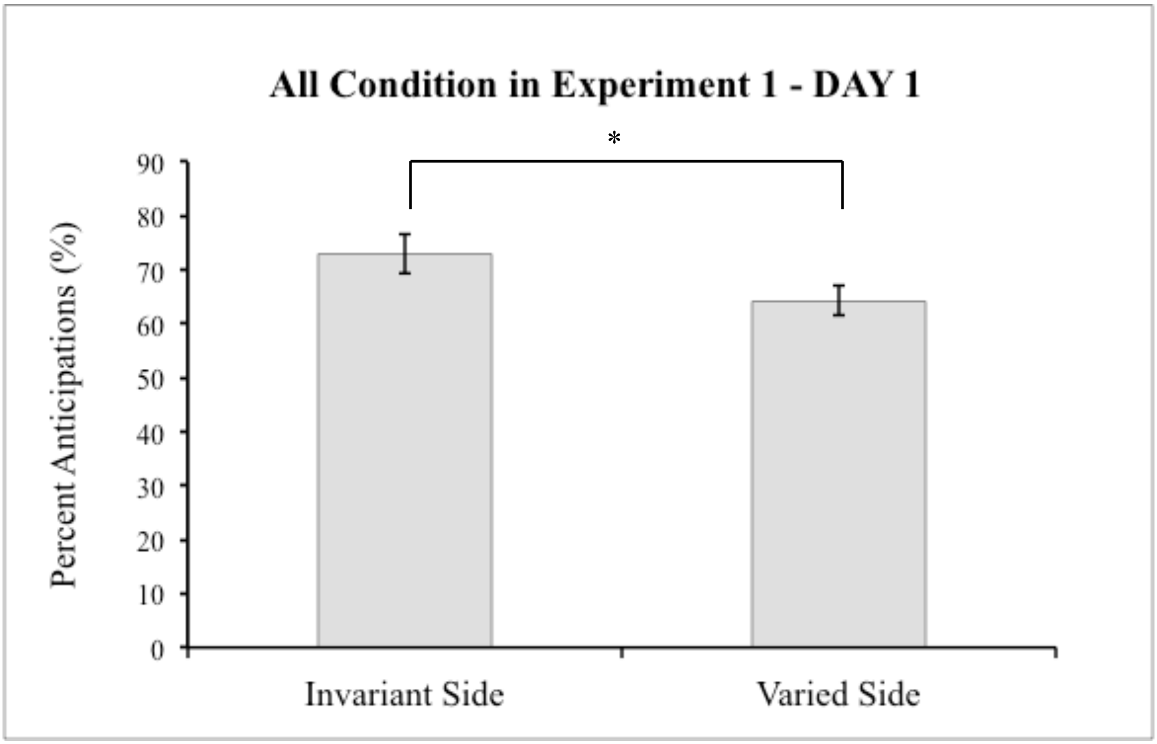

Figure 4. Mean proportion of anticipations made by infants to the invariant and varied colour combination side for all conditions in Experiment 1. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

No-change Condition. In order to investigate the infants' anticipatory behaviour in the no-change condition, infants' percent anticipations on Day 2 were examined and compared to Day 1. As expected, upon inspection of the data, it appears that similar to Day 1, infants made more anticipations to the invariant side relative to the varied side (see Figure 5). The infants still exhibited greater anticipations to the invariant side, even after a delay of 24 hours. This suggests that the information they were exposed to on Day 1 is retained and that the information that define the expect events on Day 1 are guiding infants' behaviour on Day 2. A related-samples t-test, however, found that the difference was not significant, *t*(4) = -1.69, *p* = .167. This non-significance, though, is most likely a result of our small sample size (*n* = 5), which in turn, generated a low power level.

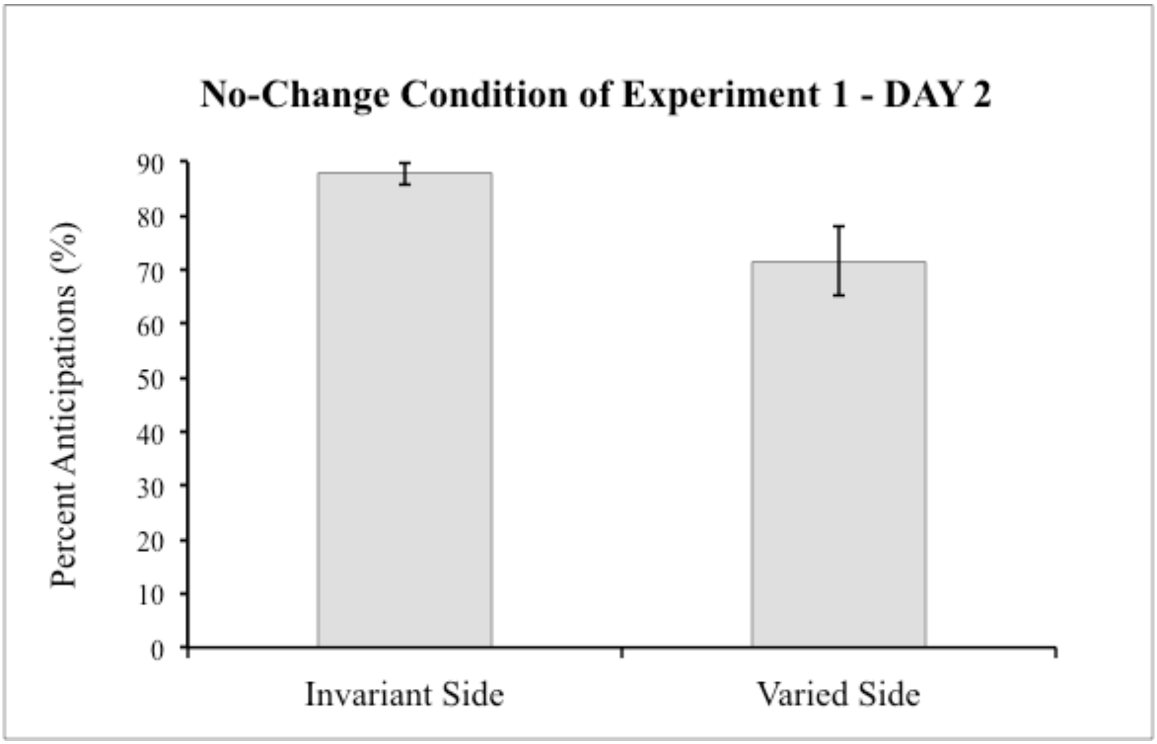

Figure 5. Mean percent anticipations made by infants to the invariant and varied colour combination side in the no-change condition of Experiment 1. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

To further investigate infants' memory for the event information encoded during expectation formation on Day 1, the mean savings measure in the no-change condition was computed. Each infants' savings measure are provided in Table 1 in Appendix B. Again, the savings measure provided a relative measure that indicated the degree of an infant's long-term retention compared to that infant's immediate retention or learning on Day 1. The resulting mean savings measure violated the assumption of parametric statistical tests. The Levene's test for equality of variance indicated unequal variances in the savings measures data for the invariant side ($F = 20.967$, $p < .005$). Consequently, a non-parametric test for independent samples, the Mann-Whitney U test, was conducted to assess if the mean savings measure of the infants in this condition were significantly different than the theoretical ratio of 1.00, which indicates memory retention. Results revealed that the infants did not make a significantly different percentage of anticipations during the first 30 trials of Day 2 relative to the last 30 trials of Day 1 on the invariant side, $U = 10$, $p > .05$, *n.s*, or on the varied side $U = 10$, $p > .05$, *n.s*. Since the stimuli on Day 2 were the same on as the stimuli presented on Day 1, infants performed the same on both days. More specifically, these results suggest that infants exhibited recognition for the event information on Day 2 even after a 24-hour delay for both invariant and varied sides (see Figure 6).

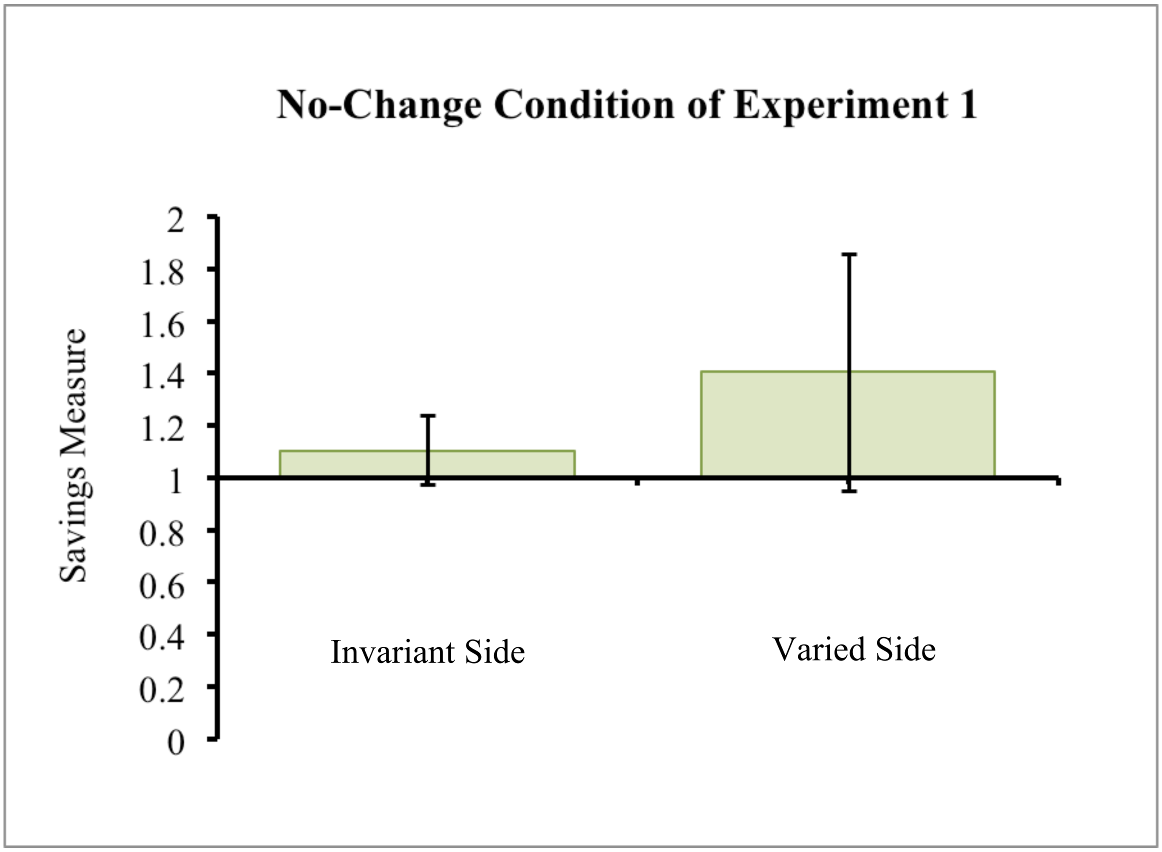

Figure 6. Mean savings measure of infants in the no-change condition of Experiment 1 for the varied and invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference from the theoretical memory retention measure of 1.00 at an alpha level of .05.

Colour-change Condition. For the colour-change condition, infants' percent anticipations on Day 2 were examined and it was found that infants made more anticipations to the invariant side in comparison to the varied side on Day 2 (see Figure 7). A related-samples t-test found that in contrast to Day 1, there was no significant difference between the percent anticipations to the two sides, $t(4) = -.470$, $p > .05$, *n.s.*

The change in the colour content of the events on Day 2, disrupted infants' anticipatory behaviour. This suggests that the colour content information infants were exposed to on Day 1 was remembered and the retrieval of this information guided their behaviour of Day 2. That is, since the colour content information of the events on Day 2 did not match what infants encoded during expectation formation on Day 1, they discriminated the Day 2 events, likely requiring them to encoded the new information and form a new expectation for the novel color events, resulting in their making a similar percentage of anticipations to the two sides on Day 2.

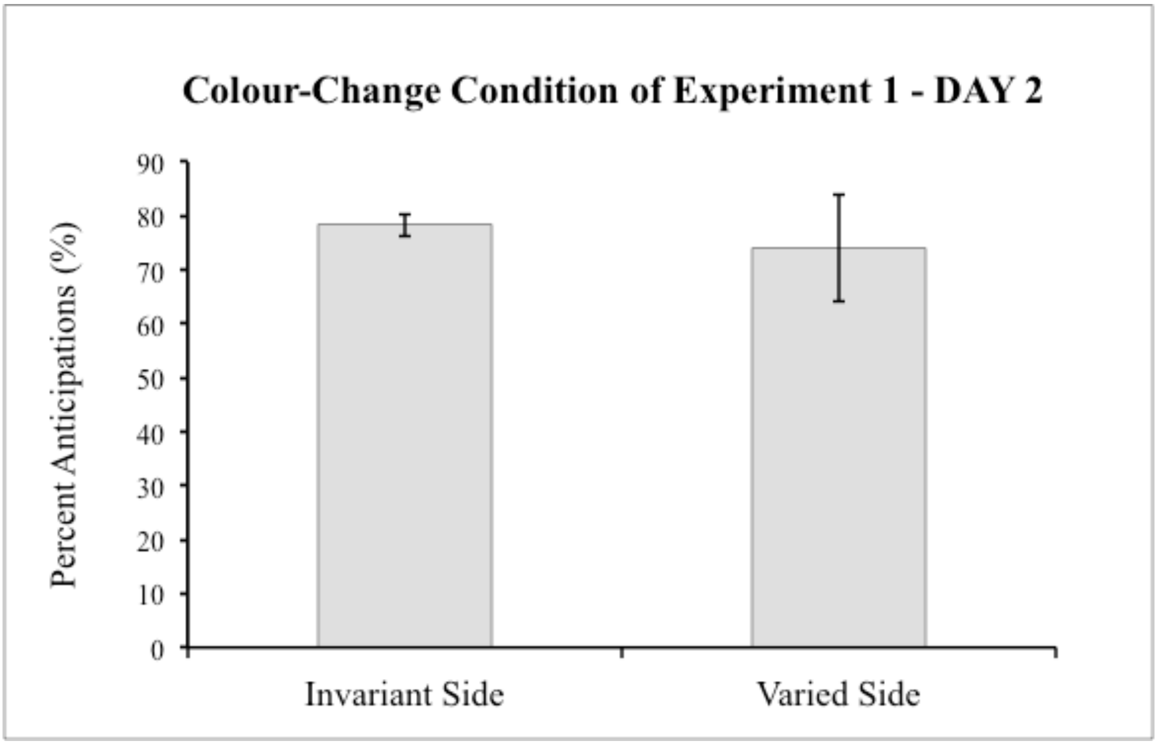

Figure 7. Mean percent of anticipations made by infants to the invariant and varied colour combination side in the colour-change condition of Experiment 1. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

Again, in order to further investigate infants' memory of expectations for the stimuli on Day 1, the savings measures for all five infants in the colour-change condition were also computed. Each infants' savings measure are provided in Table 2 in Appendix B. Similar to the no-change condition, the resulting mean savings measure violated the assumption of parametric statistical tests. The Levene's test for equality of variance indicated unequal variances in the savings measures data for both the varied side ($F = 17.58, p < .005$) and for the invariant side ($F = 11.12, p < .05$). Therefore, a non-parametric test for independent samples, the Mann-Whitney U test, was conducted to assess if the mean savings measure in this condition was significantly different than the theoretical ratio of 1.00, which indicates memory retention. Results revealed that on the invariant side, infants did make a significantly lower percentage of anticipations during the first 30 trials of Day 2 relative to the last 30 trials of Day 1, $U = .00, p < .01$ (see Figure 8). The analyses showed, however, that the infants did not make a significantly different percentage of anticipations during the first 30 trials of Day 2 relative to the last 30 trials of Day 1 on the varied side $U = 10, p > .05$, *n.s.*

These results indicate that the infants discriminated the change in event color information on the invariant side on Day 2. This is likely because infants encoded the invariant color information of the events from Day 1 in long-term memory and then discriminate similar events on Day 2 when the invariant color information does not match what they have stored in long-term memory. This results in a disruption in anticipatory behavior to the invariant side, despite the fact that the invariant is as spatially predictable on Day 2 as Day 1. These savings measure results are consistent with the percent anticipation analyses. Infants made significantly less anticipations to the invariant side on Day 2, since they discriminated that the change in content information on that side when compared to what they remember of what they were exposed to on Day 1.

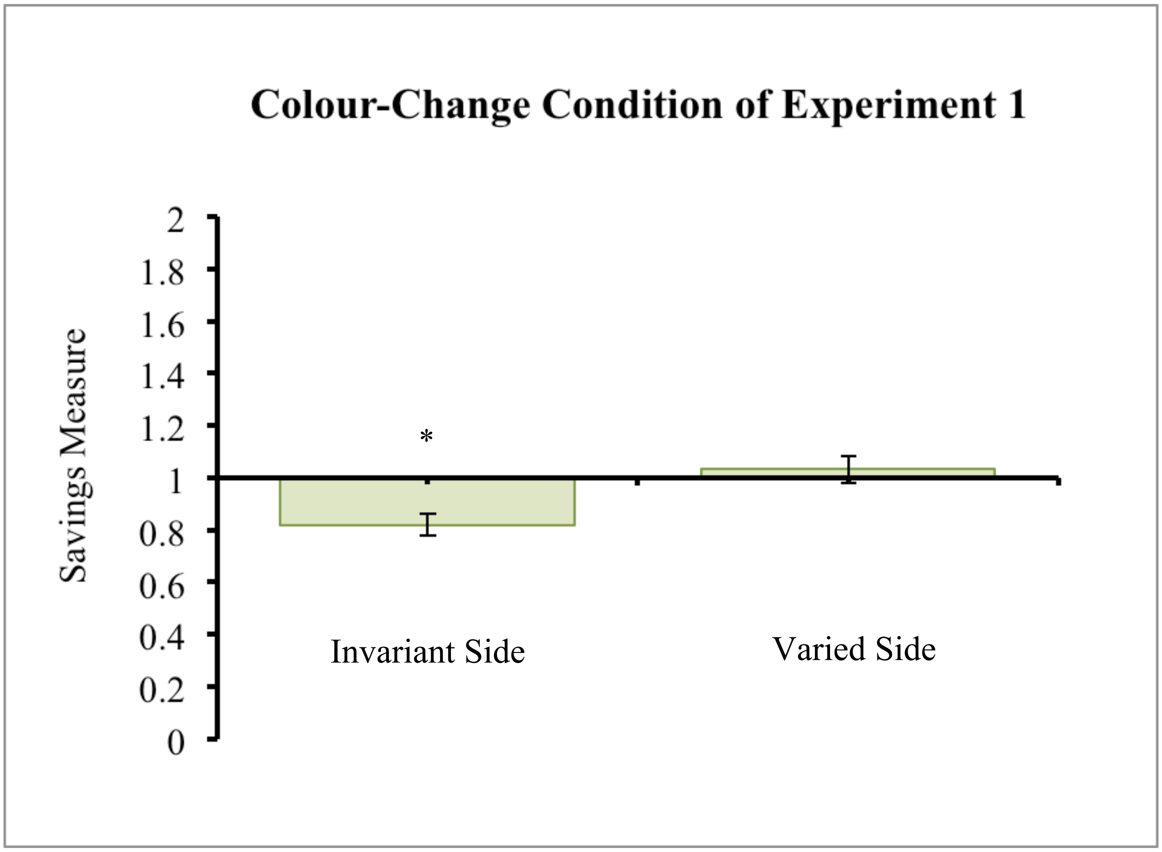

Figure 8. Mean savings measure of infants in the colour-change condition of Experiment 1 for the varied and invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference from the theoretical memory retention measure of 1.00 at an alpha level of .05.

Spatial-change Condition. For the spatial-change condition, infants' percent anticipations on Day 2 were examined. This analysis revealed that on Day 2, infants in this condition made slightly less anticipations to the invariant side in comparison to the varied side. As with the colour change condition, unlike on Day 1, infants did not make more anticipations to the invariant side relative to the varied side (see Figure 9). A related-samples t-test revealed that in comparison to Day 1 there was no significant difference between the percent anticipations on the two sides, $t(4) = 3.96$, $p > .05$, *n.s*. The change in the spatial parameter of the events on Day 2 appears to have disrupted the infants' anticipatory behaviour. This suggests that the spatial location information regarding where the expected content would appear to which infants were exposed to on Day 1 was remembered and retrieved on Day 2, thereby guiding their behaviour. That is, since the spatial information of the invariant content on Day 2 did not match what infants encoded during expectation formation on Day 1, they discriminated the events on Day 2, likely requiring the encoding of the new spatial information and formation of a new expectation, resulting in their making a similar percentage of anticipations to the events on Day 2.

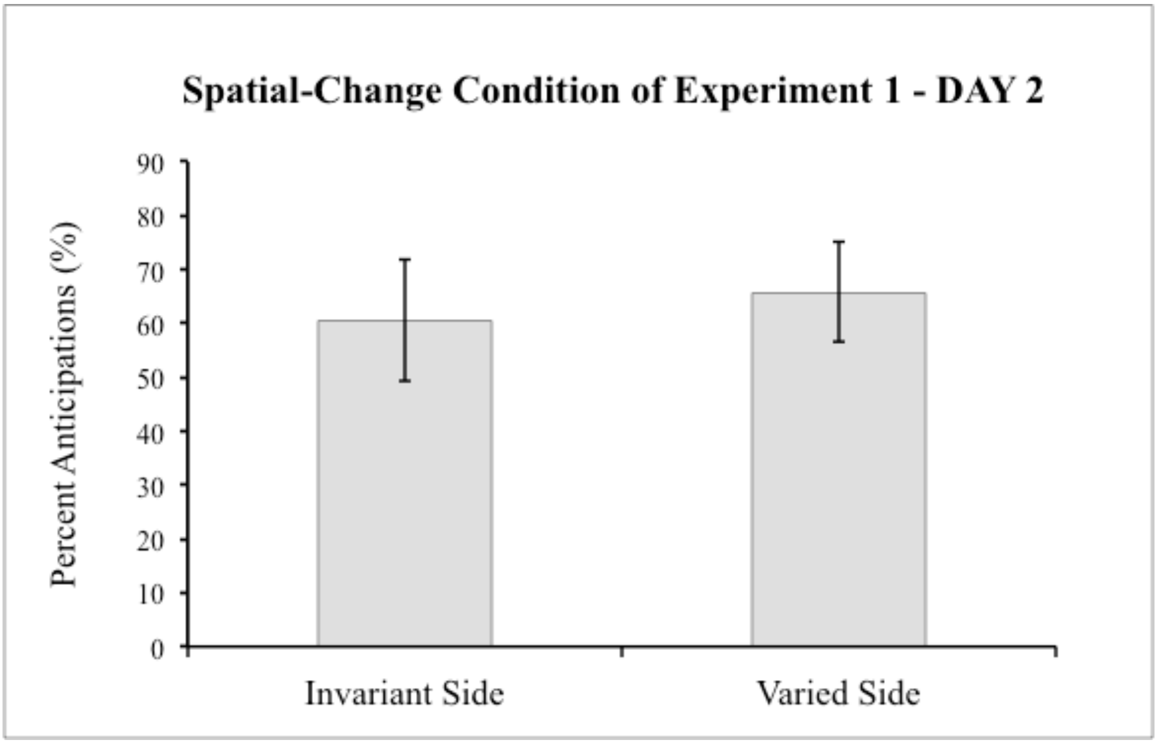

Figure 9. Mean percent of anticipations made by infants to the invariant and varied colour combination side in the spatial-change condition of Experiment 1. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

Similar to the previous conditions, the savings measures for all five infants in the spatial-change condition were also computed to further investigate the infants' memory of expectations for the stimuli on Day 1. Each infants' saving measure are provided in table 3 found in Appendix B. The resulting savings measures violated the assumption of parametric statistical tests. The Levene's test for equality of variance indicated unequal variances in the savings measures data for the varied side (F = 17.58, p = .003) and for the invariant side (F = 11.12, p = .010). Thus, a non-parametric test for independent samples, the Mann-Whitney U test, was conducted to assess if the mean savings measure of the infants in this condition were significantly different than the memory retention theoretical ratio of 1.00. Results revealed that the infants did make a significantly smaller percentage of anticipations during the first 30 trials of Day 2 relative to the last 30 trials of Day 1 on the invariant side, $U = 2.50$, $p = .032$ (See Figure 10). However, in contrast, the analyses showed that for the varied side, infants did not make a significantly different percentage of anticipations during the first 30 trials of Day 2 relative to the last 30 trials of Day 1, $U = 10$, $p = .690$, *n.s*.

Similar to the colour-change condition, these results indicate that the infants discriminated the change in spatial location information on the invariant side on Day 2. This is likely because infants encoded the invariant color information of the events from Day 1 in long-term memory and then discriminate similar events on Day 2 when the invariant color information does not match what they have stored in long-term memory. This results in a disruption in anticipatory behavior to the invariant side, despite the fact that the invariant is as spatially predictable on Day 2 as Day 1. These savings measure results are consistent with the percent anticipation analyses. Infants made significantly less anticipations to the invariant side on Day 2, since they discriminated that the change in spatial location information on that side when compared to what they remember of what they were exposed to on Day 1.

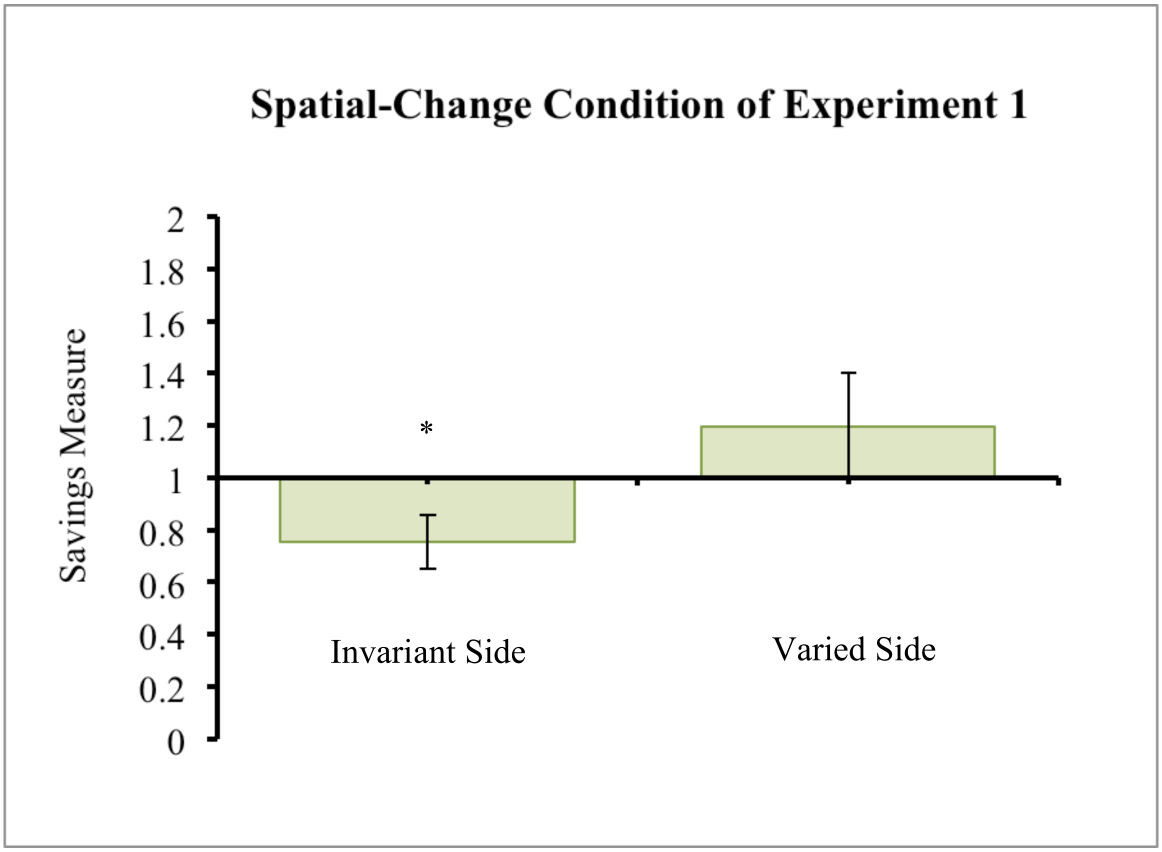

Figure 10. Mean savings measure of infants in the spatial-change condition of Experiment 1 for the varied and invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference from the theoretical memory retention measure of 1.00 at an alpha level of .05.

Pupil Diameter Changes

Comparing Day 1 to Day 2. For all conditions in this experiment, infants exhibited smaller pupil diameters on Day 2 in comparison to Day 1 for anticipations to both the invariant and varied sides (see Figures 11 and 12). Separate independent samples t-tests were conducted for each condition and each side comparing the pupil diameter at

Day 1 and Day 2 for the infants in all conditions of this experiment.

Results revealed that on although Day 2, relative to Day 1, the infants' mean pupil diameter was smaller for the invariant side, these differences were not found to be significant for neither the no-change condition ($t(4) = -1.206, p > .05$), the colour-change condition ($t(4) = -2.496, p > .05$) and the spatial-change condition ($t(4) = -0.509, p > .05$). On the varied side, infants' mean pupil diameter was smaller on Day 2, relative to Day 1. Moreover for the colour-change condition, these differences on Day 2 relative Day 1 on the varied side were found to be significant ($t(4) = 3.490, p = .025$). For the no-change condition and the spatial-change, these differences were not significant ($t(4) = 0.723, p >$ .05 for the no-change, and $t(4) = 0.480, p > .05$ for the spatial-change condition.

Although these results are not statistically significant, there still seems to be a definite trend wherein, on Day 2 all infants in the conditions in this experiment exhibited pupil diameter constriction. These results fit well with our previously mentioned eye movement VExP results (comparison of percent anticipations on Day 1 and Day 2 and savings measure). More specifically, when information is more memorable leading to discrimination of changes in event information, the mean pupil diameter was smaller on Day 2 than when viewing changes in event information that are not as memorable.

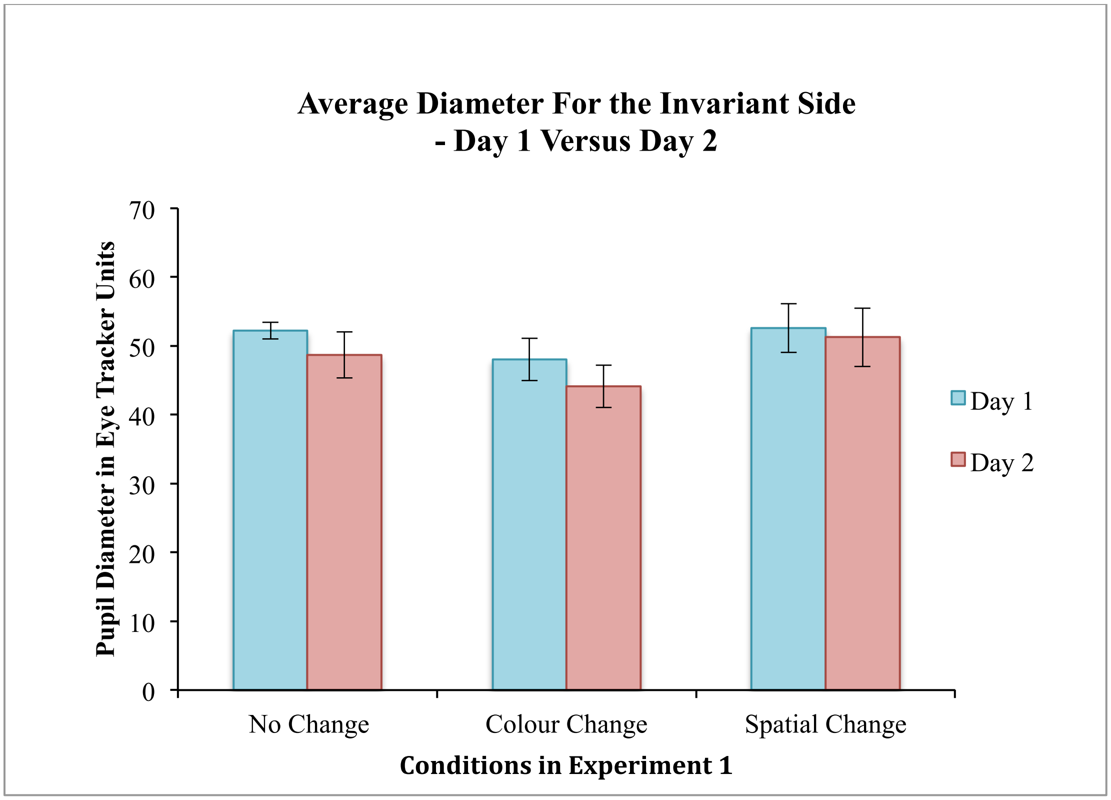

Figure 11. Mean pupil diameter of infants in the conditions of Experiment 1 on Day 1 versus Day 2, for invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

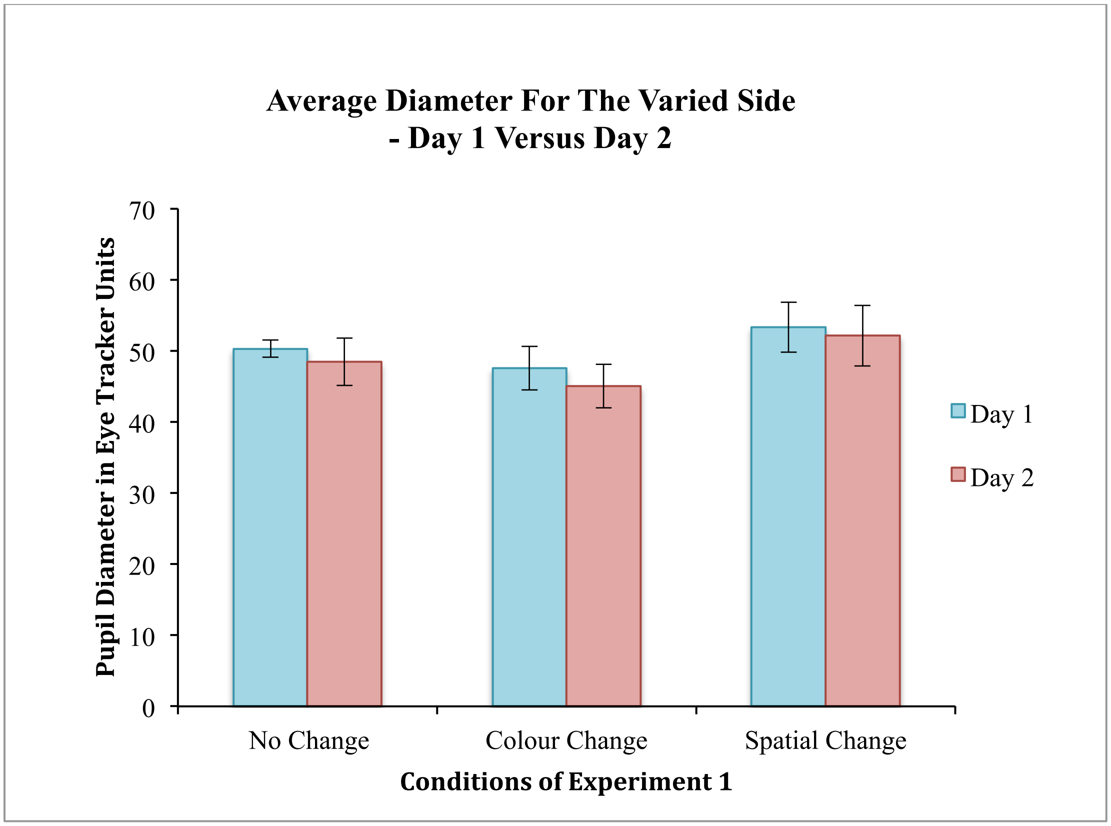

Figure 12. Mean pupil diameter of infants in the conditions of Experiment 1 on Day 1 versus Day 2, for varied colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

Discussion

This experiment aimed to investigate the relation between long-term memory and expectations in infancy. By adding a delay between the training and test phase of the VExP (Haith et al., 1988; Wenworth & Haith, 1992, 1998), this experiment investigated the theoretical relation between long-term memory and expectations in infants. Furthermore, by training infants with a particular stimulus sequence and subsequently testing them after a delay with a sequence in which either the content or spatial information has changed, the relative strengths and depth of encoding of the original perceptual information in long-term memory was explored. As a result, this study was able to assess the validity of whether anticipatory behaviours are sensitive to and guided by different event information.

Results of this experiment indicated, in line with the findings of previous studies using the VExP (e.g., Adler & Haith, 2003; Wentworth & Haith, 1992), that infants did form an expectation for the predictable event content and preferentially anticipated those events. That is, that infants made a greater percentage of anticipations to the invariant color side relative to the equally spatially predictable varied color side, suggests that the infants encoded the invariant event content and formed an expectation representation for that content. The anticipations were thus based on the expected content and guided by the formed expectation for that content.

When infants were exposed to the same sequence of events after a 24-hour delay (no-change condition), they continued to exhibit a greater percentage of anticipations to the invariant side. This suggested that the information to which they were initially exposed to was retained even after 24 hours and that the encoded information was guiding their behaviour on the second day. Moreover, infants exhibited the same pattern of content expectancy behavior to the invariant and varied sides, and did not require new learning immediately after the delay, as they did immediately before the delay (i.e. savings), when the encoding was recent and the stored representation was strongest. Together, these findings would seem to indicate that infants encoded in long-term memory the information that defined the experienced sequence of events, thereby forming an expectation for the events with predictable content. Subsequently, the information in long-term memory is retrieved to facilitate anticipatory behavior to the same events after a delay.

The second aim of this experiment was to assess the possible differences in the processing and encoding of content versus spatial information. If there were differences, one way this differential memorability might be manifested is by differential discriminability of changes in content and spatial event parameters after a retention delay. That is, if infants encode and remember content information better than spatial information then a change in content after a delay should disrupt their anticipatory behavior, whereas a change in the spatial aspect of the events after a delay should not. After the 24-hour retention delay, it was found that infants equally remembered the colour content and the spatial information, and this was evidenced by the decrease in their anticipations to the invariant side in both conditions. Had the spatial information not been as memorable as the colour combination information, the infants exposed to a change in the invariant events' spatial information would not have exhibited a disruption in their percentage of anticipations after the delay. Furthermore, in comparison to performance before the delay, infants subjected to both changes exhibited significantly less anticipations to the invariant side immediately after the retention delay. This disruption in infants' anticipatory behavior to the invariant sides after the retention delay by both color content and spatial changes suggests that infants encoded in memory not only the content information, as predicted, but also the spatial information.

Interestingly, for the varied side, the infants' anticipatory behavior was not disrupted with the retention delay, even when the spatial location of the varied events was changed. This finding was likely because the events on the varied side were constantly changing in content so there was no predictable information to store in memory and, consequently, nothing by which to discriminate after the retention delay. When the spatial location of the varied side changed, infants likely did not detect the change, even if this spatial information had been stored in memory. That is, because the varied side before the delay became the invariant side after the delay and vice versa, there was always a basis, whether from memory or currently available after the delay, of invariant information upon which to demonstrate anticipatory behavior.

Finally, this experiment also assessed any differences in infants' pupil diameters while they made anticipations. For all conditions in this experiment, infants exhibited smaller pupil diameters on the second encounter with the stimuli in comparison to their first encounter, during anticipations to both the invariant and varied sides. However, these differences between the two days were not significant. Nevertheless, although these results were not statistically significant, there still seems to be a definite trend wherein, on their second encounter with VExP, all infants in the conditions in this experiment exhibited pupil diameter constriction. More on this will be described in the general discussion section.

Experiment 2: Effects of Temporal Decay

Previous infants studies have suggested that temporal decay significantly affects the ability of infants to encode information into working memory. For example, Gilmore and

Johnson (1995) demonstrated that during an Oculomotor Delayed Response (ODR) task, 6-month-olds could only maintain information in working memory for approximately 3 seconds in order to correctly make delayed eye movements to cued locations. Three seconds and longer resulted in infants' delayed eye movements correctly localizing the cued target only by chance. Moreover, models of memory indicate that event information is first encoded in working memory before being transferred to long-term memory (Atkinson & Shiffrin, 1968; Bahrick, Hall, & Berger, 1996; Shallice & Warrington, 1970). If information is not encoded in working memory then it will not be available to be transferred to long-term memory.

In this experiment, to investigate the role of working memory and the effect of temporal decay that might limit the encoding of event information in working memory and, consequently, availability in long-term memory, we tested infants with an ISI delay of 3000 msec instead of 1000 msec. An ISI of 3000 msec was used because, based on the findings of Gilmore and Johnson (1995), this was a delay that led to temporal decay in 6-month-olds and would likely lead to temporal decay in the younger 3-month-olds used in the present study. Essentially, if the 3000 msec ISI produces temporal decay so that the information that defines the events and the event sequence are not encoded in working memory, then infants' ability to form expectations would be compromised and the information would not be available for transfer to long-term memory.

Methods

Participants

Data were collected from 15 3-month-old infants (8 males and 7 females), ranging in age from 82 days to 113 days (*M* = 98.6 days, *SD* = 7.16). All three conditions of the experiment included 5 infant participants. Infants in the sample were Caucasian (*n* = 11), Asian (*n* = 2), and African (*n* = 2), 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 14 infants participated, but insufficient data were collected from them because they cried (*n* = 6), were inattentive (i.e., disinterested, or looked at their hands or other parts of the visual field; *n* = 6), or because parents and infants did not come back on Day 2 (n = 1), or due to experimenter error (*n* = 1). The high dropout rate in this study reflected a strict criterion for inclusion of an infants' data in the analyses. However, again, the dropout rates in this experiment are well in line with the dropout rates reported in other visual expectation studies (e.g., Adler & Haith, 2003; Haith et al., 1988; Haith & McCarty, 1990; Wentworth & Haith, 1992). Participant recruitment for this experiment was exactly the same as Experiment 1.

Stimuli and Apparatus

The stimuli for this experiment were identical to those of Experiment 1 and were similarly viewed by infants on a LCD colour. The LCD monitor was mounted above a specialized crib in which the infants laid during their participation. Again similar to Experiment 1, the stimuli were 4.5° squared in size and appeared at 5.7° to the left or right of the infant's visual center.

During the experimental session, infants were placed in the same specialized crib as in Experiment 1. The equipment set-up and data collection were also the same as outline for Experiment 1 above.

Procedure

The procedure for Experiment 2 was exactly the same as the procedure of Experiment 1. On the first day of participation, families were provided with a quick overview of the procedure of the experiment that their baby would to be participating in, shown the experimental setup and given an informed consent form to fill out (See Appendix A for consent form).

The infant was placed in the specialized crib and, if available, a pacifier was offered to them as the eye tracker was focused to the infant's eye by the use of a remote control. Before the start of the experimental trials, the eye tracker was calibrated by recording the infant's fixations to known locations on the monitor at which a stimulus were presented. All subsequent eye data were filtered through these calibration values. This procedure was repeated at the outset of the second visit to account for any changes in that infant's head position relative to the monitor and eye tracker.

The procedure for stimuli presentation in each condition of Experiment 2 was identical to that used in Experiment 1, except for one difference, namely, that in these conditions the ISI was 3000 msec rather than 1000 msec. Experiment 2 also featured a no-change, colour-change and spatial change condition.

Data reduction

Similar to Experiement 1, the raw digital data recorded by the eye tracker was imported into a MATLAB toolbox called ILAB for analysis (Gitelman, 2002). With the use of ILAB, a scorer identified which of infants' eye movements were anticipatory or reactive in timing (Haith et al., 1988). 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 will judge the infant as having made a valid eye movement. Inclusion criteria for eye movements were the same as that of Experiment 1.

Our primary measure of memory recognition for expectation information in this experiment was also a savings measure. The method for calculating this measure was the same as the one found in Experiment 1. The process used to study changes in pupil diameter from Day 1 to Day 2 across all conditions were analyzed were also analogous to that of Experiment 1.

Finally, all results will be analyzed with valid statistical methodology using the statistical software program SPSS, with all tests conducted at an alpha level of $p < .05$.

Results

Preliminary Analyses

Since the invariant colour content for this experiment was also drawn from four possible combinations, and it has been previously shown that 3-month-olds have differential sensitivity to various colours (Adams, Maurer & Davis, 1986; Peeples & Teller, 1975; Teller, Peeples & Sekel, 1978), similar to Experiment 1, it was first necessary to determine whether each of the colour combinations yielded equivalent expectancy performance across infants. For these analyses, Day 1 data from all infants examined. Again, day 2 was not included since the infants viewed different stimuli with distinctive parameters across the different conditions and this could have additionally affected their anticipatory behaviour. The Levene's test for equality of variance indicated unequal variances in the anticipation data for the varied side ($F = 4.40$, $p = .029$). As such, non-parametric tests for independent samples were conducted on the percent anticipations across each possible invariant colour combination (red/green, red/blue, yellow/green and blue/yellow).

Two separate Krushall-Wallis tests were conducted for each content side (invariant and varied) for the no-change, colour-change and spatial-change conditions. Results indicated that there was no significant effect of colour combination on the median anticipations to the invariant side, $\chi^2(3, N = 15) = 3.77$, $p = .288$, or to the varied side, $\chi^2(3, N = 15) = 1.52$, $p = .679$. These preliminary analyses indicate that the specific colour combination that was featured on the invariant side did not have an effect on the infants' anticipatory performance. As such, for subsequent analyses, data were collapsed across infants who viewed different colour combinations as the invariant content.

Anticipations

Expectation Formation on Day 1. Similar to Experiment 1, before conducting analyses on memory performance on Day 2, it was essential to first assess the percent anticipations on Day 1, in order to see if the infants' anticipatory behaviours for this study were comparable to the findings of other studies of expectations for event content. Since the previous content expectation studies have demonstrated that infants form expectations for the stimuli as evidenced by a greater proportion of anticipations to the invariant side (Adler & Haith, 2003; Wentworth & Haith, 1992), it was important to first assess if this effect was also found in this study, on Day 1. A 3x2 split-plot analysis of variance was conducted comparing infants' anticipatory behaviour across the three experimental conditions (between-subjects variable – no-change, colour-change and spatial-change conditions) for the invariant color combination side and the varied color combination side (within-subjects variable). There was no significant main effect of color content side, F (1, 12) = 2.64, $p > .05$, indicating infants did not make more anticipations to the invariant color combination side (M = 84.53%, SD = 4.84) across all conditions than to the varied side (M = 77.75%, SD = 6.71). No significant interaction effect between color side and condition was found, F (2, 12) = .618, $p > .05$. However, a greater percentage of anticipations were made to the invariant side in comparison to the varied side (see Figure 13). One possible explanation is that the longer 3000 msec ISI results in a delay that is too long for the infants to keep the expectation information online in working memory before viewing additional events throughout the task (Gilmore & Johnson, 1995). As a result, the information decayed and they were unable detect the invariance of the content information and thereby form an expectation for the predictable content events. Alternatively, the small sample size of the study may have limited our power. Nonetheless, if infants did fail to encode the event information due to the information decaying from working memory, then it would not have been transferred to long-term memory (Atkinson & Shiffrin, 1968; Bahrick, Hall, & Berger, 1996; Shallice & Warrington, 1970 ; Ranganath, Johnson, D'Esposito, 2003; Axmacher, Schmitz, Weinreich, Elger & Fell, 2008). As a consequence, manipulation of event parameters on Day 2 should not disrupt these infants' performance, as they likely have no event information in long-term memory to which to compare current event information.

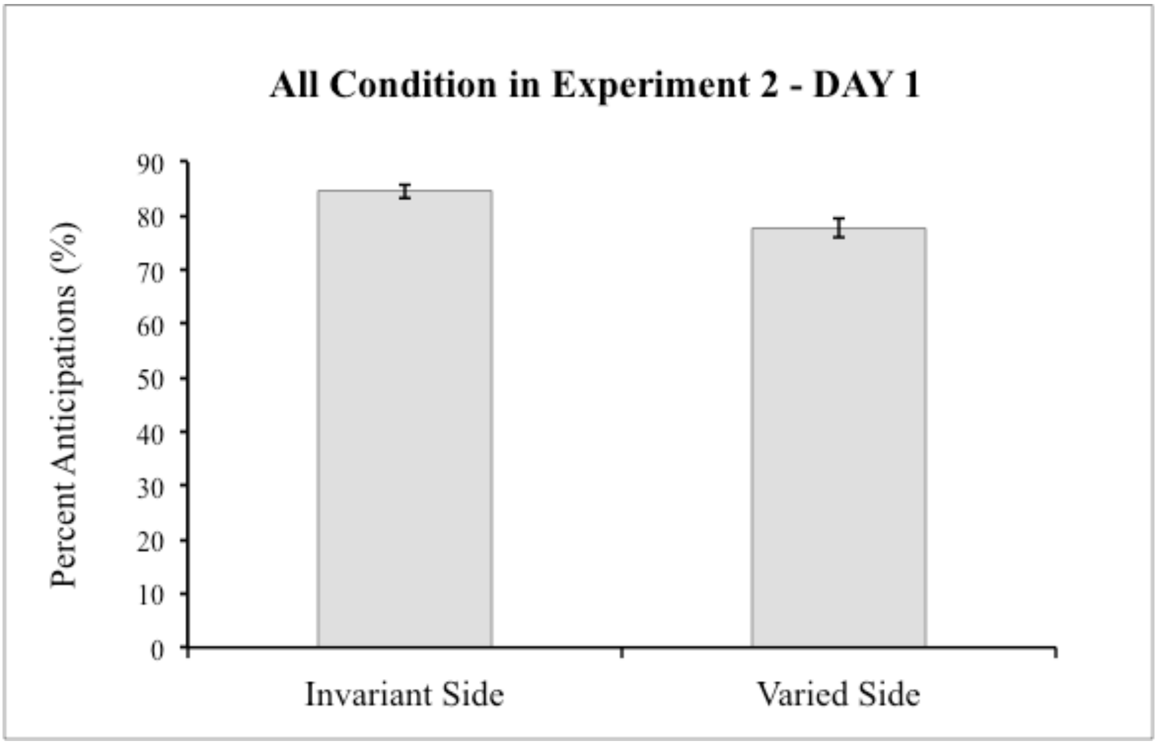

Figure 13. Mean proportion of anticipations made by infants to the invariant and varied colour combination side for all conditions of Experiment 2. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

No-Change Condition. In order to investigate the infants' anticipatory behaviour in the no-change condition, the percentage of the infants' anticipations on Day 2 were examined and compared to the proportions of Day 1. Upon assessing of the data, it appears that similar to Day 1, infants did not make more anticipations to the invariant side in comparison to the varied side (See Figure 14). This suggests that for the infants, the information they were exposed to on Day 1 was not as strongly retained as in the no-change condition, and this is likely because it was not as strongly formed on Day 1 (see Figure 2 in Anticipations--Expectation Formation on Day 1). As such, there was no expectation formation guiding the infants' behaviour of Day 2 in this no-change condition. It seems infants failed to encode the event information due to the information decaying from working memory, resulting in no information transfer to long-term memory. A related-samples t-test was also conducted and confirmed that the difference between the proportion of anticipations to the varied side was in fact not significantly different to the proportions for the invariant side, t (4) = 1.20, *p* = .295.

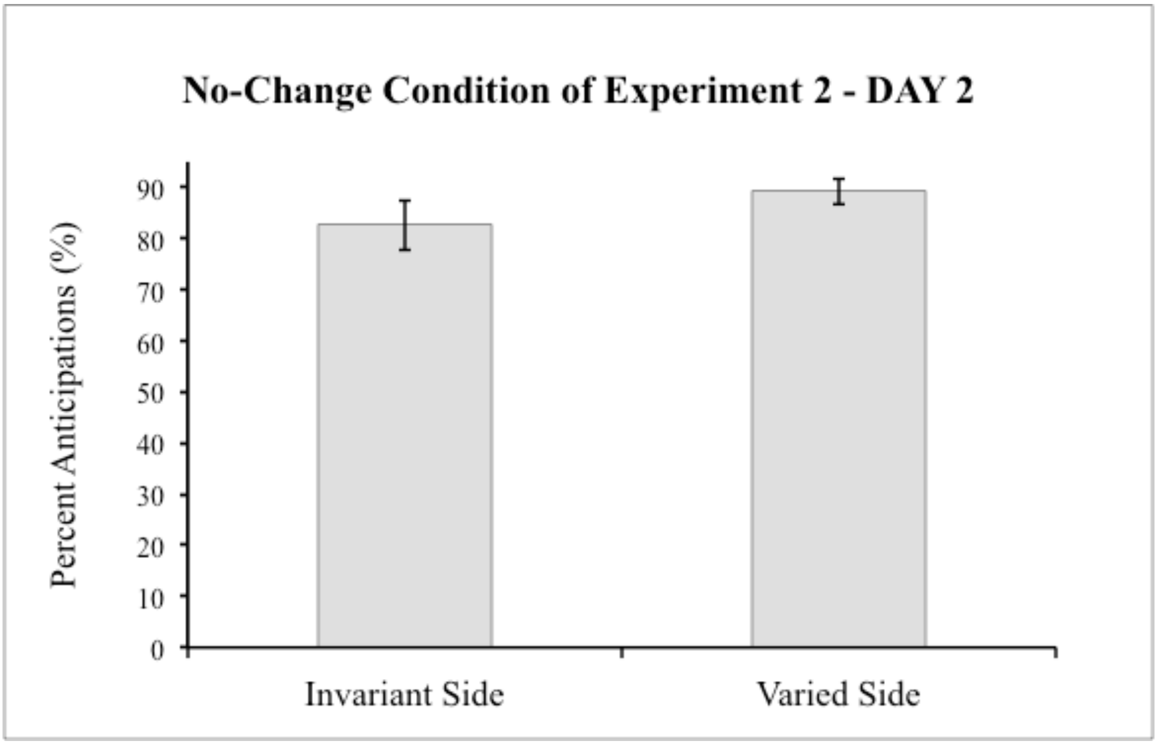

Figure 14. Mean percent of anticipations made by infants to the invariant and varied colour combination side in the no-change condition of Experiment 2. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

Similar to Experiment 1, in the interest of further investigating the infants' memory of expectations for the stimuli on Day 1, the mean savings measures in the no-change condition was also computed. Each infants' savings measure are provided in table 4 found in Appendix B. Again, the savings measure provided a relative measure that indicated the degree of an infant's long-term retention compared to that infant's immediate retention or learning on Day 1. The resulting mean savings measure violated the assumptions of parametric statistical tests. Results of the Shapiro-Wilk test indicated that the savings measure of the infants in the no-change condition deviated significantly from a normal distribution for the varied side, $S\text{-}W = .710$, $df = 5$, $p < .05$. The Levene's test for equality of variance indicated unequal variances in the savings measures data for the invariant side ($F = 5.911$, $p < .05$), and the varied side ($F = 6.568$, $p < .05$). Consequently, a non-parametric test for independent samples, the Mann-Whitney U test, was conducted to assess if the mean savings measure of the infants in this condition were significantly different than the theoretical ratio memory retention ratio of 1.00. Results revealed that the infants did not make a significantly different percentage of anticipations during the first 30 trials of Day 2 relative to the last 30 trials of Day 1 on the invariant side, $U = 5$, $p > .05$, *n.s,* or on the varied side $U = 5$, $p > .05$, *n.s* (See Figure 15). These results indicate that the infants exhibited similar anticipatory behaviour for the stimulus on Day 2 for both the invariant and varied side.

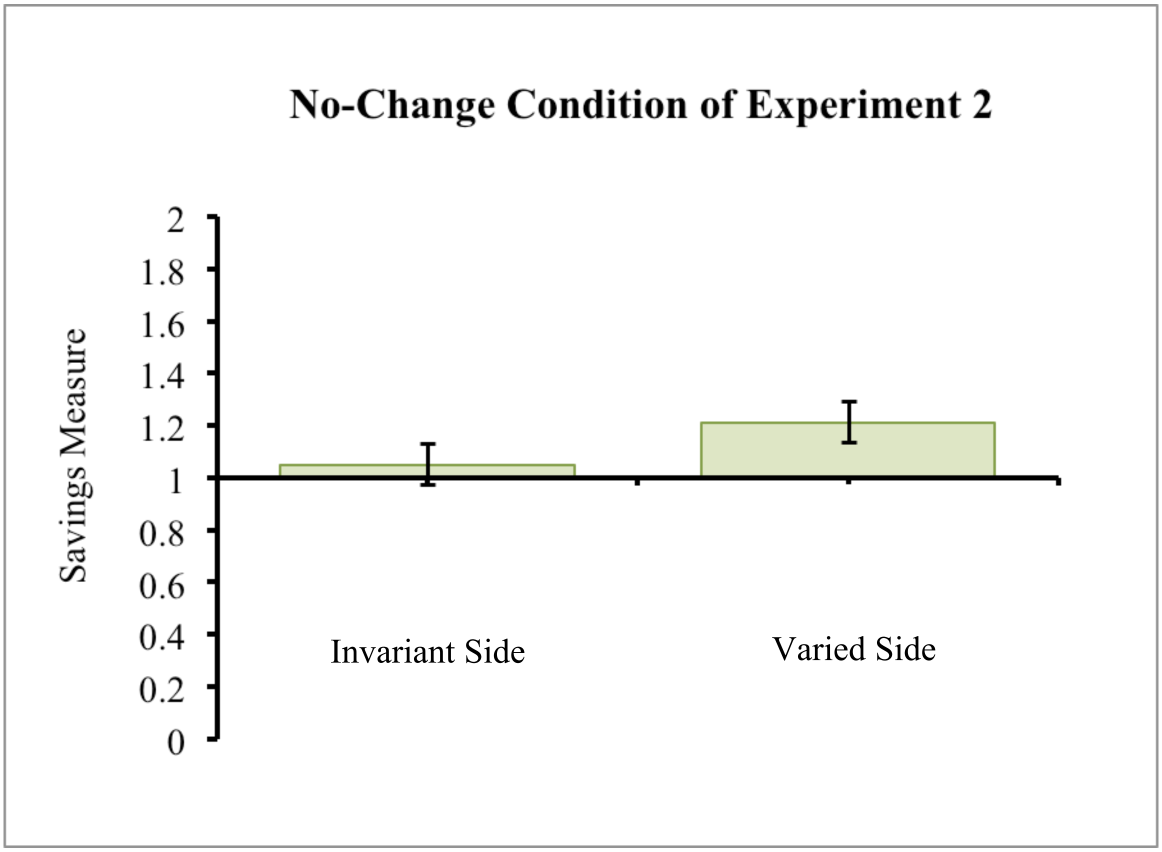

Figure 15. Mean savings measure of infants in the no-change condition of Experiment 2 for the varied and invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference from the theoretical memory retention measure of 1.00 at an alpha level of .05.

Colour-Change Condition. For the colour-change condition, the proportions of the infants' anticipations on Day 2 were also first examined and it was found that similar to

Day 1, infants did not make more anticipations to the invariant side in comparison to the varied side (See Figure 16). Although there seems to be a slightly greater proportion of anticipations made to the invariant side, a related-samples t-test confirmed that the difference between the proportion of anticipations to the varied side is not significantly different to the proportions for the invariant side, t (4) = -1.45, *p* > .05, *n.s*. This suggests that for the infants, the expectation information they were exposed to on Day 1 was not as strongly retained as in the colour-change condition in Experiment 1, likely because it was not strongly formed on Day 1 (see Figure 12 in Anticipations and Expectation Formation on Day 1). Consequently, there was no expectation formation guiding the infants' behaviour of Day 2 in this Experiment. Unlike the infants in the colour-change condition in Experiment 1, who recognized the difference between the stimuli they were exposed to on Day 2 in comparison to Day 1 (the constant colour combination of the invariant stimuli) and decreased their anticipations to the invariant side because of the difference, the infants in colour-change condition in this experiment did not.

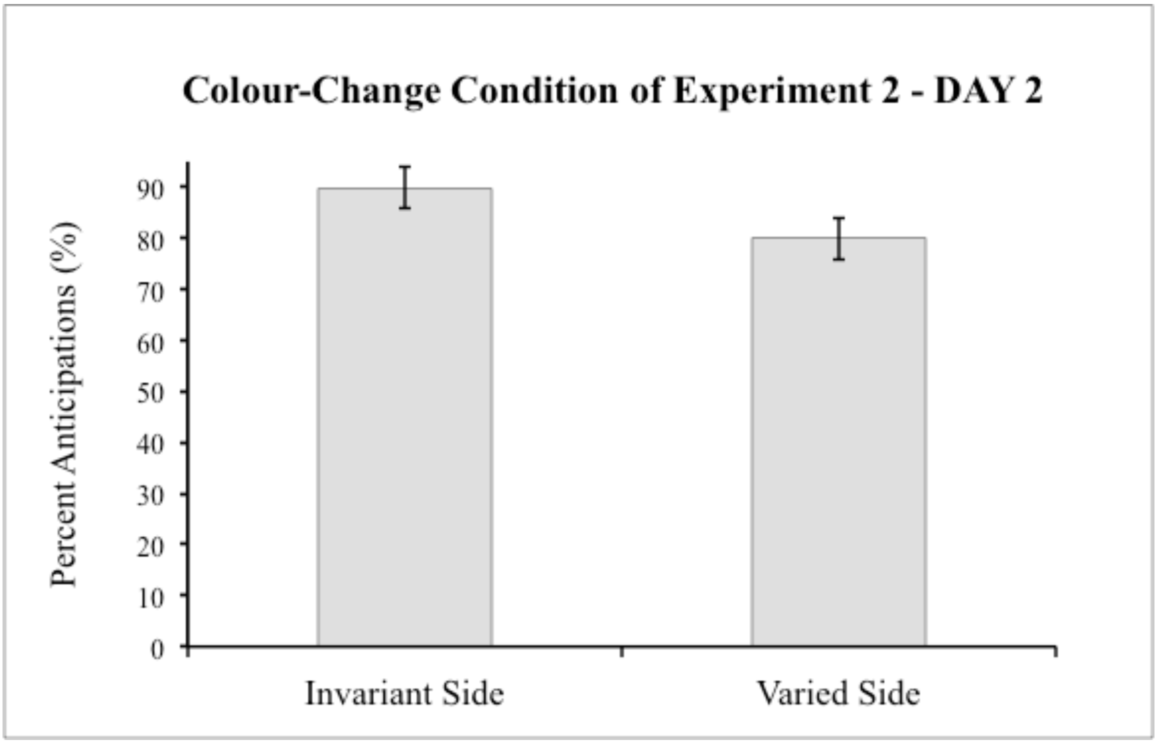

Figure 16. Mean percent of anticipations made by infants to the invariant and varied colour combination side in the colour-change condition of Experiment 2. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

Again, in order to further investigate the infants' memory of expectations for the stimuli on Day 1, the mean savings measure for all five infants in the colour-change condition were also computed. Each infants' savings measure are provided in table 5 in Appendix B. The resulting mean saving measures violated the assumptions of parametric statistical tests. Results of the Shapiro-Wilk test indicated that the savings measure of the infants in the colour-change condition deviated significantly from a normal distribution for the invariant side, S-W = .826, df = 5, *p* = .030, and for the varied side, S-W = .782, df = 5, *p* = .009. The Levene's test for equality of variance indicated unequal variances in the savings measures data for the invariant side (F = 11.12, p = .010), and for the varied side (F = 17.58, p = .003) Therefore, a non-parametric test for independent samples, the Mann-Whitney U test, was conducted to assess if the mean savings measure of the infants in this condition were significantly different than the theoretical ratio of 1.00, which indicates memory retention. Results revealed that the infants did not make a significantly different percentage of anticipations during the first 30 trials of Day 2 relative to the last 30 trials of Day 1 on the invariant side *U* = 12.50, *p* > .05, *n.s*, or on the varied side *U* = 10, *p* = .05, *n.s* (See Figure 17).

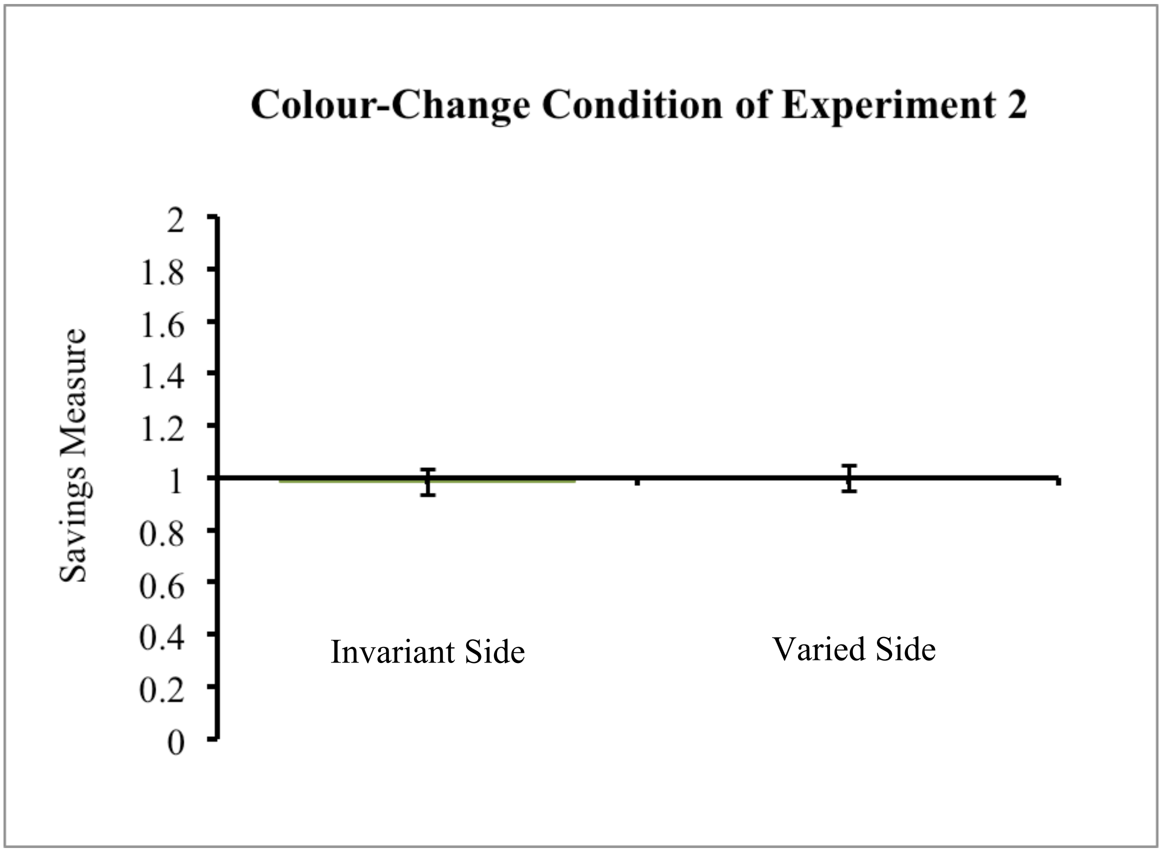

Figure 17. Mean savings measure of infants in the colour-change condition of Experiment 2 for the varied and invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference from the theoretical memory retention measure of 1.00 at an alpha level of .05.

These results indicate that the infants exhibited similar anticipatory behaviour for the stimulus on Day 2 for both invariant and varied side. Much like the anticipatory behaviour results above, these findings suggests that for the infants, the expectation information they were exposed to on Day 1 was not as strongly remembered as for the infants in the colour-change condition in Experiment 1. It seems that the longer 3000 msec ISI results in a delay that is too long for the infants to keep the expectation information online in working memory before viewing additional events throughout the task (Gilmore & Johnson, 1995). As a result, the information decayed and they were unable detect the invariance of the content information and thereby form an expectation for the predictable content events. As a consequence, manipulation of colour content parameters on Day 2 did not disrupt these infants' performance, as they likely had no event information in long-term memory to which to compare current event information.

Spatial-Change Condition. For the spatial-change condition, the proportions of the infants' anticipations on Day 2 were also first examined and it was found that similar to Day 1, infants did not make more anticipations to the invariant side in comparison to the varied side (See Figure 18). Although there seems to be a slightly greater proportion of anticipations made to the invariant side, a related-samples t-test confirmed that the difference between the proportion of anticipations to the varied side is not significantly different to the proportions for the invariant side, t (4) = -1.104, *p* = .331. This suggests that for the infants, the expectation information they were exposed to on Day 1 was not as strongly retained as in the spatial-change condition in Experiment 1, likely because it was not strongly formed on Day 1 (see Figure 2 in Anticipations and Expectation Formation on Day 1). Consequently, there was no expectation formation guiding the infants' behaviour of Day 2 in this Experiment. Unlike the infants in the spatial-change condition in Experiment 1, who recognized the difference between the stimuli they were exposed to on Day 2 in comparison to Day 1 (the constant spatial location of the invariant stimuli) and decreased their anticipations to the invariant side because of the difference, the infants in the spatial-change condition in this experiment did not.

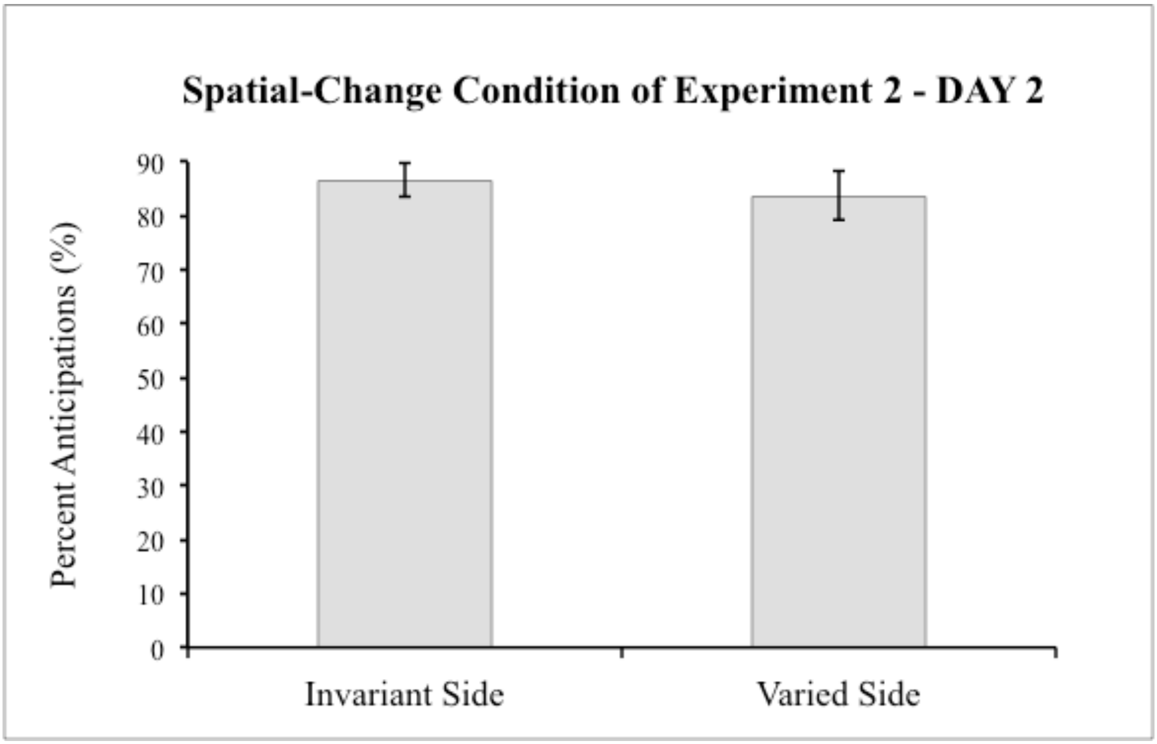

Figure 18. Mean percent of anticipations made by infants to the invariant and varied colour combination side in the spatial-change condition of Experiment 2. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

Again, in order to further investigate the infants' memory of expectations for the stimuli on Day 1, the mean savings measure for all five infants in the spatial-change condition were also computed. Each infants' savings measure are provided in table 6 in Appendix B. The resulting mean saving measures violated the assumptions of parametric statistical tests. Results of the Shapiro-Wilk test indicated that the savings measure of the infants in the spatial-change condition deviated significantly from a normal distribution for the invariant side, S-W = .826, df = 5, $p = .030$, and for the varied, S-W = .782, df = 5, $p = .009$. The Levene's test for equality of variance indicated unequal variances in the savings measures data for the invariant side (F = 11.12, p = .010) and for the varied side (F = 17.58, p = .003). Consequently, a non-parametric test for independent samples, the Mann-Whitney U test, was conducted to assess if the mean savings measure of the infants in this condition were significantly different than the theoretical ratio of 1.00, which indicates memory retention. Results revealed that the infants did not make a significantly different percentage of anticipations during the first 30 trials of Day 2 relative to the last 30 trials of Day 1 on the invariant side invariant side $U = 12.50$, $p > .05$, *n.s*, or on the varied side $U = 10$, $p = .05$, *n.s* (See Figure 19).

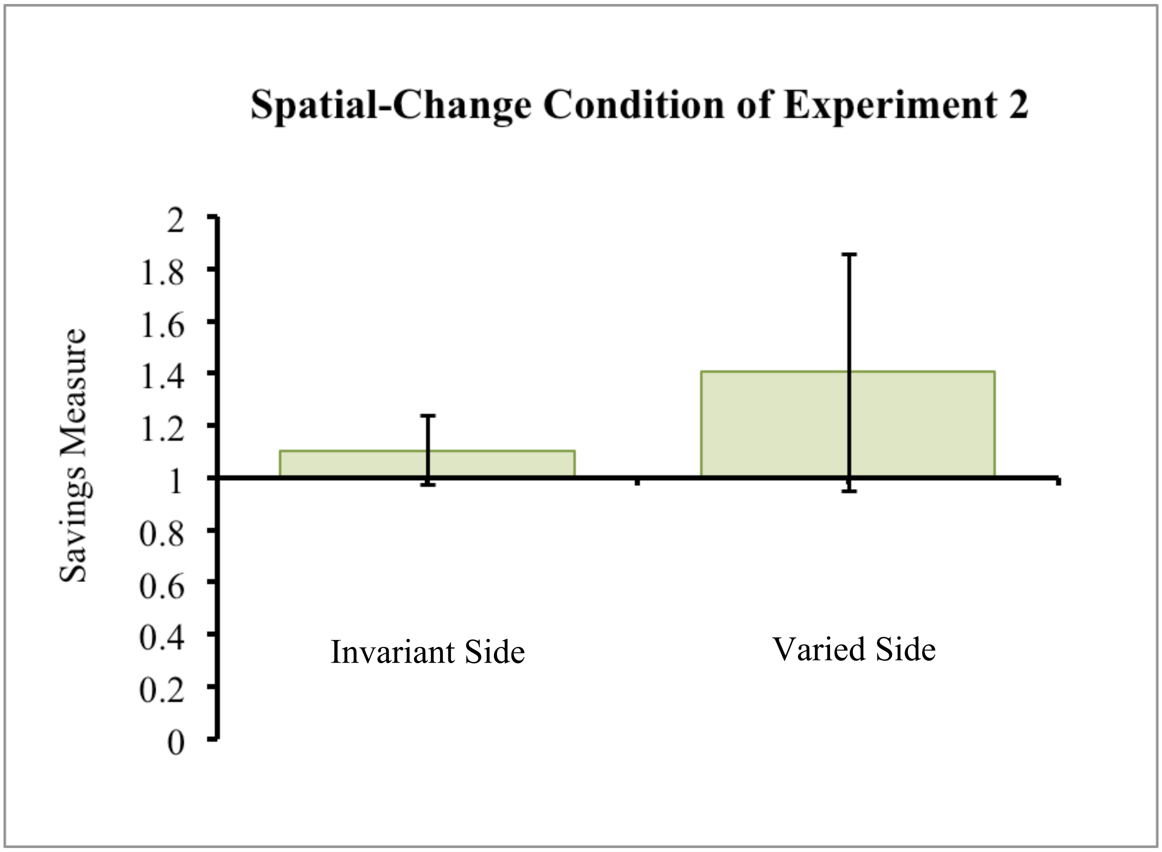

Figure 19. Mean savings measure of infants in the spatial-change condition of Experiment 2 for the varied and invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference from the theoretical memory retention measure of 1.00 at an alpha level of .05.

These results indicate that the infants exhibited similar anticipatory behaviour for the stimulus on Day 2 for both invariant and varied side. Much like the anticipatory behaviour results above, these findings suggests that for the infants, the expectation information they were exposed to on Day 1 was not as strongly remembered as for the infants in the spatial-change condition in Experiment 1. It seems that the longer 3000 msec ISI results in a delay that is too long for the infants to keep the expectation information online in working memory before viewing additional events throughout the task (Gilmore & Johnson, 1995). As a result, the information decayed and they were unable detect the invariance of the spatial location information and thereby form an expectation for the predictable content events. As a consequence, manipulation of spatial location parameters on Day 2 did not disrupt these infants' performance, as they likely had no event information in long-term memory to which to compare current event information.

Pupil Diameter Changes

Comparing Day 1 to Day 2. In comparison to Experiment 1, for all conditions in this experiment, infants exhibited larger pupil diameters on Day 2 in comparison to Day 1 for anticipations to both the invariant and varied sides (see Figures 20 and 21). Separate independent samples t-tests were conducted for each condition and each side comparing the pupil diameter at Day 1 and Day 2 for the infants in all conditions of this experiment.

Results revealed that although on Day 2, relative to Day 1, the infants' mean pupil diameter was larger for the invariant side, these differences were not found to be significant for neither the no-change condition ($t(4) = -1.206, p > .05$), the colour-change condition ($t(4) = -2.496, p > .05$) and the spatial-change condition ($t(4) = -0.509, p > .05$). On the varied side, infants' mean pupil diameter was also larger on Day 2, relative to Day 1. However again, these differences were not found to be significant for the no-change condition ($t(4) = -0.504, p > .05$), colour-change ($t(4) = -1.002, p >.05$) and the spatial-change condition ($t(4) = -0.509, p = .638$).

Although these results are not statistically significant, there still seems to be a definite trend wherein, in contrast to Experiment 1, on Day 2 all infants in this experiment exhibited pupil diameter dilation. These results also fit well with our previously mentioned eye movement VExP results (comparison of percent anticipations on Day 1 and Day 2 and savings measure). More specifically, when information is more memorable leading to discrimination of changes in event information, the mean pupil diameter was smaller on Day 2 than when viewing changes in event information that are not as memorable. The infants in this experiment did not remember the information from Day 1 whereas as the infants in Experiment 1 did.

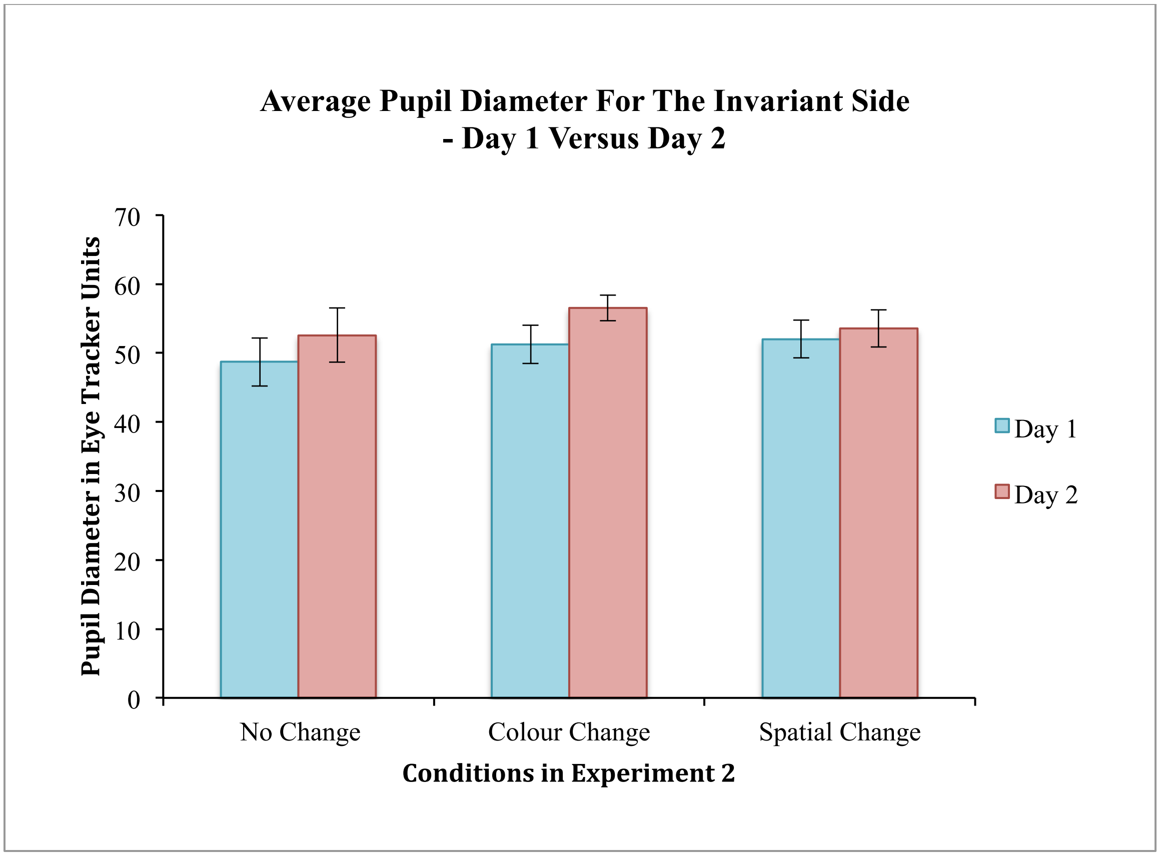

Figure 20. Mean pupil diameter of infants in the conditions of Experiment 2 on Day 1 versus Day 2, for invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

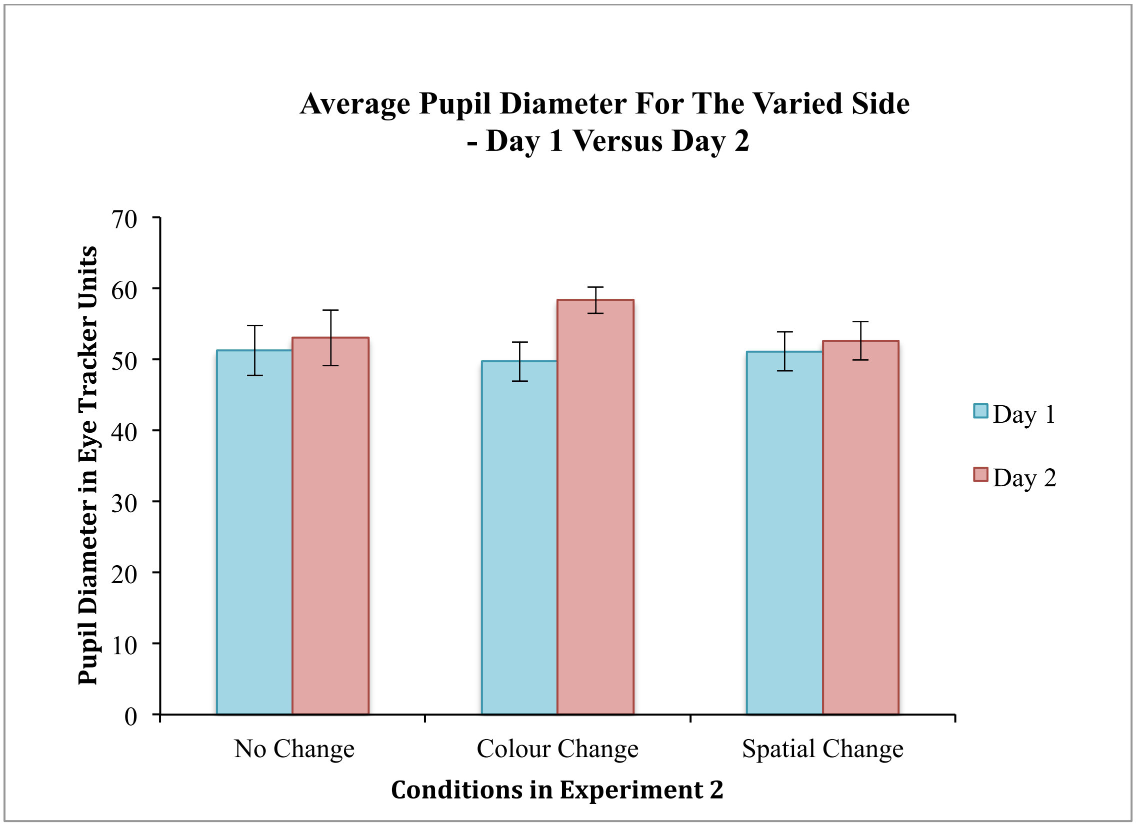

Figure 21. Mean pupil diameter of infants in the conditions of Experiment 2 on Day 1 versus Day 2, for varied colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference at an alpha level of .05.

Comparing Pupil Diameter Changes in Experiment 1 and Experiment 2

Considering the differences in the formation of expectations, as well as the memorability for the information encoded during expectation formation, across the conditions Experiment 1 in comparison to Experiment 2, whether these differences are exhibited in the pupil data was investigated. If there are differences in the memory for these conditions and pupil diameter changes are indexing these memorability differences, then one would hypothesize that pupil diameter would exhibit a similar pattern of differences across the conditions. In order to do this, the mean pupil diameter of all infants in Experiment 1 was compared to the mean pupil diameter of all infants in the Experiment 2 on both Day 1 and Day 2. Separate independent samples t-tests were conducted to assess possible differences between the mean pupil diameters of infants in Experiment 1 as compared to infants in Experiment 2 for the invariant and varied sides.

Results indicated that on Day 1, there was no significant difference between the mean pupil diameter of infants in the Experiment 1 and Experiment 2 for both the invariant, $t(28) = 0.119$, $p > .05$, n.s., and varied sides, $t(28) = -0.122$, $p > .05$, n.s. (see Figure 22). In contrast, on Day 2, infants in Experiment 2 had significantly larger pupil diameters than infants in Experiment 1 for both the invariant, $t(28) = -2.445$, $p < .025$ aand varied sides, $t(28) = -2.269$, $p < .05$ (see Figure 23).

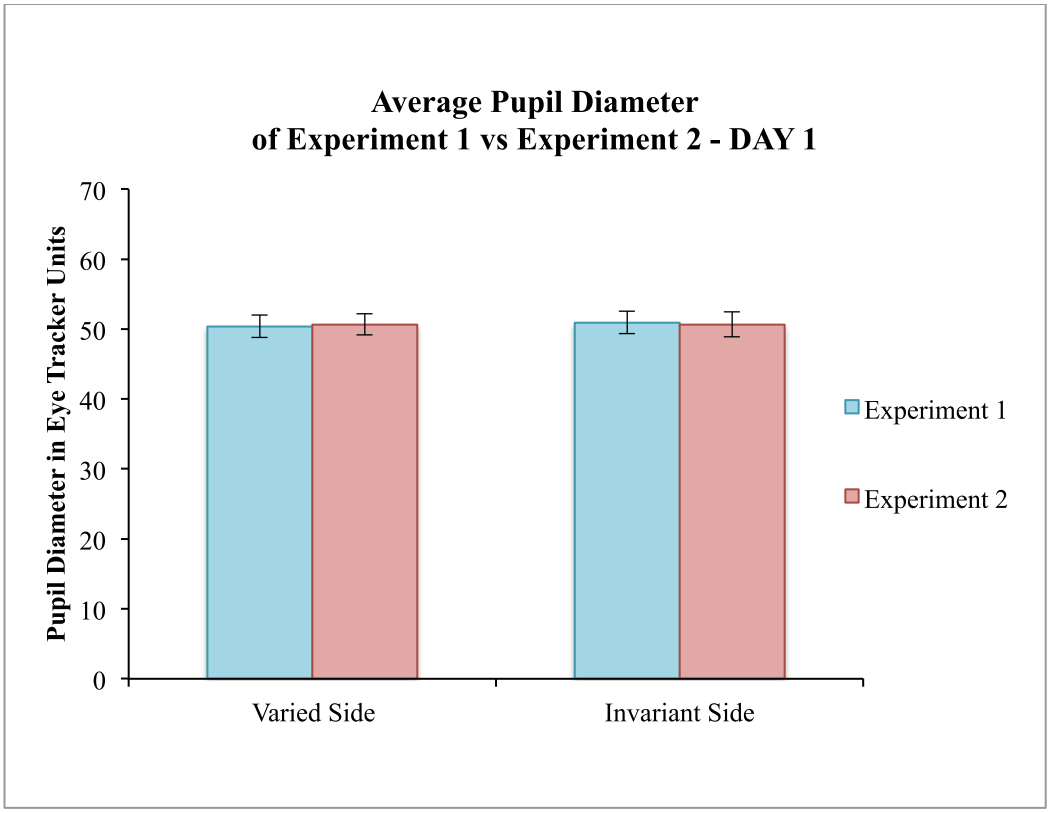

Figure 22. Mean pupil diameter of infants in the conditions of Experiment 1 and Experiment 2 on Day 1, for the varied and invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference an alpha level of .05.

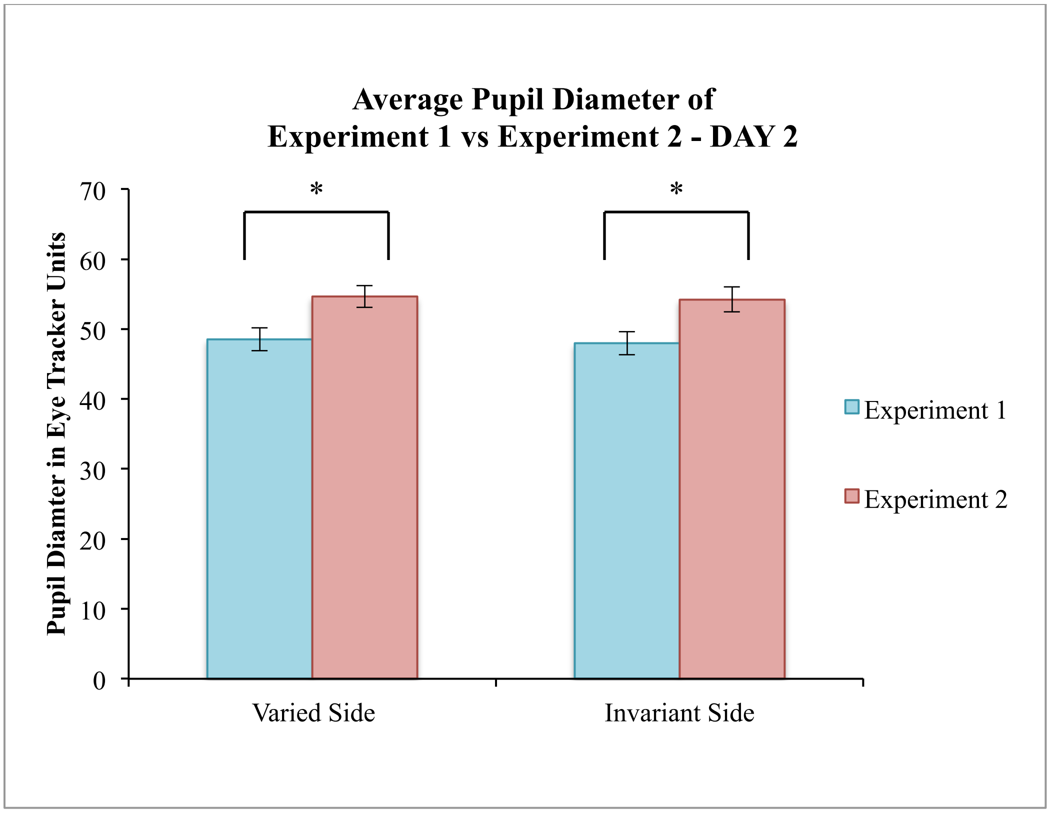

Figure 23. Mean pupil diameter of infants in the conditions of Experiment 1 and Experiment 2 on Day 2, for the varied and invariant colour combination side. Vertical error bars indicate +/- standard errors. * indicates significant difference an alpha level of .05.

These results are consistent with the comparison of percent anticipations on Day 1 and Day 2, as well as the savings measure. Here, the means of pupil diameters of infants in the two different sets of experiments exhibit the same pattern of differences. On Day 2, similar to percent anticipations and savings in which infants in Experiment 1 demonstrated retention of the stimuli on Day 1, infants in Experiment 1 also exhibited significantly smaller pupils than infants Experiment 2.

Discussion

In this experiment, the aim was to assess the effects of working memory limitations, namely, temporal decay, on long-term memory. Previous infants studies have suggested that temporal decay greatly affects the ability of infants to encode information into working memory (e.g. Gilmore &Johnson, 1995). In this experiment, to investigate the role of working memory and the process of temporal decay that might limit the encoding of event information in working memory and, consequently, availability in long-term memory, we tested infants with an ISI delay of 3000 msec, instead of 1000 msec.

Results of this experiment indicated that infants did not make significantly more anticipations to the invariant side relative to the varied side, as infants in experiment 1 did, even on Day 1. This finding revealed that an increase in the ISI from 1000 msec to 3000 msec likely produced temporal decay of the information that defined the experiment 1 infants' expectations with a 1000 msec ISI, and prevented the infants in this experiment from forming expectations for the predictable content on the invariant side. As a consequence of the temporal decay, infants in this experiment were unable to hold the relevant information (i.e. colors) online from one stimulus event to the next, thereby negating the likelihood of detecting the invariance of information in to-be-associated events (e.g. the stimuli on the invariant colour combination side).

If working memory and long-term memory are linked as proposed (Atkinson & Shiffrin, 1968; Bahrick, Hall, & Berger, 1996; Shallice & Warrington, 1970), then our findings, having demonstrated that temporal decay disrupts expectation formation, would suggest that infants' long-term memory for the information upon which the expectations should be based would likewise be disrupted. The findings of this experiment also supported this hypothesis and indicated just such an occurrence that infants who experienced the longer ISI showed no evidence that they had stored any information in long-term memory. That is, unlike the infants who experienced the 1000 msec ISI in experiment 1, infants who experienced the 3000 msec ISI in this experiment did not exhibit any difference in their percent anticipations to the invariant or varied sides as indexed overall or in their savings after the 24-hour retention delay. Further, neither did these infants exhibit a greater percentage of anticipations to the invariant side relative to the varied side after the retention delay. These findings demonstrate that the infants' memory for information experienced during expectation formation is contingent upon how well they are able to retain information in working memory thereby determining how strongly they initially formed expectations. Since the 3000 ms ISI produced temporal decay, the infants' working memory and expectation formation was affected. Consequently, the transfer of expectation formation information from working memory to long-term memory was also compromised in these infants.

With regard to the pupil diameter analysis, the results in this study were different from those found in Experiment 1. In contrast to Experiment 1, for all conditions in this experiment, infants exhibited larger pupil diameters on the second encounter with the stimuli in comparison to their first encounter, during anticipations to both the invariant and varied sides. However, statistical analyses revealed that these differences were not found to be significant. Nevertheless, although these results are not statistically significant, there still seems to be a definite trend wherein, in contrast to Experiment 1, on the second day, all infants in this experiment exhibited pupil diameter dilation. These results also fit well with our previously mentioned eye movement VExP results (comparison of percent anticipations on the first day and second day, and savings measure). More specifically, when information is more memorable leading to discrimination of changes in event information, the mean pupil diameter was smaller on second day than when viewing changes in event information that are not as memorable. Unlike the infants in Experiment 1, the infants in this experiment did not remember the information from the first encounter with the stimuli, and did not show this pupil diameter effect.

Considering the differences in the formation of expectations, as well as the memorability of this information across the conditions of Experiment 1 and Experiment 2, whether these differences are exhibited in the pupil data was also investigated. If there are differences in the memory for these conditions and pupil diameter changes are indexing these memorability differences, then one would hypothesize that pupil diameter would exhibit a similar pattern of differences across the conditions. In order to do this, the mean pupil diameter of all infants in Experiment 1 was compared to the mean pupil diameter of all infants in the Experiment 2 on both their first encounter with the stimuli and their second encounter.

Results indicated that during the first day, there was no significant difference between the mean pupil diameter of infants in the Experiment 1 and Experiment 2 for both the invariant and varied sides. However, on the second day, infants in Experiment 2 had significantly larger pupil diameters than infants in Experiment 1 for both the invariant and varied.

These results are consistent with the comparison of percent anticipations on first day and second day, as well as the savings measure. Here, the means of pupil diameters of infants in the two different sets of experiments exhibit the same pattern of differences. On the second day, similar to percent anticipations and savings in which infants in Experiment 1 demonstrated retention of the stimuli from the first day, infants in Experiment 1 also exhibited significantly smaller pupils than infants Experiment 2. More on this will be described in the general discussion below.

General Discussion

It is widely accepted by researchers, clinicians, applied professionals as well as policy makers, that the experiences that an individual encounters in the first 2 to 3 years of life has profound effects on their development and future behaviour. Yet in order for these experiences to hold such theorized long-term effects, it can be argued that it is necessary they be remembered and retrievable by the individual. Moreover, to understand how cognitive development affects behaviour in the relative immediate future, as well as later in development, the relation between infants' cognitive processes and future-oriented expectation to stimuli needs to be established.

Various studies have previously demonstrated that young infants can retain event information in memory over an extended period of time (e.g. Watson & Rayner's, 1920; Rovee & Fagen, 1976; Little, Lipstitt, & Rovee-Collier, 1984). Other studies have also 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, Arehart, Lanthier, & Haith, 2006; Wass, Lewis, & Haith, 1998) of future events. Theories have suggested that these abilities highlight our memory's function of providing a foundation upon which expectations for future events are formed (Adler et al., 2008, Adler & Haith, 2003; Haith et al., 1988). Another way to view this relation is that the function of long-term memories is not simply for the memories themselves but to inform decisions and behaviors to future events in the form of expectation representations (Grossberg, 1995; Rovee-Collier & Sullivan, 1980). The first aim of this study was to assess this hypothesis by investigating the relation between long-term memory and expectation formation in 3-month-old infants. In addition, the differences in processing and retention of content in comparison to spatial information, as well as working memory limitations such as temporal decay were investigated. Furthermore, since pupil diameter changes have also been shown to index long-term memory, this study also aimed to assess whether pupil diameter can be studied as an index of memory in infants.

Hence, the current study aimed to: 1) investigate the relation between long-term memory and expectations in infancy and memory, 2) assess the possible differences in the processing and encoding of content versus spatial information, 3) assess the effects of working memory limitations, namely temporal decay on long-term memory, 4) demonstrate the validity of the VExP as a paradigm to assess long-term memory, and 5) investigate the feasibility of using the measurement of pupil diameter changes as a mean to assess memory in infants. In order to clearly tackle these aims, this study was broken down into two experiments.

In Experiment 1, by adding a delay between the training and test phase of the VExP (Haith et al., 1988; Wenworth & Haith, 1992, 1998), this study investigated the theoretical relation between long-term memory and expectations in infants. Furthermore, by training infants with a particular stimulus sequence and subsequently testing them after a delay with a sequence in which either the content or spatial information has changed, the relative strengths and depth of encoding of the original perceptual information in long-term memory was explored. As a result, this study was able to assess the validity of whether anticipatory behaviours are sensitive to and guided by different event information. By also including the additional experiment, namely Experiment 2, where the temporal parameters of stimulus presentation were manipulated, the effect of temporal decay on memory was also investigated.

The Relation Between Memory and Expectations. Initial results of Experiment 1 indicated, in line with the findings of previous studies using the VExP (e.g., Adler & Haith, 2003; Wentworth & Haith, 1992), that infants did form an expectation for the predictable event content and preferentially anticipated those events. Specifically, that infants made a greater percentage of anticipations to the invariant color side relative to the equally spatially predictable varied color side, suggests that the infants encoded the invariant event content and formed an expectation representation for that content. The anticipations were thus based on the expected content and guided by the formed expectation for that content.

Infants' performance, however, could have been simply accounted for by the temporal predictability of the events, and not to the predictability of content or spatial information. Had this is been the case, however, infants would have exhibited an equivalent percentage of anticipations to both the invariant and varied sides when initially forming their expectation on the first day. Instead, our findings demonstrated that infants made more anticipations to the invariant side. Thus, this supports the notion that infants did encode the invariant event color content to form an expectation representation and that consequently, anticipations were based on the expected content.

If an integral characteristic of memory is the formation of expectations, as theorized (Adler et al., 2008, Adler & Haith, 2003; Haith et al., 1988), then we hypothesized that information that is encountered during the expectation formation would be remembered over the long term. Considering that our findings from Experiment 1 indicate that infants did form expectations for the events in the first place, the information encoded in that expectation representation should be stored in memory and available to guide behavior even after a significant delay. When infants were exposed to the same sequence of events after a 24-hour delay (no-change condition), they continued to exhibit a greater percentage of anticipations to the invariant side. This suggested that the information to which they were initially exposed to was retained even after 24 hours and that the encoded information was guiding their behaviour on the second day. Moreover, infants were able to exhibit the same pattern of content expectancy behavior to the invariant and varied sides, and did not require new learning immediately after the delay, as they did immediately before the delay (i.e. savings), when the encoding was recent and the stored representation was strongest. Together, these findings would seem to indicate that infants encoded in long-term memory the information that defined the experienced sequence of events, thereby forming an expectation for the events with predictable content. Subsequently, this information in long-term memory is retrieved to facilitate anticipatory behavior to the same events after a delay. Moreover, findings of the Experiment 1 demonstrated that the retention of information provided the basis for discriminating any changes in the stimuli. Infants in this experiment demonstrate this, by recognizing changes in the stimuli on the second day, as expanded on below.

Many studies featuring the VExP have previously provided evidence that infants form expectations for events that are not perceptually available and that rudimentary future-oriented processes are evident early in life (for a review, see Haith, 1994). Some of these VExP studies have also revealed that infants encode and form expectations for more complex, asymmetric (e.g., left–left–right) spatial sequences (Canfield & Haith, 1991), for the temporal parameters of visual events (Adler & Haith, 1998; Lanthier, Arehart, & Haith, 1993; Wass, Lewis, & Haith, 1998), as well as the content of events (Adler & Haith, 2003; Wentworth & Haith, 1992). This present study is unique as it extends our understanding of infants' expectations, as well as long-term memory, by demonstrating that not only can young 3-month-olds form expectations for events that are not perceptually available, but that they can also retain the information encoded during expectation formation over a 24-hour delay and use this information to guide their behaviour on a subsequent encounter with the task.

Adler and Haith (2003), as well as others (Adler et al., 2008; Grossberg, 1995;

Haith et al., 1988; Rovee-Collier & Sullivan, 1987), have previously suggested that expectations are linked to memory. They are linked, because a function of memory is to inform behavioral decisions to future events on the basis of stored representations of previously experienced events. As a consequence, upon expecting new events, information that is currently perceptually available is matched to information remembered about the past events. When this information matches, expectancy behavior is facilitated, as was the case when infants received the same event information after a 24-hour delay. When this information does not match, however, expectancy behavior is disrupted, as was the case when infants received and discriminated color and spatial event information that was different after the 24-hour delay. As a whole, these findings support the theory that the formation of expectations is an integral function of memory (Adler et al., 2008, Adler & Haith, 2003; Haith et al., 1988).

In addition to its relation to memory, the ability to form expectations is important in itself. Haith (1993) stressed this importance by suggesting that without such a capacity, infants would only be able to react to each event after its presentation and further explained that this would greatly impair efficient processing of that event and its features (Haith, 1993). Expectation formation allows infants to control their actions and behavioral responses, to increase the efficiency and effectiveness of information processing. In order for expectations to increase the efficacy of infants' information processing, infants must be able to distinguish between events that correspond with their expectation representation and events that do not. This ability is necessary to ensure that novel information from newly encountered events is correctly integrated into an existing cognitive construct or categorized into new cognitive structures. When infants are able to generalize across a set of different yet similar events, this increases the efficiency and flexibility of cognitive processing (Adler & Haith, 2003). An increase in infants' cognitive flexibility occurs because generalizing across a set of unique events that share some characteristic enables them to interact with new objects and no longer view these objects as being completely distinct from other similar events they have encountered. That infants were able to attend to and specifically encode the invariant color combination from a set of events that differed along other dimensions (e.g. pattern) which consequently guided their expectation formation and anticipatory behaviour, both immediately and after a 24-hour delay, supports the notion that expectations are an important part of cognitive efficiency and flexibility, even in infants.

Differences in Content Versus Spatial Information Processing. The second aim of this study was to assess the possible differences in the processing and encoding of content versus spatial information. This research question derived from the findings of Adler and Haith's (2003) study where infants, in forming expectations for related events that differ along some dimensions, encoded specific shared content or event features. In this same study, infants' anticipatory behaviour was affected more by the predictability of the color information than it was by the completely predictable spatial information (location at which shapes were appearing), in that infants exhibited more anticipations to color predictable events than to color unpredictable events (as they did in the current study as well). In contrast, infants' reactive behaviour (after picture onset) was unaffected by the color predictability of the pictures. Adler and Haith (2003) therefore hypothesized that these two measures are differentially sensitive to expected event content.

Adler and Haith (2003) suggested that reactive eye movements are only reactive to sensory input and, therefore, may be sensitive to the relatively primitive property of spatial location, and as a result be less sensitive to the content of events. As a result, this possibility could account for why reactive latencies did not differentiate performance, since spatial information did not vary between the two content sides (Adler & Haith, 2003). In contrast, anticipations occur prior to sensory input and involve top-down cognitive forecasting of events that are not perceptually available (Adler & Haith, 2003). Because the two content sides differed in their content predictability, Adler and Haith (2003) thus further suggested that differences in anticipations to the two sides may reflect the relative amount of cognitive processing of event's color (and pattern) content.

If Adler and Haith (2003) were correct and content information is processed at a higher cognitive level than spatial information, then in the current study content information would be more memorable than spatial information, as suggested by level-of-processing models of memory (Craik & Lockhart, 1972). One way this differential memorability might be manifested is by differential discriminability of changes in content and spatial event parameters after a retention delay. That is, if infants encode and remember content information better than spatial information then a change in content after a delay should disrupt their anticipatory behavior, whereas a change in the spatial aspect of the events after a delay should not.

In contrast to this hypothesis, infants in Experiment 1 detected when the events changed in both their colour content and their spatial parameters after the 24-hour retention delay. As a result, for both changes, infants no longer made a greater number of anticipations to the invariant side. Furthermore, in comparison to performance before the delay, infants subjected to both changes exhibited significantly less anticipations to the invariant side immediately after the retention delay. This disruption in infants' anticipatory behavior to the invariant sides after the retention delay by both color content and spatial changes suggests that infants encoded in memory not only the content information, as predicted, but also the spatial information.

Interestingly, for the varied side, the infants' anticipatory behavior was not disrupted with the retention delay, even when the spatial location of the varied events was changed. This finding was likely because the events on the varied side were constantly changing in content, and as such there was no predictable information to store in memory and, consequently, nothing by which to discriminate after the retention delay. When the spatial location of the varied side changed, infants likely did not detect the change, even if this spatial information had been stored in memory. Specifically, because the varied side before the delay became the invariant side after the delay and vice versa, there was always a basis, whether from memory or currently available after the delay of invariant information upon which to demonstrate anticipatory behavior.

The proposal that content information is processed separately, and to different levels than spatial or temporal information, has also been suggested by the findings of many adult behavioral studies (e.g., Biederman & Cooper, 1992; Craik & Lockhart, 1972; Treisman & Gelade, 1980). Moreover, studies on infants' information processing, such as their immediate perceptual discrimination (Colombo, Mitchell, Coldren, &

Freeseman, 1991) and long-term memory (Adler, Gerhardstein, & Rovee-Collier, 1998), have also demonstrated and supported the concept that informational parameters of events are processed to different cognitive levels. In the first experiment of this study, analysis of infants' anticipatory behavior after a delay was not consistent with this concept, since there was no difference found in the memorability of content information versus the spatial information. After the 24-hour retention delay, infants equally remembered the colour content and the spatial information, and this was evidenced by the decrease in their anticipations to the invariant side in both conditions. Had the spatial information not been as memorable as the colour combination information, the infants exposed to a change in the invariant events' spatial information would not have exhibited a disruption in their percentage of anticipations after the delay. This study, however, only consisted of a delay of 24 hours, and it is therefore possible that this retention delay was not long enough to capture the differences in retention of the spatial and colour content information. Studies that have demonstrated and supported the concept that informational parameters of events are processed to different cognitive levels tested infants over longer delays. For instance, in the long-term memory study by Adler, Gerhardstein and Rovee-Collier (1998), infants were tested on the mobile paradigm over a delay of one week. Perhaps if infants were tested with a change in color content or spatial location over a longer retention delay, the differences in memorability for the colour content and spatial information would have been exposed.

The Effects of Temporal Decay on Memory. The third aim of the study was to assess the effects of working memory limitations, namely, temporal decay, on long-term memory. Previous infants studies have suggested that temporal decay greatly affects the ability of infants to encode information into working memory. For instance, Gilmore and Johnson (1995) demonstrated that during an Oculomotor Delayed Response (ODR) task, 6-month-olds could only maintain information in working memory for approximately 3 seconds in order to correctly make delayed eye movements to cued locations. Three seconds and longer resulted in infants' delayed eye movements correctly localizing the cued target only by chance. Furthermore, models of memory indicate that event information is first encoded in working memory before being transferred to long-term memory (Atkinson & Shiffrin, 1968; Bahrick, Hall, & Berger, 1996; Shallice & Warrington, 1970). If information is not encoded in working memory then it will not be available to be transferred to long-term memory. In Experiment 2, to investigate the role of working memory and the effect of temporal decay that might limit the encoding of event information in working memory and, consequently, availability in long-term memory, we tested infants with an ISI delay of 3000 msec instead of 1000 msec. An ISI of 3000 msec was used because, based on the findings of Gilmore and Johnson (1995), this was a delay that led to temporal decay in 6-month-olds and would likely lead to temporal decay in the younger 3-month-olds used in the present study. Inherently, if the 3000 msec ISI produces temporal decay so that the information that defines the events and the event sequence are not encoded in working memory, then infants' ability to form expectations would be compromised and the information would not be available for transfer to long-term memory.

Results of Experiment 2 indicated that infants did not make significantly more anticipations to the invariant side relative to the varied side, as infants in Experiment 1 did with an ISI of 1000 msec, even on Day 1. As we hypothesized, this finding reveals that an increase in the ISI from 1000 msec to 3000 msec likely produced temporal decay of the information that defined infants' expectations with a 1000 msec ISI and prevented the infants from forming expectations for predictable content on the invariant side. As a consequence of the temporal decay, infants who experienced the 3000 msec ISI were unable to hold the relevant information (i.e. colors) online from one stimulus event to the next, thereby negating the likelihood of detecting the invariance of information in to-be-associated events (e.g. the stimuli on the invariant colour combination side). Because infants were incapable of maintaining the relevant information in working memory to be compared to the information in subsequent picture events, they could not form a content and spatial expectation for the color combination on the invariant side, and as such, there was no longer a greater percentage of anticipatory responding to the invariant side relative to the varied side. These results provide evidence that working memory is a crucial mechanism that enables the formation of expectations in the first place.

If working memory and long-term memory are linked as proposed (Atkinson & Shiffrin, 1968; Bahrick, Hall, & Berger, 1996; Shallice & Warrington, 1970), then our findings, having demonstrated that temporal decay disrupts expectation formation, would suggest that infants' long-term memory for the information upon which the expectations should be based would likewise be disrupted. The findings of Experiment 2, supported this hypothesis and indicated just such an occurrence that infants who experienced the longer ISI showed no evidence that they had stored any information in long-term memory. That is, unlike the infants in Experiment 1 who experienced the 1000 msec ISI, infants in Experiment 2 who experienced the 3000 msec ISI, did not exhibit any difference in their percent anticipations to the invariant or varied sides as indexed overall or in their savings after the 24-hour retention delay. Further, neither did these Experiment 2 infants exhibit a greater percentage of anticipations to the invariant side relative to the varied side after the retention delay.

These findings demonstrate that the infants' memory for information experienced during expectation formation is contingent upon how well they are able to retain information in working memory, thereby determining how strongly they initially formed expectations. Since the 3000 ms ISI produced temporal decay, the Experiment 2 infants' working memory and expectation formation was affected. Consequently, the transfer of expectation formation information from working memory to long-term memory was also compromised in these infants. If this is the case, then this predicts that any changes, color content or spatial, to the events after the retention delay would go undetected by the infants. Findings indicated that this was in fact what happened. Infants who experienced the 3000 msec ISI did not detect a change in the colour content or spatial location of the invariant side after the retention interval, in contrast to infants who experienced a 1000 msec ISI. As a consequence all of the anticipatory measures indicated that infants failed to exhibit preferential behavior to the invariant side relative to the varied side nor showed a change in their anticipatory behavior after the retention interval as compared to before. These results further support the conclusion that the increase in ISI produced temporal decay which disrupted infants' expectation formation and also compromised the transfer of the information upon which expectation formation is based from working memory to long-term memory.

This study is one of the first to establish the functioning of working memory, in the form of assessing temporal decay, in infants as young as 3 months. The finding that temporal decay as a limiting factor in infants' ability to retain information over time is very pertinent, because it may account for infants' inability to retain information over a very long period of time and namely begin to explain the phenomenon of infantile amnesia. If infants' working memory is compromised by limitations that are brought upon within their environment, information will not be available to be transferred to long-term memory. This possibility is very likely, since aside from long-term memory, working memory has also been suggested to be a critical factor in cognitive development in general (i.e Twose & Cowan, 2005; Schneider, Schumann-Hengsteler & Sodian, 2005). Researchers have demonstrated for instance, that working memory development is related to the progression of arithmetical reasoning (e.g. Henry & MacLean, 2003) and reading abilities in children (e.g. Swason & Howell, 2001).

The Validity of Using VExP to Study Memory. Findings from neuropsychological studies support the use of the VExP to study expectations and eye movements. These studies have shown that the development of specific brain pathways play important roles in the infants' ability to form expectations. For instance, Schiller (1985) defined a number of pathways that are involved in oculomotor control, stating that in early infancy, eye movements are regulated by subcortical pathways and are more exogenously controlled. However, research has since suggested that around 3-months of age infants develop cortical pathways involving the frontal eye fields, which allows for endogenously controlled eye movements as well as complex scanning patterns, that ultimately enable the formation of expectation (Richards, 2001). Johnson (1990) also suggested that the frontal eye fields in the frontal cortex undergo development between 3- and 4- months of age, influencing the infants' ability to make shifts in eye gaze, and thereby allowing them to have greater control over visual attention and the initiation and inhibition of saccadic eye movements. The findings of this study support Johnson's hypothesis by demonstrating that 3-month-olds are capable of planning and controlling their eye movements, as evidenced by their anticipations to predictable stimuli. The infants' anticipatory behaviour suggests that they encoded information from the stimuli and formed an expectation representation for that content. Infants were able to exhibit planned anticipatory eye movements that were guided by the formed expectation for that content.

Additional infant eye movement studies (e.g. Johnson, Posner & Rothbart, 1991; Johnson, 1994) have confirmed Johnson's (1990) hypothesis. For instance, Johnson, Posner, and Rothbart (1991) found that 4-month-old infants made more than double the percentage of anticipatory eye movements relative to 2- and 3-month olds toward stimuli that had been previously associated with spatial locations during a training task. Furthermore, in yet another study, 5-month-old infants were able to reduce their number of eye movements made toward a onset of a stimulus on one side of the display and redirect and delay their saccades toward another stimulus that appeared at a later time on the opposite side of the display (Johnson, 1994). These findings provide evidence that between 3- and 6-months of age, infants develop increasing control over their eye movements (Haith et al., 1988). Seeing that infants begin to gain control of their eye movements at 3-months of age, a function that is essential for the overt exhibition of visual expectations, VExP therefore seems to be an appropriate paradigm by which to study expectations and their manifestations in the control of eye movements in young 3-month-olds. VExP allows for the assessment of overt and observable anticipations that captures the cognitive construct of expectation.

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 at any age. The VExP paradigm might be well suited for examining this relation, at least in infants, because it assesses infants' expectation formation behaviour, as well as potentially their learning and memory. Findings from this study seem to support this notion since expectation formation was shown to be memorable for infants over a substantial amount of time. Moreover, results of this study also demonstrated that the VExP is sensitive to underlying characteristics of memory. For instance, temporal decay, a fundamental limitation of working memory, is captured with this paradigm. However, since this study is the first to attempt to use the VExP to study long-term memory, it would be pertinent to further investigate if the paradigm is sensitive to other aspects of long-term memory, such as cues that may aid memory retrieval. Moreover, subsequent replication would be necessary in order to fully assure the paradigm's validity in studying infant memory. Regardless, thus far, the results of this study suggest that the VExP may be especially sensitive to the function of memory and therefore might serve as a means to examine the earliest parameters of the memory system. Because the eye movement system of infants is the most adult-like of all of their behavioral responses, the VExP provides a distinctive avenue by which to examine aspects of cognitive development relative to other behavioral responses. The VExP may provide a means by which memory can be studied consistently and in an operationally similar manner over the development from infancy into childhood.

Memory in Infants. Researchers have previously suggested various pathways and developmental timelines for these pathways, to account for the development of infant memory (e.g. Schacter & Moscovitch, 1984; Bachevalier & Mishkin, 1984). The currently held viewpoint suggests that the differences in memory abilities in infants at different ages is a result of the prolonged development of the medial-temporal lobe (MTL) which is considered to be highly involved in the modulation of long-term memory (Johnson, 2011). Evidence for this perspective stemmed from observations of non-human primate infant studies, where it was found that lesions to MTL, including the hippocampus, gravely affected the infants from performing a visual paired comparison task (Johnson, 2011). Results of various other studies have suggested that the MTL is functioning to some extent in human infants as well, and accounts for their ability to hold information over time (Pascalis & Schonen, 1994; Pascalis, de Haan, Nelson & de Schonen, 1998; Johnson, book). Nelson (1995; Nelson & Webb, 2003), has further suggested that early maturing components of the MTL system allows for an immature form of explicit long-term memory and that as the MTL circuit matures and begins to fully involve the dendate gyrus and hippocampus, significant advances in explicit memory are seen in 8-month and 10-month-olds. Memory seen in young 4-month-olds in a Visual Paired Comparison task for instance, is thus suggested by Nelson (1995) to be an early form of explicit memory relying mainly on the hippocampus (Nelson, 1995).

In contrast, Rovee-Collier (e.g. Rovee-Collier & Cuevas, 2009) has instead suggested that infant memory does not involve multiple systems that develop and mature at different rates. She contested that infants do not undergo a significant transition at 8-months or 10-months of age, but rather a continuous progress throughout development. Evidence for her position derives from findings of mobile conjugate and train paradigm studies, where a linear increase in memory was established (see Figure 1 in the Introduction). However, advocates of the multi-system view have argued that the mobile conjugate and train paradigm are simply assessing implicit procedural memory, which they expect does develop gradually from an early age (e.g. Bauer, 2008; Nelson, 1995). Others have suggested, though, that these conclusions, result from misunderstandings of the methodology (see Hayne, 2004, for a complete discussion of these issues). Moreover, as previously mentioned, additional studies using the mobile conjugate paradigm have demonstrated that infants show memory specificity (Rovee-Collier, 1999). Specificity is a hallmark of explicit/declarative memory but is not typical of implicit procedural memory. This, therefore, renders it very unlikely that the mobile paradigm measures only procedural memory in infants. Instead, memory for the explicit characteristics of the cue experienced during training is likely required in order to discriminate a novel cue. Nevertheless, since the neural processes involved in the infants' memory for the mobile conjugate and train paradigm have never been explored, the debate continues to remain unresolved.

In the current study, the aim was to determine whether the VExP could be used to assess long-term memory in infants. Given our results and the nature of the task, it is not possible at this point to make claims on whether the long-term memory infants are exhibiting in our task is of an implicit or explicit nature, or support a memory development viewpoint over another. Nevertheless, given that infants were actively using previously learned information to guide their behaviour and the memory was very specific it can be suggested that it is perhaps an early form of explicit memory which would rely mainly on the hippocampus, as suggested by Nelson (1995). Since there is no means by which infants can be verbally asked if they explicitly remember the expectations, however, it could also be suggested that the learned expectations are implicit in nature, thus rendering the memory implicit as well. It can be argued that although the anticipatory behaviour infants exhibited are influenced by the information presented to them, the influence is not of explicit and overt in nature. Instead, the information infants are presented throughout each trial influences their future behaviour, through repetition and practice. Note, however, that previous studies of implicit memory in both adults and infants have relied on the analysis of reactive eye movements (e.g. Amso & Davidow, 2012), whereas the current study instead looked at anticipatory eye movements, which are modulated by different processes that govern intentional and planned behavior due to the anticipations being executed when no stimulus being present to elicit the behavior (Haith et al., 1998) These characteristics are typically associated with explicit cognition and not implicit (Rovee-Collier & Barr, 2004).

Pupil Diameter Changes in Relation to Memory. The final goal of the study was attempting to investigate the feasibility of using the measurement of pupil diameter changes as a means to assess memory in infants. When comparing average pupil diameters, infants who received an ISI of 1000 msec exhibited smaller pupil diameters after the retention interval relative to before, to both the invariant and varied color content sides. In contrast, when infants experienced a 3000 msec ISI they exhibited larger pupil diameters after the retention interval to both the invariant and varied sides. Previous research with adults has suggested that pupil diameter changes are an index of memory (e.g. Gadner, Mo & Borrego, 1974; Stanners, Coulter, Sweet & Murphy, 1979; Cansino & Trejo-Morales, 2008; Heaver and Hutton, 2011). On this basis, the current pupil diameter results suggest that there are differences in memory retention when infants experienced different ISIs.

The current pupil diameter results suggest that when infants experienced the 1000 msec ISI, they encoded and stored the information defining the expectation in long-term memory and then retrieved that information after the retention interval to guide their anticipatory behavior. When infants experienced the 3000 msec ISI, in contrast, the pupil diameter results suggest that they never encoded and stored the information defining the expectation and had to learn again the sequence of events after the retention interval. Furthermore, when comparing the mean pupil diameters of infants who received the 1000 msec ISI directly to those who received the 3000 msec ISI, results indicated that infants' pupil diameters did not differ before the retention interval but did differ after the retention interval. That differences only occur after the retention interval would further support the notion that pupil diameter changes are indexing some aspect of memory and memory retrieval. This will be more extensively discussed below.

Recent studies have demonstrated a strong correlation between pupillary diameter changes and activity in the locus coeruleus (LC) (Sara, 2009). This nucleus is found on each side of the rostal pons in the brainstem and modulates the sole source of the neurotransmitter norepinephrine (NE) to the cortex, cerebellum and hippocampus (Laeng, Sirois & Gredebäck, 2012). With regard to attention, the LC-NE system has both phasic and tonic properties that are involved in guiding attention to novel, salient or behaviorally significant stimuli, as well as additionally filtering out irrelevant stimuli during the performance of a given demanding task (Sara, 2009). Animal studies have also revealed that the two different modes in the LC-NE system, corresponds to different patterns of an animal's behaviour (Ashton-Jones & Cohen, 2005). During the phasic mode, LC cells exhibit activations when the processing of task-relevant stimuli is taking place (Aston-Jones, Rajkowski, Kubiak, & Alexinsky, 1994; Bouret, Duvel, Onat, & Sara, 2003; Usher, Cohen, Servan-Schreiber, Rajkowski, & Aston-Jones, 1999). In contrast, during the tonic mode, LC cells do not respond phasically to task events and instead show an increase in distractibility. However, during the tonic mode, it has also been found that an animal is more likely to detect novel stimuli (Laeng, Sirois & Gredebäck, 2012). Concordantly, studies have shown that the processing of task-relevant events is directed by the phasic mode and dramatic pupillary dilations are seen (Beatty, 1982; Kahneman & Peavler, 1969; Poock, 1973; Richer & Beatty, 1985; Simpson, 1969). In contrast, sustained processing yields an increase in activity of the tonic mode (e.g. Siegle, Ichikawa & Steinhauer, 2008), and as difficulty or arousal increases, performance gradually decreases and large increases in pupil diameters are concurrently seen (Beatty, 1982; Granholm, Asarnow, Sarkin, & Dykes, 1996; Howells, Stein, & Russell, 2010; Lavie, 1979; Peavler, 1974; Verney, Granholm, & Marshall, 2004).

Previous adult pupillometry studies have focused more on ‘task-evoked pupillary reflexes’ (TEPRs), which are changes in pupil diameter that correspond to cognitive effort on tasks relating to short and long-term memory access, vigilance, mental arithmetic and sentence comprehension (See Beatty & Lucero-Wagoner, 2000). During a given task, TEPRs relate to phasic activity of the locus coeruleus (Ashton-Jones & Cohen, 2005), indicating high levels of attention or cognitive effort during a task (Laeng, Sirois & Gredebäck, 2012). There are various methods that can be used to study TEPRs (see Klinger, Kuman & Hanrhan, 2008 for a review). Generally these methods involve a specific process whereby researchers begin by parsing out trials into small ‘tasks’ that last a given amount of time, and any pupil changes or the largest pupil changes that occur within the given time frame are analyzed. With regard these adult memory TEPRs studies, participants are also aware that they would subsequently tested for their memory of the items of the task they had participated in.

In the present study, we were unable to replicate the adult pupil diameter studies. One main reason for this is because infants were not aware that they would be tested again on the VExP stimuli. Moreover, the task the infants performed was also quite different from the typical remember/know memory tasks used in adult studies. Furthermore, since infants were not given specific details or instructions for the task they were participating in, our task eliminated the possibility of looking at TEPRs in relation to encoding. Traditionally, in adult memory pupillometry studies, TEPRs during encoding is the main factor analyzed. However, it has been previously been suggested that when participants not are aware that encoding for future retrieval is part of the task, it is not possible to assess phasic task related TEPRs of encoding (Kafkas & Montaldi, 2011). Instead, only tonic pupillometry changes are suggested to occur throughout this type of task (Kaskas & Montaldi, 2011). As a result, it order to explore whether there were any changes occurring in the pupil diameters of infants while they viewed the stimuli, the pupil size at the initiation of anticipations were recorded and observed instead. Our analysis centered around the physiological processes involved in online tonic changes in pupil diameter during the anticipations, where infants would require retrieval of information and as such cognitive effort.

Our study revealed that there were significant differences found between the pupil diameters of infants who received the 1000 msec ISI and the 3000 msec ISI after the retention interval. Infants who experienced the 1000 msec ISI yielded significantly smaller pupil diameters than infants who experienced the 3000 msec ISI. Moreover, given our behavioural analyses, infants who received the 1000 msec ISI found the picture events more memorable than infants who experienced the 3000 msec ISI. For infants who received the 3000 msec ISI, because the stimuli was not as memorable, the task after the retention interval required more effort and was more difficult than for infants who received the 1000 msec ISI, and as such bigger pupil diameters occurred. Initially, when first learning and encoding the elements of the task before the retention interval, these differences were not seen because it was their first encounter with the stimuli for both groups of infants, and as a result no significant differences would be exhibited in difficulty across the tasks. When first learning the task, infants who received the 1000 msec ISI would not have the ‘help’ of information (expectations) encoded previously and remembered subsequently to do the task.

All of these findings, when taken as a whole, are completely consistent with the anticipatory eye movement results and strongly support the contention the pupil diameter analysis is a viable means by which to index memory. These results are also fairly concordant with some of the adult pupillometry literature. For instance, both studies by Kafkas and Montaldi (2011), as well as van Rijn et al. (Van Rijn, Dalenberg, Borst & Sprenger, 2012), found that increases in memory strength are associated with pupil diameter constriction or reduction in pupil dilations. However, it is difficult to compare our results to those from these two studies since their methodologies are very different from ours. Perhaps future infant studies aiming to further understand the relation between memory and pupil diameter, should include methodologies comparable to adult pupillometry studies. Alternatively, studies that feature a memory task that both infants and adults can do, would allow for a more direct comparison. This is important, since it would allow for more specific inferences on the developmental changes from infancy to adulthood.

Conclusion

In summary, this study has demonstrated directly for the first time that expectations are related to memory and this was evidenced by infants being able to remember the information encoded during expectation formation over a 24-hour delay. Moreover, their memory for the expected information highly influenced their anticipatory behaviour after the retention interval. When the changes in the stimuli after the 24-hour delay did not match the infants' memory for the encoded event information encoded during initial learning, their anticipatory behaviour were disrupted. The current findings also demonstrated that the working memory limitation of temporal decay affects the formation of expectations and the memory for the expected event information.

Altogether, these findings strongly demonstrate that the VExP can be used to study infant long-term memory and likely their working memory. The paradigm not only demonstrates the capacity for the formation of future-oriented expectations in infants, which are theoretically related to memory, but can also demonstrate infants' sensitivity to characteristics of memory. Finally, and most excitingly, exploration of infants' pupil diameters suggest that pupil diameter differences may be a viable index by which to study infant memory and perhaps its underlying physiological processes. However, additional analyses comparable to the methods of the adult pupillometry literature are needed. Moreover, future studies are needed to confirm the findings of this study and to perhaps determine the longest length at which infants can remember these expectations. Specifically for this study, this is important since it could perhaps allow for the observation of differences in memorability for the colour content and spatial information. Moreover, previous long-term memory studies have tested infants over delays of up to several weeks (e.g. Rovee & Fagen, 1976; Little, Lipstitt, & Rovee-Collier, 1984; Rovee-

Collier & Barr, 2004). It would be important to test infants with the VExP at delays similar to these studies to assess if the results are concordant with one another.

Nonetheless, this study has extended our knowledge of infants' cognitive expectations and shown that expectations are in fact linked to memory.

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Appendix A

Visual & Cognitive Development Lab

Long-term Memory for Expectations in 3-Month-Old Infants

York University Department of Psychology, 4700 Keele Street, Toronto, Ontario, Canada M3J1P3
Tel: 416-736-2100, ext. 20036

Welcome! I am a Masters student at York University and a member of the Center for Vision Research.
We are required to obtain a signed consent form from the parent(s) of infants who participate in our studies.

I agree to have my child ____________________ participate in a study on cognitive development
(name of child)
in infancy.

I understand that my total time in the lab will not exceed 30 minutes, with the actual presentation to my child being only 4-5 minutes. My child will watch pictures that are displayed on a TV monitor which are usually interesting to babies near the age of my child. During this time, the movement of my child's eyes will be made under invisible infrared light. The experimenter and I (child's parent) will be present at all times. I understand that the recording of my child's visual behaviour may be analyzed at a later time. The potential risks to my child are few and include fatigue or mild frustration. In the event of either fatigue or frustration, the procedure will be stopped immediately. The benefits of participating in this study include furthering scientific knowledge about infant's cognitive development in general.

I understand that I will receive a Sears offer worth $19.99 as compensation for my time. This will be provided as compensation regardless if I withdraw from the study. I understand that I can withdraw my child from participation at any time without prejudice, that I can freely ask questions about the procedures in this study and that my child will be identified for purposes of the study by a unique code number and not by name. Also, my decision not to volunteer or to withdraw from the study will not influence the relationship with the researchers or the study staff or the nature of the relationship with York University, either now or in the future. I also understand that I will receive a summary of the findings of this study once it has been completed, and only group results, no individual results will be summarized. I understand that the researchers are interested in groups of infants and that they will not disclose any information about individual infants to any person not directly associated with the study or in any publications resulting from this study. It is assured that confidentiality will be provided to the fullest extent required by law.

The data received from your baby's participation will be securely stored on a York Server for 5 years with limited access only to those working in this lab, or scientific professionals who request to view the data temporarily. Also, the data is not linked to any of yours or your baby's personal information, but instead given a specific code. After the 5 years, data will be deleted and any consent forms or demographic information will be shredded appropriately.

If you have questions about the research in general or about your role in the study, please feel free to contact Dr. Adler either by telephone at (416) 736-5155, extension 33389 or by e-mail adler@yorku.ca). This research has been reviewed and approved by the Human Participants Review Sub-Committee, York University's Ethics Review Board and conforms to the standards of the Canadian Tri-Council Research Ethics guidelines. If you have any questions about this process, or about your rights as a participant in the study, please contact the Sr. Manager & Policy Advisor for the Office of Research Ethics, 5th Floor, York Research Tower, York University (telephone 416-736-5914 or e-mail ore@yorku.ca)."

Parent(s)' Signature(s)____

Date____________________

YORK redefine THE POSSIBLE.

Appendix B

Experiment 1: VExP and Long-Term Memory

Table 1. Savings Measure of Infants in the No-Change Condition

Infant	Savings Measure	
	Invariant Side	Varied Side
1	1.2	0.84
2	3.2	1.60
3	0.74	1.05
4	0.86	1.13
5	1.02	0.90

Table 2. Savings Measure of Infants in the Colour-Change Condition

Infant	Savings Measure	
	Invariant Side	Varied Side
1	0.67	1.14
2	0.81	0.75
3	0.95	1.33
4	0.75	1.10
5	1.91	0.83

Table 3. Savings Measure of Infants in the Spatial-Change Condition

Infant	Savings Measure	
	Invariant Side	Varied Side
1	0.97	1.33
2	0.69	0.75
3	0.45	0.89
4	0.66	1.90
5	1.00	1.11

Experiment 2: Effects of Temporal Decay of Memory

Table 4. Savings Measure of Infants in the No-Change Condition

Infant	Savings Measure	
	Invariant Side	Varied Side
1	1.00	1.21
2	0.92	1.60
3	1.15	1.13
4	0.93	1.25
5	1.25	0.89

Table 5. Savings Measure of Infants in the Colour-Change Condition

Infant	Savings Measure	
	Invariant Side	Varied Side
1	1.00	0.86
2	0.90	0.92
3	1.08	0.99
4	0.86	1.20
5	1.08	1.01

Table 6. Savings Measure of Infants in the Spatial-Change Condition

Infant	Savings Measure	
	Invariant Side	Varied Side
1	1.00	2.17
2	1.23	1.29
3	0.73	1.35
4	0.98	1.27
5	1.00	1.00