



PAPER

One-month-old human infants learn about the social world while they sleep

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Abstract

Although infants display preferences for social stimuli early in their lives, we know relatively little about the mechanisms of infant learning about the social world. In the current set of studies, 1-month-old infants underwent an adapted eyeblink conditioning paradigm to examine learning to both 'social' and non-social cues. While infants were asleep, they were presented with either a 'social' stimulus (a female voice) or one of two non-social stimuli (tone or backward voice) followed by an airpuff presented to the eyelid. Infants in the experimental groups displayed increased learning across trials, regardless of stimulus type. However, infants conditioned to the 'social' stimulus showed increased learning compared to infants conditioned to either of the non-social stimuli. These results suggest a mechanism by which learning about the social world occurs early in life and the power of ecologically valid cues in facilitating that learning.

Introduction

Much about how we think of infant abilities has changed since William James described their perception of the world as one of 'blooming buzzing confusion'. We now know that even newborn infants are capable of associative learning (for reviews, see Fitzgerald & Brackbill, 1976; Rovee-Collier & Lipsitt, 1982). Although such learning takes place within a social context very early in life, much of the research on infant associative learning has been conducted using non-social stimuli (e.g. a tone or light). There have been many studies on infants' perception and discrimination of social stimuli (i.e. faces, voices), but it is unknown whether young infants who are challenged with an associative learning task will exhibit enhanced performance when exposed to social versus non-social stimuli. Newborns display heightened interest and attention toward social stimuli shortly after birth (Cassia, Simion & Umiltà, 2001; DeCasper & Fifer, 1980; Goren, Sarty & Wu, 1975; Johnson & Morton, 1991; Macfarlane, 1975), suggesting that infants may have evolved over time to treat social stimuli in a privileged

manner compared to other stimuli (Grossman & Johnson, 2007).

Studies that directly examined the role of context on learning within the first year of life have demonstrated that the social context influences language learning (Kuhl, Tsao & Liu, 2003) as well as the development of speech production (Goldstein, King & West, 2003). In addition, changes in social context can disrupt memory formation (Learmonth, Lamberth & Rovee-Collier, 2005) as well as learning to imitate a difficult task (Nielson, 2006). Within the first weeks after birth, most infants displayed conditioned learning to social stimuli, orienting toward an odor paired with touch (Sullivan, Taborsky-Barba, Mendoza, Itano, Leon, Cotman, Payne & Lott, 1991) and a tone paired with a face and voice (Koch, 1967). However, none of these studies directly compared the efficacy of non-social versus social stimuli on learning during early infancy.

A robust learning paradigm that has emerged from the animal literature (McCormick, Clark, Lavond & Thompson, 1982; McCormick & Thompson, 1984) and has been used successfully in infants prior to 6 months of

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age is delay eyeblink conditioning (Fifer, Byrd, Kaku, Eigsti, Isler, Grose-Fifer, Tarullo & Balsam, 2010; Herbert, Eckerman & Stanton, 2003; Ivkovich, Collins, Eckerman, Krasnegor & Stanton, 1999; Klaflin, Stanton, Herbert, Greer & Eckerman, 2002; Little, Lipsitt & Rovee-Collier, 1984). In this paradigm, infants are typically presented with a tone, the conditioned stimulus (CS), followed by the unconditioned stimulus (UCS), an overlapping and co-terminating brief puff of air to the eyelid, thus eliciting a reflexive eyeblink response. Learning is demonstrated following a series of conditioning exposures, at which time the infant produces an eyeblink response when the tone is presented in the absence of the airpuff. Eyeblink conditioning has been extensively employed using nonhuman animal models to examine both the ontogeny (Brown, Pagani & Stanton, 2006; Ivkovich, Paczkowski & Stanton, 2000) and neural circuitry (for reviews see Christian & Thompson, 2003; Thompson, 2005) of associative learning. Therefore, the eyeblink conditioning paradigm may be ideal to investigate the most fundamental properties of human learning in infancy, particularly in the context of an emergent capacity to exhibit preference to social cues which may facilitate learning.

The purpose of the present set of experiments was twofold. First, we wanted to demonstrate that eyeblink conditioning can be readily adapted and used to assess learning in 1-month-old infants, even during sleep when compliance was expected to be high. Second, we wanted to examine the potential for differential learning to a CS that carries social significance compared to those that do not carry such social significance. We hypothesized that infants would show greater learning to a social CS compared to a non-social CS during eyeblink conditioning.

Experiment 1

Healthy term infants ($N = 81$) were enrolled and tested. Infants were recruited using commercially available lists of names and addresses compiled from local hospitals and infant registries. Prior to visiting the laboratory, parents completed a brief phone survey. Infants were excluded from participating in the study if they were born prior to 38 weeks of gestation, had birth complications, or were born with a serious illness, diagnosed syndromic disorder, or injury. At 1 month of age, infants and parents visited the Child Development Laboratory at the University of Maryland and participated in the current study. Associative learning was examined using an eyeblink conditioning paradigm while infants were asleep. Usable data were obtained from 57 infants ($M = 33.0$ days, $SD = 4.8$ days, $n = 34$ male) with the remaining infants being excluded because they were not able to sleep throughout the entire experiment ($n = 21$) or did not show a clear eyeblink response to the airpuff ($n = 3$). All procedures were

approved by the University of Maryland Institutional Review Board.

Infants were randomly assigned to be conditioned to a social CS (voice: $n = 22$, 15 male) versus a non-social CS (tone: $n = 22$; 13 male). In these conditions, infants were presented with either the voice of an unfamiliar female saying 'Hi Baby' or a 1000-Hz pure tone followed by a brief puff of air (~ 7 lb/in²; 100 ms duration) presented to the left eyelid. Both the tone and voice were matched on intensity (80 dB) and presentation duration (1000 ms). Control groups of infants were presented with unpaired CS-UCS presentations of either the voice ($n = 7$, five male) or tone ($n = 6$, three male) and the puff of air. The number of infants who were unable to sleep throughout the entire experiment did not differ between the different experimental groups.

Procedure

The general procedure was similar to that used by Fifer and colleagues (2010). Once the infant was asleep, 6 mm Ag/AgCl electrodes were positioned above and below the infant's left eye and one ground electrode was placed on the back of the infant's neck. The infant was then placed on his or her back in a bassinet with the head placed between two headphone speakers aligned directly with the infant's ears (Figure 1a). Custom software controlled presentation of both the airpuff UCS and auditory CS. The airpuff was delivered via an airpuff unit (San Diego Instruments, San Diego, CA). The airpuff was presented through tubing that was attached to a flexible plastic arm connected to the left speaker. The arm was positioned approximately 1 inch from the infant's left eye at a 45-degree upward angle, so that the airpuff was delivered toward the outer canthus of the left eye.

Paired trials consisted of a 1000-ms auditory CS which overlapped and co-terminated with a 100-ms airpuff UCS, yielding a 900-ms delay interval (Figure 1b). In each block of 10 trials, the 6th trial was an airpuff-alone trial to test the somatosensory response and the 10th trial was a CS-alone trial to test for a conditioned response (Figure 1c). Random inter-trial and inter-block intervals ranged between 6 and 10 seconds and each block lasted 89 s. Criteria were established prior to the present study, in which only infants who completed a total of 15 blocks (120 CS-US pairings, 15 airpuff-alone trials, 15 CS-alone trials) were included in the analyses.

During unpaired trials, control infants were presented with the same number of stimuli as the paired groups in a pseudorandom sequence. Stimuli were presented separately with an inter-trial interval ranging between .5 to 8.5 seconds and block length was equal to block length for paired trials (89 s). A Gellerman series (Gellerman, 1933) was used to determine the order of the presentation of the CS and UCS such that the same stimulus would not be presented on more than three consecutive trials and no block contained more than nine stimulus changes in stimulus type on consecutive trials.

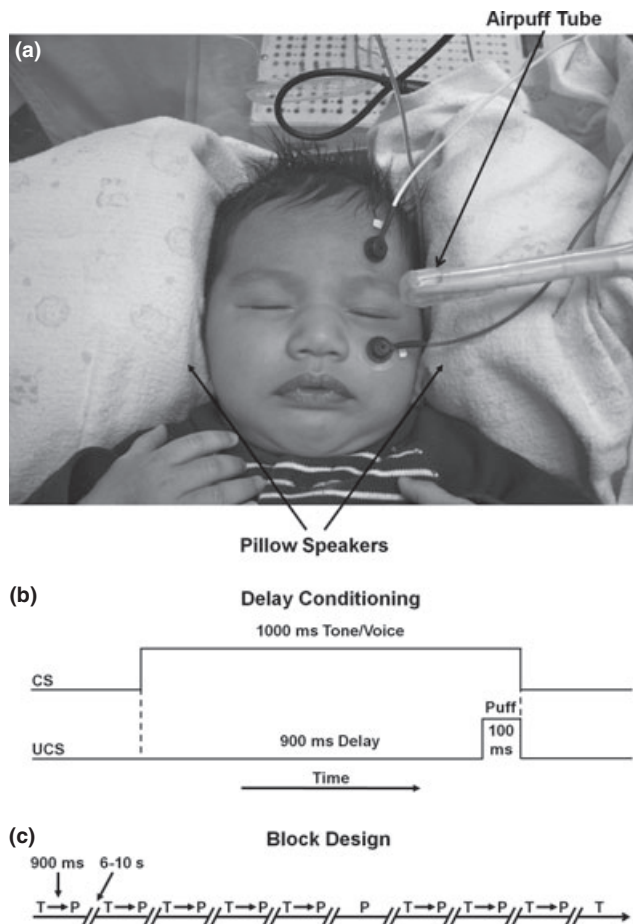


Figure 1 Experimental methods. (a) Sleeping 1-month-old infant during eyelid conditioning. Two electrodes placed above and below the infant's left eye recorded eyelid movements during conditioning. Tone or voice stimuli were presented through speakers located inside pillows that were aligned with the infant's ears. The airpuff stimulus was presented through tubing attached to a plastic arm. (b) Experimental design for paired conditioning trials. The conditioned stimulus (CS), either a tone or voice, was presented for 1000 ms. The unconditioned stimulus (UCS), a 100 ms puff of air, overlapped and coterminated with the CS. (c) Example of the block design for conditioning to the tone stimulus. In each block, infants were presented with five tone-airpuff trials (T→P) followed by an airpuff-alone trial (P), three additional tone-airpuff trials, and concluding with a tone-alone trial (T). Random inter-trial and inter-block intervals between 6 and 10 seconds. Infants were presented with a total of 15 blocks. For data analysis, blocks were aggregated into five separate bins, each comprising data from the tone-alone trials of three consecutive blocks.

Stimulus collection and reduction

The raw electrooculogram (EOG) signal was amplified using a custom bioelectric amplifier (SA Instruments, San Diego, CA) with a gain of 1000 Hz and filtered using high and low pass filters of 1 and 1250 Hz, respectively. The amplified signal was digitized at a sampling rate of 512 Hz using a 12-bit A/D converter (± 2.5 V input range) and Snap-Master data acquisition software

(HEM Data Corporation, Southfield, MI). Prior to recording EOG from each participant, a 50 μ V 10 Hz signal was input into the channel and the amplified signal was recorded for calibration purposes.

The raw EOG signal was processed and analyzed off-line using the EMG Analysis System from James Long Company (Caroga Lake, NY). The signal was digitally filtered offline with a high-pass filter of 28 Hz, a low-pass filter of 250 Hz, and a digital band-stop filter (50–70 Hz) was used to remove 60-Hz noise. The signal was rectified and smoothed by using moving averages with a 20-ms window. Baseline EOG value was defined as the average activity recorded during the 20 ms prior to CS onset.

Response analysis

Conditioned responses (CRs) were examined as eyeblink responses that occurred during CS-alone trials. Eyeblink responses that occurred between 800 and 1500 ms after tone onset and reached at least 1 *SD* above the mean of the baseline (Little *et al.*, 1984) during CS-alone trials were counted as CRs. Peak latency and amplitude during CS-UCS, UCS-alone, and CS-alone trials were examined to determine if any differences in blink morphology existed between the groups.

Statistical analysis

The percentage of CRs across conditioning trials was used as the primary measure of learning. The percentage of CRs was computed across the 15 blocks in aggregates of three trials for a total of five three-trial bins. Peak latency and amplitude during CS-UCS trials were separately averaged across the 15 blocks in aggregates of 15 trials for a total of eight 15-trial bins. Data were analyzed using a repeated measures analysis of variance (ANOVA) with Bin as the within-subjects measure and Group as the between-subjects measure. Preliminary analyses were conducted to examine the influence of age and sex on learning variables, and no significant main or interaction effects were found, therefore sex and age were excluded from further analyses.

Results and discussion

To validate our learning paradigm as well as to demonstrate replication of previous associative learning findings in awake (Herbert *et al.*, 2003; Ivkovich *et al.*, 1999; Klaflin *et al.*, 2002; Little *et al.*, 1984) and asleep infants (Fifer *et al.*, 2010), we first examined whether infants in the voice or tone paired groups showed increased learning compared to infants in the unpaired groups. A significant Bin by Group interaction effect was found for the infants who received the tone ($F(1, 26) = 9.039$, $p = .006$, $d = .590$) as well as the infants who received the voice ($F(1, 27) = 61.281$, $p < .001$, $d = 1.507$). Specifically, infants who received the tone or the voice paired with an airpuff displayed increased conditioning across trials

compared to infants who received unpaired presentations of the tone and airpuff (Figure 2).

Next, we examined whether infants' learning differed if they were presented with the voice or tone CS. We found a significant Bin by Group interaction effect (linear trend) showing that infants who were conditioned using a voice CS displayed significantly greater learning across trials compared to infants who were conditioned to a tone CS ($F(1, 42) = 4.314, p = .044, f = .320$). A main effect of Group revealed that infants conditioned to the voice displayed a significant increase in %CR compared to those conditioned to the tone ($F(1, 42) = 4.487, p = .040, f = .327$; Figure 3a).

To examine whether paired groups differed in their rate of increase in conditioned responses over the course of the experiment, the slope of the learning curve was computed for each infant by regressing %CR on Bin. A one-way ANOVA on the obtained slopes revealed that the voice-conditioned infants increased in learning more rapidly across Bins compared to tone-conditioned infants ($F(1, 42) = 4.629, p = .037, f = .332$; Figure 3b). There was no difference between groups in the obtained intercepts ($F(1, 42) = 2.421, p = .127$).

Table 1 displays the average peak latency and amplitude for all groups during the CS-UCS trials. To determine whether infants responded differentially to the voice compared to the tone presentations, we examined eyeblink response amplitude to airpuff presentations during the CS-UCS trials. We found a significant main effect of Bin showing that all groups (paired and unpaired) showed a significant decrease in eyeblink response amplitude across trials ($F(4, 212) = 13.231,$

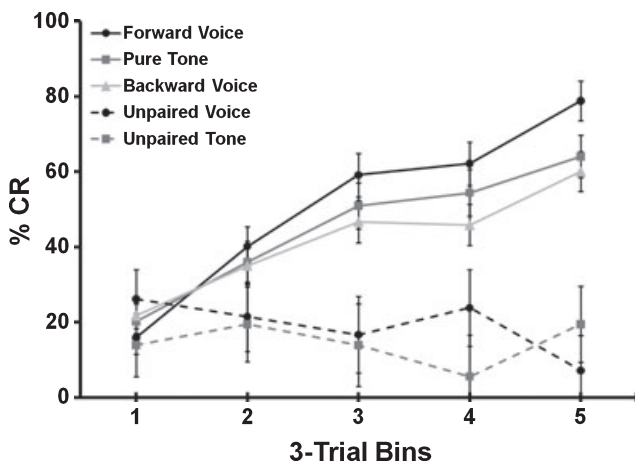


Figure 2 Infants display conditioning to both social and non-social stimuli. One-month-old infants showed increased percentage of conditioned responses (%CR) across CS-alone trials when presented with social (forward voice: solid black line) or non-social stimuli (pure tone: solid dark gray line; backward voice: solid light gray line) that was paired with an airpuff. Infants who received unpaired trials (forward voice: dashed black line; pure tone: dashed dark gray line) did not show an increase in %CR across CS-alone trials. Data are presented as a function of three-trial bins. Error bars represent Mean \pm SEM.

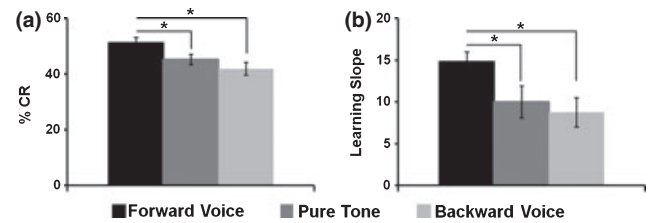


Figure 3 One-month-old infant learning is facilitated by social cues. Infants conditioned to a forward female voice (black bars) showed (a) a greater percentage of conditioned responses (%CR) and (b) a more rapid increase in learning across Bins (learning slope) compared to infants conditioned to a pure tone (dark gray bars) or backward female voice (light gray bars). Error bars represent Mean \pm SEM. * $p < .05$.

$p < .001, f = .500$) suggestive of a general pattern of habituation to the airpuff. Interestingly, infants who were conditioned with the voice stimulus tended to show greater eyeblink response amplitude compared to infants who were conditioned with the tone stimulus, though the difference was marginally significant ($F(42) = 3.107, p = .085, f = .272$). The voice and tone groups did not differ on eyeblink response amplitude during airpuff-alone trials ($p > .20$), suggesting that the increased eyeblink response observed in the voice group was not due to an increased sensory sensitivity to airpuff presentations.

The results of Experiment 1 suggest that 1-month-old infants differentially learn to a social versus non-social stimulus during sleep. Specifically, infants conditioned to a social stimulus, a female voice, showed greater learning compared to infants who were conditioned to a non-social stimulus, a pure tone. In addition, infants had a tendency to show greater blink responses to the voice compared to the tone. These results suggest that social stimuli may facilitate learning during early infancy.

Experiment 2

Because the tone and voice differ on acoustic properties such as complexity and frequency, it may be that potential learning differences observed to the voice compared to the tone are the result of differential learning to complex versus simple sounds rather than social versus non-social stimuli. Therefore, we included an additional control group ($n = 20$, nine male; age: $M = 33.3$ days, $SD = 4.5$ days) that was presented with a temporally reversed version of the voice stimulus paired with presentations of the airpuff. The backward voice stimulus matches the forward voice stimulus on several acoustic properties such as frequency and complexity, but does not have the same prosodic patterns as the forward voice. Several studies have shown that preverbal infants respond differentially and show preference to forward compared to backward speech presentations (Dehaene-Lambertz, Dehaene & Hertz-Pannier, 2002; Pena, Maki, Kovacic, Dehaene-Lambertz, Koizum,

Table 1 Peak latency (in ms) and amplitude (in μV) during CS-UCS trials

	Forward voice (<i>n</i> = 22)	Unpaired voice (<i>n</i> = 7)	Pure tone (<i>n</i> = 22)	Unpaired tone (<i>n</i> = 6)	Backward voice (<i>n</i> = 20)
Latency	973.63 (8.2)	982.04 (7.1)	974.64 (11.9)	990.19 (7.7)	974.640 (12.2)
Amplitude	3.47 (1.6)	2.60 (.85)	2.63 (1.5)	2.41 (7.68)	3.51 (.38)

ms = milliseconds; μV = microvolts.

Bouquet & Mehler, 2003; Ramus, Hauser, Miller, Morris & Mehler, 2000). Therefore, we hypothesized that infants would display greater learning to the forward voice compared to the backward voice. Data collection and analysis was similar to that described in Experiment 1. All procedures were approved by the University of Maryland Institutional Review Board.

Results and discussion

We conducted repeated measures ANOVA with Bin as the within-subjects factor to determine whether the backward voice group demonstrated conditioning across CS-alone bins. We found that, similar to the paired forward voice and paired tone groups, the paired backward voice group also showed an increase in %CR across bins ($F(4, 76) = 8.799, p < .001, f = 0.681$; Figure 2). Separate repeated measures ANOVAs were conducted to compare learning to the backward voice with learning to the forward voice and the tone. A significant Bin by Group interaction effect (linear trend) revealed that infants who were conditioned using a forward voice CS displayed significantly greater learning across trials compared to infants who were conditioned to a backward voice CS ($F(1, 40) = 8.414, p = .006, f = .459$). A main effect of group showed that infants who were conditioned to the forward voice displayed significantly greater average %CR compared to infants who were conditioned to the backward voice ($F(1, 40) = 8.975, p = .005, f = 0.474$; Figure 3a). Infants conditioned to the backward voice did not differ significantly in their learning from infants conditioned to the tone ($p > .20$). Groups did not differ on either peak amplitude or peak latency observed during CS-alone trials ($ps > .20$; Table 2).

Independent sample *t*-tests were used to examine differences in the slope of the learning curve between the backward voice group and the tone and forward voice groups. The forward voice-conditioned infants learned

more rapidly across the Bins compared to the backward voice-conditioned infants ($t(40) = 2.933, p = .006, d = 0.927$; Figure 3b). No difference in learning slope was found between the tone and backward voice groups ($p > .20$).

When differences in blink response amplitudes during CS-UCS trials were examined, we found that infants conditioned to the backward voice displayed increased blink amplitude responses compared to the tone-conditioned group ($t(40) = 2.482, p = .017, d = .785$). No difference in blink response amplitude was found between the forward or backward voice groups ($p > .20$).

The results of Experiment 2 further suggest that learning is increased in the context of social stimuli compared to non-social stimuli. Infants conditioned to a forward voice showed increased learning compared to those infants conditioned to a backward voice. This result suggests that increased learning to a social stimulus is not dependent upon the complexity or frequency of the auditory stimulus. Similar to results in Experiment 1, we found an increase in blink response amplitude in the backward voice group compared to the tone group, suggesting that perhaps infants' blink amplitude is related to increased auditory complexity and not differences in learning.

General discussion

During the first weeks of life, much of the infant's time is spent sleeping. Moreover, infants are capable of rapidly learning associations between external stimuli while sleeping (Fifer *et al.*, 2010). However, little is known about what conditions may be optimal for infant learning during the first weeks of life. Here, we tested the hypothesis that infants' learning is enhanced to social versus non-social cues. In the current set of experiments, we examined 1-month-old infants' learning using a 'social' cue (human female voice) and compared it to learning to two different non-social cues (pure tone or backward female voice). We found that infants displayed learning to both the social and non-social cues but the learning was greater for the social cue compared to both of the non-social cues. Overall, these results suggest that infants can learn more readily in the context of stimuli that are both ecologically relevant and socially salient.

Shortly after birth, infants show preference toward various social cues such as faces (Cassia *et al.*, 2001; Goren *et al.*, 1975; Johnson & Morton, 1991), voices

Table 2 Peak latency (in ms) and amplitude (in μV) during CS-alone trials

	Forward voice (<i>n</i> = 22)	Pure tone (<i>n</i> = 22)	Backward voice (<i>n</i> = 20)
Latency	1257.60 (114.3)	1206.7 (94.1)	1167.57 (172.2)
Amplitude	1.23 (0.7)	1.04 (0.2)	1.37 (.5)

ms = milliseconds; μV = microvolts.

(Beauchemin, Gonzalez-Frankenberger, Tremblay, Vannasing, Martinez-Montes, Belin, Béland, Francoeur, Carceller, Wallois & Lassonde, in press; DeCasper & Fifer, 1980; deRegnier, Nelson, Thomas, Wewerka & Georgieff, 2000; Vouloumanos & Werker, 2007), and odors (Delaunay-El, Marlier & Schaal, 2006; Macfarlane, 1975). These preferences toward social stimuli may be the result of a neural system that has developed over the course of evolution to treat social stimuli as more privileged in comparison to other non-social stimuli (Grossman & Johnson, 2007). Therefore, these intrinsic preferences then may guide the infant to attend more readily to important information within the immediate environment which, in turn, may help facilitate learning (Meltzoff, Kuhl, Movellan & Sejnowski, 2009). The increased learning observed in the infants conditioned to social (voice) versus non-social (tone, backward voice) stimuli provides evidence in line with this hypothesis.

Fifer and colleagues recently found that newborn infants learn the association between a tone and airpuff during sleep (Fifer *et al.*, 2010). Our results expand upon these basic findings from newborns to 1-month-olds showing that, using the same paradigm, infants continue to display learning during sleep 1 month after birth. Most importantly, our results show that infants display greater learning during the presentation of social stimuli during sleep. Previous research has shown that infants are capable of discriminating various auditory social stimuli such as syllables (Cheour, Martynova, Näätänen, Erkkola, Sillanpää, Kero, Raz, Kaipio, Hiltunen, Aaltonen, Savela & Hämäläinen, 2002) and words (deRegnier *et al.*, 2000; Sambeth, Ruohio, Alku, Fellman & Huotilainen, 2008) during sleep, suggesting that the infants in the current set of experiments were capable of processing auditory information while sleeping, thus leading to differential learning to social versus non-social stimuli.

In the current study, we found that 1-month-old infants display greater learning to a forward voice compared to a backward voice. Although the forward and backward voices were matched on some acoustic properties such as frequency and complexity, they differ on prosody (i.e. stress and intonation patterns). Previous studies have demonstrated that, even within the first months of life, infants display a preference for and differential neural responses to the prosodic pattern of forward versus backward speech (Dehaene-Lambertz *et al.*, 2002; Pena *et al.*, 2003; Ramus *et al.*, 2000). Therefore, it is likely that the increased learning to the forward voice in our study was facilitated by using a stimulus which was potentially more salient given its preferred prosodic pattern. However, it may be this characteristic of the voice that may be considered most 'social' given that infants use the prosody of speech in order to understand others' communicative intentions (Fernald, 1989).

It has been well established that infants learn about auditory stimuli, particularly voices, while in the womb

(for review see Moon & Fifer, 2000). Given that the infants in the current study had experience with forward speech prenatally as well as an additional 4 weeks postnatally, the results of the current study may be due to increased familiarity with the natural acoustic properties of a forward voice compared to those of a tone or backward voice. With the design of the current study, we are unable to address this issue. Future studies should examine how familiarity of auditory stimuli, particularly voices, affects learning abilities in young infants.

It should be noted that we did not find differences in blink response amplitudes or latencies between the forward and backward voice groups. Dehaene-Lambertz and colleagues (2002) have shown that infants show greater differences in brain activation between forward and backward speech when they are awake compared to when they are asleep. This finding may help explain the lack of difference in blink response amplitude between the forward and backward voice groups given that the infants in our study were conditioned while they were asleep.

The current findings demonstrate that young infants' learning can be enhanced when ecologically valid and salient social cues are used. In addition, we have designed a learning tool that may be used to examine infant social learning during the first month of life, a period during which infants demonstrate limited capacities for higher-level social cognition (as reviewed in Rochat & Striano, 1999; Striano & Reid, 2006). Our strategy may be used to examine the emergence of individual differences in learning within a social context, which may be related to the development of subsequent social behaviors. More importantly, given that atypical eyeblink conditioning responses have been observed in individuals with autism spectrum disorder (ASD) (Sears, Finn & Steinmetz, 1994), this new paradigm may allow one to examine social learning abilities in infants who are at higher risk for developing social disorders, such as infant siblings of children with ASD.

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