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Effects of stimulus repetition rate on ABR threshold, amplitude and latency in neonatal and adult Mongolian gerbils [1]

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Summary The ABR wave forms of 16-day-old and adult Mongolian gerbils were evoked by click stimuli presented at rates ranging from 1 to 80/sec. Wave I and wave IV thresholds were determined for each of 5 click rates. Amplitudes and latencies of waves I and IV were measured at each of 7 click rates and 3 intensity levels (15, 40 and 65 dB above threshold). Thresholds for waves I and IV in the adult gerbil and wave I in the 16 day gerbil were unaffected by changes in stimulus repetition rate. Neonatal wave IV thresholds were unaffected by click rate for rates below 25/sec but increased approximately 7 dB/decade increase in click rate when rate exceeded 25/sec. Increasing click rate produced greater reductions in ABR amplitude among neonates than adults for both waves I and IV. Decreases in amplitude due to increasing rate were independent of intensity level in both neonatal and adult subjects. Increasing rate produced similar increases in wave I latency among 16 day and adult subjects, but produced much greater increases in wave IV latency among neonates. Stimulus intensity level and click rate acted independently on wave I and wave IV latency in adult subjects and wave I latency in neonates. However, an interaction between rate and intensity was observed with respect to neonatal wave IV latency.

Key words: Auditory brain-stem response; Rate effects; Development; Gerbil

The auditory brain-stem response (ABR) is widely used in both clinical and experimental applications to assess peripheral hearing sensitivity and/or brain-stem auditory nervous system function. Three parameters of the ABR response are routinely measured: threshold, amplitude and latency.

The effects of stimulus repetition rate on the ABR warrant investigation for several reasons. First, ABR rate effects vary according to the maturational and neurological characteristics of the subject, with immature and/or neurologically impaired subjects exhibiting greater rate effects than others (Fujikawa and Weber 1977; Pratt et al. 1981; Gerling and Finitzo-Hieber 1983; Lasky 1984b). Consequently, knowledge of rate effects can both facilitate assessments of neurological function and potentially increase understanding of auditory system development. Second, stimulus presentation rate directly affects data collection time for ABR testing. As hundreds or thousands of stimuli are involved in a single ABR assessment, it is desirable to use the most rapid stimulation rate which is appropriate for a given application. However, appropriate rates can be determined only when the effects of increasing rate have been characterized for all relevant measures of the ABR. Third, while the effects of repetition

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rate on ABR latency have been studied extensively among normal adult and infant populations as well as in neurologically and/or hearing-impaired subjects, the effects of repetition rate on ABR amplitude and threshold have received little attention.

Increased stimulus repetition rate is associated with increased latency, decreased amplitude and alterations of wave form morphology in the ABRs of adult humans (Thornton and Coleman 1975; Don et al. 1977; Weber and Fujikawa 1977; Chiappa et al. 1979) and mammals (Jewett and Romano 1972; Shipley et al. 1980; Church et al. 1984; Burkard and Voigt 1989; Kelly et al. 1989). In human adults, it has been shown that latency-rate functions are approximately linear for click rates ranging from 10 to 100/sec (Don et al. 1977) and that increasing rate has a greater effect on absolute latency for later waves of the ABR as compared to earlier ones (Yagi and Kaga 1979; Debruyne 1986). Similar findings with respect to ABR latency-rate functions have also been reported in the adult gerbil (Burkard and Voigt 1989).

Only a few investigators have systematically examined the effects of stimulus rate on ABR component wave amplitudes or the possible interaction between stimulus intensity level and either latency-rate or amplitude-rate functions. The findings of Pratt and Sohmer (1976) in children and Yagi and Kaga (1979) in adults suggest that amplitude-rate effects, in contrast to latency-rate effects, are greater for early waves of the ABR than for later waves. Studies in which both stimulus repetition rate and intensity level have been varied suggest that the effects of increasing rate on adult wave V latency are independent of stimulus intensity over a moderate range of intensity levels (30–70 dB HL; Don et al. 1977; Weber and Fujikawa 1977; Gerling and Finitzo-Hieber 1983; Paludetti et al. 1983). However, little is known about the relationship between repetition rate and intensity level for other parameters of the response.

The effects of increased stimulus repetition rate on ABR latency and amplitude are more pronounced among infants than adults. For a fixed stimulus intensity level, the slopes of wave V latency-rate functions increase with decreasing subject age (Fujikawa and Weber 1977; Despland and Galambos 1980; Dey-Sigman et al. 1984; Lasky 1984a, b; Zimmerman et al. 1987). Dey-Sigman et al. (1984) have further suggested that wave V latency-rate effects in newborns vary as a function of stimulus intensity level, with increases in rate causing the greatest increases in latency at high stimulus intensities. Newborn amplitude-rate functions have proven difficult to characterize, partly because amplitude measures are highly variable among infant populations (Dey-Sigman et al. 1984; Lasky 1984b). However, the findings of Dey-Sigman et al. (1984) again suggest that stimulus repetition rate and intensity level may interact non-linearly in their effects on newborn ABR amplitude.

In the present study, we evaluated the auditory brain-stem response in 16 day and adult Mongolian gerbils. Gerbils are well suited to developmental studies of hearing as they begin hearing postnatally (12–13 days after birth) and have a readily accessible cochlea. In addition, substantial baseline data have been collected with respect to the development of hearing in this species (Finck et al. 1972; Harris and Dallos 1984; Woolf and Ryan 1984, 1985a, b; Yancey and Dallos 1985; Smith and Kraus 1987). At each age, we determined the effects of increasing click rate on ABR threshold and characterized the effects of increasing rate on both amplitude and latency of the response at several stimulus intensity levels. ABR thresholds for waves I and IV were evaluated as a function of click rate. Amplitudes and latencies for waves I and IV were measured as a function of click rate and suprathreshold intensity level.

Materials and methods

Subjects were Mongolian gerbils (*Meriones unguiculatis*). Pilot experiments, used to develop experimental methods and determine appropriate stimulus parameters, involved approximately 50 gerbils of various ages. Data presented herein were subsequently collected from 14-16 day (neonatal) and 13-10 week (adult) gerbils. Approximately equal numbers of male and female subjects of each age were used.

Animals were anesthetized with sodium pentobarbital (Nembutal), 40–60 mg/kg i.p. initially and 10 mg/kg thereafter as needed to maintain a very light level of anesthesia throughout the experiment. Atropine sulfate, 0.6 mg/kg i.m., was also administered to all subjects. Adult gerbils were tracheotomized. Core body temperature was maintained at 37.5°C in both neonatal and adult animals by a feedback-controlled heating pad.

The right ear was routinely stimulated in all experiments. The pinna was resected and, in young animals, the opening of the external auditory canal was gently expanded using blunt forceps. Grass type E2 platinum subdermal electrodes were positioned as follows: active electrode (+) at dorsal midline just posterior to the plane of the pinnae; reference electrode (−) at dorsal midline approximately 1.5 cm anterior to the active electrode; ground electrode in left thigh muscle. Interelectrode impedance always fell between 2 and 5 kΩ.

Acoustic rarefaction clicks were generated by 0.1 msec square waves and presented to the ear via a closed sound delivery system consisting of a Telex 1470 earphone coupled to a speculum. The speculum was sealed to the external ear canal with True Mold (Hal-Hen Co., Long Island City, NY) impression compound. The acoustic spectrum of the click was limited to frequencies below 6.5 kHz and was approximately 3 msec in duration. Sound pressure near the tympanic membrane was monitored using a Knowles BL-1830 microphone input to a B and K Type 2031 Narrow Band Spectrum Analyzer.

Evoked responses were amplified (20,000 or 50,000 ×), bandpass filtered (100–2000 Hz, 24 dB/octave rolloff) and averaged on-line by a DEC PDP 11/23 computer. The ongoing averaged response was monitored on an oscilloscope and the final wave form was plotted on an XY recorder and stored on disk. Recording windows were 10 msec for experiment 1 and 20 msec for experiment 2. Two wave forms were collected under each stimulus condition to ensure wave form repeatability. In addition, threshold level responses at the 10/sec click rate were recorded periodically during the course of each experiment to ensure that the animal's physiological status had not degraded.

Experiment 1: threshold data

Threshold data were collected from 5 neonatal and 5 adult gerbils. Five click repetition rates were used: 1, 10, 25, 50 and 80 clicks/sec. For each click rate, wave I and wave IV thresholds were determined by systematically attenuating the stimulus from a moderate intensity level (where the response could clearly be seen) to a level where no response was observed. Stimuli were decremented in 5 dB steps near threshold. Threshold was defined as the lowest intensity level for which a repeatable wave I or wave IV response could be visually detected in the plotted wave form. The number of click stimuli used to elicit each wave form varied according to repetition rate as follows: 200 stimuli at 1 click/sec, 500 stimuli at 10 clicks/sec and 1000 stimuli at 25, 50 and 80 clicks/sec. Pilot experiments which compared responses comprised of 200 vs. 500 stimuli at 1 click/sec and 500 vs. 1000 stimuli at 10 clicks/sec revealed no consistent differences in threshold, amplitude or latency measures as a function of stimulus number. Thus, we felt justified in using fewer total stimuli to elicit wave forms at the slower click rates.

Experiment 2: amplitude / latency data

Amplitude and latency data were collected from 9 neonatal and 8 adult gerbils. For each animal, wave I threshold was determined as previously described using a stimulus rate of 10 clicks/sec. (Data from experiment 1 had shown wave I thresholds to be equally sensitive at 1 and 10 clicks/sec for both age groups.) ABR wave forms were then collected for each of 3 intensity levels (15, 40 and 65 dB above wave I threshold). At each level, 7 click rates were examined (1, 2, 5, 10, 25, 50 and 80 clicks/sec). Peak-to-trough amplitudes and peak latencies for waves I and IV were measured directly from the plotted wave forms for each of the 21 stimulus conditions.

Results

Representative wave forms from an adult and a 16 day gerbil are shown in Fig. 1. The adult gerbil ABR consists of 6 vertex-positive peaks which occur within 7 msec of stimulus onset (Smith and Kraus 1987), with waves I and IV being most prominent. The 16 day gerbil ABR differs from the adult wave form in morphology, but consistently demonstrates clear wave I and wave IV components corresponding to those which characterize the adult response.

Threshold

Threshold data are presented in Fig. 2. At slow click rates, thresholds for 16 day gerbils were 10–20 dB poorer than adult thresholds, consistent with the fact that auditory sensitivity is still somewhat immature at 16 days (Finck et al. 1972; Harris and Dallos 1984; Woolf and Ryan 1984; Woolf and Ryan 1985a; Smith and Kraus 1987). Increasing click rate had no effect on adult wave I or wave IV thresholds, or on wave I thresholds in 16 day animals. Wave IV thresholds in 16 day gerbils became elevated with increasing click rate, but differed significantly from baseline (1/sec) values only when very fast click rates (50 or 80/sec) were used.

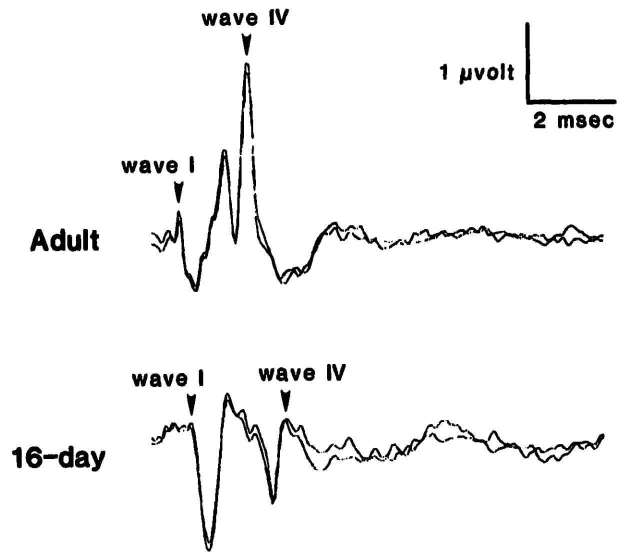

Fig. 1. Representative ABR wave forms from a 16 day and an adult gerbil. Positive potentials are plotted as upward deflections.

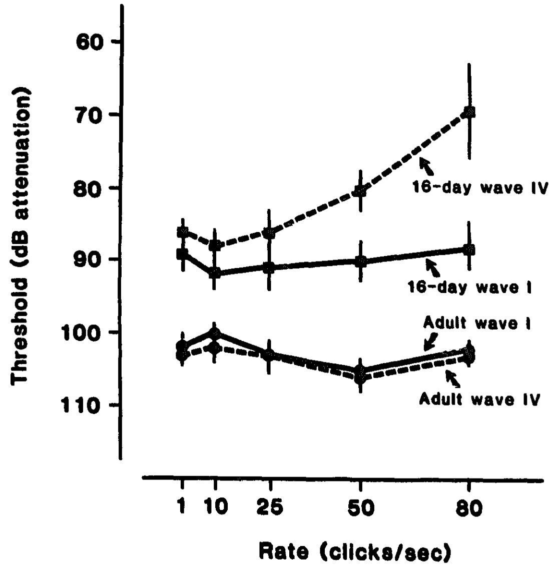

Fig. 2. Mean thresholds for waves I and IV as a function of click rate for 16 day and adult gerbils. Bars indicate standard error of the mean.

Wave I and wave IV thresholds were analyzed using separate age (2) × rate (5) repeated measures analyses of variance (ANOVAs). For wave I thresholds, both the age × rate interaction and the main effect of rate failed to reach significance, confirming that increasing click rate had no reliable effect on wave I thresholds in either young or adult gerbils. As expected, the analysis of variance for wave IV thresholds yielded a significant age × rate interaction (F (4, 32) = 3.18, $P < 0.001$), with follow-up one-way analyses confirming that rate had a significant effect on wave IV thresholds in 16 day animals (F (4, 32) = 23.32, $P < 0.001$), but not in adults. For 16 day subjects, mean wave IV thresholds at 50 and 80 clicks/sec differed significantly from the mean 1/sec baseline threshold and from each other (Newman-Keuls paired comparisons, $P < 0.05$), while thresholds obtained at 10 and 25 clicks/sec were similar to baseline values.

Absolute amplitude

Wave I and wave IV amplitude-intensity functions are plotted in Fig. 3 for representative slow and fast click rates. Amplitudes always increased with increasing intensity level over the range of intensities we examined (15–65 dB above threshold). Because there were no statistically significant differences between amplitudes recorded at 1, 2 and 5 clicks/sec for any of the 4 age × wave categories (Newman-Keuls paired comparisons, $P > 0.05$), amplitude data were pooled across these 3 click rates. The slopes of wave I amplitude-intensity functions were steeper for 16 day subjects than for adults, indicating that similar increases in suprathreshold stimulus intensity level caused greater increases in wave I amplitude for the younger animals. Absolute wave I amplitudes for neonatal and adult animals were similar in size near threshold, but became relatively larger for the neonates as stimulus intensity was raised above threshold. Increasing click rate had little effect on the shape of wave I amplitude-intensity functions in either 16 day or adult animals. Absolute wave IV amplitudes were larger in adult subjects than in neonates, but neonatal and adult wave IV amplitude-intensity functions had similar slopes. The shapes of wave IV amplitude-intensity functions were largely unaffected by increasing click rate in both 16 day and adult subjects.

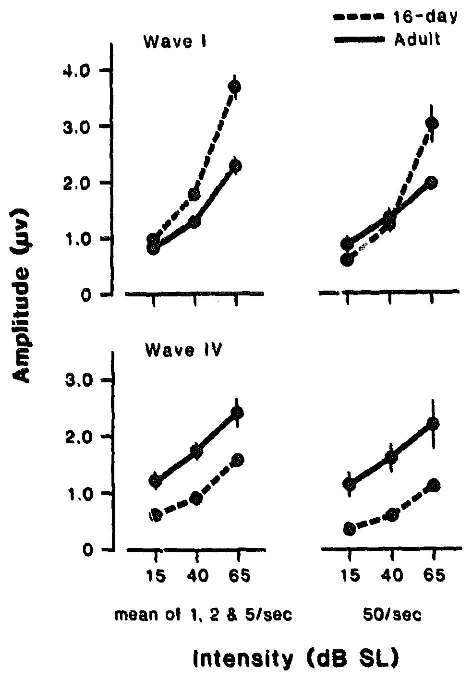

Fig. 3. Mean amplitudes for waves I and IV as a function of stimulus intensity level. Data from 16 day and adult gerbils are plotted for representative slow and fast click rates. Bars indicate standard error of the mean.

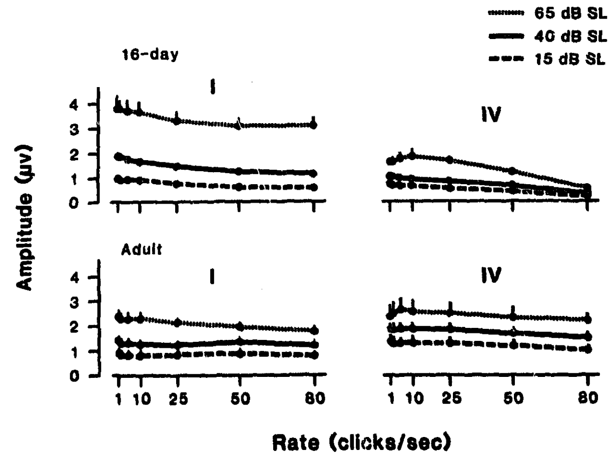

Fig. 4. Mean amplitudes for waves I and IV as a function of click rate and stimulus intensity level for 16 day and adult gerbils. Bars indicate standard error of the mean.

The effects of increasing click rate on wave I and wave IV absolute amplitudes are summarized in Fig. 4. In general, amplitudes decreased or remained constant as rate increased, and amplitude-rate functions were approximately linear.

Increasing click rate resulted in decreased wave I amplitudes in neonates but not adults. Neonatal wave I amplitude-rate functions obtained 15, 40 and 65 dB above threshold were roughly parallel, indicating that increasing click rate caused similar decreases in wave I amplitude at all 3 intensity levels. Adult wave I amplitudes were constant as a function of click rate at all stimulus intensities. Wave IV absolute amplitudes decreased with increasing click rate in both neonates and adults. However, wave IV amplitude-rate effects were greater for the neonatal subjects. For 16 day animals, the effect of increasing click rate on wave IV amplitudes appeared to be greater at 65 dB SL than at lower stimulus intensities; however, this interaction of rate and intensity was not confirmed by statistical analysis. Adult wave IV amplitude-rate functions were approximately parallel across intensity level.

Separate age (2) × rate (7) × intensity (3) repeated measures ANOVAs were performed on wave I and wave IV absolute amplitude data. The wave I analysis yielded a significant age × intensity interaction (F (2, 24) = 8.94, $P < 0.005$), which confirmed that increasing intensity level produced greater increases in wave I amplitude among neonates than adults, and a significant age × rate interaction (F (6, 90) = 12.67, $P < 0.001$), which confirmed that increases in click rate reduced wave I amplitude to a greater extent in neonatal animals than in adults. Neither the age × rate × intensity nor the rate × intensity interactions for wave I amplitude approached statistical significance. The wave IV analysis of variance yielded a significant age × rate interaction (F (6, 90) = 6.21, $P <$ 0.001), which confirmed that increasing click rate reduced wave IV amplitudes more among neonates than adults. The age × rate × intensity, rate × intensity, and age × intensity interactions for wave IV amplitude all failed to approach significance.

Percentage change in amplitude

At slow click rates, different combinations of age, wave and intensity level produced mean absolute amplitudes of different sizes. In order to evaluate the effects of increasing click rate on amplitude across these variables, we transformed absolute amplitude data into units of percentage change in amplitude based on amplitude values obtained at the slowest click rate. Amplitude data associated with each of the 12 combinations of age, wave and intensity level were considered separately. Within each data set, amplitudes obtained using click rates faster than 1/sec were expressed as percentages of the amplitude obtained using a 1/sec click rate. The resultant values are presented in Table I. Increasing click rate resulted in greater percentage reductions of wave I and wave IV amplitude among neonates than adults. While increasing click rate produced equivalent decreases in neonatal wave I absolute amplitude across intensity level, increasing rate appeared to cause the greatest reductions in neonatal wave I percentage amplitude at low intensity levels. This contrast can be explained by the fact that wave I absolute amplitudes in 16 day animals were much smaller when elicited by low intensity as compared to high intensity stimuli. Thus, a similar decrease in absolute amplitude represented a much greater percentage change in amplitude for responses near threshold. Among adult subjects, a percentage reduction in wave I amplitude with increasing click rate was apparent only at the highest stimulus intensity level. Percentage changes in wave IV amplitude due to increasing click rate appeared to be independent of stimulus intensity level for both 16 day and adult subjects.

Amplitude ratios

Mean wave IV/wave I amplitude ratios are presented in Table II. Such amplitude ratios provide a means of comparing wave I and wave IV absolute amplitudes and the relative effects of increasing click rate and/or intensity level on each. For adult subjects, wave IV amplitudes were generally larger than wave I amplitudes, resulting in mean amplitude ratios which were always

TABLE I

Mean (S.E.) percentage amplitudes of the ABR by age, wave, intensity level and click rate. Within each age × wave × intensity category, 100% amplitude represents the mean amplitude measured at 1 click/sec.

Age	Wave	Intensity (dB SL)	Rate (click/sec) 1	2	5	10	25	50	80
16 days	I	15	100	92.3 (2.5)	92.8 (4.4)	89.1 (5.2)	73.8 (3.5)	61.9 (3.4)	57.3 (4.7)
		40	100	99.9 (2.3)	96.5 (2.1)	89.9 (2.1)	81.1 (3.2)	67.4 (3.6)	62.0 (2.4)
		65	100	101.1 (4.5)	98.8 (5.1)	96.9 (4.3)	89.8 (6.7)	82.9 (5.8)	83.8 (6.6)
	IV	15	100	101.5 (9.4)	85.4 (12.0)	93.3 (13.7)	84.1 (11.9)	56.6 (9.5)	32.3 (12.6)
		40	100	102.5 (9.1)	89.9 (5.2)	84.2 (3.6)	79.1 (6.8)	60.7 (5.9)	27.8 (7.4)
		65	100	97.5 (7.4)	104.6 (9.6)	109.9 (9.3)	100.3 (4.3)	70.9 (3.4)	28.1 (4.5)
Adult	I	15	100	91.2 (5.6)	87.0 (5.2)	92.9 (5.5)	99.6 (6.2)	100.1 (6.9)	94.8 (8.3)
		40	100	92.6 (2.8)	100.1 (4.6)	96.4 (5.7)	92.8 (7.1)	100.3 (6.5)	91.2 (6.3)
		65	100	97.0 (6.1)	98.4 (5.6)	101.4 (8.2)	94.8 (9.2)	85.9 (7.4)	77.3 (5.8)
	IV	15	100	99.6 (8.9)	101.0 (9.7)	101.3 (13.2)	106.8 (12.9)	102.7 (17.9)	84.6 (9.9)
		40	100	107.3 (10.0)	110.9 (10.1)	108.7 (7.1)	108.5 (7.0)	95.6 (3.6)	89.1 (5.7)
		65	100	106.7 (5.5)	113.8 (4.3)	109.5 (7.7)	106.5 (4.5)	95.4 (4.7)	91.8 (6.2)

TABLE II

Mean (S.E.) wave IV/wave I amplitude ratios of the ABR by age, intensity level and click rate.

Age	Intensity (dB SL)	Rate (clicks/sec)						
		1	2	5	10	25	50	80
16 days	15	0.70 (0.09)	0.69 (0.05)	0.64 (0.07)	0.64 (0.08)	0.72 (0.08)	0.60 (0.09)	0.64 (0.22)
	40	0.56 (0.06)	0.55 (0.05)	0.51 (0.04)	0.52 (0.04)	0.52 (0.03)	0.52 (0.03)	0.31 (0.04)
	65	0.46 (0.05)	0.43 (0.03)	0.48 (0.05)	0.50 (0.03)	0.51 (0.04)	0.39 (0.04)	0.16 (0.03)
Adult	15	1.41 (0.21)	1.49 (0.22)	1.55 (0.18)	1.42 (0.15)	1.40 (0.14)	1.28 (0.11)	1.19 (0.13)
	40	1.43 (0.35)	1.57 (0.35)	1.46 (0.28)	1.56 (0.34)	1.55 (0.26)	1.30 (0.26)	1.34 (0.29)
	65	1.02 (0.16)	1.15 (0.22)	1.19 (0.21)	1.19 (0.21)	1.17 (0.21)	1.17 (0.24)	1.18 (0.15)

greater than 1. Mean amplitude ratios for 16 day subjects were always less than 1, reflecting the fact that wave I amplitudes were typically larger than wave IV amplitudes for these subjects. For both the adult and 16 day groups, amplitude ratios decreased or remained constant as click rate increased. This reflects the fact that increasing rate produced greater reductions in absolute amplitude for wave IV than for wave I.

An age (2) × rate (7) × intensity (3) repeated measures ANOVA revealed that increasing click rate caused similar decreases in amplitude ratio among neonatal and adult subjects, as indicated by a significant main effect of rate (F (6, 90) = 8.49, $P < 0.0001$) combined with an age × rate interaction which failed to reach significance. However, Newman-Keuls paired comparisons showed that amplitude ratios decreased significantly from baseline (1/sec) values only at the 80/sec rate. While there was a clear trend for amplitude ratios to decrease with increasing intensity level among neonatal subjects, statistical analysis revealed no significant effect of intensity on amplitude ratio or interaction of intensity level with age or rate. As expected, amplitude ratios varied significantly as a function of subject age (F (1, 15) = 27.26, $P < 0.0001$).

Absolute latency

In Fig. 5, latency-intensity functions for waves I and IV are plotted for representative slow and fast click rates. Latencies always decreased or remained constant with increasing intensity level over the range of intensities we examined (15–65 dB above threshold). As there were no statistically significant differences in the absolute latencies recorded at 1, 2 and 5 clicks/sec for any of the 4 age × wave categories (Newman-Keuls paired comparisons, $P > 0.05$), latency data were pooled across these 3 click rates.

Both wave I and wave IV latencies were substantially longer in 16 day subjects than in adults. Neonatal wave I latencies were approximately 0.5 msec longer than adult wave I latencies and this difference did not change with increasing click rate. Neonatal wave IV latencies were about 2.1 msec longer than adult wave IV latencies at slow

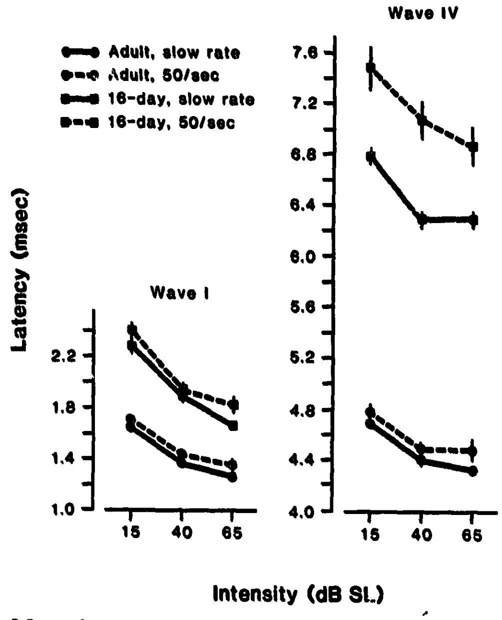

Fig. 5. Mean latencies for waves I and IV as a function of stimulus intensity level. Data from 16 day and adult subjects are plotted at representative slow and fast click rates. Slow rate values represent pooled data for the 1, 2 and 5 click/sec conditions. Bars indicate standard error of the mean.

click rates. However, the increase in wave IV latency associated with increasing click rate was much greater among neonatal subjects than adults, such that the difference between 16 day and adult wave IV latencies increased to about 2.6 msec at fast click rates.

Based on the 3 intensity levels we examined (15–65 dB above threshold), both neonatal and adult wave I latency-intensity functions were somewhat non-linear, with a 25 dB increase in intensity near threshold (from 15 to 40 dB SL) producing larger decreases in wave I latency than a 25 dB increase in intensity well above threshold (from 40 to 65 dB SL). The slopes of wave I latency-intensity functions were somewhat steeper for neonates than for adults, indicating that similar increases in stimulus intensity level were associated with greater decreases in wave I latency for the younger subjects. Both neonatal and adult wave I latencies increased slightly when stimuli were presented at fast repetition rates. However, increasing click rate had little effect on the shape of wave I latency-intensity functions in either neonatal or adult animals.

Wave IV latency-intensity functions were nonlinear for both neonatal and adult subjects with the largest latency decreases associated with near-threshold increases in intensity (15–40 dB SL). While the slopes of wave IV latency-intensity functions appeared to be steeper among 16 day subjects than adults, this observation was not supported by statistical analysis. For 16 day subjects, increasing intensity caused a greater reduction in wave IV latencies at fast click rates than at slow ones. Thus, the shape of neonatal wave IV latency-intensity functions depended on stimulus repetition rate. Adult wave IV latency-intensity functions maintained a relatively constant shape as click rate was varied, indicating that increases in stimulus intensity caused decreases in wave IV latency that were independent of stimulus presentation rate.

Normalized latency data are presented in Fig. 6. Absolute latencies always increased with increasing click rate and all latency-rate functions were approximately linear. Wave I latency-rate functions had similar shallow slopes for neonates and adults and these slopes did not vary as a function of stimulus intensity level. Wave IV latency-rate functions, however, were much steeper in neonates than adults, indicating that increasing click rate prolonged wave IV latencies much more in the immature subjects. The slopes of adult wave IV latency-rate functions did not vary with stimu-

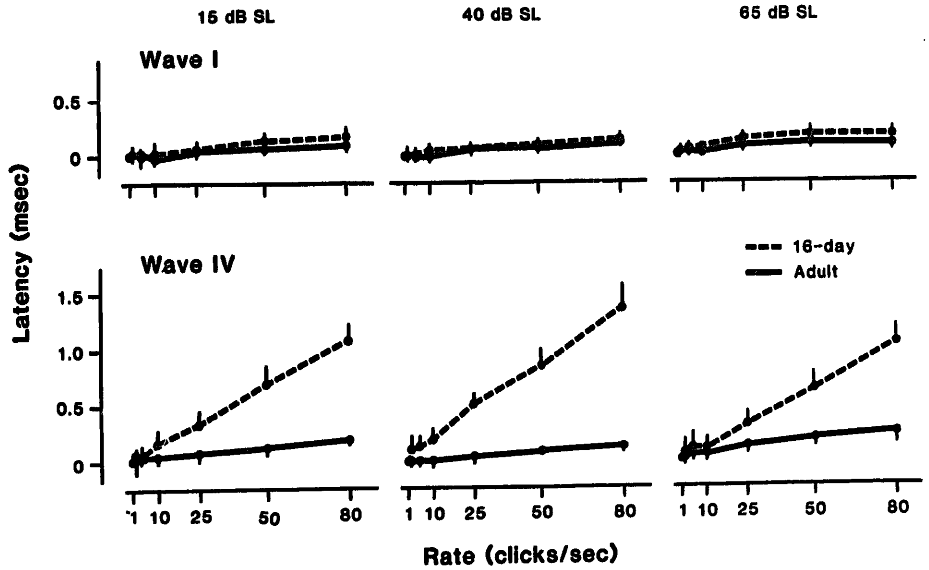

Fig. 6. Mean shifts in latency for waves I and IV as a function of click rate. Data from 16 day and adult gerbils are plotted for 3 stimulus intensity levels. Bars represent standard error of the mean.

lus intensity level. In neonates, increasing click rate prolonged wave IV latencies slightly more at 40 dB SL than at 15 or 65 dB SL.

Separate age (2) × rate (7) × intensity (3) repeated measures ANOVAs were performed on wave I and wave IV absolute latencies. The wave I analysis yielded a significant age × intensity interaction (F (2, 24) = 5.54, $P < 0.05$), confirming that the slopes of wave I latency-intensity functions were steeper for the younger subjects. For wave I latencies, a significant main effect of rate (F (6, 90) = 22.44, $P < 0.001$), paired with an age × rate interaction which failed to reach significance, confirmed that increasing click rate caused a similar increase in wave I latencies for neonates and adults. Neither the age × rate × intensity nor the rate × intensity interactions for wave I latency approached significance. The analysis of wave IV latencies yielded a highly significant age × rate interaction (F (6, 90) = 94.92, $P < 0.001$), reflecting the fact that increasing click rate prolonged wave IV latencies much more among neonates than adults. Because the age × rate × intensity interaction for wave IV was also significant (F (12, 180) = 1.88, $P < 0.05$), we evaluated the rate × intensity interactions for each age group separately. The rate × intensity interaction was significant for neonatal subjects (F (12, 180) = 2.60, $P < 0.005$) but not for adults. This confirmed that the shape of wave IV latency-intensity functions varied with click rate among neonates but not adults, and also that the slopes of wave IV latency-rate functions varied with stimulus intensity in neonatal but not adult subjects.

Interpeak latency

Wave I–IV interpeak latency data are presented in Table III. At slow click rates, interpeak latencies for 16 day animals were approximately 1.4 msec longer than those in adults. Neonatal interpeak latencies increased dramatically with increasing click rate, reflecting the fact that wave IV latencies were prolonged much more than wave I latencies as rate was increased. Adult interpeak latencies were unaffected by changes in click rate since increasing rate prolonged adult wave I and wave IV latencies about equally. Interpeak latencies did not appear to vary meaningfully with stimulus intensity level in either neonatal or adult subjects.

An age (2) × rate (7) × intensity (3) repeated measures ANOVA was performed on interpeak latency data. A significant age × rate × intensity interaction was present (F (12, 180) = 2.41, $P < 0.01$), even though we did not observe a meaningful difference in interpeak latency as a function of intensity level in either adult or neonatal subjects. Both the main effect of rate and the age × rate interaction were significant (F (6, 90) = 64.68, $P < 0.001$, and F (6, 90) = 46.78, $P < 0.001$, respectively). Follow-up analysis of the simple effects of rate within each age group confirmed that neonatal interpeak latencies increased with increasing click

TABLE III

Mean (S.E.) wave I–wave IV interpeak latencies of the ABR by age, intensity level and click rate.

Age	Intensity (dB SL)	Rate (clicks/sec)						
		1	2	5	10	25	50	80
16 days	15	4.50 (0.09)	4.50 (0.09)	4.54 (0.07)	4.66 (0.08)	4.78 (0.09)	5.08 (0.14)	5.15 (0.16)
	40	4.33 (0.07)	4.40 (0.07)	4.44 (0.07)	4.48 (0.06)	4.75 (0.06)	5.10 (0.13)	5.50 (0.18)
	65	4.59 (0.07)	4.63 (0.10)	4.65 (0.10)	4.59 (0.10)	4.77 (0.10)	5.03 (0.14)	5.47 (0.17)
Adult	15	3.03 (0.06)	3.09 (0.05)	3.05 (0.06)	3.09 (0.04)	3.06 (0.05)	3.06 (0.06)	3.13 (0.03)
	40	3.01 (0.05)	3.04 (0.05)	3.04 (0.05)	3.02 (0.05)	3.01 (0.05)	3.04 (0.06)	3.04 (0.05)
	65	3.06 (0.04)	3.04 (0.03)	3.07 (0.04)	3.09 (0.06)	3.09 (0.05)	3.14 (0.05)	3.19 (0.06)

rate (F (6, 90) = 110.58, $P < 0.001$), while adult interpeak latencies did not.

Discussion

Our primary goal in this study was to examine age-related differences in the effects of stimulus repetition rate on ABR threshold, amplitude and latency. Thus, while we have presented additional results which relate to, for example, the specific effects of intensity on amplitude and latency, we will focus this discussion on the differential effects of stimulus repetition rate in neonatal and adult subjects.

Threshold-rate effects

In the present study, absolute thresholds for ABR waves I and IV in the adult gerbil and wave I in the 16 day gerbil were unaffected by changes in stimulus repetition rate between 1 and 80 clicks/sec. Neonatal wave IV thresholds were unaffected by differences in click rate for rates below 25 clicks/sec, but increased approximately 7 dB/decade increase in click rate when rate exceeded 25/sec. To the extent that gerbils are representative of other species, these findings suggest that relatively rapid click rates can be used to estimate auditory brain-stem evoked potential thresholds in young subjects.

Sininger and Don (1989) recently reported that ABR wave V thresholds in normal-hearing human adults did not vary as click rate was increased from 21 to 48/sec. While they examined only a limited range of click rates, their data are consistent with our finding that increasing rate has no effect on wave IV threshold in the adult gerbil. We are unaware of other studies which have addressed the effects of stimulus repetition rate on auditory evoked potential thresholds in either adult or developing subjects. Because many experimental and clinical procedures require estimations of threshold sensitivity, it would be useful to characterize threshold-rate functions in other species.

Amplitude-rate effects

We found that increasing click rate produced greater reductions in ABR amplitude among neonates than adults for both waves I and IV. Furthermore, decreases in ABR amplitude due to increasing click rate did not vary significantly as a function of stimulus intensity level in either neonatal or adult subjects. These findings are generally consistent with the reports of other investigators. Dey-Sigman et al. (1984) evaluated the effects of click rate on wave V amplitude in human newborns and adults. Stimuli were presented 40, 60 or 80 dB above adult perceptual threshold for the click stimulus. For both the newborn and adult groups, wave V amplitude decreased with increasing rate at 60 and 80 dB HL but was relatively unaffected by changes in rate at 40 dB HL. Increasing rate produced greater decreases in amplitude among newborns than adults at both the 60 and 80 dB HL intensity levels. Lasky (1984b) also examined the effects of increasing stimulus presentation rate on ABR amplitude in human newborn (32, 36 or 40 weeks gestational age) and adult subjects, with stimuli presented 70 dB above adult perceptual threshold. He found that term newborns exhibited steeper amplitude-rate functions for waves I, III and V than either the gestationally younger newborns or the adults. This finding is difficult to interpret, particularly in light of the fact that stimuli were presented at a constant intensity level with respect to adult thresholds while absolute sensitivity in preterm infants may be changing with age. Shipley et al. (1980) evaluated ABR amplitude-rate effects in a small sample of developing kittens. They reported that amplitude-rate effects were greatest early in development and were more apparent for wave V than for earlier components of the response. Sanes and Constantine-Paton (1985) examined changes in the amplitude of auditory evoked potentials recorded from the eighth nerve and inferior colliculus of developing mice using click stimuli presented 20 dB above each subject's evoked potential threshold. These investigators found that the amplitudes of both VIIIth nerve and IC recorded evoked potentials decreased as click rate increased and that the slopes of amplitude-rate functions decreased with increasing subject age. Smith and Kraus (1987) assessed the rate resistance of ABR component waves in developing and adult gerbils by analyzing the percent detectability of each wave in response to a suprathreshold stimulus (50dB SL re: adult ABR threshold) as a function of click rate. Adult wave form amplitudes were largely unaffected by increasing stimulus rate, with only minor changes noted at the fastest rate examined (40/sec). However, the detectability of ABR responses in developing subjects showed significant effects of increasing click rate until postnatal days 14 and 20 for waves I and IV, respectively.

In summary, data from several laboratories suggest that increases in stimulus repetition rate reduce the amplitude of brain-stem auditory evoked potentials to a greater degree in young subjects than in adults. This appears to be true both for wave I and later components of the ABR. Among newborn subjects, there may also be an interaction between the effects of stimulus repetition rate and stimulus intensity level on the amplitude of later waves. Dey-Sigman et al. (1984) demonstrated that the reduction of wave V amplitudes with increasing rate in human newborns was most severe at high stimulus intensities, a finding which may reflect the contribution of immature auditory neurons to the newborn evoked response. Specifically, the decreased sensitivity of immature neurons would result in their maximal activation at high stimulus intensities, where their reduced resistance to stimulus rate effects would become apparent in the overall evoked response. In the present study, we observed a tendency for wave IV amplitude-rate effects to increase with stimulus intensity in neonatal gerbils, but were unable to confirm a rate-intensity interaction by statistical analysis.

Latency-rate effects

Our data indicate that increases in click repetition rate produce similar increases in wave I latency for 16 day and adult subjects, but produce much greater increases in wave IV latency among neonates. These findings are consistent with those of Lasky (1984a), who reported that the slopes of wave I and wave III latency-rate functions were similar for human newborns and adults, while the slopes of wave V latency-rate functions were steeper among the newborns. Zimmerman et al. (1987) examined latency-rate effects in a group of normal newborns who were followed longitudinally from birth through 6 months of age, and in adults. At each age, latency-rate functions demonstrated increasing slopes from wave I to wave III to wave V. The slopes of infant wave I latency-rate functions were constant as a function of subject age with infant and adult functions having similar slopes. In contrast, latency-rate functions for waves III and V demonstrated systematic decreases in slope with increasing subject age. Other investigators who have studied the effects of stimulus repetition rate on wave V latency in human subjects have likewise found that the slopes of wave V latency-rate functions decrease with increasing subject age (Despland and Galambos 1980; Dey-Sigman et al. 1984; Lasky 1984b).

In the present study, the effects of stimulus intensity level and click repetition rate were found to be independent for wave I and wave IV latency in adult subjects and wave I latency in neonatal subjects. Increasing click rate produced slightly greater increases in neonatal wave IV latency at 40 dB SL than at 15 or 65 dB SL, suggesting a possible interaction between stimulus repetition rate and intensity level. Dey-Sigman et al. (1984) examined the effects of click rate on wave V latency-intensity functions in human newborns and adults. They found that newborn latency-intensity functions were rate dependent, with increasing click rate prolonging wave V latency most at high intensity levels, while adult latency-intensity functions did not vary as a function of click rate. This result reinforces their findings with respect to amplitude-rate effects (see above) which suggest that rate effects in the newborn are most severe at high stimulus intensities. In human adults, both Don et al. (1977) and Gerling and Finitzo-Hieber (1983) have reported that increases in wave V latency due to increasing click rate are independent of stimulus intensity over a moderate range of intensity levels.

Thus, there is general agreement among investigators that increasing click rate prolongs wave I latency similarly for newborn and adult subjects, while increasing rate prolongs the latency of later waves to a greater extent in young subjects. The available data further suggest that stimulus intensity level and repetition rate act independently on adult ABR latencies and wave I latency in newborn subjects. However, the present data together with that of Dey-Sigman et al. (1984) suggest that, in young subjects, stimulus repetition rate may alter the latency of later waves, or more rostral generator sites, differentially as a function of stimulus intensity level. Since latency-intensity measures are frequently employed in assessing auditory function, this issue warrants further investigation.

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References

Burkard, R. and Voigt, H.F. Stimulus dependencies of the gerbil brain-stem auditory-evoked response (BAER). I. Effects of click level, rate and polarity. J. Acoust. Soc. Am., 1989, 85: 2514–2525.

Chiappa, K.H., Gladstone, K.J. and Young, R.R. Brainstem auditory evoked responses. Studies of wave form variations in 50 normal human subjects. Arch. Neurol., 1979, 36: 81–87.

Church, M.W., Williams, H.L. and Holloway, J.A. Brain-stem auditory evoked potentials in the rat: effects of gender, stimulus characteristics and ethanol sedation. Electroenceph. clin. Neurophysiol., 1984, 59: 328–339.

Debruyne, F. Influence of age and hearing loss on the latency shifts of the auditory brain-stem response as a result of increased stimulus rate. Audiology, 1986, 25: 101–106.

Despland, P.-A. and Galambos, R. The auditory brainstem response (ABR) is a useful tool in the intensive care nursery. Pediat. Res., 1980, 14: 154–158.

Dey-Sigman, S.E., Ruth, R.A. and Rubel, E.W. ABR rate/intensity interaction: developmental effects. Paper presented before the American Speech and Hearing Association, San Francisco, CA, November, 1984.

Don, M., Allen, A. and Starr, A. The effect of click rate on the latency of auditory brain-stem responses in humans. Ann. Otol. Rhinol. Laryngol., 1977, 86: 186–195.

Finck, A., Schneck, C.D. and Hartman, A.F. Development of cochlear function in the neonate Mongolian gerbil (*Meriones unguiculatis*). J. Comp. Physiol. Psychol., 1972, 78: 375–380.

Fujikawa, S.M. and Weber, B.A. Effects of increased stimulus rate on brain-stem electric response (BER) audiometry as a function of age. J. Am. Audiol. Soc., 1977, 3: 147–150.

Gerling, I.J. and Finitzo-Hieber, T. Auditory brainstem response with high stimulus rates in normal and patient populations. Ann. Otol. Rhinol. Laryngol., 1983, 92: 119–123.

Harris, D.M. and Dallos, P. Ontogenetic changes in frequency mapping of a mammalian ear. Science, 1984, 225: 741–743.

Jewett, D.L. and Romano, M.N. Neonatal development of auditory system potentials averaged from scalp of rat and cat. Brain Res., 1972, 36: 101–115.

Kelly, J.B., Kavanagh, G.L. and Picton, T.W. Brainstem auditory evoked response in the ferret (*Mustela putorius*). Hear. Res., 1989, 39: 231–240.

Lasky, R.E. Developmental adaptation and rate effects on auditory evoked brain-stem responses. Poster at the Int. Conf. on Infant Studies, New York, April, 1984a.

Lasky, R.E. A developmental study on the effect of stimulus rate on the auditory evoked brain-stem response. Electroenceph. clin. Neurophysiol., 1984b, 59: 411–419.

Paludetti, G., Maurizi, M. and Ottaviani, F. Effects of stimulus repetition rate on the auditory brainstem responses (ABR). Am. J. Otol., 1983, 4: 226–234.

Pratt, H. and Sohmer, H. Intensity and rate functions of cochlear and brain-stem evoked responses to click stimuli in man. Arch. Otorhinolaryngol., 1976, 212: 85–92.

Pratt, H., Ben-David, Y., Peled, R., Podoshin, L. and Scharf, B. Auditory brain stem evoked potentials: clinical promise of increasing stimulus rate. Electroenceph. clin. Neurophysiol., 1981, 51: 80–90.

Sanes, D.H. and Constantine-Paton, M. The development of stimulus following in the cochlear nerve and inferior colliculus of the mouse. Dev. Brain Res., 1985, 22: 255–268.

Shipley, C., Buchwald, J.S., Norman, R. and Guthrie, D. Brain stem auditory evoked response development in the kitten. Brain Res., 1980, 182: 313–326.

Sininger, Y.S. and Don, M. Effects of click rate and electrode orientation on threshold of the auditory brain-stem response. J. Speech Hear. Res., 1989, 32: 880–886.

Smith, D.I. and Kraus, N. Postnatal development of the auditory brain-stem response (ABR) in the unanesthetized gerbil. Hear. Res., 1987, 27: 157–164.

Thornton, A.R.D. and Coleman, M.J. The adaptation of cochlear and brain-stem auditory evoked potentials in humans. Electroenceph. clin. Neurophysiol., 1975, 39: 399–406.

Weber, B.A. and Fujikawa, S.M. Brainstem evoked response (BER) audiometry at various stimulus presentation rates. J. Am. Audiol. Soc., 1977, 3: 59–62.

Woolf, N.K. and Ryan, A.F. The development of auditory function in the cochlea of the Mongolian gerbil. Hear. Res., 1984, 13: 277–283.

Woolf, N.K. and Ryan, A.F. Ontogeny of neural discharge patterns in the ventral cochlear nucleus of the Mongolian gerbil. Dev. Brain Res., 1985a, 17: 131–147.

Woolf, N.K. and Ryan, A.F. Development of cochlear and brain-stem auditory evoked potentials in the Mongolian gerbil. Neurosci. Abst., 1985b, 11: 450.

Yagi, T. and Kaga, K. The effect of the click repetition rate on the latency of the auditory evoked brain stem response and its clinical use for a neurological diagnosis. Arch. Otorhinolaryngol., 1979, 222: 91–97.

Yancey, C. and Dallos, P. Ontogenic changes in cochlear characteristic frequency at a basal turn location as reflected in the summating potential. Hear. Res., 1985, 18: 189–195.

Zimmerman, M.C., Morgan, D.E. and Dubno, J.R. Auditory brain stem evoked response characteristics in developing infants. Ann. Otol. Rhinol. Laryngol., 1987, 96: 291–299.