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Behavioral and Electrophysiological Indices of Memory in Typically Developing and Hypoxic-Ischemic Injured Infants

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Behavioral and electrophysiological indices of memory were examined in 12-month-old typically developing control infants (CON) and infants with history of perinatal hypoxic-ischemic injury (HII) across 2 days. Using a visual paired comparison (VPC) procedure, novelty preference was tested

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immediately after a familiarization period and then after delays of 2 min and 24 h. Both groups showed a significant novelty preference only for the no-delay condition. On day two, event-related potentials (ERPs) were recorded while infants viewed the VPC familiar face, a more recently familiarized face, and a novel face, and mean amplitude for components thought to reflect memory (positive slow wave, PSW) and attention (negative central, Nc) were computed. In temporal regions, HII showed a diminished Nc and enhanced PSW to the recently familiarized face, while CON showed a similar trend for the PSW only. Overall, infants showed the largest PSW over left scalp regions. Finally, a positive correlation between VPC novelty preference after 24 h and PSW was found in CON, and preliminary results suggest that this association differs as a function of group. Therefore, in comparison with CON, HII showed both similarities and differences on individual tasks of memory as well as potentially disparate relations between the behavioral and neural mechanisms underlying memory performance.

BACKGROUND

The capacity to transform a new experience into a lasting memory is essential to human learning and development. The study of memory in infants can provide an early window into this process of cognitive development. Although infants are nonverbal, their memory can be evaluated through the use of both behavioral and electrophysiological measures. Visual paired comparison (VPC) is the behavioral task that is most often used to evaluate nonverbal visual recognition memory in infants. This task involves familiarizing the infant to a visual stimulus for a fixed period of time and subsequently testing the infant by showing the familiarized stimulus next to a novel stimulus such that the infant simultaneously views both the familiar and novel stimuli. Memory is inferred if the infant shows preferential looking, greater than is expected by chance, to one stimulus over the other, typically a preference toward the novel stimulus (Bauer, San Souci, & Pathman, 2010). Prior studies have used the VPC task to demonstrate visual recognition memory across time delays at various infant ages. Geva, Gardner and Karmel (1999) demonstrated novelty preference after a short delay in 4-month-olds, Pascalis, de Haan, Nelson and de Schonen (1998) demonstrated novelty preference after a 24-h delay in 6-month-olds, and Morgan and Hayne (2011) demonstrated novelty preference in 12-month-olds when tested immediately but not after 24-h delay. Through use of the VPC task, all of these studies demonstrated the presence of visual recognition memory in infants from ages 4 to 12 months,

and although the overall trend is toward retention over progressively longer time delays after shorter periods of familiarization with increasing age, the precise retention intervals at various ages during infancy remain to be identified (Rose, Feldman, & Jankowski, 2004). There are also implications beyond infancy as visual recognition memory performance by 6 and 12 months of age is predictive of childhood IQ (Colombo, 1993; Rose & Feldman, 1995).

The electrophysiological responses used to study memory are event-related potentials (ERPs), which are a subset of the continuous electroencephalogram (EEG) that reflects transient changes in the brain's electrical activity in response to a discrete event. The ERP components related to attention and memory in infants and children are the negative central (Nc) and late slow waves, which include the negative slow wave (NSW) and positive slow wave (PSW), all of which are located over frontocentral brain regions (Nelson & McCleery, 2008). The Nc component, in studies of 4.5-, 6- and 7-month-olds, has been shown to be larger during periods of attention than inattention (Richards, 2003) and larger for novel than familiar stimuli (Reynolds & Richards, 2005). The late slow waves, also in studies of 4.5, 6 and 7-month-olds, were shown during periods of attention to be manifest as a NSW over frontal regions in response to a novel stimulus and as a PSW over temporal regions in response to a infrequent-familiar stimulus (Reynolds & Richards, 2005). The manifestation of the late slow waves have also been shown to change with development, as another study demonstrated that during periods of attention to a novel stimulus, the PSW was present in 4.5-month-olds, but by 7.5 months of age the NSW appeared and the PSW was no longer present (Richards, 2003). These studies indicate that by 7.5 months of age, the Nc reflects attention and may also play a role in novelty detection, the NSW reflects novelty detection, and the PSW reflects memory updating of partially encoded stimuli (Nelson & McCleery, 2008).

A newly emerging field in the study of infant memory is the integration of visual behavioral and electrophysiological measures. (Reynolds & Guy, 2012). A study on 4.5- to 7.5-month-olds showed that overall preference for the novel stimulus on VPC correlated with larger Nc response to the novel stimulus (Reynolds, Courage, & Richards, 2010). In 6-month-olds, the amplitude of a late slow wave component over the right-central and temporal brain regions during familiarization to a stimulus predicted subsequent performance on the immediately following VPC test (Snyder, 2010). This integration of measures is also beginning to be used to examine the influence of pre- and perinatal experience on infant memory. A study on infants of diabetic mothers (IDM), who are at increased risk of perturbations in hippocampal development due to the adverse effects

of metabolic fluctuations during pregnancy, found that even though IDM and control infants performed similarly on the visual paired comparison task, there was a difference in their ERP responses (Nelson et al., 2000). Integrating behavioral and electrophysiological tools may allow for the detection of subtle memory impairments during infancy following potentially adverse pre- or perinatal experience.

Hypoxic-ischemic injury (HII), which occurs in 1–6/1,000 live full-term births, results in increased risk for neonatal mortality and later neurodevelopmental disabilities (Volpe, 2008). The hippocampus is particularly susceptible to perinatal HII (Nyakas, Buwalda, & Luiten, 1996). Many previous animal and human studies have demonstrated atrophy of the hippocampus and memory impairments following HII (Isaacs et al., 2003; Maneru et al., 2003; Mikati et al., 2005; Quamme, Yonelinas, Widaman, Kroll, & Sauve, 2004; Yonelinas et al., 2002). One particular study by Vargha-Khadem and colleagues reported decreased hippocampal volumes of 39–57% below normal on volumetric MRI analysis of adolescents who experienced HII either during infancy or early childhood. Furthermore, although these children all had IQs within the normal range, they exhibited impairments in both their episodic memory and their delayed verbal and visual memory (Vargha-Khadem et al., 1997). Adults who experienced HII very early in life showed impairment on the VPC task in comparison with controls (Munoz, Chadwick, Perez-Hernandez, Vargha-Khadem, & Mishkin, 2011).

The memory impairments in persons who experienced HII early in life have previously not been noted to occur until school age, at the earliest. One explanation for this could be that the hippocampus does not reach maturity until 5–7 years of age, so it is not until this point that the memory impairments become evident (Bachevalier & Vargha-Khadem, 2005). Conversely, memory impairments in children who have experienced perinatal HII may be present from the time of the injury, but may go unnoticed until they enter school because relatively few demands are placed on memory during infancy or early childhood. No prior studies have tested infants with a history of perinatal HII for memory impairments while they are still in infancy.

This study examined visual behavioral and electrophysiological measures of memory independently as well as in relation to one another in both typically developing infants and a small group of infants with a history of perinatal HII at 12 months of age. Our aims were to both better elucidate the relationship between behavioral and electrophysiological measures of memory in typically developing 12-month-old infants as well as to explore any potential differences between typically developing infants and those with a history of HII.

METHODS

Participants

The final sample consisted of 34 12-month-old infants: 25 control infants (CON; mean age = 381 days, *SD* = 15 days; 14 female infants) and nine infants who experienced a hypoxic-ischemic injury in the perinatal period (HII; mean age = 383 days, *SD* = 15; three female infants). Inclusion criteria for all infants were birth at greater than or equal to 35-week gestational age and weight less than 10 pounds. HII infants were recruited from the neonatal neurology clinic at Boston Children's Hospital. To qualify for the present study, HII infants had to have exhibited clinical signs and/or symptoms of perinatal hypoxic injury, which included seizures or two of the following symptoms lasting for greater than 24 h: Abnormal consciousness, difficulty maintaining respiration, abnormal tone or reflexes, or feeding difficulty of presumed central origin. The HII infants included in our study suffered mild-to-moderate severity of illness as evidenced by Sarnat stage ranging from I–II. Additional information on severity of illness for the HII group, including number of subjects who required therapeutic hypothermia and/or suffered seizures, 1-min and 5-min Apgar scores and initial blood pH, is detailed in Table 1. Exclusion criteria were any chronic fetal or infant factors such as IUGR, maternal drug use, maternal diabetes, metabolic disorder, congenital malformations, or severe quadriplegia or significant abnormality in vision or eye movements. Typically developing participants were recruited from the Research Participant Registry of the Laboratories of Cognitive Neuroscience at Boston Children's Hospital.

Hypoxic-ischemic injury and CON participants were included in the final sample if they had sufficient data from either the eye-tracking or the ERP paradigm. Four infants (3 CON and 1 HII) were excluded because they missed their Day 2 appointment (and therefore had neither Day 2

TABLE 1
Hypoxic-Ischemic Injury (HII) Severity of Illness

	HII (n = 9)
Therapeutic hypothermia (*n*)	5
Sarnat stage I–III (range)	I–II
Seizures (*n*)	3
1-min Apgar score (range)	0–5
5-min Apgar score (range)	2–8
Initial pH (range)	6.6–7.3

eye-tracking nor ERP data to analyze). An additional 21 infants were excluded (17 CON and four HII) because they did not meet criteria for inclusion in the eye-tracking analysis (criteria described under data analysis—visual paired comparison) and they did not provide the minimum number of artifact-free trials in the ERP task. Further, two HII infants were excluded from subsequent analyses due to severe motor and visual impairment. Project approval was obtained from the Institutional Review Board of Boston Children's Hospital, and informed consent was obtained by the parents of each infant participant. The CON and HII groups were matched on both age ($t(32) = .27$, $p = .79$, $d = 0.14$) and socioeconomic status, as estimated by parental income ($t(28) = .42$, $p = .68$, $d = 0.16$). Additionally, the Mullen Scales of Early Learning (Mullen, 1995) was administered to assess cognitive ability. An early learning composite score (ELC) was calculated for each participant based on performance across four subscales: Visual reception, fine motor, receptive language, and expressive language. No difference was found between HII and CON infants on the ELC ($t(31) = .36$, $p = .72$, $d = 0.13$; see Table 2, for each group's mean and standard deviation for age in days, income index, and Mullen ELC).

Stimuli

Stimuli for the eye-tracking and ERP tasks consisted of color photographs of female faces displaying neutral expressions. Each woman was seated in front of a gray background and wearing a gray cloth to cover their clothing. Face images were taken from a database of women who participated in other studies with their infants and signed a release for use of their image in future research. All of the faces used were Caucasian females with neutral expressions and none contained glasses or other

TABLE 2
Demographic Information for HII and CON Infants Contributing Useable VPC or ERP Data

	CON *Mean (SD)*	*HII* *Mean (SD)*
Age (days)	381 (*15*)	383 (*15*)
Income index	9 (2)	9 (2)
Mullen ELC	103 (12)	102 (10)

Note. Income Index assessed on a 1–10 scale, with each point corresponding to a $10,000 increment; Mullen ELC refers to the Early Learning Composite Standard Score. CON = control infants; HII = hypoxic-ischemic injured infants; VPC = visual paired comparison; ERP = event-related potential.

accessories. Size and luminance of all stimuli were also matched to ensure that high- and low-level stimulus differences could not influence this study (see Figure 1).

Apparatus

Visual paired comparison

Participants were seated on a chair in front of a 17" TFT Tobii T60 monitor. Images were presented on the monitor using Tobii Studio software (Tobii Technology AB; www.tobii.com). During stimulus presentation, the Tobii monitor recorded gaze location for both eyes based on the reflection of near-infrared light from the cornea and pupil. Gaze information was sampled at a frequency of 60 Hz. Monitor specifications included an accuracy of 0.5 degrees of the visual angle and a tolerance of head movements within a range of 44 × 22 × 30 cm.

Event-related potentials

Electroencephalogram (EEG) was recorded continuously throughout the ERP task using a 128-channel HydroCel Geodesic Sensor Net (HCGSN), which was referenced on-line to vertex (Cz). For a subset of participants, a NetAmps 200 amplifier was used and the electrical signal was amplified with a .1- to 100-Hz band pass filter. For the remaining infants, a NetAmps 300 amplifier was used with no band pass filter (an analysis using amplifier type as a between-subjects factor is described in the ERP results section). All data were digitized at a 500 Hz sampling rate and stored on a computer disk for further processing and analysis.

Figure 1 Examples of stimuli used in the visual paired comparison (VPC) and event-related potential paradigms. For these three images, each appeared as the VPC face, the recent familiar face, and the novel face, across different test versions.

E-Prime software was used for stimulus presentation, and NetStation software was used for EEG data acquisition and postprocessing.

Procedure

The eye-tracking and ERP tasks took place over a 2-day period for each infant. On Day 1, infants completed the initial portions of the eye-tracking task. Participants were seated in a chair in front of a Tobii T60 monitor in an electrically and sound-shielded testing room with dim lighting. The chair was positioned such that each participant's eyes were approximately 60 cm from the monitor. Before beginning the eye-tracking experiment, participants completed a calibration procedure to ensure the eye-tracker was adequately tracking gaze. In this calibration procedure, a red dot appeared at 5 locations: Each of the four corners of the monitor and the center of the screen. Following calibration, the Tobii Studio program reported whether the eye-tracker successfully picked up gaze at the five locations. If calibration was successful, the experimental procedure was begun. If calibration was unsuccessful, the monitor and chair were adjusted and the calibration procedure was rerun until it successfully picked up on all five locations of gaze.

Following calibration, infants began the Day 1 portion of the visual paired comparison task (VPC). During all phases of the VPC, faces were presented side by side, each measuring 14 × 14.5 cm on the screen and separated by a distance of 3.5 cm. With infants positioned at 60 cm from the screen, this resulted in each face subtending a visual angle of 13 degrees. During the first phase of the VPC, infants were presented with the same unfamiliar female face on both the left and right side of the screen for 25 sec. This constituted the VPC familiarization phase. Infants were then tested using the VPC at three delays: (1) immediately following familiarization ("Imm"), (2) two minutes following familiarization ("2 min"), and (3) 1 day after familiarization ("Day 2"). For each of these three VPC tests, infants were shown the familiar face next to a novel face for a total of 20 sec and the left or right position of the faces switched sides after 10 sec. At each VPC comparison test, infants saw a unique face paired with the familiarization face.

The Day 2 visit began with the final portion of the eye-tracking experiment and concluded with the ERP paradigm. Infants were again calibrated to ensure successful gaze tracking with the Tobii monitor and then presented with the third and final VPC test comparison (Day 2). After the eye-tracking portion of the experiment, the ERP task began. Before fitting the child with the HCGSN, infants were familiarized to a new face. This face was presented 20 times for 500 ms in the center of

the screen with a variable intertrial interval of no less than 1,500 ms. EEG was then recorded as infants saw this newly familiarized face ("recent familiar"), the VPC familiarization face from Day 1 and Day 2 ("VPC") and a third never-before-seen face ("novel") presented in a semi-randomized order such that for every three stimuli presented, these three faces each appeared once (so they were randomized within every set of three). This ensured an even number of presentations of each of the three stimuli. Stimuli were counterbalanced across participants, such that the "VPC" face for one set of infants would serve as the "recent familiar" face for a second set of infants and the "novel" face for a third set of infants.

From a separate room, an experimenter observed infant's eye movements and attentiveness through a video camera mounted on top of the experimental monitor. Stimulus presentation was initiated only when the child was attending to the screen, and any trial where an infant's attention shifted during image presentation was flagged and removed from later analysis. Images were presented until infants saw a maximum of 126 trials or until the infant became too fussy to continue.

Data processing and analysis

Visual paired comparison

Gaze data were collected at a sampling rate of 60 Hz throughout the testing session. Before the eye-tracking data were exported from the Tobii Studio program, areas of interest (AOIs) were drawn onto the stimuli, enabling the subsequent analysis of gaze data within these particular AOIs. A single AOI was created for each picture that encompassed the face and gray background and was labeled as familiar face or unfamiliar face. Each participant's eye-tracking data were exported from Tobii Studio, with time samples identified in which gaze fell within one of the faces. These exported data files were run through a custom-made Python script (Python Programming Language; www.python.org), which extracted and summed gaze duration for each face on each trial. Using gaze duration to the familiar face during familiarization, two variables were calculated for each infant to determine whether they had sufficient and unbiased looking during this initial phase: (1) total time on familiarization face (summing familiarization face on left and right), and (2) side bias, calculated as total time on familiarization face on the left side divided by total time on the familiarization face on the left plus the right. Based on criteria used in previous work (e.g., Ferroni, Menon, Rigato, & Johnson, 2007; Taylor & Herbert, 2013; Tenenbaum, Shah, Sobel, Malle, & Morgan, 2013), infants

were included in subsequent analyses if they looked to the familiarization faces greater than 30% of the time (i.e., 7.5 sec out of the 25 sec length of familiarization) and had a side bias no greater than 85% to either side. A measure of novelty preference was calculated for each of the three VPC tests by summing total time on the novel face and dividing by the total time on the novel and familiar faces combined. This resulted in a variable for proportion of time on the novel face for the three comparison delays: Imm, 2 min, and Day 2. Infants were included in the single-task VPC analysis if they looked to the faces for more than 30% of the time at each delay (i.e., 6 sec out of the 20 sec length of each comparison). Table 3 details attrition for the VPC task at each phase of data analysis. For both groups, 50% of infants who were successfully familiarized contributed to the VPC single-task analysis.

Event-related potentials

The data were analyzed offline with NetStation EEG analysis computer software (EGI: Electrical Geodesics, Inc.). The continuous EEG was digitally filtered and then segmented to 1,500 ms after stimulus presentation, with a baseline period beginning 100 ms before stimulus onset. The filter settings were based on the amplifier used during session recording. For infants tested using a NetAmps 200, a 30-Hz low-pass filter was applied; for infants tested using a NetAmps 300, a 0.3- to 30-Hz bandpass filter was applied. Amplifier was included as a between-subjects variable in subsequent analyses to examine differences due to this change in equipment (see Results). After filtering and segmentation, data were then baseline corrected to the mean amplitude of the 100 ms baseline period. Artifact detection was then run to identify trials containing eyeblinks (defined by a voltage exceeding $\pm$ 140 μV), and these trials were excluded

TABLE 3
VPC Attrition Information

	Less than 30% looking in familiarization	*Side bias (>85%) in familiarization*	*Less than 30% looking and >85% side bias*	*Successful familiarization*	*More than 30% looking in Imm, 2 min, and Day 2*
CON	5	1	1	36	18
HII	1	0	1	12	6

Note. CON = control infants; HII = hypoxic-ischemic injured infants; VPC = visual paired comparison.

from further analysis. The remaining segments were visually examined by an experimenter to identify bad channels and other artifacts (e.g., eye movements, body movements, or high-frequency noise). The whole trial was excluded from further analysis if more than 10% of channels were marked bad for that trial.

Average waveforms for each individual participant within each experimental condition were generated and re-referenced to the average reference. Participants with fewer than eight good trials per condition were excluded from further analysis. While typical infant ERP studies create average waveforms for subjects with a minimum of 10 good trials, because the recruitment of full-term HII infants with only mild-to-moderate HII injury was especially limited (as, for example, HII is much more common in premature infants), we used more liberal exclusionary criteria at this stage in processing. Average waveforms were then visually examined by an experimenter with expertise in infant ERP who was blind to participant group, and infants were excluded if the averaged waveforms showed excess noise for at least one of the three conditions. The number of subjects lost at each phase of ERP processing is described in Table 4. Of subjects who wore the EEG net for at least 20 trials per condition, 57% of CON (16/28) and 75% of HII (6/8) were accepted into the final analysis. For the final sample, the mean number of accepted trials did not differ between CON ($M = 37.13$, $SD = 6.93$) and HII ($M = 42.67$, $SD = 11.62$); $t(20) = -1.39$, $p = .18$, $d = 0.67$).

Analyses focused on two regions: (1) frontocentral electrodes, which were grouped into left (19, 24, 29, 30), middle (5, 6, 12, 13, 112, VREF), and right (4, 105, 111, 124) regions of interest, and (2) temporal electrodes, which were grouped into left (34, 38, 44, 45, 46) and right (102, 108, 114, 116, 121; see Figure 2). Mean amplitude values for the Nc and

TABLE 4
ERP Attrition Information

	Hat refusal	*Did not complete (<60 trials viewed)**	*>60 trials viewed <8 good per condition*	*>8 good per condition reject for noisy average*	*>8 good per condition accepted average*	*Percent accepted after >60 trials*
CON	1	12	5	7	16	0.57
HII	2	3	1	1	6	0.75

Note. *Consistent with past work in our Laboratory, no child who viewed less than 20 trials per condition had 8+ good trials per condition. We therefore classify this as exclusion due to task refusal as opposed to exclusion due to noisy ERP data. CON = control infants; HII = hypoxic-ischemic injured infants; ERP = event-related potential.

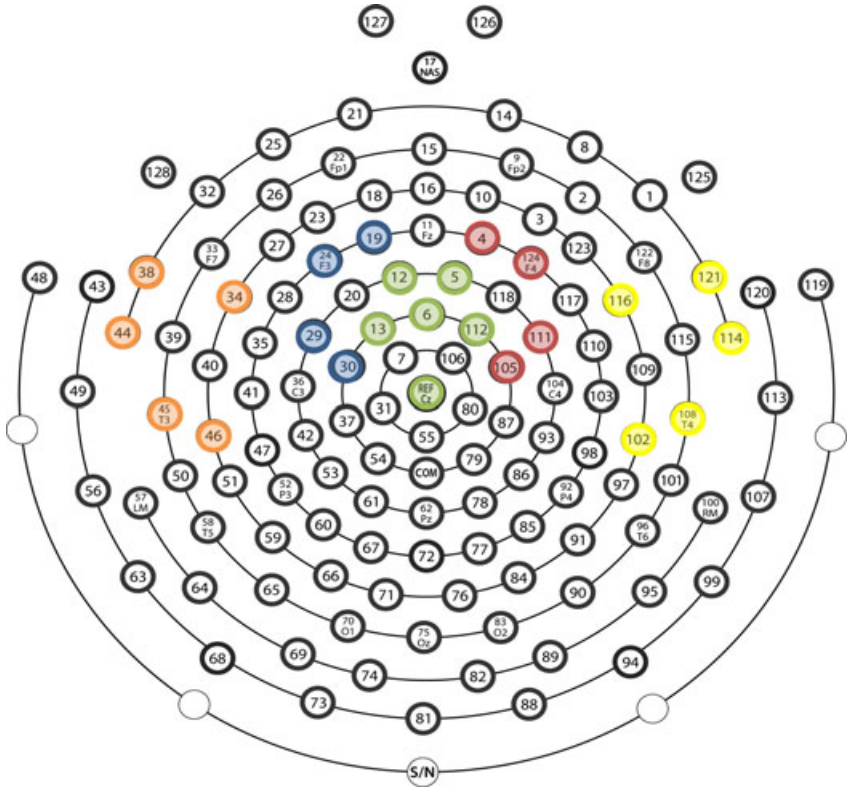

Figure 2 Electrode groupings for 128-channel HydroCel Geodesic Sensor Net. Frontocentral groupings consisted of left (19, 24, 29, 30), middle (5, 6, 12, 13, 112, VREF), and right (4, 105, 111, 124). Temporal groupings consisted of left (34, 38, 44, 45, 46) and right (102, 108, 114, 116, 121).

PSW components were extracted for each individual participant for each stimulus condition at each of the scalp regions (averaging each amplitude value within the specified time window). The time windows for the Nc and PSW were determined, using prior work on infant ERP waveforms as a guide (de Haan, Johnson, & Halit, 2003; Nelson & McCleery, 2008), by examining the grand mean average waveforms for all CON and HII subjects, collapsed across condition, to narrow in on the time windows encompassing the components of interest in our group of infants (see also Figures 3 and 4). Nc mean amplitude was calculated to include the negative deflection occurring between 175 and 650 ms following stimulus onset, and the PSW mean amplitude was calculated to include the subsequent

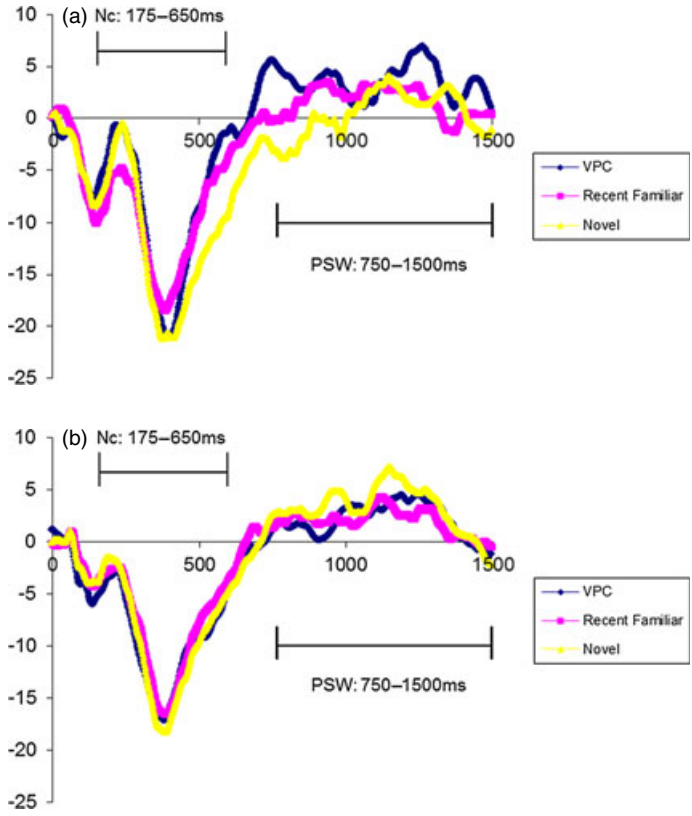

Figure 3 Grand averaged waveform for negative central and positive slow wave to visual paired comparison (VPC) face, recent familiar face, and novel face in (a) hypoxic-ischemic injured infants and (b) control infants collapsed across the 14 frontocentral electrode sites.

positive deflection occurring between 750 and 1,500 ms following stimulus onset.

RESULTS

Visual paired comparison

For the 18 CON and six HII that contributed sufficient data from the VPC familiarization phase and all three test delays, there was no difference in total looking during familiarization (CON: $M = 15.8$ sec, $SD = 3.8$ sec; HII: $M = 16.8$ sec, $SD = 3.4$ sec; $t(22) = -0.55$, $p = .59$,

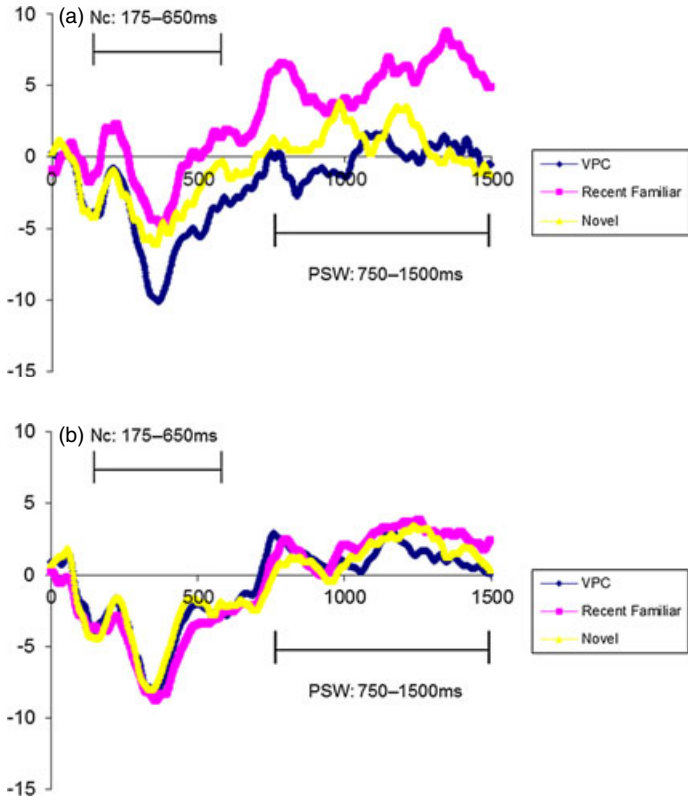

Figure 4 Grand averaged waveform for negative central and positive slow wave to visual paired comparison (VPC) face, recent familiar face, and novel face in (a) hypoxic-ischemic injured infants and (b) control infants collapsed across the 10 temporal electrode sites.

d = .28). A preliminary ANOVA including test version as the between-subjects factor revealed no main effects of this variable, and the present analysis therefore collapsed across this factor. To examine novelty preference during the VPC, a 3 (Delay: Imm, 2 min, Day 2) × 2 (group: CON, HII) repeated-measures ANOVA was performed with the within-subjects factor of delay and the between-subjects factor of group. There was no effect of group or interaction between group and delay; however, a main effect of delay was revealed ($F(2, 44) = 5.47$, $p = .008$, $\eta_p^2 = .20$), with infants showing a significantly greater proportion of time on the novel face at the Imm delay ($M = .57$; $SD = .08$) as compared to the 2-min delay ($M = .51$; $SD = .13$; $t(23) = 2.56$, $p = .017$, $d = 1.2$); novelty

preference on Imm was also marginally greater than Day 2 (M = .53; SD = .08; $t(23) = 1.82$, $p = .08$, $d = 0.86$). No significant difference was found between novelty preference at 2 min and Day 2 ($t(23) = .86$, $p = .40$, $d = 0.41$). One-sample t tests revealed that proportion of time on the novel face was significantly different from chance (.50) only for Imm delay ($t(23) = 4.46$, $p < .001$, $d = .91$). This held true for each group individually as well, with significantly more time on the novel face during the Imm delay than would be expected by chance for both CON ($t(17) = 3.27$, $p = .004$, $d = 0.77$) and HII ($t(5) = 3.5$, $p = .017$, $d = 1.42$; see Table 5, for complete details of VPC novelty preference at each delay separated by group).

Event-related potentials

Figures 3 and 4 show grand averaged ERP waveforms of the three faces presented (VPC, recent familiar, and novel) for CON and HII for frontocentral electrodes and temporal electrodes, respectively. The present analyses examined mean amplitude of the Nc and PSW components. Of the 22 infants (16 CON, six HII) who contributed a sufficient number of artifact-free trials during the ERP task, 16 infants (12 CON, four HII) were run with a NetAmps 200 EEG amplifier and the remaining six infants (four CON, two HII) were run with a NetAmps 300 amplifier. An initial omnibus ANOVA examined this between-subjects variable of amplifier on the Nc and PSW, as well as the between-subjects variable of test version. No main effects of amplifier or test version were found for the Nc or PSW mean amplitude analyses at frontocentral electrode sites and temporal electrode sites and subsequent results therefore collapse across these variables.

TABLE 5
Mean Proportion of Time on Novel Face for CON and HII

	CON (n = 18)			*HII (n = 6)*		
	Mean (SD)	*t-Value*	*p-Value*	*Mean (SD)*	*t-Value*	*p-Value*
Imm	.55 (*.07*)	3.27	.004*	.62 (*.08*)	3.50	.017*
2 min	.52 (*.11*)	0.71	.49	.47 (*.19*)	−0.44	.68
Day 2	.53 (*.09*)	1.33	.20	.53 (.06)	1.27	.26

Note. *t*- and *p*-values are results from one-sample *t* test comparing each value to chance (50%). *Denotes significance of $p < .05$. CON = control infants; HII = hypoxic-ischemic injured infants.

Frontocentral electrodes

Nc mean amplitude. To examine the mean amplitude of the Nc component, a 3 (condition: VPC, recent familiar, novel) × 3 (region: Left, middle, right) × 2 (group: CON, HII) repeated-measures ANOVA was run using condition and region as the within-subjects factors and group as the between-subjects factor. There were no significant main effects or interactions for mean amplitude of the Nc component.

PSW mean amplitude. A 3 (condition: VPC, recent familiar, novel) × 3 (region: Left, middle, right) × 2 (group: CON, HII) repeated-measures ANOVA with condition and region as the within-subjects factors and group as the between-subjects factor examined the mean amplitude of the PSW component and found a main effect of region ($F(2, 40) = 10.57$, $p < .001$, $\eta_p^2 = .35$), but no other main effects or interactions. The region effect revealed a greater (more positive) PSW amplitude on the left ($M = 4.92$, $SD = 3.82$) as compared to both the middle region ($M = 2.37$, $SD = 3.43$; $t(21) = 3.04$, $p = .006$, $d = 1.46$) and the region on the right ($M = -.40$, $SD = 4.13$; $t(21) = 3.98$, $p = .001$, $d = 1.91$; see Figure 5). The middle region also showed significantly greater PSW amplitude than the right region ($t(21) = 3.32$, $p = .003$, $d = 1.59$).

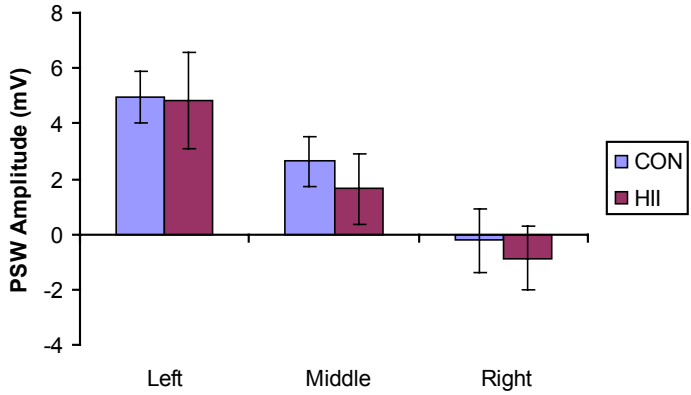

Figure 5 Mean positive slow wave amplitude response for control infants and hypoxic-ischemic injury infants in left, middle, and right frontocentral regions. A main effect of region was found ($p < .001$), with the left region response significantly larger than the middle and right regions ($ps < .01$), and the middle region significantly greater than the right region ($p < .005$). A similar main effect of region was found for temporal electrode sites, with left greater than right ($p = .003$). Error bars ± SEM.

Temporal electrodes

Nc mean amplitude. To examine the mean amplitude of the Nc component in the temporal region, a 3 (condition: VPC, recent familiar, novel) × 2 (region: Left, right) × 2 (group: CON, HII) repeated-measures ANOVA was run using condition and region as the within-subjects factors and group as the between-subjects factor. This analysis revealed a significant interaction between condition and group ($F(2, 40) = 4.12$, $p < .024$, $\eta_p^2 = .17$). Follow-up t tests revealed that for CON, mean amplitude of the Nc did not differ across the three conditions (VPC: $M = -3.98$, $SD = 3.93$; recent familiar: $M = -4.86$, $SD = 4.01$; Novel: $M = -3.59$, $SD = 2.92$; all ps > .14). For HII, the Nc response to the VPC face ($M = -5.03$, $SD = 3.64$) was significantly greater (more negative) than to the recent familiar face ($M = -.58$, $SD = 3.00$; $t(5) = 2.62$, $p = .047$, $d = 1.46$) and marginally greater than to the novel face ($M = -2.93$, $SD = 3.63$; $t(5) = 2.02$, $p = .099$, $d = .63$); Nc responses to recent familiar and novel faces did not differ for HII ($p = .29$). No other main effects or interactions were significant.

PSW mean amplitude. A 3 (condition: VPC, recent familiar, novel) × 2 (region: Left, right) × 2 (group: CON, HII) repeated-measures ANOVA with condition and region as the within-subjects factors and group as the between-subjects factor examined the mean amplitude of the PSW component for the temporal electrode sites and, consistent with results at frontocentral electrode sites, found a main effect of region ($F(1, 20) = 11.15$, $p = .003$, $\eta_p^2 = .36$), with PSW mean amplitude greater (more positive) over the left region ($M = 5.11$, $SD = 4.12$) as compared to the right ($M = -1.42$, $SD = 5.17$),

A main effect of condition was also revealed ($F(2, 40) = 8.84$, $p = .001$, $\eta_p^2 = .31$), with a significantly greater PSW for the recent familiar condition ($M = 3.15$, $SD = 3.67$) as compared to the VPC condition ($M = .93$, $SD = 3.05$; $t(21) = 2.94$, $p = .008$, $d = .67$) and marginally greater responding to the recent familiar as compared to novel ($M = 1.45$, $SD = 2.94$; $t(21) = 1.97$, $p = .063$, $d = .52$). PSW responses to VPC and novel faces did not significantly differ ($p = .5$). A significant interaction between condition and group ($F(2, 40) = 8.84$, $p = .001$, $\eta_p^2 = .31$) was also found. Follow-up t tests revealed that for HII, PSW to the recent familiar condition ($M = 5.56$, $SD = 3.42$) was significantly greater as compared to the VPC ($M = -.10$, $SD = 3.59$; $t(5) = 3.03$, $p = .029$, $d = 1.77$) and marginally greater as compared to novel ($M = 1.13$, $SD = 3.04$; $t(5) = 2.40$, $p = .06$, $d = 1.5$); for CON, PSW to recent familiar ($M = 2.25$, $SD = 3.43$)

was marginally greater than to VPC (M = 1.32, SD = 2.85; $t(15)$ = 1.86, p = .08, d = .3), while there was no difference between PSW to novel (M = 1.57, SD = 2.99) and the other two conditions for CON (ps > .4).

Cross-task analyses: VPC and ERP

A final set of analyses were run to examine the relations between performance on the VPC eye-tracking task and the ERP task for the CON and HII infants. The VPC measures included the proportion of time spent on the novel face at each comparison delay: Imm, 2 min, and Day 2. ERP measures included Nc and PSW amplitude. For the present analyses, Nc variables and PSW variables were each collapsed across condition, then an average Nc (Nc-all), and an average PSW (PSW-all) was calculated from the average for frontocentral electrode sites and temporal electrode sites. Due to the main effect of region found for the PSW in both the frontocentral and the temporal analyses, responses were averaged from left frontocentral electrodes and left temporal electrodes to create a PSW-left variable that focused on the region of highest amplitude. Infants were included in a correlation if they had (1) met minimum criteria for the VPC familiarization, (2) met criteria for inclusion in the ERP analysis, and (3) met minimum criteria for at least one of the three VPC delay conditions (i.e., if an infant spent greater than 30% of the time on the images during Imm test, but not 2 min or Day 2, they would be included in the correlation only for Imm test). Table 6 details the number of infants contributing to each analysis, including the number of infants contributing data to all five sets of analyses (CON = 9, HII = 3).

For CON, correlations were performed examining novelty preference at each comparison delay with the three ERP variables (Nc-all, PSW-all, PSW-left). For the VPC Imm delay condition (13 CON) and the VPC

TABLE 6
Numbers of Subjects Contributing Data to Each Analysis

	VPC analysis	*ERP analysis*	*Correlation ERP/VPC Imm*	*Correlation ERP/VPC 2 min*	*Correlation ERP/VPC Day 2*	*Included in all analyses*
CON	18	16	13	13	12	9
HII	6	6	4	3	6	3

Note. A subset of infants were in ERP analysis as well as at least one correlation, but not in VPC analysis. This subset can be calculated by subtracting the infants included in all analyes from infants included in a given correlation. CON = control infants; HII = hypoxic-ischemic injured infants; VPC = visual paired comparison; ERP = event-related potential.

2-min delay condition (13 CON), no significant relations were found with the ERP measures (*p*s > .37). When examining relations with the VPC Day 2 test (12 CON), a significant positive correlation between novelty preference and PSW-all was found ($r(10) = .73$, $p = .007$; see Figure 6) and a marginal correlation with PSW-left ($r(10) = .51$, $p = .092$).

Correlations for HII infants were not conducted due to limited sample size (3, 4, and 6 infants for Imm, 2 min, and Day 2, respectively). However, we conducted a preliminary analysis to examine the influence of group on these cross-task relations. A univariate ANOVA was conducted for each VPC delay that included novelty preference as the dependent variable with group and PSW-all as potential explanatory variables. For novelty preference, the model showed no main effects or interactions when the dependent variable was VPC Imm (*p*s > .24) and VPC 2 min (*p*s > .84). In the model using Day 2 VPC novelty preference as the dependent variable, an interaction between group and PSW-all was found ($F(1, 14) = 4.60$, $p = .05$, $\eta_p^2 = .25$), suggesting that the relation between PSW mean amplitude and Day 2 novelty preference is different for the two groups. Figure 6 shows the relation between Day 2 VPC novelty preference and PSW amplitude across all regions (PSW-all) for both HII and CON.

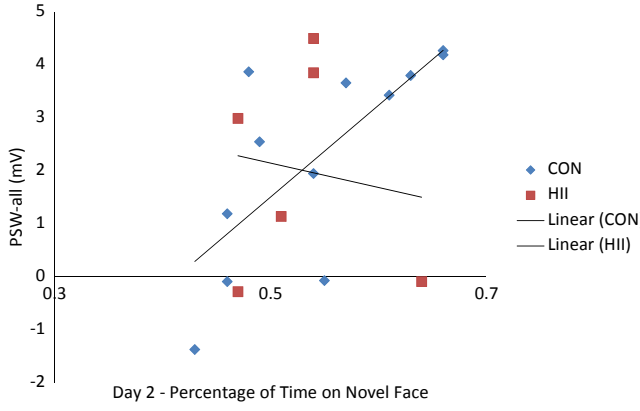

Figure 6 Correlation between visual paired comparison Day 2 novelty preference (proportion of time spent on novel face) and mean amplitude of the positive slow wave (PSW) component across all electrode sites (the mean for PSW over frontocentral electrode sites and the mean for PSW over temporal electrode sites were averaged together to obtain this PSW-all component). Control infants showed a significant positive relation between these variables ($r = .73$, $p = .007$) while hypoxic-ischemic injury showed no relation between these variables ($r = -.16$, $p = .76$).

DISCUSSION

The present study examined behavioral and electrophysiological measures of memory independently as well as in relation to one another in 12-month-old typically developing infants and a small group of infants with a history of perinatal HII. Our analyses revealed five major findings: (1) HII and CON show similar behavioral indices of memory as indexed by VPC novelty preference across three delays, (2) PSW responses were greatest over left scalp regions, (3) over temporal electrode sites HII infants show differential patterns of Nc responses to the three faces as compared to CON, (4) at temporal electrode sites, the PSW showed largest responses to the recent familiar face condition, and (5) in examining the relation between the VPC and ERP measures, CON showed a significant positive correlation between VPC novelty preference after a 24-h delay and PSW mean amplitude.

The first two findings mentioned demonstrate the similarities found between infants who have experienced HII and typically developing infants in the present study. With regard to the VPC task, both groups exhibit a VPC novelty preference only when tested immediately after familiarization but not after a 2-min or 24-h delay. This result is similar to the findings of Morgan and Hayne (2011), who used 3D pictures of cartoon-like faces, and also showed that 1-year-olds exhibited a VPC novelty preference immediately after familiarization but not after 24-h delay. Furthermore, they found it was not until age 2 years when their participants exhibited novelty preference after 24-h delay; their study did not evaluate a 2-min delay. In contrast to our findings, studies on younger infants using slightly different testing methods than our own found novelty preference after varying time delays. One study, which similarly used pictures of female faces but differed in their familiarization methods, found that 6-month-olds exhibited a novelty preference after both a 2-min and 24-h delay (Pascalis et al., 1998). Another study, which used pictures of black-and-white sunburst and diamond patterns, found that 4-month-olds exhibited a novelty preference after a short delay lasting approximately the length of a feeding (Geva et al., 1999). It is difficult to compare these studies, as their VPC testing methods were slightly different from one another and from our own, but based on our study and that of Morgan and Hayne (2011), 12-months-old infants appear to demonstrate visual recognition memory retention on behavioral testing of less than 2 min.

A second finding that showed no group differences was greater PSW mean amplitude over the left region. For the temporal electrode sites, this meant greater PSW over the left as compared to the right region, and for the frontocentral electrode sites, greater PSW over left as compared to

right and middle regions. The regionalization of PSW to the left or right hemisphere has been under debate in prior studies. The PSW regionalization finding in our study is consistent with one prior study that found when 6-month-olds viewed pictures of unfamiliar faces, their PSW was more positive in the left than right hemisphere (de Haan & Nelson, 1997). However, other studies showed slightly different findings: A study of 6- and 7.5-month-old infants found a greater PSW amplitude at right temporal and midline frontal regions when viewing pictures of novel as compared to familiar objects (Reynolds, Guy & Zhang 2010); another study of 6-month-olds showed no difference in PSW amplitude between hemispheres when viewing pictures of both familiar and unfamiliar faces (de Haan & Nelson, 1999); a third study of 6-month-olds demonstrated a PSW localized only over the right hemisphere when viewing upright faces (de Haan et al., 2003). Thus, there remains some controversy surrounding regional localization of the PSW during face processing, and future work should continue to explore these hemispheric differences.

In the ERP analyses focused on frontocentral electrode sites, the present study found no influence of group or condition on Nc and PSW amplitude. On the other hand, ERP analyses focused on temporal sites revealed several significant findings relating to both group and condition for both components. Mean amplitude for Nc was similar for the VPC, recent familiar, and novel face for CON, but in contrast, HII showed a diminished Nc response to the recent familiar face as compared to the VPC face. With greater Nc thought to reflect greater attention (Nelson & McCleery, 2008), this suggests that HII might devote less attentional processing to the recent familiar face, the face they were familiarized to just before the ERP session, as compared to the VPC face. This diminished attention in relation to other stimuli in HII as compared to the consistent attention across conditions in CON necessitates further study, but suggests an atypical pattern of attention to familiar and unfamiliar stimuli in the HII group.

Positive slow wave analyses over temporal electrode sites revealed a main effect of condition, with greater responses to recent familiar as compared with VPC and novel faces. Past work has identified a role for the PSW in memory updating (Nelson & McCleery, 2008), and the larger PSW in the present analysis could reflect that the recent familiar face is the most remembered face for these 12-month-olds. This finding is consistent with the current VPC findings, as on Day 2, neither HII nor CON show a novelty preference during the VPC, suggesting that their memory for the VPC face was not strong on Day 2, the day of ERP testing. Thus, infants might show the greatest PSW to the recent familiar face while

treating the VPC and novel face as new and not remembered. On a group level, both HII and CON showed greater PSW responding to the recent familiar face as compared to the VPC face, but this difference was more pronounced for HII.

Although two prior studies reported significant relations between VPC performance and ERP responses (Reynolds, Courage, & Richards, 2010; Snyder, 2010), the present study is the first to show that in 12-month-old typically developing infants, greater VPC novelty preference after a 24-h delay correlates with greater PSW mean amplitude, despite these infants not exhibiting a significant novelty preference at this delay. One explanation could be that the cortical processes that are actively working to update the familiar stimulus in their memory represent enhanced memory processes that could be seen in the VPC as well, that is, a more robust or greater PSW as the reflection of memory updating could relate to a greater novelty preference. However, as a group, memory for the familiar stimulus after a 24-h delay is not yet solidified to the point that it is visible on behavioral testing alone. Although the HII sample was too small for testing similar relations, a preliminary analysis revealed that group and PSW interact to influence Day 2 novelty preference, suggesting that different mechanisms might be underlying the relations between behavioral and electrophysiological measures of memory in the two groups.

While prior studies have found both adolescents and adults with a history of early HII to be impaired on measures of visual recognition memory, delayed recall, and tests of attention and executive function (Maneru, Junque, Botet, Tallada, & Guardia, 2001; Vargha-Khadem et al., 1997), the present preliminary findings for this group of infants experiencing mild-to-moderate HII suggest that while behaviorally (both on the VPC and on standardized cognitive assessment), these infants do not differ from typically developing infants at 12 months, the underlying neural mechanisms for memory and attention might be atypical. However, despite this pattern of similarities and differences between groups in the present study, an important set of limitations must be considered. First and foremost, the HII results need to be interpreted with caution due to the small sample size. To increase the power of the present statistics for the VPC and ERP, a larger sample size is needed that can help elucidate how these tasks might differ as a function of perinatal HII. Further, perinatal HII is not a homogenous experience, as can be surmised from Table 1, and therefore, a further limitation of the present work is that it was unable to more precisely group these infants into potentially meaningful subgroups, such as separating infants who did or did not undergo therapeutic hypothermia shortly after birth. With these limitations in mind, future work with this

important population of infants is needed to expand the present findings and further explore the neural mechanisms underlying memory that might develop differently as a result of perinatal HII.

In conclusion, the present work with 12-month-old typically developing infants and infants experiencing HII revealed similar behavioral markers of memory, and similar electrophysiological markers of memory and attention in frontocentral electrode sites, alongside differential attentional processing in temporal sites. Additionally, while typically developing infants showed a positive relation between novelty preference at the longest delay and PSW responses, preliminary analyses reveal that infants experiencing HII show a different pattern. Taken together, this work highlights the benefit of evaluating behavioral recognition memory in conjunction with ERP responses in hopes of revealing more subtle differences in memory and attentional processing in both HII and typically developing infants. Future work studying early infant memory should continue this approach, examining behavioral and brain responses independently as well as side by side, to better understand brain–behavior relations during development.

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