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The Ontogeny of Human Learning in Delay, Long-Delay, and Trace Eyeblink Conditioning

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The ontogeny of associative learning in delay (750-ms conditional stimulus [CS], 650-ms interstimulus interval [ISI]), long-delay (1,350-ms CS, 1,250-ms ISI), and trace (750-ms CS, 500-ms trace interval, 1,250-ms ISI) eyeblink conditioning was examined in 5-month-old human infants and adults. Infants and adults showed different acquisition rates but reached equivalent asymptotes of conditional responses (CRs) in standard delay conditioning. In long-delay and trace conditions, infants exhibited less robust conditioning than adults and minimal ability to appropriately time CRs. During infancy, the ISI, rather than the conditioning procedure, predicted rate and effectiveness of CRs. These findings suggest that higher order cognitive abilities begin emerging early in development. Across ontogeny, however, there are changes in the limits and parameters that support associative learning.

In humans and animals, learning performance in eyeblink conditioning procedures is sensitive to the effects of postnatal development (e.g., Ivkovich, Paczkowski, & Stanton, 2000; Little, Lipsitt, & Rovee-Collier, 1984; Nicholson & Freeman, 2000; Stanton, 2000) and aging (e.g., Coffin & Woodruff-Pak, 1993; Graves & Solomon, 1985; Powell, Buchanan, & Hernandez, 1981; Weiss & Thompson, 1991; Woodruff-Pak, Lavond, Logan, & Thompson, 1987). Furthermore, changes in learning performance in these procedures have functioned as indicators of possible changes in brain functioning accompanying human disorders such as early Alzheimer's (Solomon & Pendlebury, 1988; Woodruff-Pak, 1988), severe mental retardation (Ohlrich & Ross, 1968; Ross, 1972), autism (Sears, Finn, & Steinmetz, 1994; Sears & Steinmetz, 2000), dyslexia (Coffin & Boegle, 2000), and premature birth (Herbert, Eckerman, Goldstein, & Stanton, in press). Together, these data suggest that eyeblink conditioning procedures can facilitate one's understanding of changes in learning and memory across ontogeny and, in the context of the well-defined neural circuitry responsible for the behaviorally observed learning (e.g., Stanton, 2000; Stanton & Freeman, 2000; Steinmetz, 2000; R. F. Thompson, 1986; Woodruff-Pak & Steinmetz, 2000a, 2000b), can facilitate the formation of hypotheses concerning the maturation of underlying neural structures and circuitry that might be responsible for these developmental changes.

In delay conditioning, a conditional stimulus (CS) overlaps and coterminates with a response-eliciting unconditional stimulus (US). Acquisition of standard delay conditioning is possible without involving any brain regions above the level of the cerebellum. Damage to the cerebellum or its associated circuitry yields deficits in the ability of humans to form associations in delay eyeblink conditioning (e.g., Daum et al., 1993; Solomon, Stowe, & Pendlebury, 1989; Topka, Valls-Sole, Massaquoi, & Hallett, 1993; Woodruff-Pak, Papka, & Ivry, 1996), whereas damage to the bilateral hippocampus does not (Gabrieli et al., 1995). In adult rabbits, cerebellar lesions can prevent the acquisition of delay conditioning or abolish a previously acquired delay conditional response (CR; e.g., Lincoln, McCormick, & Thompson, 1982; McCormick et al., 1981; for recent reviews, see Steinmetz, 2001; Woodruff-Pak & Lemieux, 2001). Similarly, developmental studies with infant rats have determined that delay eyeblink conditioning emerges postnatally and is due to maturation of the cerebellum (Stanton & Freeman, 2000). Learning in this procedure fails to develop normally in rats subjected neonatally to localized cerebellar lesions (Freeman, Barone, & Stanton, 1995) or to antimitotic agents that disrupt cerebellar maturation (Freeman, Carter, & Stanton, 1995).

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In trace conditioning, a stimulus-free "trace" interval is incorporated between the CS and the US. This temporal separation between the stimuli requires the organism to maintain a memory "trace" of the CS to form an association with the US. Acquisition of trace conditioning appears to require the cerebellum and the additional involvement of higher cortical structures such as the hippocampus and prefrontal cortex (e.g., James, Hardiman, & Yeo, 1987; Moyer, Deyo, & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson, & Weisz, 1986; Weible, McEchron, & Disterhoft, 2000). Stimulation of the hippocampus can facilitate trace conditioning (Prokasy, Kesner, & Calder, 1983), whereas hippocampal lesions produce deficits in the acquisition of trace conditioning (e.g., Moyer et al., 1990; Port, Romano, Steinmetz, Mikhail, & Patterson, 1986; Solomon et al., 1986). In adult humans with temporal lobe amnesia, the acquisition of trace conditioning also appears to be impaired (Clark & Squire, 1998; McGlincheyBerroth, Carrillo, Gabrieli, Brawn, & Disterhoft, 1997; Woodruff-Pak, 1993).

Thus, comparisons of acquisition on delay and trace conditioning have the potential to facilitate one's understanding of the maturation of cerebellar and hippocampal circuitry across human ontogeny. To date, there has been some suggestion of age-related changes in the limits and parameters that support associative learning in these procedures at different ages. College-age adults exhibit no significant differences in levels of conditioning obtained under trace and delay procedures (e.g., Ross & Ross, 1971), whereas 4- to 6-year-old children acquire higher levels of conditioning in delay compared with trace procedures (Werden & Ross, 1972). At 2 months of age, infants are able to learn a delay CR after extensive training, but they are unable to learn the trace CR (Little, 1973). Although these comparisons are made across procedural variations including CS duration, trace interval, and number of training trials, they provide some suggestion that performance differences in delay and trace conditioning can help assess changes in neurocognitive functioning across human development.

Previously, we have shown that infants' learning of delay conditioning is particularly sensitive to changes in the interstimulus interval (ISI) between tone onset and airpuff onset (Claflin, Stanton, Herbert, Greer, & Eckerman, 2002; Ivkovich, Paczkowski, & Stanton, 2000; Ivkovich & Stanton, 2001). At 5 months of age, human infants exhibit optimal learning when the delay interval is 650 ms in duration and fail to exhibit delay conditioning at much shorter ISIs (250 ms). At longer ISIs (1,250 ms), which have typically been a feature of trace conditioning, infants show only minimal learning of well-timed CRs following two 50-trial training sessions (Claflin et al., 2002). The sharp ISI function for 5-month-old infants during delay conditioning is in stark contrast to younger infants who only exhibit learning in delay conditioning at extremely long ISIs (1,500 ms; Little et al., 1984) and adults who exhibit learning in delay conditioning across a broad range of ISIs (e.g., Kimble, 1947; Ross & Ross, 1971). It is therefore possible that the inability to learn effectively at long ISIs contributes, at least in part, to the inability of very young infants to learn in trace conditioning. This possibility has been supported in research with developing rats, in which long-delay and trace conditioning (matched in ISI interval) emerge later in postnatal development (postnatal day [PND] 30) than standard delay conditioning (PND 23; Ivkovich, Paczkowski, & Stanton, 2000). However, although early hippocampal lesions (PND 10) impair the subsequent acquisition of both long-delay and trace conditioning at PND 25 when performance typically begins to develop, trace conditioning is much more impaired than long-delay conditioning (Ivkovich & Stanton, 2001). These findings suggest a relative contribution of both the ISI and the short-term memory component in the acquisition of trace conditioning early in development.

Our developmental research to date has highlighted the importance of examining the emergence of delay and trace conditioning at matched ISIs to provide independent confirmation of differential development of responding on the basis of stimulus overlap (delay conditioning) compared with a stimulus-free interval (trace conditioning). Here, in two experiments, we examine the performance of human adults and 5-month-old infants on standard delay, long-delay, and trace eyeblink conditioning. Experiment 1 focuses on the acquisition of standard delay eyeblink conditioning. This experiment provides an age-related comparison on the simplest associative learning procedure and provides a basis for interpreting any subsequent differences that might emerge in learning performance in Experiment 2, when infants and adults are tested on long-delay and trace eyeblink conditioning. Together these two studies compare and contrast the behavioral expression of learning across three conditioning procedures in infants and adults and provide the opportunity to consider the underlying neural maturation that may affect learning performance across human development.

Experiment 1

The purpose of this experiment was to compare the acquisition pattern of human adults and 5-month-old infants in standard delay conditioning. Comparisons of delay eyeblink conditioning across laboratories and experimental designs provide some suggestion that there may be a gradual decrease in optimal ISI across ontogeny. Learning is optimal at ISIs of 1,500 ms in 10- to 30-day-old infants (Little et al., 1984), 650 ms in 6-month-old infants (Claflin et al., 2002), 800 ms in 4- to 6-year-old children (Werden & Ross, 1972), and 500 ms in adults (Kimble, 1947). In this experiment we provide a direct comparison of the rate of acquisition and response timing in basic associative learning during infancy and adulthood by providing virtually identical standard delay conditioning paradigms at both ages.

Method

Participants. The participants were 15 (7 male and 8 female) undergraduate students enrolled in introductory psychology classes at Duke University (average age = 18.6 years; range = 18–20 years) and 14 (9 male and 5 female) 5-month-old (± 10 days) healthy full-term infants.[1] Infants and their parents were recruited by mail from local county birth records and were primarily from non-Hispanic White families (89%). In 97% of the families, both parents had graduated from high school, and in 73% of the families, both parents had completed 4 or more years of college.

Participants were randomly assigned to the standard delay group ($n = 10$ infants, 8 adults) or the unpaired control group ($n = 4$ infants, 7 adults per

[1] Data from infants in the standard delay condition and the Unpaired 650 control condition (see later) were reported in Claflin et al. (2002).

Table 1
Number of Participants and Gender Distribution in Each Session of Experiments 1 and 2 as a Function of Conditioning Group

	Experiment 1	Experiment 2	
Condition	Sessions 1 and 2	Sessions 1 and 2	Session 3
Infant			
Delay 650	10; 6 m, 4 f	—	—
Unpaired 650/trace	4; 3 m, 1 f	4; 3 m, 1 f	2; 1 m, 1 f
Long-delay	—	10; 6 m, 4 f	7; 3 m, 4 f
Trace	—	10; 6 m, 4 f	7; 4 m, 3 f
Unpaired 1,250	—	4; 2 m, 2 f	2; 1 m, 1 f
Adult			
Delay 650	8; 4 m, 4 f	—	—
Unpaired 650/trace	8; 4 m, 4 f	8; 4 m, 4 f	—
Long-delay	—	8; 4 m, 4 f	—
Trace	—	8; 5 m, 3 f	—
Unpaired 1,250	—	7; 3 m, 4 f	—

Note. Dashes indicate groups or sessions that were not conducted for each experiment. m = male; f = female.

group)[2] and experienced two conditioning sessions 6−8 days apart. Number of participants and gender distribution per group in Experiment 1 are presented in Table 1. An additional 4 adults and 9 infants were excluded from the final analysis because of the following reasons: They failed to meet the criterion number of conditioning trials ($n = 6$ infants), they were unresponsive to the airpuff US (the mean percentage of nonresponding on US-alone trials exceeded 43% across the two sessions; $n = 1$ infant), the data were not usable as a result of technical difficulties ($n = 4$ adults), or there were rescheduling conflicts that precluded the completion of all sessions ($n = 2$ infants). Chi-square analysis revealed no evidence of selective attrition based on gender, ethnic origin, or conditioning group.

Procedure. The general procedure was identical to that used in our prior studies of infant eyeblink conditioning (for full method details, see Ivkovich, Collins, Eckerman, Krasnegor, & Stanton, 1999; Ivkovich, Eckerman, Krasnegor, & Stanton, 2000). Infant participants were seated on a parent's lap and were entertained by a visual display of brightly colored moving objects. Adult participants sat in a chair and were entertained by a video (*Milo and Otis*) presented without sound on a TV screen. A custom-built eyeblink conditioning system (U.S. Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, NC) controlled presentation of the tone CS (1 kHz, 80 dB), delivered through two small 7-Ohm speakers positioned to the left and right above the participant's head, and the airpuff US (approximately 1/20 lb/in.^{2}) delivered to the participant's right eye. A camera positioned about 1 m to the front and right of the participant yielded a video record of the participant's head, and a signal box with a trial counter and lights illuminated to indicate the presentation of the tone and airpuff stimuli.

Paired training sessions. Paired training trials consisted of a 750-ms tone CS preceding, overlapping, and coterminating with a 100-ms airpuff US, yielding a 650-ms delay interval ("Delay 650"; see Figure 1, top panel). The intertrial interval varied from 8 to 16 s (average 12 s). Every 6th trial in a block of 10, as well as Trial 1 and 2 at the start of the session, was an air-alone trial to test for somatosensory responsiveness. Every 10th trial was a tone-alone trial to test for conditional responding. A maximum of 50 trials (38 CS–US pairings) were presented for paired sessions (about 12−15 min); however, the total number of trials per session was determined by the participant's cooperativeness. Participants were required to reach a criterion of at least 32 trials during each paired training session to be included in the present analyses.

Unpaired training sessions. During unpaired training sessions, participants experienced the same 43 tones and 45 airpuffs as used for the paired training session, but the stimuli were presented explicitly unpaired, 4−8 s apart (average 6 s) in a manner that matched the paired condition for stimulus density. This group served to control for nonassociative increases in responding to the tone. Participants were required to reach a criterion of at least 64 trials during unpaired training to be included in the present analyses.

Response measures. Frame-by-frame video coding was used to evaluate each trial for the occurrence and timing of a blink.[3] A blink occurring after the airpuff, within 1 to 30 video frames (500 ms) after airpuff onset, was considered a UR. CRs included any blink occurring at least 300 ms after tone onset and prior to airpuff onset. Adaptive CRs included only those blinks initiated within 21 frames (350 ms) prior to airpuff onset. The adaptive CR measure provides a conservative measure of conditional responding by including only those responses that are well timed to reduce the impact of the upcoming airpuff (see Claflin et al., 2002; Ivkovich, Paczkowski, & Stanton, 2000) and eliminating voluntary responses that potentially occur early in the CR period (Spence & Ross, 1959). On trials when no airpuff was presented (tone-alone test trials), any response within the comparable CR and UR periods combined was considered a CR. Responses that occurred in the first 300 ms after the tone onset were coded as alpha, or startle, responses (SR). These responses were considered to be either reflexive reactions to the tone or voluntary eyeblink responses that are often observed in human adult eyeblink conditioning. The 300-ms alpha period used in the present study is consistent with the alpha period we have used in our previous studies of human infant eyeblink condition-

[2] As in our previous research with 5-month-old human infants (Claflin et al., 2002; Herbert et al., in press), we included only a small number of participants in the unpaired control conditions. This reflects the consistently low levels of "conditional" responding produced by infants in nonassociative groups.

[3] Electromyographic (EMG) records of eyeblinks were also recorded to enable direct age comparisons in motor performance and somatosensory effectiveness. In general, however, EMG recordings were sensitive to head and facial movements, such as sucking and chewing by infants, and showed large intersubject variability in signal-to-noise ratio. Measurement errors may also have arisen from age differences in muscle mass or skin conductance, contributing to any observed age differences in CR or unconditional response (UR) amplitudes. We therefore used only latency measures analyzed from video records to determine whether there were differences in performance across age and group that could contribute to differences in learning.

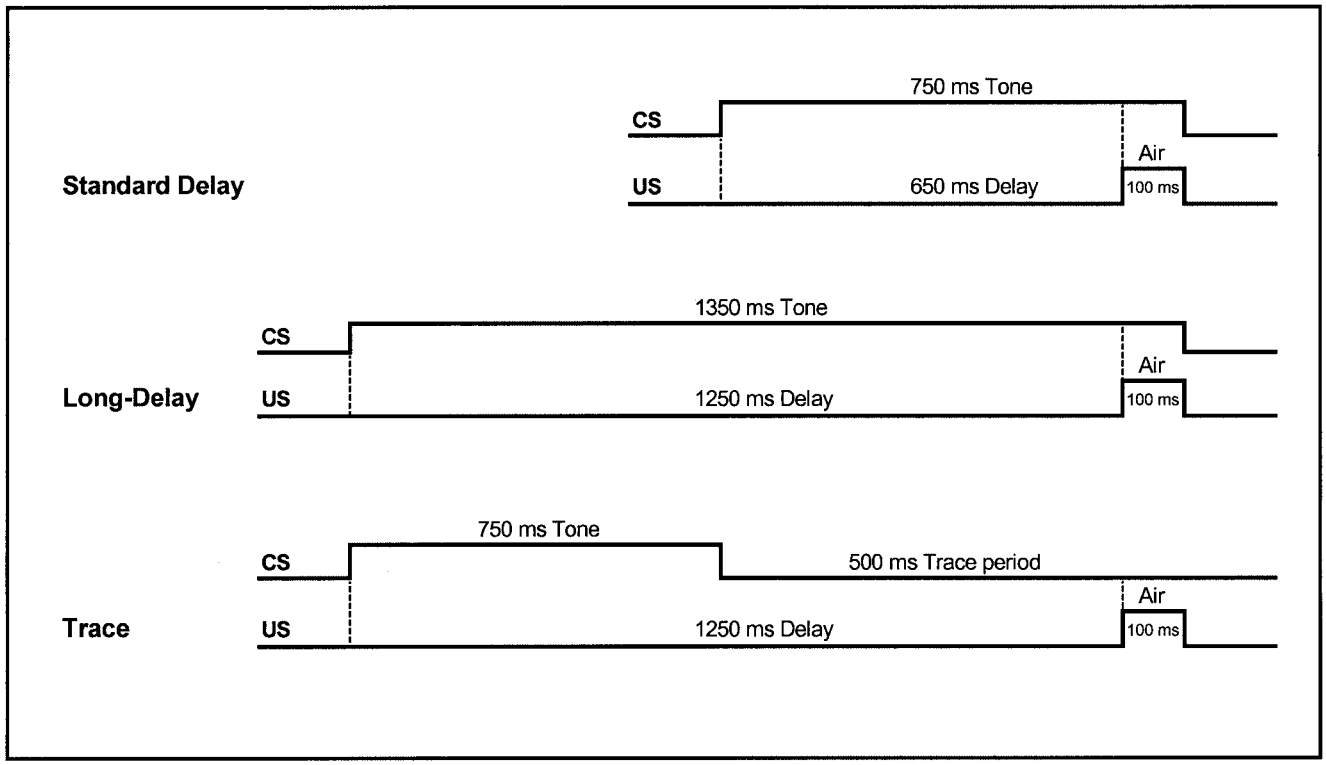

Figure 1. Experimental design for Delay 650 (standard delay; Experiment 1), long-delay, and trace (Experiment 2) paired training procedures. CS = conditional stimulus; US = unconditional stimulus.

ing (Claflin et al., 2002; Ivkovich et al., 1999; Ivkovich, Eckerman, Krasnegor, & Stanton, 2000). Although the traditional duration of the alpha period in studies with human adults is 100−200 ms (e.g., Gabrieli et al., 1995; McGlinchey-Berroth et al., 1997; Woodruff-Pak, 1993), the increased duration in our developmental studies reflects the potentially slower response latencies of infants. Definition and scoring of CRs in unpaired participants was exactly analogous to scoring of paired participants. For unpaired trials corresponding (by trial sequence) to paired trials, the 650-ms CS period was divided into a 300-ms alpha period followed by a 350-ms CR period (designated "Unpaired 650"). For unpaired trials corresponding to CS-alone test trials, the combined CS and US periods were used.

If it was not possible to make a determination because the participant's right eye was out of view, the trial was treated as uncodable and excluded from analyses. A second independent observer established video coding reliability on 70% of the sessions. Percentage agreement by the two observers on CR and UR responses within one frame was 95% and within two frames was 99%.

The primary measure of learning was the percentage of CRs across training sessions. For paired sessions, the percentage of CRs was calculated for blocks of six paired trials for a minimum of six blocks. For unpaired training sessions, the percentage of CRs was based on six-trial blocks of corresponding tone-alone trials. Average CR and UR onset latencies for each block also were obtained from the frame-by-frame video codings (16.7 ms/frame). In a small number of instances (5 out of 168 total observations) with infants, data from a particular trial block were missing for a particular participant. In these rare instances, the average of the preceding and succeeding block was substituted for the missing data. Data were analyzed using mixed analysis of variance (ANOVA). Post hoc Newman–Keuls comparisons were performed as needed.

Results

Infant and adult learning in standard delay conditioning. Infants and adults demonstrated robust conditioning across two sessions compared with the performance of their unpaired control groups. At both ages, CRs emerged across Session 1 and reached an asymptote during Session 2. Figure 2 suggests that adults exhibited more rapid learning than infants in Session 1 but that there were no age-related differences in the asymptote of learning. These observations were supported by a 2 (infant vs. adult) × 2 (Delay 650 vs. Unpaired 650) × 2 (session) × 6 (blocks) repeated measures ANOVA of percentage CRs. This ANOVA revealed significant three-way interactions of Session × Block × Pairing, $F(5, 125) = 2.98$, $p < .05$, and Session × Age × Pairing, $F(1, 25) = 4.30$, $p < .05$. Post hoc (Newman–Keuls) analysis of the Session × Block × Pairing interaction revealed that higher levels of percentage CRs emerged in paired groups compared with unpaired groups during Session 1 from Block 3 onward (all ps $<$ .05). Higher levels of percentage CRs by paired groups compared with unpaired groups were maintained throughout all blocks of Session 2 (all ps $< .001$). Analysis of the Session × Age × Pairing interaction showed that, collapsed across blocks, adults who experienced paired training produced consistently higher levels of CRs than unpaired groups in Session 1 (ps $< .05$). In contrast, infants who experienced paired training failed to show significantly higher levels of CRs in Session 1 compared with infants and adults in unpaired groups (ps $> .1$), although their level of CR production was not significantly different from adults in paired

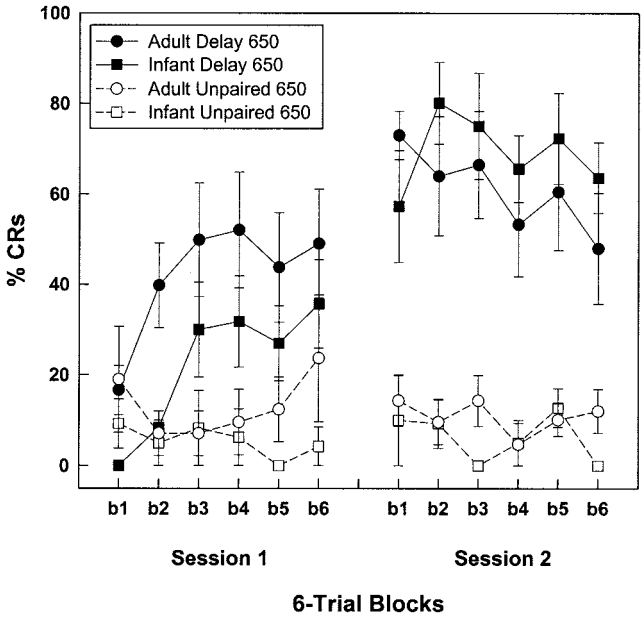

Figure 2. Mean (± *SEM*) percent adaptive responses for 5-month-old infants and adults. Data are presented as a function of six-trial blocks (b) across two training sessions. CRs = conditional responses.

groups ($p > .08$). In Session 2, the level of responding by both infants and adults in paired groups was significantly higher than infants and adults in unpaired groups ($ps < .001$).

UR onset latency was analyzed at both ages to identify whether differences in conditional responding across paired and unpaired groups were secondary to differences in US efficacy or motor performance. No such differences were found when paired and unpaired groups were compared. Overall, infant URs were initiated later on US-alone trials than adult URs. Mean (± *SE*) UR onset latency on US-alone trials for the infant standard delay condition was 126.1 (± 18.8) ms, and for the unpaired group was 163.4 (± 24.3) ms. Mean (± *SE*) UR latency for the adult standard delay group was 61.9 (± 2.5) ms, and for the unpaired group was 73.8 (± 10.8) ms. A 2 (infant vs. adult) × 2 (Delay 650 vs. Unpaired 650) ANOVA on UR onset latency failed to find a main effect or interaction involving pairing ($Fs < 2.22$). However, there was a significant main effect of age (adults < infants), $F(1, 28) = 21.63$, $p < .001$.

Measures of mean UR blink duration from onset to full closure on US-alone trials also revealed age but not pairing differences. Mean (± *SE*) UR duration from onset to full closure for the infant standard delay condition was 66.9 (± 5.0) ms, and for the unpaired group was 74.7 (± 9.1) ms. Mean (± *SE*) UR duration for the adult standard delay group was 46.2 (± 3.3) ms, and for the unpaired group was 40.8 (± 2.9) ms. A 2 (infant vs. adult) × 2 (Delay 650 vs. Unpaired 650) ANOVA on UR duration from onset to full closure failed to find a main effect or interaction involving pairing ($Fs < 1.7$). However, there was a significant main effect of age, $F(1, 28) = 28.53$, $p < .001$, with infants producing URs that were longer in duration from onset to full closure than adult URs.

This age difference in UR onset latency and duration could reflect a difference in US efficacy or motor performance across age. This possibility was not borne out in the percentage CR measure, however, because there were no age differences in the asymptotic level of learning (CRs) in the paired condition or in baseline responding in the unpaired condition. In this study all participants were conditioned using the same intensity airpuff. Although our previous research has suggested that at 4−5 months of age the amplitude of the UR is not predictive of learning rate (Ivkovich et al., 1999), additional studies manipulating airpuff intensity are needed to directly address the possibility. In any case, there were no differences in performance between the paired and unpaired groups, within each age, that could contribute to differences in learning observed in the delay condition.

Comparing infant and adult CR timing. We have previously observed that 5-month-old infants show alterations in CR timing in long-delay procedures that are not obvious in the CR percentage measure (Claflin et al., 2002). In the present analysis we assessed the possibility that alterations in the onset latency of CRs may also occur in standard delay conditioning and that there may be age differences in this effect. The sampling period in this descriptive analysis incorporated both the alpha (SR or orienting period) and the adaptive CR period for both the paired and unpaired conditions (see *Method* section above).

The distribution of responses in each group was determined by dividing the period from CS onset to US onset into thirteen 50-ms bins. The mean number of responses initiated in each response bin is presented as a function of age, condition, and session in Figure 3.

Comparisons of the adaptive period (the final 350 ms of the trial epoch) generally support the findings from the percentage CR analysis (see earlier). Onset of adaptive CRs by infants in the paired group typically occurred early in adaptive period (the final 350 ms of the trial epoch) and increased in number between Session 1 and Session 2. Onset of adaptive CRs by adults in the paired condition were well distributed across the adaptive period but were at the highest frequency late in the adaptive period, closer to the time of US onset. In the alpha period (the first 300 ms of the trial epoch), infants initiated few responses in either the paired or the unpaired group. In comparison, adults in both groups produced alpha responses across both sessions.

A 2 (infant vs. adult) × 2 (paired vs. unpaired) × 2 (Session 1 vs. Session 2) × 13 (50-ms CR bins) repeated measures ANOVA of CRs confirmed that there were significant interactions of Age × Pairing, $F(1, 25) = 12.60$, $p < .01$; Session × Pairing, $F(1, 25) = 17.80$, $p < .001$; Age × Bin, $F(12, 300) = 2.90$, $p < .001$; and Pairing × Bin, $F(12, 300) = 6.32$, $p < .0001$. The Age × Session × Bin interaction also approached significance, $F(12, 300) = 1.65$, $p < .08$. Post hoc tests of the Age × Bin interaction confirmed that adults responded much more than infants during bins −650 ms, −600 ms, −550 ms, and −400 ms of the alpha period ($p < .01$). Age differences in the adaptive CR period were observed only in the final bin of the trial epoch (bin −50 ms, $p < .05$).

The large effect of pairing in the above analyses was consistent with the observation that only the groups experiencing paired conditioning initiated responses in the adaptive period (see Figure 3). Therefore, a subsequent 2 (infant vs. adult) × 2 (Session 1 vs. 2) × 13 (50-ms CR bins) repeated measures ANOVA was performed to analyze the timing of CRs across only the paired conditions. This ANOVA confirmed a significant Age × Session × Bin interaction, $F(12, 192) = 2.61$, $p < .01$. Post hoc Newman–Keuls of this interaction revealed that the differences

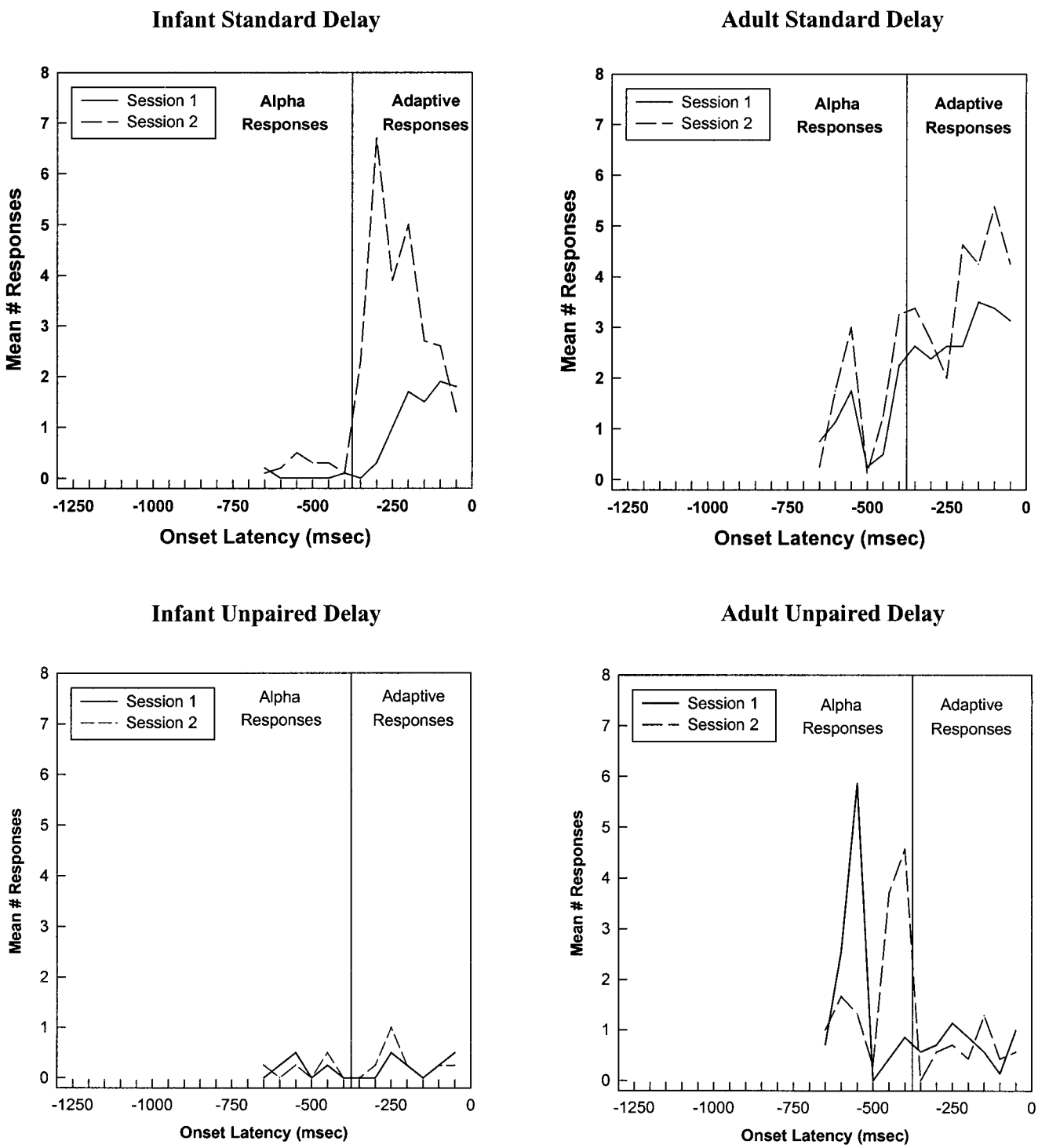

Figure 3. Mean distribution of the onset latency for responses during the conditional response period for infants and adults in the Delay 650 (standard delay) and Unpaired 650 conditions, as a function of training session. Tone onset occurs at −650 ms. Airpuff onset occurs at 0 ms. (Latencies of up to −1,250 ms are plotted for comparison with Figures 5 and 6.)

between infants and adults during Session 1 were present in most alpha bins (−650 ms, $p < .06$; −600 ms, $p < .06$; −550 ms, $p < .01$; −450 ms, $p < .06$; −400 ms, $p < .01$) and in early adaptive bins (−350 ms, $p < .05$; −300 ms, $p < .05$). During Session 2, adults initiated significantly more responding than infants in only two alpha bins (−600 ms, $p < .06$; −400 ms, $p < .01$) and in the final adaptive bin prior to airpuff onset (−50 ms, $p < .001$), reflecting an increase in infant responding across sessions.

Finally, we examined the timing of SRs that were a feature of adult responding that was not observed in the infant conditions. A 2 (paired vs. unpaired) × 2 (Session 1 vs. 2) × 13 (50-ms CR bins) repeated measure ANOVA of adult responding confirmed significant main effects of pairing, $F(1, 13) = 43.66$, $p < .0001$, and bins, $F(12, 156) = 3.86$, $p < .0001$, but not session, $F(1, 13) = 3.11$, *ns*. There was also a significant Bin × Pairing interaction, $F(12, 156) = 4.38$, $p < .0001$. Post hoc Newman–Keuls of this interaction revealed that differences between adult groups reflected high levels of SRs in both the paired and unpaired groups followed by high levels of adaptive CRs only in the paired group. There were no significant differences between the mean number of CRs initiated in the paired and unpaired groups during the early alpha period (−650 ms to −450 ms, *p*s >.1), demonstrating that these SRs were not a product of learning per se. The paired group subsequently produced significantly higher levels of responding in almost all remaining alpha and CR bins (all *p*s < .05, except −250-ms bin, $p < .09$). The largest differences in CR initiation between the paired and unpaired group were observed in the final bins of the adaptive period (−100 ms, $p < .001$; −50 ms, $p < .001$), supporting the conclusion that adaptive CRs by adults in the paired condition were well timed to coincide with airpuff onset.

As in our previous research with human infants, we have used the onset of the blink response rather than peak latency as our measure of CR timing. As a final analysis of differences in conditional responding across age, we examined the duration of CRs from onset to full closure, produced at both ages on paired trials and matched unpaired trials. A 2 (infant vs. adult) × 2 (paired vs. unpaired) ANOVA on CR duration from onset to full closure failed to find a main effect or interaction involving age (infant = adult, *F*s < 1.46). However, there was a significant main effect of pairing, $F(1, 26) = 5.98$, $p < .05$. Overall, the onset to full-closure duration of CRs was longer in paired conditions ($M = 78.69$, $SE = 5.0$) than in unpaired conditions ($M = 56.16$, $SE = 6.5$). On the basis of CR duration findings, we can conclude that although slightly different estimates of percentage CRs will be obtained by the use of onset compared with peak measures, these differences do not vary as a function of age. More importantly, however, the duration measure reveals that the rate of the blink response to the tone changes as a function of type of training in both infants and adults.

Discussion

Infant and adult performance on standard delay conditioning differs in terms of the size of the UR amplitudes, the production of alpha responses, and the initial rate of CR acquisition. Age-related differences in rate of learning are in line with findings of even slower acquisition by younger human infants in delay eyeblink conditioning (Little et al., 1984). However, the slower learning rate in the present study did not preclude 5-month-old infants from reaching and maintaining the same learning asymptotes as adults across two sessions of delay conditioning. The 1-week retention interval between acquisition sessions did not appear to interfere with infant or adult learning or the subsequent savings of conditional responding.

The observation of SRs by adults in both paired and unpaired groups across both sessions suggests that these early "orienting" responses are a nonassociative feature of eyeblink conditioning. In comparison, infants produced very few responses in the alpha period, irrespective of conditioning paradigm or amount of training. The subsequent learning of infants in paired conditions, despite the absence of SRs, suggests that the production of orienting responses is not crucial for the development of conditional responding on standard delay eyeblink procedures. Orienting responses may, however, be a factor that supports the faster acquisition rates observed in adults compared with infants. The role of orienting responses in this type of learning remains to be determined.

Knowing the similarities and subtle differences in learning by infants and adults in the standard delay conditioning procedure provides a critical basis for interpreting findings in more complex conditioning procedures (e.g., trace, discrimination, reversal). In Experiment 2, we compare and contrast infant and adult learning in trace conditioning. This is the simplest of the higher order conditioning phenomena that critically depend on an interaction of forebrain systems with the basic cerebellar-brainstem learning circuit.

Experiment 2

We have previously established that infant rats have difficulty learning a trace conditioning procedure (e.g., Ivkovich, Paczkowski, & Stanton, 2000; Ivkovich & Stanton, 2001). However, infant rats also have difficulty learning in a long-delay conditioning procedure that involves the same ISI as the trace conditioning procedure (Ivkovich, Paczkowski, & Stanton, 2000). On the basis of these findings, we argued that the inability of developing rats to acquire trace conditioning may be due to the much longer ISI between the tone onset and the airpuff onset and not necessarily due to deficits in short-term memory processes that enable associations over the trace interval. The purpose of the present experiment was to compare the acquisition patterns of human adults and 5-month-old infants in trace conditioning and in a delay conditioning procedure that matched the longer ISI that is a feature of trace conditioning procedures.

Method

Participants. The participants were 23 (13 male and 10 female) undergraduate students enrolled in introductory psychology classes at Duke University (average age = 19.3 years; range = 18–25 years) and 24 (15 male and 9 female) 5-month-old (± 10 days) healthy full-term infants.[4]

Participants were randomly assigned to the long-delay or trace groups ($n = 10$ infants, 8 adults per group) or to an unpaired control group ($n = 4$ infants, 7 adults). To establish an unpaired control group for the trace procedure, we included the data from the unpaired participants ($n = 4$ infants, 7 adults) in Experiment 1 in the present study but recoded them to correspond to the longer interval following tone offset. In all groups, participants experienced a minimum of two conditioning sessions, 6−8 days apart. Gender distribution and number of participants per session in Experiment 2 are presented in Table 1. An additional 9 infants and 1 adult were excluded from the final analysis for the following reasons: They failed to meet the criterion number of conditioning trials ($n = 5$ infants), they were unresponsive to the airpuff US ($n = 3$ infants), or there were rescheduling conflicts that precluded the completion of all sessions ($n = 1$ infant, 1 adult). Chi-square analysis revealed no evidence of selective attrition based on gender, ethnic origin, or conditioning group.

[4] Session 1 and Session 2 data for infants in the paired and unpaired long-delay conditions were reported in Claflin et al. (2002).

Procedure. The procedure was identical to that used in Experiment 1, except where otherwise noted. Depending on scheduling availability, infants in both paired and unpaired groups received an additional third session of conditioning because of their low levels of adaptive CRs following two sessions. Of the original 32 infants in Experiment 2, 21 participated in a third training session (see Table 1). Chi-square analysis revealed no evidence of selective attrition between the second and third session based on gender, ethnic origin, or conditioning group.

Paired training sessions. In the long-delay condition, the 1,350-ms tone CS preceded, overlapped, and coterminated with the 100-ms airpuff, yielding a 1,250-ms ISI ("Delay 1250"; see Figure 1, middle panel). In the trace condition, the 750-ms tone was followed by a 500-ms stimulus-free period prior to the 100-ms airpuff, yielding a 1,250-ms ISI ("Trace 500"; see Figure 1, bottom panel).

Unpaired training sessions. In the unpaired long-delay condition ("Unpaired 1250"), the same 43 tones (1,350 ms in duration) and 45 airpuffs (100 ms in duration) as used for the paired training session were presented explicitly unpaired, and CRs were scored with respect to a 1,250-ms CS period. In the unpaired trace condition, the data from Experiment 1 in which participants experienced unpaired presentations of the 750-ms tone and the 100-ms airpuff were recoded to correspond to the longer ISI (1,250 ms) in the trace condition (i.e., 750-ms tone, 500-ms trace period, and 350-ms UR period).

Response measures. For the purpose of direct comparison across delay intervals, the alpha and adaptive CR periods in long-delay and trace conditioning were identical to the periods we reported in standard delay conditioning. As in Experiment 1, responses that occurred in the first 300 ms after the tone onset were coded as SRs, and responses that were initiated within 21 frames (350 ms) prior to airpuff onset were coded as adaptive CRs. A blink occurring after the airpuff, within 1 to 30 video frames (500 ms) after airpuff onset, was considered a UR.

Results

Infant and adult learning in long-delay and trace conditioning. Infants exhibited minimal levels of adaptive CRs in the long-delay and trace procedure following two training sessions (see Figure 4). In contrast, adults demonstrated robust conditioning across two sessions in comparison with the performance of their unpaired control groups. As in the standard delay condition in Experiment 1, CRs by adults in the long-delay and trace condition emerged in Session 1 and reached an asymptote during Session 2. These observations were supported by a 2 (infant vs. adult) $\times$ 2 (paired vs. unpaired) $\times$ 2 (long delay vs. trace) $\times$ 2 (session) $\times$ 6 (blocks) repeated measures ANOVA of percentage CRs. This ANOVA revealed significant interactions of Age $\times$ Pairing, $F(1, 50) = 14.27$, $p < .001$, and Session $\times$ Pairing, $F(1, 50) = 17.15$, $p < .001$. No other interactions were significant (all Fs < 2.65, ps $> .10$). Post hoc Newman–Keuls tests of the Age $\times$ Pairing interaction confirmed that adults in paired conditions exhibited learning relative to their respective unpaired controls ($p < .0001$), whereas infants did not. Post hoc analysis of the Session $\times$ Pairing interaction revealed that there was a significant increase in the levels of percentage CRs produced by paired groups across sessions (M % CRs Session 1 = 30.9, Session 2 = 65.4, $p < .0001$), whereas there was no such increase in the unpaired groups (M % CRs Session 1 = 10.4, Session 2 = 9.1). There was no main effect or any interactions involving group (long delay = trace; all Fs < 1.77, ps $> .20$).

Analyses of UR onset latencies failed to reveal differences in unconditional responding that could account for differences in learning across paired and unpaired groups. There were, however, differences in the onset of URs as a function of type of paired conditioning and age. A 2 (infant vs. adult) $\times$ 2 (paired vs. unpaired) $\times$ 2 (trace vs. long delay) ANOVA on UR onset latency revealed a significant interaction of Age $\times$ Group, $F(1, 57) = 5.27$, $p < .05$. Post hoc comparisons of this interaction revealed that, overall, infants produced significantly later URs in trace conditions ($M = 179.59$, $SE = 20.9$) compared with long-delay conditions ($M = 123.75$, $SE = 13.5$). Given that there was no difference in the percentage CRs demonstrated by infants in the long-delay and trace paired groups, it is unclear what these UR differences might reflect or whether these differences might influence subsequent conditioning rates. Adults exhibited no group differences in their onset of URs (trace: $M = 67.07$, $SE = 6.7$; long delay: $M = 64.31$, $SE = 3.8$).

When the duration from onset to full closure was analyzed, infants did exhibit differences in URs as a function of paired conditioning. A 2 (infant vs. adult) $\times$ 2 (paired vs. unpaired) $\times$ 2 (trace vs. long delay) ANOVA on UR duration revealed a significant interaction of Age $\times$ Pairing, $F(1, 57) = 4.53$, $p < .05$. Post hoc comparisons of this interaction revealed that, overall, infants produced significantly longer URs in unpaired conditions ($M = 73.9$, $SE = 5.2$) compared with paired conditions ($M = 63.8$, $SE = 3.3$). Adults did not exhibit differences in UR duration as a function of pairing (unpaired: $M = 39.6$, $SE = 1.9$; paired: $M = 43.4$, $SE = 2.0$). Determining the significance of the longer latency responses by infants is beyond the scope of the present article.

Comparing infant and adult CR timing. To determine whether there were alterations in CR timing in long-delay conditions compared with trace conditions in infants and adults following two training sessions, the ISI for each session was divided into twenty-five 50-ms bins. The mean number of responses initiated per bin is presented as a function of age, condition, and session in Figure 5. Mean onset latencies in the corresponding unpaired conditions are presented in Figure 6.

Across sessions and conditions, infants initiated few responses in the alpha period or in the adaptive period. CRs by infants in paired conditions typically occurred early in the CR period, immediately after the alpha period, and increased in number between Session 1 and Session 2. In comparison, adults produced SRs in both paired and unpaired conditions but CRs only in the paired condition. The onset of CRs by adults in the paired long-delay condition was generally well timed to anticipate airpuff onset. Adults in the paired trace condition also exhibited an additional spike in CR initiation that appeared to be associated with CS offset. Infants did not appear to show a corresponding increase in responding immediately following CS offset.

To determine whether there were age-related differences in onset latencies across the long-delay and trace groups, we performed a 2 (infant vs. adult) $\times$ 2 (paired vs. unpaired) $\times$ 2 (long-delay vs. trace) $\times$ 2 (Session 1 vs. Session 2) $\times$ 25 (50-ms CR bins) repeated measures ANOVA on the mean number of responses initiated across the trial epoch. This ANOVA revealed a significant three-way interaction of Bin $\times$ Age $\times$ Group, $F(24, 1200) = 2.10$, $p < .01$. This interaction reflected differences in peak CR initiation across age and across the delay and trace group in adults. There were no differences in infant CR peaks across delay and trace procedures. Infants produced a spike in CR initi-

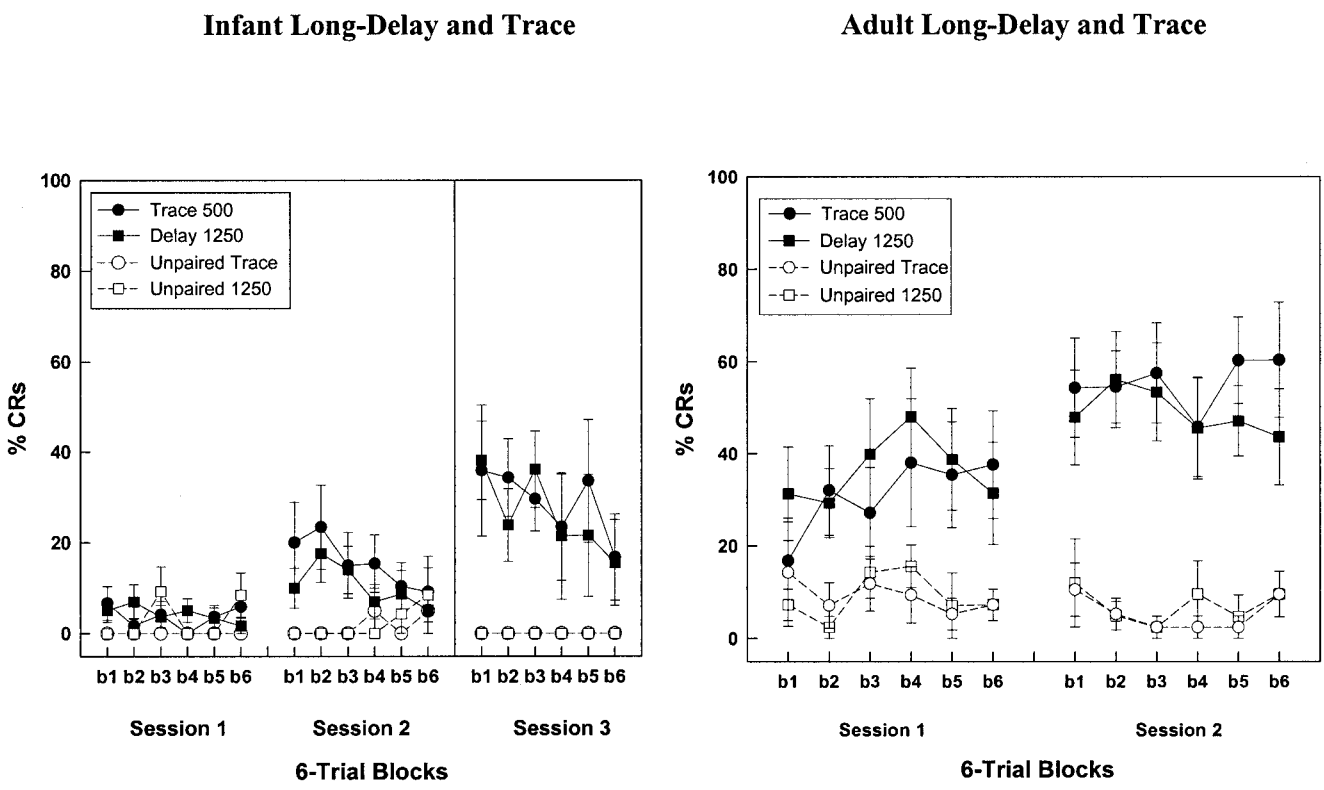

Figure 4. Mean (± *SEM*) percentage of adaptive responses for 5-month-old infants and adults in the paired trace (Trace 500) and paired long-delay (Delay 1250) conditions and from the matched unpaired long-delay and unpaired trace conditions. Data are presented as a function of six-trial blocks (b) across two training sessions and across a third training session for a subgroup of infants (Session 3, left panel). CRs = conditional responses.

ation at approximately −950 to −800 ms prior to airpuff onset. However, only in the −850-ms bin did infant CR initiation approach significantly greater levels than adult CR initiation ($p < .058$). Adults initiated significantly more CRs than infants in all alpha period bins (−1,250 ms to −1,000 ms; all ps $< .01$) and in all bins from −650 ms to airpuff onset (all ps $< .01$). Unlike infants, adults exhibited differences in the CR timing as a function of the type of paired condition they experienced. Adults in the long-delay condition initiated a greater number of SRs at −1,200 ms than adults in the trace condition ($p < .05$). In contrast, the performance of adults in the trace condition was distinguished by a spike in CR initiation approximately −450 ms prior to airpuff onset, approximately 300 ms after CS offset, that was not observed in the adult long-delay condition ($p < .05$; −400 ms, $p < .06$). This spike in CR production that appeared to correspond to tone offset was not observed in the infant trace condition ($p < .001$).

These group differences were observed only in the paired groups, as confirmed by a significant Bin × Age × Pairing interaction, $F(24, 1200) = 3.67$, $p < .0001$. Within the unpaired groups there was only an Age × Bin effect, $F(24, 1200) = 4.35$, $p < .0001$, reflecting the alpha spike (−1,250 ms to −1,200 ms) in adults that was not present in any of the unpaired infant groups (ps $< .05$). The CR peak at approximately −450 ms, which distinguished trace from long-delay in adults in the paired analyses reported above, was not observed in any of the unpaired groups. This difference in CR peak across the adult long-delay and trace conditions, and the presence of SRs exclusively in adult groups and regardless of pairing, resulted in a four-way interaction of Bin × Age × Pairing × Group that approached statistical significance, $F(24, 1200) = 1.52$, $p < .053$.

Does infant learning benefit from additional paired training? Given the low level of adaptive CR production by infants following two training sessions, we assessed the impact of a third training session on acquisition in long-delay and trace conditions. Across acquisition sessions, infants exhibited moderate increases in their production of adaptive CRs (see Figure 4, right panel). Given the lack of any significant differences in previous long-delay and trace comparison analyses and the small number of participants in the two unpaired groups, the data were collapsed across groups for the subsequent analysis. A 2 (paired vs. unpaired) × 3 (sessions) × 6 (blocks) repeated measures ANOVA of percentage CRs by infants revealed a significant Session × Pairing interaction, $F(2, 34) = 4.49$, $p < .05$. There were no other interactions (Fs < 2.19, ps $> .1$). Post hoc analyses of this interaction revealed no difference in mean percentage CRs in paired and unpaired groups during Session 1 (paired $M = 4.01$, $SE = 0.7$; unpaired $M = 2.16$, $SE = 0.97$) but significantly higher levels of adaptive CRs in paired groups compared with unpaired groups in Session 2 (paired $M = 12.99$, $SE = 1.75$; unpaired $M = 1.90$, $SE = 0.81$; $p < .001$) and Session 3 (paired $M = 27.61$, $SE = 3.07$; unpaired $M = 0.00$; $p < .0001$).

The distribution of responses across the CR sampling period for the three training sessions were then subjected to a 2 (paired vs.

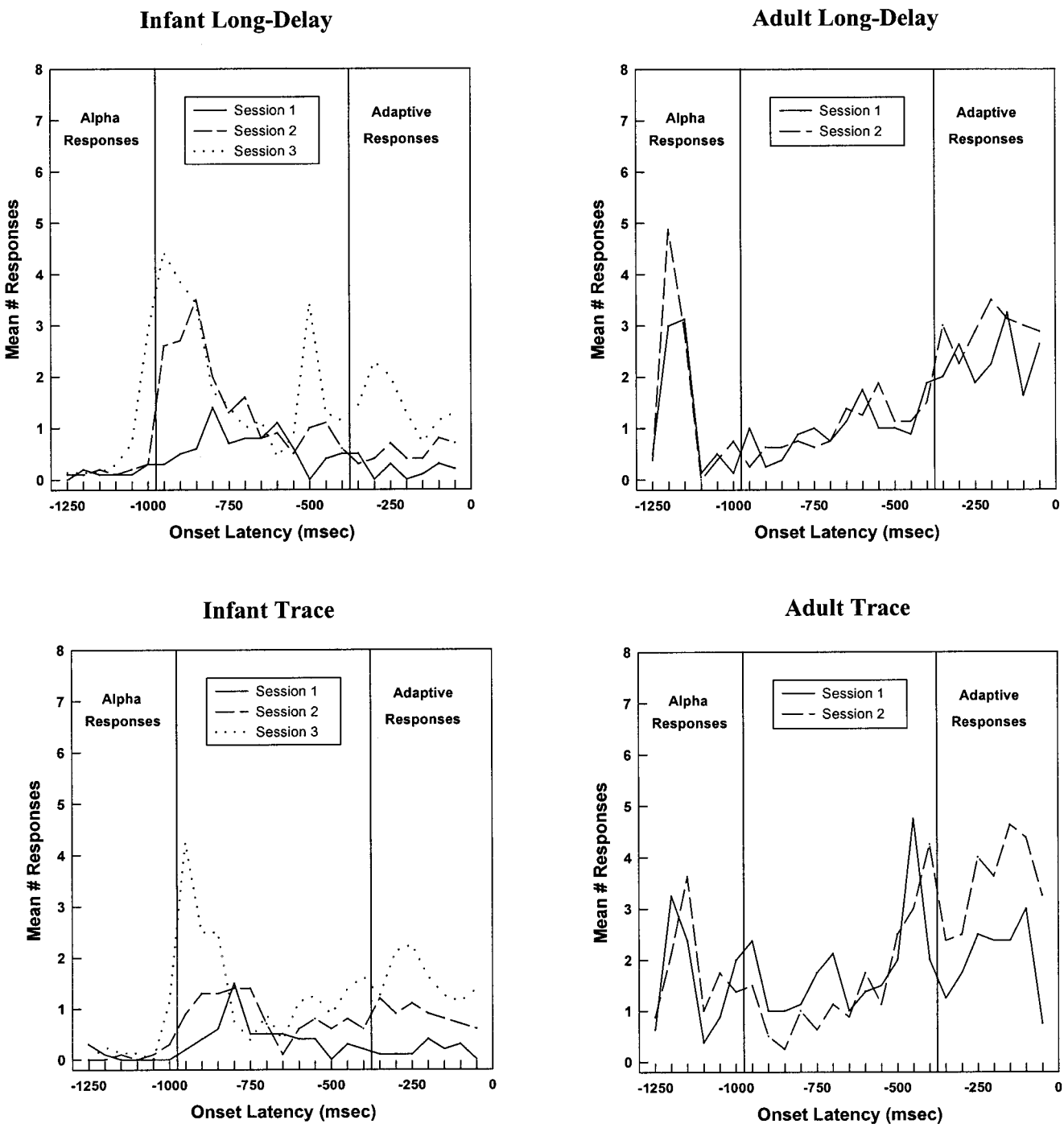

Figure 5. Mean distribution of the onset latency for responses during the conditional response period for 5-month-old infants and adults in the paired long-delay and paired trace conditions, as function of training session. In both the conditions, tone onset occurs at −1,250 ms and airpuff onset occurs at 0 ms. In the trace condition, tone offset occurs at −750 ms and is followed by a 500-ms stimulus-free period prior to airpuff onset.

unpaired) × 3 (sessions) × 25 (50-ms CR bins) repeated measures ANOVA. This ANOVA confirmed a significant interaction of Session × Pairing, $F(2, 34) = 4.56$, $p < .05$. No other interactions reached significance (Fs < 1.3, ps $> .2$). Therefore, analyses of CR onset generally support the findings of the previous analyses that paired groups exhibit learning compared with unpaired groups across sessions. By failing to find an interaction of Session × Pairing × Bin ($F < 0.5$), there was no evidence to suggest significant changes in the timing of infant CRs following this additional third training session.

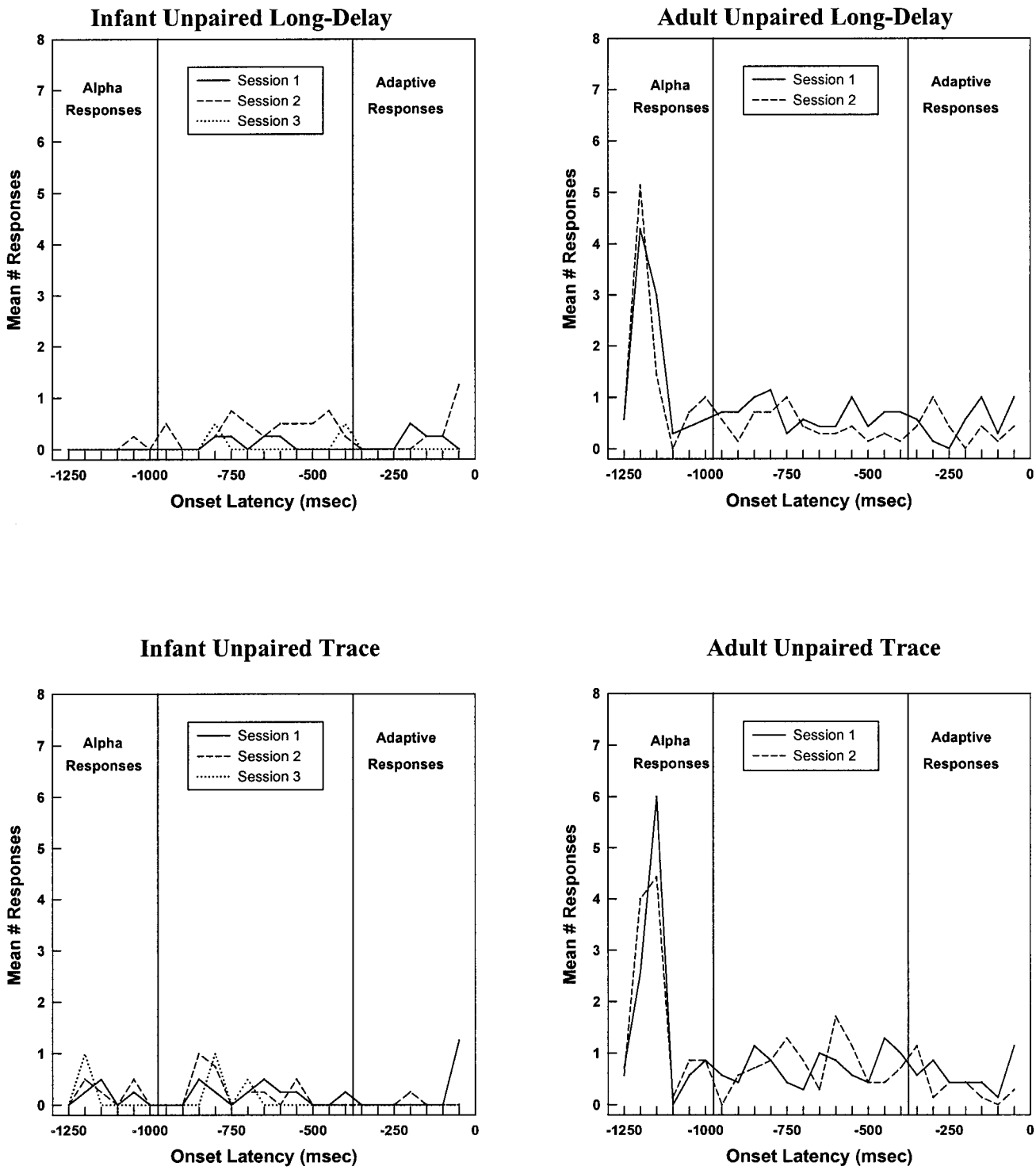

Figure 6. Mean distribution of the onset latency for responses during the conditional response period for 5-month-old infants and adults in the unpaired long-delay and unpaired trace condition, as function of training session. In both the long-delay and trace conditions, tone onset occurs at −1,250 ms and airpuff onset occurs at 0 ms. In the trace condition, tone offset occurs at −750 ms and is followed by a 500-ms stimulus-free period prior to airpuff onset.

General Discussion

The present study examined the acquisition of delay, long-delay, and trace conditioning in human adults and 5-month-old infants. In adults, type of conditioning procedure did not differentially affect rate of associative learning, confirming previous findings (e.g., Hansche & Grant, 1960; Kimble, 1947; Reynolds, 1945; Ross & Ross, 1971). Infants exhibited slower learning rates in even the simplest conditioning procedure, but this effect was greatly magnified in the more demanding conditioning paradigms. Under optimal learning conditions (standard delay conditioning), 5-month-old infants demonstrated patterns of acquisition that were indistinguishable from adults in terms of asymptote of learning and timing of responding. For infants in long-delay and trace procedures, ISI rather than trace interval determined the effectiveness of conditioning. These findings are consistent with our previous research with infant animals (e.g., Ivkovich, Paczkowski, & Stanton, 2000; Ivkovich & Stanton, 2001). Increasing the ISI impairs infants' ability to appropriately time CRs to precede airpuff onset, regardless of the type of conditioning procedure (trace or long delay). On the basis of these findings, we believe that the difficulty infants experience acquiring trace conditioning is at least partially the result of the much longer ISI between the tone onset and the airpuff onset and not necessarily due to deficits in short-term memory. Increases in the duration of the ISI within the delay procedure undoubtedly change the demands made on the basic cerebellar-brainstem learning circuit, particularly the cerebellar cortex and possibly also the deep nuclei of the cerebellum. Given that cerebellar structures undergo prolonged postnatal development (Bayer, Altman, Russo, & Zhang, 1993; Spreen, Risser, & Edgell, 1995), it is therefore possible that changes in the rate of learning as a function of ISI reflect changes in synaptic contact and efficacy within these structures. The increased cerebellar demands of long ISIs on immature cerebellar circuitry may actually overshadow the hippocampal demands of trace conditioning and result in similar initial learning rates across long ISI procedures, as we observed with infants in Experiment 2.

In the only other study of delay and trace eyeblink conditioning in infants, Little (1973) reported that 1.5- to 2.5-month-old infants were able to reach levels of 80% CRs in delay conditioning following six daily sessions of 50 CS–US pairings but failed to exhibit evidence of learning in trace conditioning. As in the present study, the two tasks were matched in ISI (1,500 ms); however, in Little's delay condition infants experienced a 1,500-ms CS whereas in the trace condition infants experienced only a 100-ms CS prior to the stimulus-free interval. On the basis of our previous research (Claflin et al., 2002) and those of Little et al. (1984), demonstrating infants' failure to exhibit CRs in delay procedures with short CSs, the inability of infants to learn a trace response to a 100-ms CS is not surprising. Although the infants in Little (1973) could be trained to produce CRs in trace conditions, this was observed only following the acquisition of delay conditioning, and only as the result of gradually modifying the delay procedure to create the trace procedure. Through successive decrease of the length of the tone and increase in the gap between the offset of the tone and the onset of the airpuff, thereby creating the stimulus-free interval, infants were able to maintain high levels of percentage CRs with CS durations from 1,250 to 100 ms (trace intervals from 250 to 1,400 ms). However, it is possible that the gradual shaping of delay to the trace procedure may have altered task demands in such a way that it is difficult to ascertain the psychological and neurological determinants of learned performance. In contrast, the present design revealed that although 5-month-old infants can learn a delay response with a 650-ms CS (Experiment 1), they have difficulty learning a trace response when the same CS is followed by a 500-ms stimulus-free interval (Experiment 2). There does not appear, however, to be a developmental dissociation in performance on delay and trace procedures during infancy, as seen in the comparisons of long-delay and trace conditioning in Experiment 2. In developing rats, the short-term memory component appears to be more closely linked to the hippocampus than the ISI component is (Ivkovich & Stanton, 2001). Whether there are also deficits in trace conditioning in human infants resulting from the short-term memory component remains to be determined with ISIs and CS durations that provide sufficient opportunity for learning to emerge.

How do these findings from human infant eyeblink conditioning studies contribute to our understanding of normative brain development in the first year of life? Although cerebellar-brainstem circuitry is sufficiently developed within the first 6 months of life to allow processing of association formation, the results from Experiment 2 suggest that there is considerable further development of associative learning across subsequent ontogeny. Changes in synaptic efficacy within the cerebellar-brainstem circuitry may play a role in the developmental changes in learning rates we observe at longer ISIs in delay conditions (see also Solomon, Groccia-Ellison, Levine, Blanchard, & Pendlebury, 1990). Cerebellar development (Stanton & Freeman, 2000) and plasticity (Freeman & Nicholson, 2001; Nicholson & Freeman, 2000) are implicated in the ontogeny of eyeblink conditioning in rats.

Even under optimal learning conditions in the present study, infants exhibited slower learning rates than adults. This delayed learning rate appears even more exaggerated in long ISI conditions. It is important to consider, however, the interplay between response expression and response timing (Garcia & Mauk, 1998; Mauk & Ruiz, 1992; Ohyama & Mauk, 2001). Mauk and his colleagues have demonstrated in adult rabbits that the anterior interpositus nucleus is critically involved in the production of CRs, whereas the ipsilateral cerebellar cortex influences the amplitude and timing of responding (Garcia, Steele, & Mauk, 1999; Ohyama & Mauk, 2001). There is also evidence that temporal lobe structures are involved in CR timing (McGlinchey-Berroth, Brawn, & Disterhoft, 1999). The poor response timing reported here of infants in long-delay and trace conditions may therefore reflect the differential development and interconnectivity of elements of cerebellar circuitry and/or its interactions with temporal lobe structures. Although infants clearly begin to exhibit adaptive responses when given additional training (a third acquisition session), it remains to be determined whether their inappropriately timed responses give way to adaptive responding or whether the two patterns of responding will coexist in their CR production. The occurrence of high levels of poorly timed, but nonetheless associatively based, CRs during early infancy underscores the importance of examining CR timing versus CR expression in developmental studies of eyeblink conditioning.

We found no evidence of differences in the rate of CR acquisition in long-delay and trace procedures in the performance of adults or in the performance of infants. These findings are consistent with our previous research with infant rat pups (Ivkovich, Paczkowski, & Stanton, 2000) in which performance on long-

delay is more similar to trace than standard delay conditioning. There are, however, at least two studies with young adult (4- to 6-month-old) rabbits that suggest similarities in some measures of responding on long-delay and standard delay conditioning (Solomon & Groccia-Ellison, 1996; L. T. Thompson, Moyer, & Disterhoft, 1996). In the present study we did observe subtle differences in response timing across long-delay and trace procedures in adults. In the long-delay condition, CRs generally increased in number across the CR period, with a spike in CR initiation approximately 200 ms prior to airpuff onset. In the trace condition, there was an additional spike in the initiation of CRs approximately 450 ms prior to airpuff onset. This finding suggests that adults in the trace condition may have been using CS offset as an indicator for initiating CR production. Infants in our trace condition did not show a similar spike in their initiation of CRs following tone offset. Our infant results are in contrast to those reported by Little (1973), who observed differences in CR response timing by younger infants as a function of procedure. Infants in that trace condition produced comparatively fewer CRs than in the delay condition, but they also produced a spike in early CR production approximately 625 ms after CS onset (525 ms after CS offset) not observed in the delay condition. On the basis of these findings, Little suggested that CS offset in trace conditions may influence the timing of CRs by eliciting orienting responses from infants. Without the comparison latency data from infants in matched unpaired conditions, it is difficult to determine whether these early responses were orienting or associative responses. The present infant data suggest that 5-month-old infants do not show orienting responses at either CS onset or CS offset, providing tentative support for the conclusion that early CRs reported by Little (1973) in the trace condition may have been associative. However, we found no corresponding increase in CR initiation in response to CS offset in our own trace data to address this issue directly. There may be changes in responding to CS offset during the infancy period that can account for the differences in response latencies reported here and in Little (1973). On the other hand, these differences may reflect performance changes across infancy in response to procedural variations such as the CS duration and trace interval. Whether infants and adults are differentially affected by variables such as CS offset, in addition to the longer ISI interval addressed here, clearly warrants further investigation.

In summary, this comparative study of classical eyeblink conditioning in infants and adults begins to create a developmental framework for understanding changes in association formation across ontogeny. The ability to form these simple associations provides a fundamental cornerstone for the development of learning and more advanced cognitive abilities such as memory development and concept formation. The simple sensory and response demands and the well-characterized underlying neural circuitry of eyeblink conditioning procedures provide the opportunity to not only report on changes in human learning between at least 10 days and 80 years of age but also to test hypotheses about the underlying causes for the observed behavioral changes. Our data provide important information on the typical developmental trajectory of cerebellar and hippocampal learning and, as such, also create an important normative comparison for studies with infant and child populations who may be at risk for damage to these systems.

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