Maturation of the auditory event-related potentials during the first year of life

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This study examined the maturation of cortical auditory event-related potentials (ERPs) from birth until 12 months of age. In the 15 infants studied, all ERP peaks observable at 12 months of age, the P150, N250, P350, and N450 were identifiable already at birth. As in previous studies, the amplitudes of the ERP peaks increased and latencies shortened with increasing age. In addition, the time courses of the amplitude growth of these peaks differed from each other. It was concluded, that the generators of all the infantile ERP peaks are functional already at birth, and that the maturational changes in the waveform morphology can mostly be accounted for by the changing relative strengths of the different generators. *NeuroReport* 13:47–51 © 2002 Lippincott Williams & Wilkins.

Key words: Auditory; ERP; Event-related potentials; infants; Maturation; N250; Newborns

INTRODUCTION

The ERP methodology is especially useful in investigating central sensory processing in infants, since at this age, behavioral methods provide rather limited information about their perceptual capacities. In adults, the long-latency responses consist of the P1, N1, P2 and N2 components [1,2]. However, the auditory ERPs of newborns and infants have no resemblance to the adult ERP waveform.

The majority of full-term healthy newborns show ERPs with a predominant positive component at the midline electrode, and a negative component at the temporal electrodes [3]. This midline positivity of the newborn ERPs has most commonly been labeled as the infantile P2 [4–9]. Novak *et al*. [10] have, however, discerned two positive peaks in the latency range of the infantile P2 (100–300 ms). The discontinuity (a negative trough) between these two positivities had a peak latency of about 200 ms, and was termed N1 by the authors. Barnet [4], Molfese [11] and Ohlrich *et al*. [5] also labeled the infantile negativity at 200 ms N1, whereas other authors called it N2 [6,7,12] on the basis of its peak latency at 200 ms. However, still other authors have defined as the N2 a negativity at about 400–600 ms which follows the infantile P2. Barnet [4] defined the P2 (100–300 ms) and N2 (500 ms) as the response landmarks of the infant auditory ERP, since only these two peaks were present in all subjects of her study. Indeed, the P2 and N2 peaks can be identified in all the above-mentioned studies despite the differences in nomenclature, paradigm, and interstimulus interval (ISI).

There is lack of fine-graded longitudinal studies, following the development of the ERP waveform until it attains the childhood morphology. The results of the previous longitudinal studies are difficult to summarize due to the different peak nomenclatures used and problems in replicating of some results. In general, the above-mentioned studies have demonstrated the increase in waveform complexity, the enhancement of the amplitudes and the decrease of the latencies with age (for review see [13]). Some authors suggested that the ERPs of 3-month-old infants have a morphology similar to that in adults [6], but the problem is that the waveform structure of even 7- to 9-year-old children is different from that in adults. In children, the auditory ERP consists of the P1, N250 (N2), and the N450 (N4) peaks [2,14], when sounds are presented with ISIs < 1 s. Only with longer ISIs can an adult-like N1 wave be recorded in children, in addition to the N2 and the N4 waves 14–16. The correspondence between the child and infant ERPs has not yet been established. One reason being that different paradigms have been used at different ages. In infants, long ISIs (≥ 1 s) have been traditionally used, whereas in children the ISIs have often been shorter.

The purpose of the present study was to examine the development of the ERP waveform structure from birth to 12 months of age in the same infants, using a paradigm comparable to our earlier studies [17]. We chose the ISI of 700 ms that we also used in our previous study [14] with 7- to 9-year-old children in order to be able to compare the infants' and children's ERPs. Our purpose was to examine obligatory ERP components; therefore a relatively short ISI

was used in order to avoid the elicitation of attention/orienting-related components evoked when sounds are infrequently presented against a silent background. By applying tones of three different pitches we could use relatively short ISIs and yet obtain large enough ERP amplitudes, since presenting three different tones in the same sequence reduces frequency-specific neuronal refractoriness.

MATERIALS AND METHODS

The ERPs of 15 infants were recorded 2–4 days after birth and thereafter every 3 months until the age of 12 months. All infants were born at term (gestational age 38–42 weeks, Apgar score 8–10, weight 3060–4040 g), passed hearing screening by evoked otoacustic emissions (EOAE), and were considered healthy by a neonatologist.

The 100 ms three-partial harmonic tones of 500, 625 and 750 Hz fundamental frequencies were equiprobably presented with a 700 ms ISI (offset-to-onset). The second and third partials of each tone were lower in intensity by 3 dB and 6 dB, respectively.

The experiments were conducted in a silent room in the Hospital for Children and Adolescents of the Helsinki University Central Hospital. The sounds were presented through two loudspeakers placed 20 cm from both sides of the infant's head. Stimulus intensity at the infant's head was 70 dB SPL (sound-pressure level). The study protocol was approved by the Ethical Committees of the Department of Obstetrics and Gynecology and the Hospital for Children and Adolescents. Informed parental consent was obtained before recruitment.

The EEG was recorded (bandpass 0.1–30 Hz, sampling rate 250 Hz) using the NeuroScan 3.0 acquisition software. Disposable electrodes were attached to eight scalp sites: F3, F4, C3, C4, P3, P4, T3, and T4 according to the International 10–20 system. Eye movements were monitored with two electrodes, one placed below and the other at the outer corner of the right eye. The duration of the analysis epoch was 900 ms, including a 100 ms pre-stimulus time. The epochs were filtered (bandpass 1.0–15 Hz), baseline-corrected with respect to the mean amplitude of the pre-stimulus interval, and separately averaged for each tone.

As the auditory ERPs of newborns do not significantly differ from each other when obtained during active sleep or wakefulness [3,18], the data recorded during active sleep in newborns and during wakefulness in older infants are presented here. Active sleep was characterized by closed eyes, irregular respiration, rapid eye, and occasional body movements, and mixed or low-voltage irregular continuous EEG patterns.

The responses to the three tones were grouped together as no significant differences were observed at the group level. The number of the epochs in the individual and group averages was comparable across the ages (range 310–420; mean 389).

The ERP peaks were labeled according to their representative latencies at all ages (Fig. 1). The amplitudes of the P150 and P350 peaks were measured 50–250 and 250–400 ms from stimulus onset, and those of the N250 and N450 peaks at 180–280 and 350–600 ms from stimulus onset, respectively. Two-way ANOVAs (age

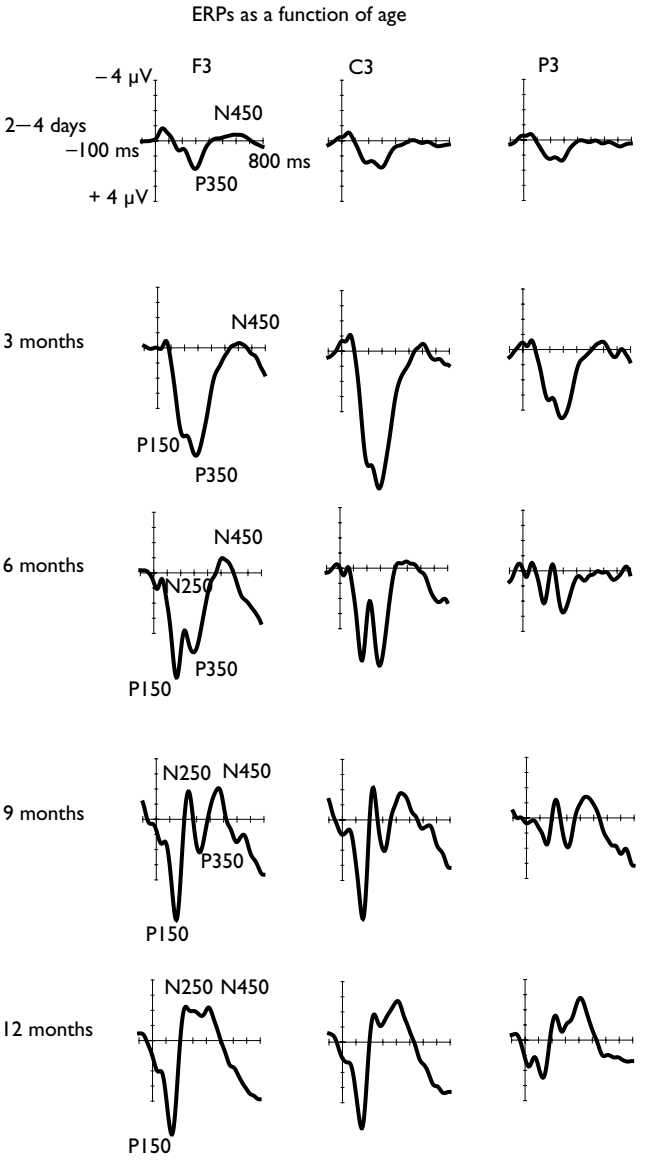

Fig. 1. Group-average ERPs obtained in response to 100 ms harmonic tones at birth (2–4 days) and at 3, 6, 9, and 12 months of age. The development of the P150-N250-P350-N450 peaks could be seen from their low-amplitude precursors at birth to the robust and well-defined peaks at 9 and 12 months of age.

(5) × electrodes (8)) were performed to examine the age effects for each peak separately. The sources of the significant ANOVA effects were determined by using the LSD (least significant difference) *post hoc* test. Greenhouse–Geisser adjustments were performed when applicable (ε values are reported).

RESULTS

Figure 1 shows the group-average ERPs obtained in 15 infants from birth to 12 months of age. At birth (top row), a positivity peaking at about 300 ms was the predominating peak, which was followed by a low-amplitude negative deflection at 450–600 ms (here called N450). A small

negative deflection (here called N250) could be seen about 200–250 ms between the two positive deflections, here called the P150 and the P350. Figure 2 demonstrates a distinct N250 at the age of 2–4 days and 3 months in individual infants.

The age effect on the P150 amplitude was significant ($F(4,56) = 7.55$, $p <= 0.0001$, $\varepsilon = 0.62$). The *post hoc* LSD test revealed that the P150 amplitude was significantly smaller at birth than at 3, 6, 9 and 12 months of age ($p < 0.003$–0.00001; the grand-average peak amplitudes at C3: 2.18, 8.76, 7.39, 8.91, 6.29 μV, for the newborn, 3-, 6-, 9-, and 12-month-old infants, respectively). No significant growth of the P150 amplitude was, however, revealed from the age of 3 months onwards. The P150 latency decreased from 3 to 6 ($p < 0.02$) and, further, to 9 months of age ($p < 0.0002$; main age effect $F(4,56) = 14.83$, $p < 0.00001$, $\alpha = 0.68$; grand-average peak latencies at C3: 179.6, 192.4, 158.5, 139.3 and 142.5 ms, for the newborn, 3-, 6-, 9-, and 12-month-old infants, respectively).

The main age effect on the N250 amplitude was significant at $F(4,56) = 12.11$ ($p < 0.00002$, $\varepsilon = 0.67$), the N250 being more negative at 9 months of age than at birth ($p < 0.002$), 3 months ($p < 0.00001$) or 6 months ($p < 0.02$; grand-average peak amplitudes at C3: 1.44, 6.87, 0.348, −2.45, −3.56 μV, for the newborn, 3-, 6-, 9, and 12-month-old infants, respectively). The N250 latency remained stable with age (main age effect non-significant; grand-average peak latencies at C3: 226.6, 234.9, 218.8, 225.7 and 238.1 ms for the newborn, 3-, 6-, 9-, and 12-month-old infants, respectively).

The main age effect on the P350 amplitude ($F(4,56) = 10.56$, $p < 0.0001$, $\varepsilon = 0.64$) originated from its significant growth from birth to 3 months ($p < 0.001$) and a decrease from 6 to 9 ($p < 0.01$) and 12 months of age ($p < 0.0003$; grand-average peak amplitudes at C3: 2.32, 7.84, 8.27, 3.97, and 2.14 μV for the newborn, 3-, 6-, 9-, and 12-month-old infants, respectively). No significant differences were found for the P350 peak latency (grand-average peak latencies at C3: 292.4, 326.3, 291.5, 308.0, and 332.9 ms, for the newborn, 3-, 6-, 9-, and 12-month-old infants, respectively).

The N450 amplitude was significantly smaller at birth and at 3 months of age than at 6, 9 and 12 months of age (main age effect $F(4,56) = 8.58$, $p < 0.0001$ $\varepsilon = 0.77$, *post hoc* tests $p < 0.05$–0.0001; grand-average peak amplitudes at C3: −0.71, −1.37, −3.28, −4.02, and −4.57 μV, for newborn, 3-, 6-, 9-, and 12-month-old infants, respectively). The N450 peak latency was found significantly shorter at 9 and 12 months than at any younger age (main age effect $F(4,56) = 2.88$, $p < 0.04$, $\varepsilon = 0.77$, *post hoc* tests $p < 0.05$–0.009; the grand-average peak latencies at C3: 504.0, 494.7, 497.2, 451.9, and 433.5 ms, for newborn, 3-, 6-, 9-, and 12-month-old infants, respectively.

DISCUSSION

The present study investigated the development of the long-latency auditory ERP to a repetitive harmonic tone from birth to 12 months of age. The amplitudes of all peaks increased during the first year of life, although the amplitude–age relationship was not linear. Notably, the precursors of all peaks seen at the age of 12 months were discernible already at birth.

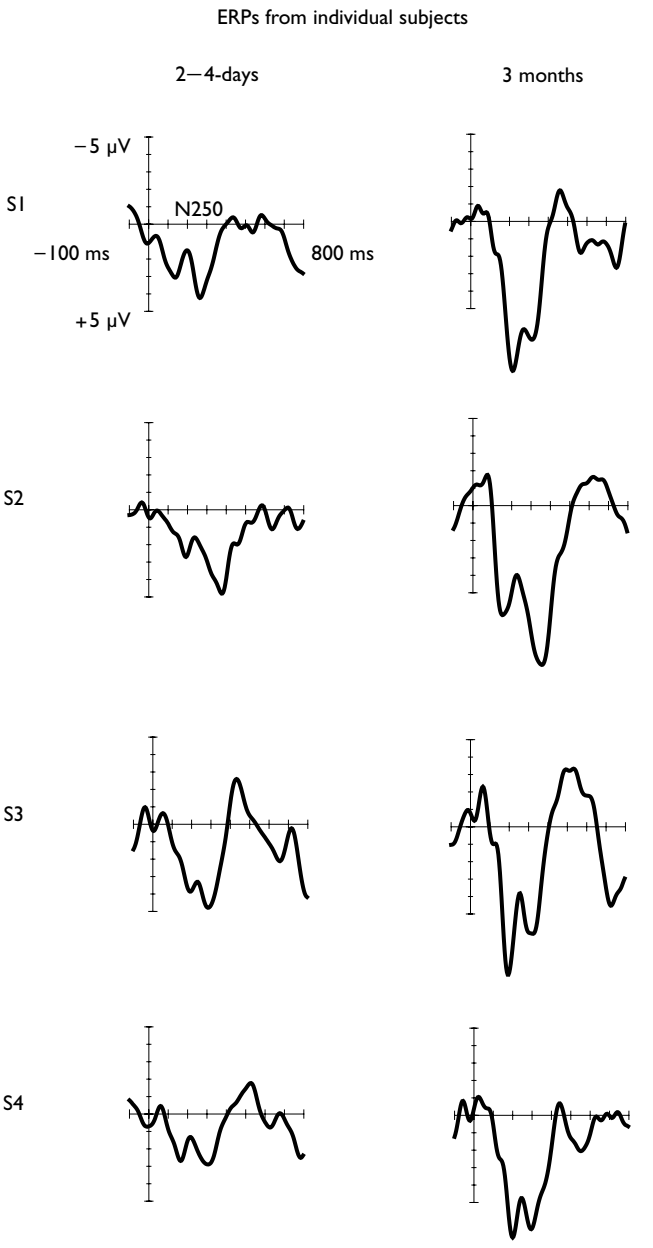

Fig. 2. The ERPs of four individual infants (S1–S4) demonstrating the presence of the N250 peak at birth (left column) and at 3 months of age (right column) in the individual data.

At birth, the ERP was predominated by a broad positivity at about 300 ms, followed by a negativity at 450–600 ms, corresponding to the infantile P2–N2 response landmarks according to Barnet [4]. The N250 peak, obtained in newborns in the present study, has been described previously as well [4,5,10–12,19]. However, in newborns it has not been reported as consistently as the positive–negative P2–N2 complex, most probably due to its low amplitude and greater latency variation at birth. Indeed, at birth and at 3 months of age in the group-average waveforms (Fig. 1) the N250 was just a protuberance in the positive-going slope, whereas in the individual records it

was much better defined (Fig. 2). This was probably caused by the inter-individual peak-latency jitter, which is substantial in newborns and is largest during the first 6 months of life [7]. Another possible reason for the poor expression of the N250 at birth is its overlap by the robust P150/P350 positivities. The present study demonstrated the emergence of the N250 peak from this small-amplitude negative trough: its latency remained constant across the age range studied, and its amplitude greatly increased, mostly between 3 and 9 months of age. Most likely, this can be attributed to the increasing strength of the N250 generators during this period. Consequently, the present data suggest that the generators of the N250 peak, clearly detectable in the grand-average waveforms from 6 months of age onwards, are active already at birth.

The N250 divided the infantile P2 into the earlier P150 and the later P350 peaks, which in the present study were regarded as separate peaks. However, the possibility that a single infantile P2 was subdivided into two peaks by an overlap with the emerging N250 needs to be considered. This assumption is supported by the finding that the P150 and P350 amplitudes increased to the same extent from birth to 3 months of age. However, thereafter their maturational trajectories started to dissociate. The P150 amplitude remained unchanged from 6 to 12 months of age, whereas the P350 amplitude significantly diminished from 6 to 9 months of age. This finding replicates the results of Kurtzberg *et al*. [20] who found an increase in the P2 amplitude until 6 months, followed by a decrement to 12 months. Accordingly, in the group-average waveforms of the present study, at 12 months of age, the P350 could only be seen as a discontinuity between the N250 and N450 peaks. Therefore, the present differential maturational courses of the P150 and P350 suggest the existence of at least two components of the infantile P2, which most probably reflect separate neural processes already at birth. This hypothesis supported by Novak *et al*. [10], who showed that the first peak of the double-peaked positive wave was of maximum amplitude frontally, whereas the second peak was largest more centrally.

The growth of the peak amplitudes, sharpening of their morphology, and the decrease in their latencies have been traditionally accounted for by the advancing myelination, increase in synaptic density, efficacy, and synchronization [21,22]. However, it should be also taken into consideration that the surface-recorded ERPs reflect the sum of the superimposed activity from multiple generators, which may have different maturational courses.

The present study demonstrated the mutual dependence of the amplitudes of the negative and positive ERP peaks during the first year of life. That is, the predominant growth of the positive peaks during the first 3 months of life might have obscured a weaker activity of the N250 generators during this period. The probable increase in the N250 generator strength by the 6 months of age resulted in the emergence of the robust N250 peak recorded at the scalp; in contrast, the amplitude of the P150 generally remained stable from 6 months onwards. The growth of the N250 peak during the second half of the first year of life was paralleled by the decrease in the P150 peak latency, presumably due to an overlap with the growing N250.

The significant diminution of the P350 amplitude from 6 to 12 months of age was accompanied by the growth of the N250 and N450 amplitudes and the decrease of the N450 peak latency. It can therefore be suggested that the P350 amplitude decrease during this period was caused by its temporal overlap with the increasing N250 and N450 generator activities.

On the basis of the present data, it is difficult to unambiguously conclude whether the infantile peaks are analogous to any of the adult ERP peaks. However, the ERP observed at 12 months of age (the P150–N250–N450 complex) seems to correspond to the P100-N250-N450 complex described in 3- to 7–9-year-old children [14,23]. The persistence of the P150 peak during the infancy, with a stable amplitude and steadily decreasing latency from 3 months of age suggest that it is the precursor of the childhood P100 which, in turn, develops into the mature adult P1 response [24] by further decreasing in latency and amplitude from 6 to 20 years of age [2]. In contrast, the infantile N250 remained at the same latency from at birth and can be considered the correlate of the childhood N250 (N2) at 12 months of age.

CONCLUSION

The present data indicate that the generators of the infantile auditory ERP components have different maturational courses during the first year of life. The main maturational change from birth to 3 months of age was a remarkable increase in the positive amplitudes (the P150 and P350 peaks), suggesting considerable strengthening of their generators. By 6 months of age, the growth of these positivities was terminated whereas the N250 peak became robust. From 6 to 9 months of age, the ERP maturation was characterized by a strong growth of the negativities (the N250 and N450). Finally, between 9 and 12 months of age, the infant ERP attained a morphology that is seen throughout the next 10 years of life: the P150-N250-N450 complex. Importantly, all the peaks observed at the age of 12 months were identifiable already at birth.

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