

Electrophysiological Brain Responses of Six-Month-Old Low Risk Premature Infants

Margarita Stolarova

University of Konstanz, Konstanz, Germany

Heather Whitney, Sara J. Webb, Raye-Ann deRegnier,
Michael K. Georgieff, and Charles A. Nelson

*Institute of Child Development
University of Minnesota*

This study sought to examine the electrophysiological correlates of visual perceptual priming in a sample of low risk preterm infants. We compared the ERP data of 20 healthy preterm infants at the age of 6 months (corrected age: 4 months) to those of 20 six-month-old and 20 four-month-old full term infants. The comparison groups represented the preterm infants' chronological and corrected ages respectively. The results showed that the brain responses of the preterm infants at the age of 6 months, specifically the topography and the latency of the Nc component, are more similar to those of their corrected age peers than to those of the chronological age controls.

In this study, we examined the electrophysiological brain responses of a group of low risk premature infants. Premature delivery interrupts a period of rapid brain growth (Bourgeois, 2001), and confronts an immature system with a set of atypical experiences, more characteristic of full-term (mature) infants. Studying a population of healthy preterm infants gives us an opportunity to examine the relative influence of experience versus maturation on the processes of neural and behavioral development, and gives us information about developmental pre-programming and developmental plasticity of the infant brain (van Hof-van Duin, Heersema, Groenendaal, Baerts, & Fetter, 1992). We compared a group of low

risk premature infants to two control groups of healthy full-term infants representing the corrected and chronological ages of the premature group respectively (4- and 6-month olds).¹ We assumed that the corrected age peers (4-month-old full-term infants) were at a similar level of biological maturation as the premature infants in this study, while the 6-month-old full-term infants had had approximately the same duration of postnatal experience as the premature infants. By comparing the premature group to each of the two full-term control groups, we hoped to determine the relative developmental stage of the preterm infants. The results reported here are part of a larger study on infant memory development in low and high risk premature infants.

In order to study neural functioning, we recorded event-related potentials (ERPs). ERPs have been employed increasingly in the study of infant cognition for the last 20 years and have been used to interpret the underlying processes for different cognitive tasks, predominantly in areas such as attention (Alho, Sainio, Sajaniemi, Reinikainen, & Naatanen, 1990), speech discrimination, and early word meaning (Molfese, 1990; Novak, Kurtzberg, Kreuzer, & Vaughan, 1989), and recognition memory (Nelson, 1994). This methodology has also been successfully applied to the study of clinical populations, such as infants with Down Syndrome (Karrer, Karrer, Bloom, Chaney, & Davis, 1998), and children with autism (Dawson, Carver, Maltzoff, Pangiotides, McParland, & Webb, 2002). We focus here on the middle latency *negative central* (Nc) component, a widely distributed component, maximal over fronto-central leads (e.g., de Haan & Nelson, 1997; for a recent review see Snyder, Webb, & Nelson, 2002). The Nc has been shown to reflect a basic orienting process, part of the obligatory attention allocation response (Richards, 2002). As such, it is suitable as a measure of developmental changes in very young infants.

In relation to our goals, we hypothesized that by comparing the ERP patterns of the premature infants, specifically the morphology and topography of their Nc, to those of the two control groups, we would be able to better understand the roles of biological maturation and experience in infant brain development. In particular, there are four possible outcomes to this comparison: The brain responses of the premature infants will (a) be more similar to those of their corrected age counterparts (here full-term 4-month olds), and different in terms of morphology and/or topography from the ERPs of the 6-month-old full-term infants, if a cognitive process is more strongly driven by biological maturation, (b) be more similar to those of their chronological age peers, if experience is the main driving force and the immature systems are capable of benefiting from it, (c) fall "in between"

¹The term *chronological age* refers to the infants' age since birth. The term *corrected (for prematurity) age* describes the infants' age since their projected due date for delivery following a full-term gestation. In this study, the premature infants were tested at 4-months corrected age and approximately 6-months chronological age.

4- and 6-month-old control infants, if both experiential and maturational factors are operating at the same time in an interactive pattern, and (d) be different from both 4- and 6-month-old full-term infants, without showing a tendency for an intermediate developmental stage, if some aspect of the infants' brain organization is altered by the experience of prematurity itself. If this is the case, we would expect the ERPs (here specifically the Nc) of the premature infants to differ in morphology or topography from those of both full-term comparison groups.

Previous research has demonstrated that the development of preterm infants' visual evoked potentials (VEPs) is more closely related to their corrected than to their chronological age (Atkinson, Anker, Rae, Weeks, Braddick, Rennie 2002; Roy, Barsoum-Homsi, Orquin, & Benoit, 1995). The ERP responses, investigated here using a visual priming paradigm with faces (based on Webb, 2001; Webb & Nelson, 2001, 2002), depend strongly on basic visual orienting. Thus, we hypothesized that the ERPs of the premature infants would resemble those of the 4-month-old full-term controls in terms of amplitude and latency of the Nc, and would differ from the ERP responses of the 6-month-old full-term infants.

METHODS

Participants

Preterm group. Preterm infants qualified for this study if they had been born between the 31st and the 33rd week of gestation (assessed using the best available obstetric evidence and confirmed by physical examination of the newborn), were appropriate for gestational age (AGA) in size and weight at birth, and had Apgar scores >7 at 5 min (Apgar & James, 1962). Premature infants were excluded if they had experienced an intraventricular hemorrhage (IVH), had been mechanically ventilated for more than 24 hr, had neurobiological risk scores (Brazy, Eckerman, Oehler, Goldstein, & O'Rand, 1991; Contractor, Leslie, Bowen, & Arnold, 1996) higher than three (scores of six or more place an infant at increased risk for developmental impairments), or if the pregnancy had been complicated by diabetes or maternal alcohol or drug abuse. These inclusion and exclusion criteria ensured that the infants participating in this experimental group did not have a history of significant medical complications other than prematurity.

A total of 59 preterm infants were enrolled in this study. Each premature infant was tested within a week of the infants' 4-month corrected birthday, which means, 4 months after the estimated delivery date ($M = 17$ weeks, range 16 to 18 weeks) at approximately six months chronological age ($M = 25$ weeks, range 24 to 26 weeks), see Table 1. Of the 59 premature infants tested, 39 were excluded due to: (a) over- or underestimation of their gestational age at birth ($n = 8$), (b) medical complications not apparent at the time of enrollment, but revealed by an additional

TABLE 1
Mean Age at Testing in Weeks and Mean Number of Trials Per Condition Included in the Individual Averages for Each Group

	Age at Testing		Mean Number of Trials Per Condition		
	<i>M</i>	Range	<i>M</i>	<i>SD</i>	Range
4-month-old controls ^a	17 weeks	16–18	14.2	2.6	10–20
Premature infants ^a					
<i>corrected age:</i>	17 weeks	16–18			
<i>chronological age:</i>	25 weeks	24–26	13.2	2.7	10–20
6-month-old controls ^a	25 weeks	25–26	15.6	3.0	11–20

Note. The two control groups represented the corrected (4 months) and the chronological (6 months) ages of the premature infants. No differences between the groups with regard to trial number were found. Infants with fewer than 10 artifact trials were not included in the grand averages computed for each group and condition. The averages for the different conditions included an equal number of trials per channel within the individual subject's data set.

^a*n* = 20.

review of the infants' medical records by the experimenters (*n* = 9), (c) excessive data artifact generally caused by infant movement or fussiness (*n* = 22). There were no significant differences between the included and excluded premature infants on any of the collected demographic variables or developmental status. The present sample consisted of 20 preterm infants (4 males), born between the 31st and 33rd week of gestation, who met the inclusion criteria and had more than 10 artifact-free trials per channel and condition (*M* = 15, range 10 to 20 trials). The small percentage of included male infants reflects the fact that male infants tend to have more medical complications than female infants (Lauterbach, Raz, & Sander, 2001), and, thus, fewer boys met our inclusion criteria.

Full term control groups. The 4- and 6-month-old full-term infants were recruited from a community volunteer participant pool. The full-term 4-month olds serve as a comparison group for the preterm infants' corrected for prematurity age, while the full-term 6-month olds serve as a chronological age comparison group. Infants in the full-term groups were born within 10 days of their estimated delivery date, and were tested within a week of their 4- or 6-month birthdays, respectively, with the mean age at testing: 4-month-olds: *M* = 17 weeks, range 16 to 18 weeks; 6-month olds *M* = 25 weeks, range 25 to 26 weeks (see Table 1). They were included in the final sample only if they had no history of pre- or perinatal medical complications as reported by their parents at test. A total of 138 full-term control infants were tested (69 four- and 69 six-month olds). The data for 81 infants were excluded due to: (1) medical complications, most often gestational diabetes (*n* = 16) and (2) excessive data artifact due

to movement or fussiness (*n* = 65). Out of a total of 57 full-term infants without any history of medical complications and with more than 10 artifact-free trials per channel and condition (see Table 1 for mean number of trials per group), a subsample of 40 infants (20 four- and 20 six-month olds) were matched to the preterm group according to test order and gender (7 infants with recognition memory task first, 4 males in each group).

With regard to the demographic background of the infants in the three groups (Table 2), when subjected to an overall ANOVA, no significant differences emerged in terms of socioeconomic status (SES) as measured by household income and maternal education. No group differences were found with regard to non-parental care the infants received, their feeding history, or primary caregiver. The only variable differentiating the preterm infants and the two control groups was the number of twins included (40% in the preterm group, 0% in the two control groups). Multiple birth tends to be a common cause of prematurity in the gestational age population of interest here (Gardner, Goldenberg, Cliver, Tucker, Nelson, & Copper, 1995). In contrast, full-term twin gestations are rarer and are more often accompanied by pre- and perinatal medical complications than singleton gestations (Buscher, Horstkamp,

TABLE 2
Demographic Characteristics

Demographic Variables		4-Month Olds (%)	Preterm Infants (%)	6-Month Olds (%)
Household income per year	< \$25,000	0	15.8	0
	\$25,000–\$65,000	52.6	36.8	40
	> \$65,000	47.4	47.4	60
Maternal education	High school degree or less	10.5	5.3	5
	2-year college degree or some college	21.1	15.8	15
	4-year college degree	36.8	47.4	45
	Postgraduate studies	31.6	31.6	35
Care type	Home	88.9	63.2	66.7
	Day-care	11.1	36.8	33.3
Primary caregiver	Mother	78.9	73.7	58.8
	Father	0	10.5	5.9
	Both	21.1	15.8	35.3
Breastfeeding for 2 months or more	Yes	83.3	78.9	94.4
	No	16.7	21.1	5.6
Multiple birth**	Singleton	100	60	100
	Twin	0	40	0

Note. The full-term controls were matched with the preterm infants in terms of gender (4 males in each group) and test order (7 infants with recognition task first). The only significant difference (**) was found in regard to the number of twins in each group.

Wessel, Chen, & Dudenhausen, 2000), which would have led to exclusion from the present sample. We can assume that the asymmetries in regard to the number of twins included in the 3 groups reflect the selection criteria used.

Stimuli

The stimuli were full screen color video images of female faces. The visual angle subtended by the stimuli was approximately 12°. When shown to the participants, each image filled a 13-in. (33 cm) monitor. The models posed a neutral facial expression in front of a gray background with a gray scarf covering their clothing.

ERP Recording Procedure

Infants were seated at a distance of approximately 60 cm from a computer screen in a darkened room. Each testing session consisted of two experiments: a visual priming paradigm (based on Webb, 2001; Webb & Nelson, 2003), presented in this analysis, and a recognition paradigm (based on de Haan & Nelson, 1997), not reported here. The test order was counterbalanced across subjects. The recording ended when the maximum number of available trials (102 in the priming and 100 in the recognition paradigm) was reached or when the participants became too tired or too fussy to continue. Infants were presented with a series of pictures of unfamiliar female faces, each displayed for 500 ms, and followed by 1700–2200 ms gray screen presentation. In the priming experiment, a total of 62 novel images were shown, 20 of these pictures were primed immediately (the primed stimulus followed immediately after the initial presentation), 20 were primed with a delay (the primed stimulus followed after four intervening trials), and 22 were not repeated. When the infant was not attending, the EEG was not recorded, and the stimuli were repeated until the participant looked back at the screen.

Electrophysiological Recording, Data Reduction and Data Analysis

The electroencephalogram (EEG) was recorded from 14 scalp locations using silver-silver-chlorided (Ag-Ag-Cl) electrodes and Grass EC2 electrode paste. The electrodes were placed over midline (Fz, Cz, Pz, Oz) and lateral (F3, F4, C3, C4, T3, T4, T5, T6, P3, P4) scalp positions according to the international 10–20 system (Jasper, 1958). The data were referenced to Cz during recording and re-referenced off line to linked mastoids. A ground electrode was placed on the forehead, the electrooculogram (EOG) was recorded from bipolar electrodes placed

vertically above and below the infant's right eye. Impedances were accepted if they were less than or equal to 10 k Ω .

The EEG and EOG were recorded using a Grass Neurodata Acquisition system with Model 12A5 amplifiers. The EEG gain was set to 20,000, the EOG to 5,000. A bandpass filter of 0.1–30 Hz was applied and a 60 Hz notch filter was engaged. The EEG was sampled at 100 Hz. Each trial consisted of 100 ms baseline recording, 500 ms stimulus presentation, 1200 ms post stimulus recording, and a randomized intertrial interval (ITI) ranging from 500 to 1000 ms.

Individual artifact-free trials were combined to form average waveforms for each subject and each condition (novel vs. primed immediately vs. primed with a delay). Artifact rejection procedures were similar to those described in previous reports (e.g., Webb & Nelson, 2001, 2002). Infants with fewer than 10 artifact free trials per channel and condition were not included in the grand averages computed for each group and stimulus type. The averages for the different conditions included an equal number of trials per channel within the individual subject's data set (Table 1).

Attention was focused on the middle latency negative component Nc (400–770 ms). Frontal, central, and parietal electrodes were subject to an omnibus repeated-measures ANOVA with the within-subject factors CONDITION (Novel, Primed immediately, Primed delayed) and LOCATION (Frontal with Fz, F3 and F4, Central with Cz, C3 and C4, Parietal with Pz, P3 and P4), as well as the between-subject factor GROUP (premature infants, four-month and six-month full-term control groups). Follow-up ANOVAs were conducted for significant main effects and interactions ($p < .05$), the Greenhouse-Geisser method was applied to correct for violation of sphericity, post hoc tests employed the Bonferroni correction for multiple comparisons.

RESULTS

The analysis of the average Nc amplitude revealed a significant main effect of LOCATION ($F(2, 114) = 59.3, p < .01$). The follow-up pairwise comparisons showed that the mean average amplitude at frontal and central recording sites was significantly more negative than the average amplitude at parietal sites. A LOCATION \times GROUP interaction, $F(4, 354) = 7.3, p < .01$, was also observed. The follow-up ANOVAs revealed significant location effects for all three groups, preterm infants, $F(2, 38) = 50.5, p < .001$, 4-month-old control group, $F(2, 38) = 14.6, p < .001$, and 6-month-old full term infants, $F(2, 38) = 5.6, p < .05$. In the 2 less mature groups, the average amplitude at parietal electrodes was significantly less negative than at frontal, $p < .001$, and at central, $p < .001$, leads. In the 6-month-old group, however, there was no significant difference between parietal and central leads, only between parietal and frontal leads, with parietal leads again showing less negativity. This means that while the Nc demonstrated the expected distribution with maximal amplitude over fronto-central electrodes in all three

groups, there is a tendency for decreased negativity at frontal and increased negativity at parietal leads across all conditions in the 6-month-old control group compared to both less mature groups.

The analysis of latency to peak of the Nc demonstrated main effects of GROUP, $F(2, 57) = 6.1, p < .05$, CONDITION, $F(2, 114) = 3.2, p < .05$, and LOCATION, $F(2, 114) = 5.5, p < .01$, as well as CONDITION \times GROUP, $F(4, 354) = 2.9, p < .05$, and LOCATION \times GROUP, $F(4, 354) = 6.1, p < .01$, interactions. In order to clarify the contributions of the fronto-central versus the parietal electrodes to the group differences of interest, two ANOVAs with CONDITION and LEAD as within-subject factors and GROUP as the between-subject variable for a combined fronto-central sensor group (F3, F4, Fz, C3, C4, and Cz) and for the parietal electrodes (P3, P4 and Pz) were conducted. For the fronto-central leads both the preterm group ($M = 582.4$ ms) and the four-month-old full-term group ($M = 584.3$ ms) demonstrated significantly longer latencies to peak minimal amplitude than the 6-month-old full-term group ($M = 522$ ms). There were no differences between the preterm infants and the four-month-old full-term infants. For parietal electrodes, no group differences were found (see Figure 1 and Figure 2).

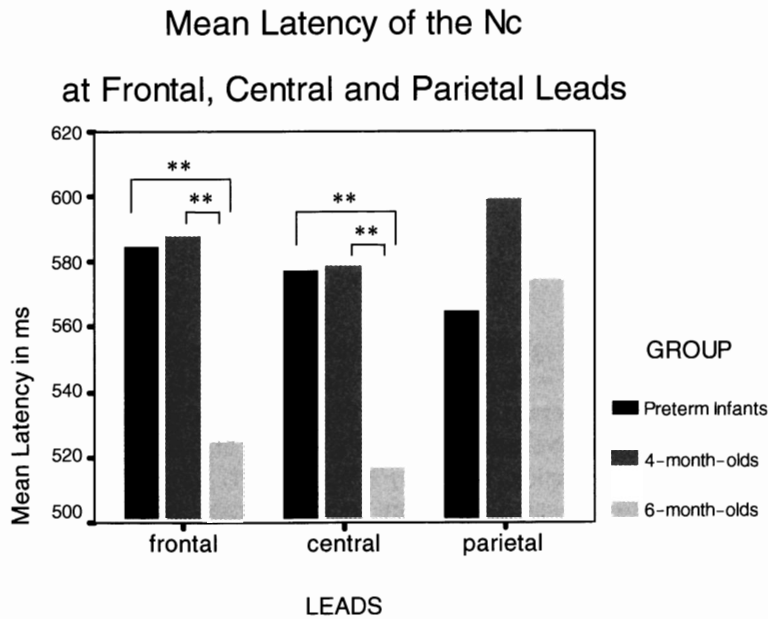


FIGURE 1 Mean latency of the Nc at frontal, central, and parietal leads across conditions. The group differences (**) in regard to mean latency to peak amplitude are carried by the frontal and central electrodes, while there are no significant group differences in latency of the Nc at parietal leads.

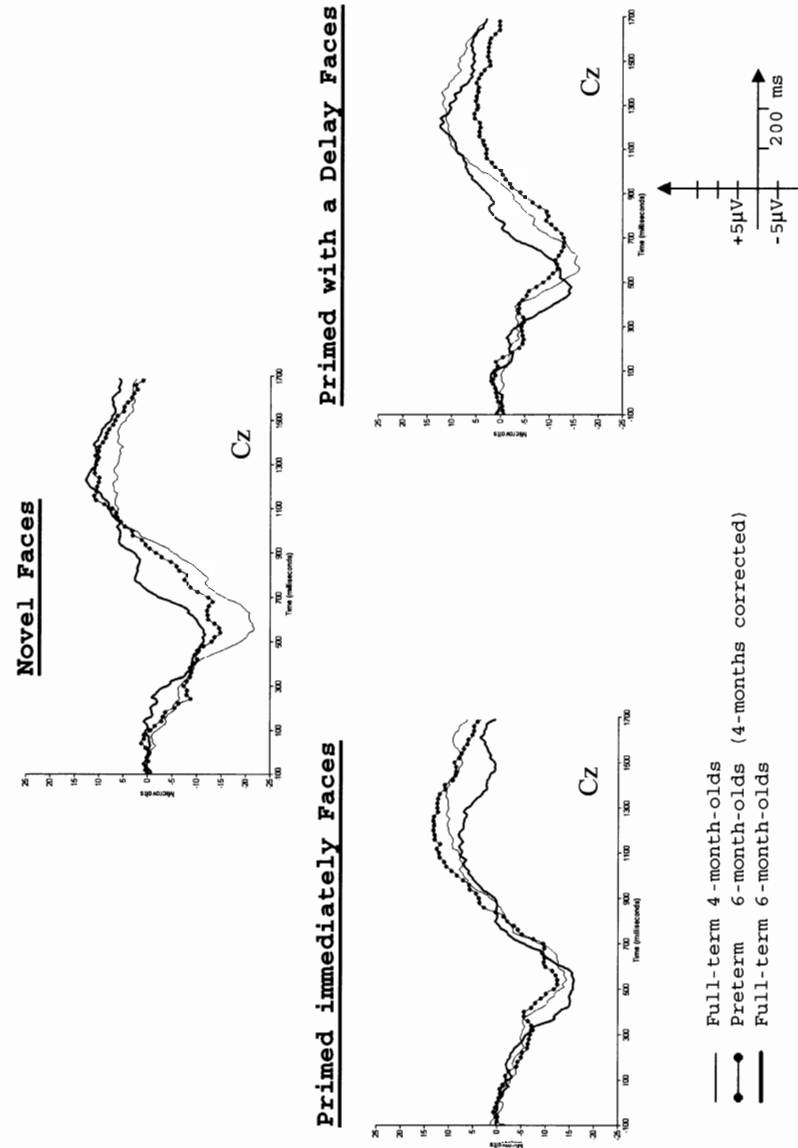


FIGURE 2 Sample ERPs for all groups and conditions at Cz. The preterm infants and the 4-month-old full-term infants demonstrate on average longer latencies to peak amplitude of the Nc compared to the 6-month-old full-term infants.

Follow-up ANOVAs revealed that the condition effect is carried by the 6-month-old group, main effect of CONDITION, $F(2, 38) = 12.9, p < .01$. Faces primed with a delay elicited on average significantly longer latencies ($M = 637.4$ ms) compared to both novel faces ($M = 534.2$ ms) and primed immediately faces ($M = 548.6$). A CONDITION \times LOCATION interaction, $F(4, 76) = 11.5, p < .01$, showed that the latency to peak amplitude at parietal electrodes was very similar across conditions, while for the fronto-central sensor group, the primed with a delay faces elicited a significantly slower response than the other two conditions. No significant main effects or interactions with CONDITION were found for the preterm infants or the 4-month-old full-term group.

In summary, the preterm infants demonstrated longer latencies to peak amplitude at frontal and central leads in comparison with the 6-month-old full-term infants (Figure 1). No significant differences between the preterm and the 4-month-old full-term groups were found. In addition, in the 6-month-old control group, a condition effect of priming was observed: the primed with a delay faces elicited a slower response than the two other conditions. No differences between novel and primed-immediately faces were found. The 2 post-conceptionally less mature groups did not show significant condition effects.

DISCUSSION

The main goal of this study was to examine the Nc component of the ERP in a group of 6-month-old healthy preterm infants in comparison to those of 2 full-term control groups. The control groups were chosen so that they represented the chronological (6 months) and the corrected for prematurity (4 months) ages of the preterm infants. We aimed to determine whether the preterm infants' brain responses were commensurate with their corrected or chronological age. In addition, we hypothesized that the differences between these 3 groups would contribute to our understanding of the relative influence of maturation and experience, with regard to early visual processing.

The most apparent and stable group differences were found in latency to peak amplitude of the Nc. Across frontal and central leads, the 6-month-old full-term group demonstrated shorter latencies (by an average 60 ms) than the 2 less mature groups (Figure 1 and Figure 2). The mean latencies in the preterm group and the 4-month-old full-term group were nearly identical ($M = 582.4$ ms and $M = 584.3$ ms, respectively). Previous research has shown that the latency to peak amplitude for early ERP components decreases throughout childhood (Barnet, 1975; Courchesne, 1978; Eggermont, 1988; Ponton, Eggermont, Kwong, & Don, 2000). This development toward faster processing has been demonstrated for the auditory, visual, and somatosensory modalities across a variety of experimental designs, and has been interpreted as a general, task-independent, speed of information

processing increase, possibly due to the progressing myelination of the corresponding brain areas and their interconnections, or due to an increase in efficiency of the synapses (Eggermont & Don, 1986).

Our results show that in terms of processing speed, the 6-month-old preterm infants are similar to their corrected age peers and slower than their chronological age controls. It is noteworthy that the latencies obtained in the preterm group were not longer than those in the corrected age control group (4-month-old full-term infants). This finding suggests that moderately premature infants with no additional medical complications do not show evidence of developmental delays in their electrophysiological response to visual stimuli, but appear to develop at the same rate as full-term infants of the same post-conceptual age. Thus, with regard to speed of processing, brain development at this early postnatal stage seems to follow its maturational trajectory, and depend less on experience, regardless of the premature birth.

The analyses of the Nc amplitude also revealed group differences. Specifically, the 6-month-old full-term group showed decreased negativity across frontal electrodes and increased negativity across parietal leads, leading to a reduction of the differences between anterior and posterior recording sites, when compared with each of the 2 less-mature groups. There were no differences between the 4-month full-term controls and the preterm group. The pattern of group differences observed for the amplitude of the Nc is similar to the group differences for the latency of the Nc in that the 2 less-mature groups showed very similar responses, while differing significantly from the 6-month-old full-term controls.

In electrophysiological studies of adult cognition, it has been hypothesized that a decrease in amplitude reflects a reorganization towards more automated and efficient processing (Desimone, 1998). Greater negativity of the Nc at fronto-central leads in infants has previously been interpreted as a sign of greater allocation of attention (Courchesne, Ganz, & Norcia, 1981). Recently, Richards (2002) provided additional evidence for an increase of the Nc amplitude during attention in infants at ages 4.5, 6, and 7.5 months. Karrer et al. (1998) have shown that 6-month-old infants with Down Syndrome demonstrate larger Nc areas and greater peak amplitudes compared to 6-month-old typically developing infants in an 80/20% oddball paradigm with female faces as stimuli. The authors interpreted this as a sign of slower and less efficient stimulus encoding in the Down Syndrome group. It is likely that the patterns described by Karrer et al., reflect a developmental delay, while the ERPs reported here, for the 2 less mature groups, represent an earlier stage of development compared with the 6-month-old full-term infants. Thus, the change in amplitude of the Nc likely reflects a developmental progression towards greater efficiency.

The electrophysiological methodology used here has proved suitable for tracking developmental changes across the relatively short time span of 2 months. It

has also helped evaluate indirectly the relative stage of brain development in a clinical infant population. The results of this study indicate that at the chronological age of 6 months, the brain responses of low risk premature infants develop according to their post-conceptual age. At an early postnatal developmental stage this phenomenon seems to be task-independent, and probably reflects general maturationally driven developmental processes, such as increasing myelination and connectivity of the infant brain. It can be expected that, with age, the interaction of maturational and experiential factors will become increasingly important (Katz & Shatz, 1996; Singer, 1986; Whitney, 2003).

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