

Evidence of altered central nervous system development in infants with iron deficiency anemia at 6 mo: delayed maturation of auditory brainstem responses¹⁻⁴

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ABSTRACT Iron deficiency anemia has long been thought to have effects on the central nervous system (CNS). Finding direct evidence of this in human infants, however, has been challenging. Auditory brainstem responses (ABRs) provide a noninvasive means of examining an aspect of the CNS that is rapidly maturing during the age period when iron deficiency is most common. ABRs represent the progressive activation of the auditory pathway from the acoustic nerve (wave I) to the lateral lemniscus (wave V). The central conduction time (CCT, or wave I–V interpeak latency) is considered an index of CNS development because myelination of nerve fibers and maturation of synaptic relays lead to an exponential reduction in the CCT from birth to 24 mo. In 55 otherwise healthy, 6-mo-old Chilean infants (29 with iron deficiency anemia and 26 nonanemic control infants), the CCT was longer in those who had been anemic at 6 mo, with differences becoming more pronounced at 12- and 18-mo follow-ups despite effective iron therapy. The pattern of results—differences in latencies but not amplitudes, more effects on the late ABR components (waves III and V), and longer CCTs (as an overall measure of nerve conduction velocity)—suggested altered myelination as a promising explanation, consistent with recent laboratory work documenting iron's essential role in myelin formation and maintenance. This study shows that iron deficiency anemia in 6-mo-old infants is associated with adverse effects on at least one aspect of CNS development and suggests the fruitfulness of studying other processes that are rapidly myelinating during the first 2 y of life. *Am J Clin Nutr* 1998;68:683–90.

KEY WORDS Iron deficiency anemia, nutritional anemia, auditory brainstem responses, central nervous system development, myelination, infants, central conduction time

INTRODUCTION

For years, there has been speculation that iron deficiency during infancy has long-lasting effects on the central nervous system. Iron deficiency is considered to be the world's most common single-nutrient disorder, with a peak prevalence in infancy. An estimated 25% of babies worldwide have iron deficiency anemia (1, 2), and an even higher proportion have iron deficiency without anemia. However, because of the many challenges of studying the central nervous system in human infants, direct evi-

dence of central nervous system effects has come from animal studies. That evidence is increasingly compelling. In addition to older research on iron's role in central nervous system neurotransmitter function (for reviews, *see* references 3–5), more recent work shows that brain iron is essential for normal myelination (6, 7). In rats, there is an influx of transferrin and iron into the brain in the immediate postnatal period. As iron and its transport and storage compounds are redistributed in the brain, myelinogenesis and iron uptake are at their peak. Iron and its related proteins concentrate in oligodendrocytes and become more concentrated in white than in gray matter (most brain iron is found in this myelin fraction). Oligodendrocytes require iron to synthesize fatty acids and cholesterol for myelin production (8). Furthermore, animal studies have consistently found a lasting deficit in brain iron when iron deficiency occurs early in development (9–13). Although only 2 studies of iron deficiency in animal models examined myelination directly, both found iron-deficient rats to be hypomyelinated (8, 14).

The results of these and other animal studies indicate that iron deficiency during brain growth has long-lasting effects on the central nervous system. Yet, obtaining evidence of similar effects in human infants has posed many methodologic challenges. Over the past 20 y, research on the effects of iron deficiency and iron therapy on infant development has depended heavily on standardized tests of infant development, which have important limitations and bear unknown relations to central nervous system func-

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²Preliminary results of this study were presented as a poster at the Pediatric Academic Societies' Annual Meeting, Washington, DC, 1996 (*Pediatr Res* 1996;39:20A) and at the 14th International Congress of EEG and Clinical Neurophysiology, Florence, Italy, 1997 (*Electroenceph Clin Neurophysiol* 1997;103:63).

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tions. By measuring brainstem auditory evoked potentials in the present study, we provide more direct evidence of central nervous system alterations in iron-deficient anemic infants. Such neurophysiologic measurements have not been conducted previously in iron-deficient infants. They were possible in this study because of the presence of a highly sophisticated infant neurophysiology laboratory in Chile, a rapidly industrializing country in which iron deficiency in infancy has been widespread.

Changes in auditory brainstem evoked potentials or responses (ABRs) are particularly relevant to study in infants with iron deficiency. ABRs consist of a succession of 5–7 waves recorded at the scalp within the first 10 ms of stimulation (15, 16). ABRs seem to represent the progressive activation of different levels of the auditory pathway, from the distal part of the acoustic nerve (wave I) to the lateral lemniscus (wave V) (17). Developmental changes in ABRs have been studied carefully. There are well-established developmental progressions—decreases in absolute and interpeak latencies, decreases in duration, and increases in amplitude—of the waves from birth until age 18–24 mo, when stable values are reached (18). Latency changes have been related to increases in conduction velocity during axonal myelination (19–21). Other changes, such as increases in amplitude and reductions in duration, are probably due to improvements in synchronization at the axonal or synaptic levels (22). Thus, these developmental progressions are occurring during the age period when iron deficiency is most common.

The purpose of the present study was to determine whether iron deficiency anemia impairs the neuromaturation of the auditory pathway. At the time the study was designed in 1990, we wondered whether there might be alterations in ABR development in iron-deficient anemic infants, because previous work by our group and others had shown that protein-energy malnutrition during infancy produced dramatic alterations in morphology, amplitude, and latency of ABRs (23, 24) that persisted after nutritional recovery. However, we did not have specific predictions about which components would be altered. In the intervening years, iron's role in myelinogenesis became clearer. In light of this new information, we considered that the parameters of ABRs that show rapid developmental changes as a result of axonal myelination (eg, latency indexes, which reflect the speed of nerve conduction) should be most affected. Preliminary results of this study were presented previously (25, 26).

SUBJECTS AND METHODS

Subjects

This study was conducted in Chile, a South American democracy with a highly literate population and a comprehensive health care system. Infant health is generally excellent in Chile; generalized undernutrition, hemoglobinopathies, hookworm infection, and elevated lead concentrations are virtually absent. However, dietary iron deficiency is common. According to Chilean custom, almost all babies are breast-fed in the early months and ≈50% are breast-fed up to 6 mo of age. Unmodified powdered cow milk, which may contribute to iron deficiency, is distributed free of charge at all health maintenance visits as part of a long-standing, highly effective program to prevent generalized undernutrition. Solid foods (fruit and cereal) are usually introduced at 4 mo of age, with meat and vegetables added at 6 mo and legumes and eggs at 9 mo. This diet provides ≈5 mg Fe,

mostly of vegetable origin and of poor bioavailability (27). Previous studies have consistently shown that 27–35% of Chilean infants fed powdered cow milk develop iron deficiency anemia at 9–18 mo of age, and biochemical evidence of iron deficiency is present in 43–65% of infants (28, 29). Routine pediatric care and infant feeding practices in Chile during this study did not include iron supplementation, although a national program of iron fortification of milk for infants is scheduled to start soon.

This neurophysiology study was conducted between 1991 and 1996 in conjunction with a clinical trial of the developmental effects of preventing iron deficiency. Potential study participants for both the preventive trial and the neurophysiology study were identified during routine pediatric visits at community clinics of the national health system in 4 contiguous urban communities on the southeastern outskirts of Santiago, Chile. The communities, with running water, sewage facilities, and electricity, are located at an elevation of 600 m and are inhabited by primarily lower middle class residents, of generally homogeneous ethnic origin.

At the 4-mo clinic visit, infants were evaluated to ensure that those considered for study participation were healthy according to the following entrance criteria: birth weight ≥3.0 kg, singleton birth, no major congenital anomalies, no major birth or neonatal complications, no emergency cesarean delivery, no jaundice requiring phototherapy, no hospitalization for other than an uncomplicated problem, no chronic illness, no iron therapy, and no evidence in a pediatric physical examination of failure to thrive, specific nutrient deficiency, or other conditions that could interfere with development. Other entrance criteria were specific to successful completion of the study: residence within the identified neighborhoods; a stable, literate caregiver who was available to accompany the infant to project appointments; no other infant <12 mo of age in the household; and infant not in daycare (30).

Between 5 and 6 mo a finger-stick hemoglobin determination was performed [HemoCue; Leo Diagnostics, Helsingborg, Sweden (31)]. Infants with finger-stick hemoglobin concentrations >103 g/L entered the preventive trial (30, 32). For those few children in whom finger-stick hemoglobin values were ≤103 g/L, a venipuncture was performed immediately for determination of iron status. Iron-status measures were performed by the Hematology Unit of the Institute of Nutrition and Food Technology, which serves as the reference laboratory for these assays for all of Chile and for other parts of South America. Measures included hemoglobin, hematocrit, mean cell volume (MCV) [model ZBI; Coulter, Hialeah, FL (33)], serum ferritin (34), and erythrocyte protoporphyrin (EP) [Hematofluorometer; Helena Laboratories, Beaumont, TX (35)]. Anemia at 6 mo was defined as a venous hemoglobin concentration ≤100 g/L (36). (The concentration for the screening finger-stick hemoglobin value was chosen to be somewhat higher to increase the chances that all infants with venous hemoglobin concentrations ≤100 g/L would be identified and removed from the preventive trial.) Iron deficiency was defined as ≥2 iron measures in the deficient range [MCV < 70 fL (37), EP > 1.77 μmol/L (100 μg/dL) red blood cells (38), serum ferritin < 12 mg/L (38)], an increase in hemoglobin of ≥10 g/L after 6 mo of iron therapy, or both (37). Cut-offs for anemia and iron measures were based on the consensus of the hematologists of the project (Tomas Walter) and the External Advisory Committee (Peter Dallman, Fernando Viteri, and Ray Yip), who used normative data to establish ≈2 SDs from the mean for 5–6-mo-olds. For each infant with iron deficiency anemia, an infant with a normal finger-stick hemoglobin value was

selected randomly to receive a venipuncture. Those who were clearly nonanemic (hemoglobin ≥ 115 g/L), regardless of iron status, constituted the comparison group (subsequent analyses considered the effects of iron deficiency within the nonanemic group). These 6-mo-old infants with iron deficiency anemia and the nonanemic control infants did not enter the preventive trial. They were not at a disadvantage compared with those in the preventive trial with respect to relevant factors such as birth weight and growth at 6 mo.

All infants in the neurophysiology study were treated orally for 1 y with 15 mg elemental Fe/d as ferrous sulfate (Fer-in-Sol; Mead Johnson Nutritionals, Evansville, IN). This dose was intended to be therapeutic for anemic infants and prophylactic for nonanemic infants during the period between 6 and 12 mo and prophylactic for all infants thereafter. Adherence was monitored through weekly home visits and monthly pediatric clinic visits. A venipuncture was repeated at 12 mo to determine response to therapy with respect to hemoglobin concentration and iron measures; a finger-stick hemoglobin concentration was obtained at 18 mo to monitor maintenance of improvement. Project personnel and parents understood that all infants would receive oral iron, but neither parents nor project personnel were informed of a child's hematologic status until study completion.

Project physicians provided routine pediatric health maintenance and care for illnesses between regular checkups for all infants who met the entrance criteria. Regular examinations were given monthly between 4 and 12 mo of age. For infants in the neurophysiology study, checkups continued bimonthly between 12 and 18 mo. At each visit, infants received routine pediatric physical examinations, including otoneuroscopy. Thus, the project offered more than usual health maintenance and prompt attention to any intercurrent illnesses. If an infant was sick at the time of a scheduled neurophysiology assessment, the evaluation was postponed until the child recovered. ABR measures (described in the next section) were made before treatment (at 6 mo) and were repeated at 12 and 18 mo of age.

All aspects of the study were explained to parents of qualifying infants, and signed, informed consent was obtained. Ninety-seven percent agreed to participate. The research protocol was approved by the institutional review boards of the University of Michigan Medical Center, Ann Arbor; INTA, University of Chile, Santiago; and the Office of Protection from Research Risks, National Institutes of Health, Bethesda, MD.

This report compares ABR variables in 29 infants who had iron deficiency anemia at 6 mo and 26 infants who had normal hemoglobin concentrations. With the exception of sex, there were no significant differences between the anemic and nonanemic groups in characteristics related to birth or growth (Table 1). These were well-nourished infants, whose growth was at the US 50–75th percentile (39), on average, both at birth and on entry into the neurophysiology study at 6 mo. Because a greater proportion of the anemic group was male, all findings are reported controlling for sex. Of the infants, 85% were restudied at 12 mo and 71% at 18 mo. Attrition was similar in both groups. There were no significant differences between children who did and those who did not complete the 1-y study with respect to factors such as sex, birth weight, or mother's education.

ABR procedures

The infants were tested during a spontaneous afternoon nap, generally while in quiet sleep. No sedatives were used. ABR

TABLE 1
Characteristics of infants at 6 mo of age¹

	Infants with iron deficiency anemia (<i>n</i> = 29)	Nonanemic control infants (<i>n</i> = 26)
Percentage male (%)	83	54 ²
Birth weight (kg)	3.47 \pm 0.08	3.53 \pm 0.08
Gestational age (wk)	39.1 \pm 0.2	39.4 \pm 0.2
Growth at 6 mo		
Weight (kg)	8.13 \pm 0.16	8.29 \pm 0.16
Length (cm)	67.5 \pm 0.4	67.0 \pm 0.4
Head circumference (cm)	43.7 \pm 0.2	44.0 \pm 0.2

¹Values of continuous variables are $\bar{x} \pm$ SEM, adjusted for sex.

²Significantly different from infants with iron deficiency anemia, *P* < 0.05 (Fisher's exact test).

recording (a noninvasive technique) was carried out in a quiet, dimly lit, and electrically shielded room with an integrated Nicolet Compact Four (C4) machine (Nicolet Biomedical Instruments, Madison, WI). ABRs were recorded with silver-silver chloride disk electrodes placed, according to the 10-20 International System, on the vertex (active electrode) and earlobe (reference electrode) ipsilateral to the stimulation (40–42). An electrode placed on the forehead was used as a ground. Interelectrode impedance was kept at <5 k Ω .

The ABRs were elicited monaurally by stimulating the ipsilateral ear with a series of square wave rarefaction clicks (0.1 ms) through TDH-39 headphones (Telephonics Corp, Farmingdale, NY) at 75 and 85 dB above normal hearing level. The contralateral ear was masked by white noise of 35 dB. The repetition rate of stimuli was 11.1/s. The bioelectrical activity recorded at the vertex was amplified, filtered (150–3000 Hz), and automatically averaged in the Nicolet C4 system. The recording window was 10.2 ms from click onset, and each trial consisted of 1500 artifact-free individual responses. The data acquisition program automatically rejected any traces contaminated by high-amplitude artifacts. The files generated by the acquisition program were transferred to a personal computer in ASCII format for subsequent analysis.

A second program, specially designed in our laboratory, worked off-line to read the stored data and display the averaged responses on the computer screen. With the aid of cursors, the individual waves of each response were identified and marked, and the program automatically transferred the following data to a spreadsheet: absolute latency and amplitude for waves I, III, and V, and interpeak latencies I–III, III–V, and I–V. These values were processed by the commercial software program EXCEL (version 5.0; Microsoft, Redmond, WA). In keeping with usual practice, the latency and amplitude values obtained for the right and left ears were averaged so that each child is represented by one value in any given group mean. No child had ABR findings indicative of hearing loss.

All ABR measures were obtained and processed without knowledge of whether a given child was in the anemic or nonanemic group. ABRs recorded from one infant with iron deficiency anemia and one nonanemic control infant at 6 mo of age are shown in **Figure 1** to illustrate the quality and pattern of the recordings.

Statistical analysis

Analysis of variance was the primary statistical technique used to identify differences between infants who had iron defi-

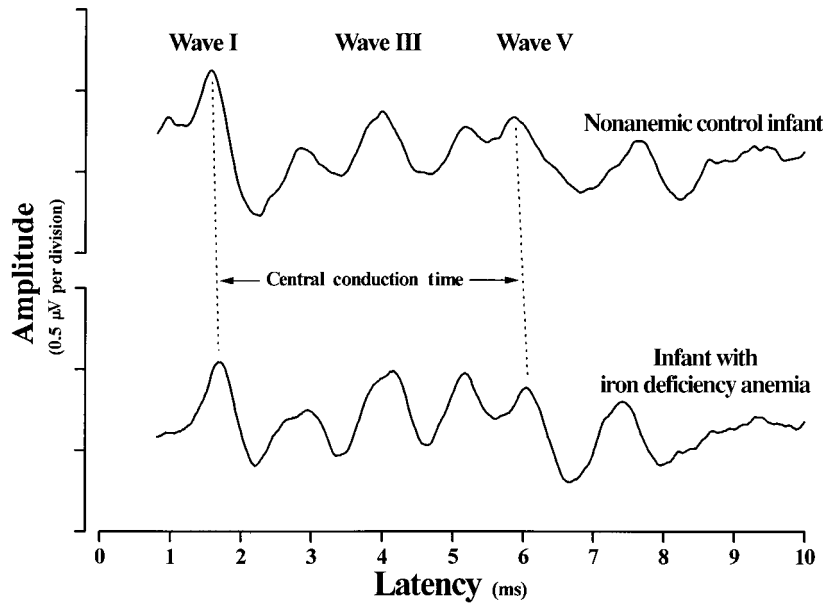


FIGURE 1. Representative auditory brainstem responses at 6 mo. Recordings at 85 dB from one nonanemic control infant are shown in the top trace and from one infant with iron deficiency anemia in the bottom trace. The principal component waves are indicated with roman numerals. Central conduction time (wave I–V interval) values are 4.52 and 4.76 ms in the nonanemic control infant and the infant with iron deficiency anemia, respectively.

ciency anemia at 6 mo and the nonanemic control group. Two-tailed tests of significance were used, with an alpha level of 0.05. Sex was a covariant in all analyses. This approach was chosen because there was a higher proportion of males in the anemic group and males have been reported to have longer ABR latencies (43) (a pattern also noted in our data).

A preliminary step in analyzing the ABR results was to determine whether iron deficiency without anemia affected ABR variables. We approached this question 3 ways: 1) the 14 nonanemic infants with 2 iron measures in the deficient range were compared with the 12 nonanemic infants with entirely normal iron measures or only a single alteration, 2) the 6 nonanemic infants who showed the most marked response to iron therapy (increase in hemoglobin ≥ 10 g/L) were compared with the rest of the nonanemic group, and 3) the 16 nonanemic infants who showed any increase in hemoglobin ≥ 6 g/L were compared with the 8 nonanemic infants who had little or no change in hemoglobin concentration. Of the 81 comparisons involved (at 85 dB), only 1 was statistically significant at $P < 0.05$ (amplitude of wave V at 12 mo). Thus, we found no evidence of altered ABR measures in nonanemic infants with iron deficiency, and further analyses contrasted infants with iron deficiency anemia with the entire nonanemic comparison group. However, the question of effects of iron deficiency without anemia on ABRs remains open because the number of nonanemic infants in the different iron-status categories was small.

Because ABR studies yield several variables, we identified major outcome measures on conceptual grounds. We emphasized measures that provided summary information of several individual ABR variables. The central conduction time [(CCT) wave I–V interpeak latency] was considered to be the most important measure. Nerve conduction between waves I and V reflects the overall integrity of the auditory pathway, with latency decreasing with increasing age in infancy and with improved myelination (18, 44). Thus, if myelination was

impaired, the CCT would be longer. The central-to-peripheral ratio (wave III–V interval divided by wave I–III interval) (21) was a second important summary measure. This ratio indicates whether the more central or more distal components of the auditory pathway are differentially affected. With a disorder of myelination, the central-to-peripheral ratio should be increased as a result of the centripetal direction of the myelination process (21). Our third summary measure determined whether latency measures were more affected than amplitude measures. If myelination was impaired by early iron deficiency anemia, we reasoned that latency would be more affected than amplitude. Therefore, we compared the proportion of latency and amplitude measures showing statistically significant ($P < 0.05$) or suggestive (P value between 0.05 and 0.10) differences between infants who had iron deficiency anemia at 6 mo and the nonanemic comparison group.

To help understand differences in these overall measures (and to derive some of the them), we also examined individual ABR variables. Such comparisons of individual ABR measures were considered to be secondary analyses. Results at 75 and 85 dB were similar. In the figures and tables we show responses at 85 dB because of the better resolution at this level; findings at 75 dB are noted in the text.

RESULTS

Data on the infants' initial hematologic status and the changes after oral iron therapy are provided in **Table 2**. Infants who had iron deficiency anemia at 6 mo showed an excellent response to iron therapy. At 12 mo, the average hemoglobin concentration had increased >25 g/L in the anemic group, compared with 7 g/L in the nonanemic group. Similarly, there were marked improvements in MCV and EP in infants who were anemic at 6 mo and modest changes in the nonanemic group. Serum ferritin concentrations improved in both groups.

TABLE 2
Initial iron status and hematologic change after 6 mo of oral iron therapy¹

	Infants with iron deficiency anemia at 6 mo (n = 29)	Nonanemic control infants (n = 26)
Finger-stick hemoglobin at 6 mo (g/L)	93.1 ± 1.6	120.0 ± 1.7 ²
Hemoglobin (g/L)		
6 mo	94.7 ± 1.2	122.5 ± 1.2 ²
12 mo	120.3 ± 1.7	129.9 ± 1.9 ²
18 mo	122.1 ± 2.5	131.4 ± 2.4 ³
Erythrocyte protoporphyrin [μmol/L (μg/dL) red blood cells]		
6 mo	3.68 ± 0.33 (207.7 ± 18.4)	1.91 ± 0.35 ² (107.9 ± 19.5)
12 mo	1.73 ± 0.10 (97.7 ± 5.4)	1.34 ± 0.10 ³ (77.2 ± 5.8)
Mean cell volume (fL)		
6 mo	65.2 ± 0.9	73.9 ± 0.9 ²
12 mo	72.5 ± 0.8	77.0 ± 0.8 ²
Ferritin (μg/L)		
6 mo	11.4 ± 2.4	15.3 ± 2.6
12 mo	20.8 ± 3.9	28.2 ± 3.9

¹ $\bar{x} \pm \text{SEM}$, adjusted for sex. Hemoglobin, erythrocyte protoporphyrin, mean cell volume, and ferritin measurements were made in venous blood specimens with the exception of hemoglobin at 18 mo, which was measured in finger-stick samples.

^{2,3}Significantly different from infants with iron deficiency anemia (analysis of covariance with sex as the covariant); ² $P < 0.001$, ³ $P < 0.01$.

The CCT, our single most important measure, was longer in infants who had iron deficiency anemia at 6 mo than in nonanemic control infants. A longer CCT indicates slower nerve conduction velocity. Differences between infants who were anemic at 6 mo and the nonanemic comparison group were statistically significant at 12 and 18 mo at both 75 and 85 dB and showed a suggestive trend at 6 mo at 85 dB (**Figure 2**). Because of the suggestion of differences at 6 mo, we also performed analyses of covariance controlling for CCT at 6 mo (**Table 3**). The differences at 12 and 18 mo remained significant.

Over the 1 y of follow-up, the nonanemic group showed the expected developmental decrease in CCT as conduction velocity improved (**Figure 2**). Infants who had initially been anemic also showed developmental progression but did not catch up with the nonanemic group. In fact, at 12 mo their CCT values had not decreased as much as those of nonanemic infants (group \times time interaction: $F_{[1,36]} = 4.41$, $P < 0.05$), and the difference between groups became larger. There was no differential change between 12 and 18 mo; the degree of difference observed at 12 mo was maintained. Thus, after 6 mo of iron therapy effective in improving hematologic status and 6 mo of prophylactic iron, the differences in nerve conduction velocity between infants who entered the study with iron deficiency anemia and the nonanemic comparison group became more marked.

At 85 dB, the central-to-peripheral ratio was significantly different between groups at 12 mo, with a higher ratio in infants who had been anemic at 6 mo (**Table 3**). At 75 dB, this ratio was higher in anemic infants at both 6 and 12 mo (0.91 ± 0.02 compared with 0.86 ± 0.02 in the anemic and nonanemic control groups at 6 mo, respectively, $F_{[2,52]} = 3.86$, $P = 0.06$; 0.92 ± 0.03 compared with 0.85 ± 0.03 in the anemic and nonanemic control groups at 12 mo, respectively, $F_{[2,44]} = 4.25$, $P < 0.05$). Our other summary measure also differed in the predicted direction. At both 75 and 85 dB, 44% of the 18 latency or interpeak latency measures (those specified in **Table 4** plus the 3 CCT measures) were significantly different or suggestive between anemic and nonanemic infants, compared with 0% of the 9 amplitude measures.

Analyses of individual ABR variables, other than the CCT and central-to-peripheral ratio, showed that wave III–V interpeak latencies, as well as some isolated absolute latencies, were longer in infants with iron deficiency anemia at 6 mo than in nonanemic infants. At 85 dB, differences between anemic and nonanemic infants in wave III–V interpeak latencies were statistically significant at 12 and 18 mo, as was the absolute latency of wave V at 12 mo; the wave I–III interpeak latency and the absolute wave latency of wave V at 18 mo showed suggestive trends (**Table 4**). Findings at 75 dB (not shown) revealed statistically significant differences between anemic and nonanemic infants in the wave III–V interpeak latencies at 12 and 18 mo, with a suggestive trend at 6 mo; the absolute latencies at 12 mo were all different between groups, significantly so for waves I and V and suggestive for wave III.

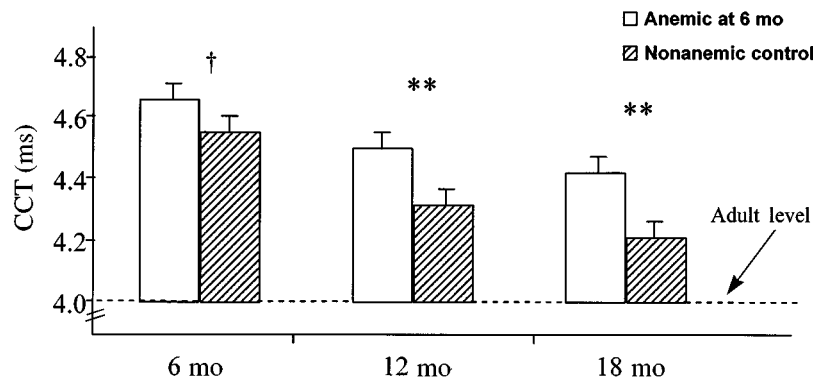


FIGURE 2. Differences in maturation of the central conduction time (CCT) in infants. CCT values at 85 dB are shown at study entry (6 mo) and at 12 and 18 mo follow-ups after iron treatment (see **Table 3** for n values in each group). Higher CCT values in infants who had iron deficiency anemia at 6 mo reflect slower nerve conduction of the auditory pathway in the brainstem. Values are means \pm SEMs, controlling for sex. †, **: Difference between the anemic and nonanemic groups, † $P < 0.10$, ** $P < 0.01$ (analysis of covariance with sex as the covariant).

TABLE 3
Summary auditory brainstem response measures at 85 dB¹

	Infants with iron deficiency anemia at 6 mo	Nonanemic control infants
Central conduction time (ms)		
6 mo	4.65 ± 0.04	4.55 ± 0.04 ²
12 mo, controlling for 6-mo value	4.47 ± 0.03	4.36 ± 0.03 ³
18 mo, controlling for 6-mo value	4.37 ± 0.04	4.24 ± 0.04 ³
Central-to-peripheral ratio		
6 mo	0.91 ± 0.02	0.89 ± 0.02
12 mo	0.91 ± 0.02	0.83 ± 0.02 ³
18 mo	0.90 ± 0.02	0.87 ± 0.02

¹ $\bar{x} \pm \text{SEM}$, adjusted for sex. At 6 mo, $n = 29$ anemic infants and 26 nonanemic control infants; at 12 mo, $n = 23$ infants who had been anemic at 6 mo and 24 control infants; at 18 mo, $n = 19$ infants who had been anemic at 6 mo and 20 control infants. Central conduction time is wave I–V interpeak latency; central-to-peripheral ratio is wave III–V interpeak latency divided by wave I–III interpeak latency.

²Suggestive trend, $P < 0.10$.

³Significantly different from infants with iron deficiency anemia, $P < 0.01$ (analysis of covariance).

DISCUSSION

The present study assessed the effects of early iron deficiency anemia on the functional status of one sensory pathway of the central nervous system—the auditory system. Like many parts of the brain, the auditory pathway is still developing and maturing during the first years of life. Our data show that infants who had iron deficiency anemia at 6 mo of age had less mature ABRs, particularly evidenced by longer CCT values than in control infants. Our findings that these differences remained as the infants got older, despite effective iron therapy, raise the concern that there may be long-lasting effects of iron deficiency anemia on the central nervous system during brain growth. With 1 y of follow-up, this study could not determine whether formerly anemic infants ultimately catch up with their nonanemic counterparts.

We postulate that impaired myelination is the most likely explanation for our findings. Any genetic or pathophysiologic factor that alters the normal progression of myelination in the central nervous system would produce slower nerve conduction, here indexed by the development of the auditory system. For instance, previous research showed altered auditory evoked potentials in infants with another nutritional disorder—severe malnutrition (23, 24, 45, 46). A differential effect on the late ABR components (waves III and V) would also be compatible with altered myelination. Jiang (21) noted, “Because of the centripetal direction of the myelination process, delayed myelination affects the late components earlier and more significantly than early ABR components, producing, for example, a prolonged III–V interval and an increased III–V/I–III ratio.”

Work with a myelin mutant, the taiep rat, supports this summary (47, 48). The myelin disorder that characterizes this mutant is due to a defect in oligodendrocytes, leading to a specific disruption in the development and maintenance of more central myelin. Electrophysiologic and electron microscopy examinations in taiep rats reveal that the peripheral portions of the auditory nerve have a normal response (wave I) and adequate myelination (Schwann cells). In contrast, the brainstem portion of the auditory pathway becomes completely demyelinated in adult rats, leading not only to prolonged CCT values, but also to loss of the

TABLE 4
Individual auditory brainstem response measures at 85 dB¹

	Infants with iron deficiency anemia at 6 mo	Nonanemic control infants
Absolute latencies (ms)		
Wave I		
6 mo	1.81 ± 0.05	1.78 ± 0.06
12 mo	1.81 ± 0.06	1.67 ± 0.06
18 mo	1.68 ± 0.06	1.68 ± 0.06
Wave III		
6 mo	4.25 ± 0.06	4.19 ± 0.06
12 mo	4.18 ± 0.07	4.04 ± 0.07
18 mo	4.00 ± 0.07	3.93 ± 0.06
Wave V		
6 mo	6.45 ± 0.07	6.33 ± 0.07
12 mo	6.32 ± 0.07	6.00 ± 0.07 ²
18 mo	6.09 ± 0.08	5.88 ± 0.07 ³
Interpeak latencies (ms) ⁴		
Wave I–III interval		
6 mo	2.44 ± 0.03	2.41 ± 0.04
12 mo	2.37 ± 0.03	2.37 ± 0.03
18 mo	2.32 ± 0.03	2.24 ± 0.03 ³
Wave III–V interval		
6 mo	2.20 ± 0.03	2.14 ± 0.03
12 mo	2.15 ± 0.04	1.96 ± 0.04 ⁵
18 mo	2.09 ± 0.04	1.96 ± 0.04 ⁶
Amplitudes (μV)		
Wave I		
6 mo	0.34 ± 0.03	0.36 ± 0.03
12 mo	0.39 ± 0.03	0.41 ± 0.03
18 mo	0.43 ± 0.03	0.44 ± 0.03
Wave III		
6 mo	0.27 ± 0.02	0.29 ± 0.02
12 mo	0.33 ± 0.03	0.37 ± 0.02
18 mo	0.38 ± 0.03	0.38 ± 0.03
Wave V		
6 mo	0.23 ± 0.02	0.26 ± 0.02
12 mo	0.27 ± 0.02	0.31 ± 0.02
18 mo	0.31 ± 0.02	0.32 ± 0.02

¹ $\bar{x} \pm \text{SEM}$, adjusted for sex. At 6 mo, $n = 29$ anemic infants and 26 nonanemic control infants; at 12 mo, $n = 23$ infants who had been anemic at 6 mo and 24 control infants; at 18 mo, $n = 19$ infants who had been anemic at 6 mo and 20 control infants.

^{2,5,6}Significantly different from infants with iron deficiency anemia (analysis of variance): ² $P < 0.01$, ⁵ $P < 0.001$, ⁶ $P < 0.05$.

³Suggestive trend, $P < 0.10$.

⁴Wave I–V interval (central conduction time) is given in Table 3.


next ABR waves. The pattern of results in the present study—differences in latencies but not amplitudes, more effects on the late ABR components (waves III and V), and longer CCTs (as an overall measure of nerve conduction velocity)—supports the hypothesis that altered myelination is the most likely explanation.

However, in addition to its role in the production and maintenance of myelin, iron is apparently involved in the function of such neurotransmitters as dopamine, serotonin, and γ -amino butyric acid (for review, see references 3–5). Some of these neurotransmitters are involved in the transmission of the auditory pathway (49). For instance, substances that deplete serotonin increase the amplitude of some ABR components (50). Thus, iron deficiency could interfere directly with neurotransmission in the auditory pathway or indirectly by altering certain

processes that modulate brainstem auditory activity. The pattern of findings in this study, with differences in latencies but not amplitudes, seems to fit best with a direct effect on neurotransmission through an alteration in myelination.

We found no differences, other than iron deficiency anemia, that might account for our findings. Infants who were anemic at 6 mo were similar to nonanemic control infants with respect to birth weight, gestational age, growth, and health. Nonetheless, the possibility cannot be dismissed that some unidentified factor closely linked to iron deficiency anemia was responsible for our findings. We could not determine whether iron deficiency in the absence of anemia affects ABR measures. We found no evidence of such effects in our preliminary analyses of nonanemic infants with iron deficiency, but the number of infants studied was small. On the basis of iron's role in myelin formation and maintenance, it seems reasonable that iron deficiency without anemia should be associated with ABR measures intermediate between those observed in iron deficiency anemia and those observed in iron sufficiency. This will be an important question to address in future studies with larger samples of nonanemic infants who differ in iron status.

Our results have several implications. Although the CCT values in infants who had iron deficiency anemia at 6 mo were broadly within the normal range, albeit longer than those in control infants, the differences could have functional significance for language development. Iron deficiency anemia was associated with alterations in the properties of the auditory message and its transmission through brainstem structures during an age period when iron deficiency is most common and language is emerging. Further follow-up of these children will be necessary to determine effects on language acquisition.

Perhaps the most important implication of our findings, however, is that they may generate further specific, testable hypotheses about the effects of iron deficiency on the developing central nervous system. Many parts of the brain are being myelinated in the first 2 y of life, when iron deficiency is widespread. Increasingly, we have direct and indirect noninvasive measures of myelination in humans. With the hypothesis of impaired myelination in early iron deficiency, it should be possible to design studies with specific measures, using techniques such as imaging, evoked and spontaneous potentials, and, it is hoped, behavioral progressions known to depend on myelination. Such hypothesis-driven research would be an advance over previous studies of iron-deficient infants, which depended largely on global tests of development. Thus, this study suggests new, promising directions for understanding some specific central nervous system mechanisms by which iron deficiency could alter infant behavior and development. 

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