Perinatal Brain Iron Deficiency Increases the Vulnerability of Rat Hippocampus to Hypoxic Ischemic Insult1,2,3

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ABSTRACT Fetal brain iron deficiency occurs in human pregnancies complicated by diabetes mellitus or intrauterine growth retardation. Because neurocognitive deficits are more common in the offspring of these pregnancies, we tested the hypothesis that perinatal brain iron deficiency predisposes the neonatal hippocampus, a structure important for memory processing, to injury. Brain iron concentration was reduced by 45% in 45 neonatal rats by maternal dietary iron restriction during gestation. Right-sided neuronal injury in four hippocampal subareas was induced by hypoxic-ischemic insult (ipsilateral carotid artery ligation and subsequent hypoxia on postnatal d 7) and was quantified histochemically on d 8 by cytochrome c oxidase activity (n = 30), and on d 14 by Nissl staining (n = 15). Acute right-sided cytochrome c oxidase activity loss occurred in CA1 (P = 0.02), CA3c (P < 0.001) and dentate gyrus (P < 0.001) in the iron-deficient group, whereas only CA1 (P = 0.003) was affected in the iron-sufficient group. Long-term right-sided Nissl substance loss occurred in CA1 (P < 0.001), CA3a.b (P < 0.001) and dentate gyrus (P = 0.008) in the iron-deficient group, but only in CA1 (P = 0.004) in the iron-sufficient group. No increase in right-sided free-iron staining was present in either group. Perinatal iron deficiency predisposes the neonatal hippocampus to a greater acute loss of neuronal metabolic activity after an hypoxic-ischemic event, suggesting compromised cellular energetics. The subsequently greater loss of hippocampal neuronal integrity suggests poorer recoverability after injury in the perinatal iron-deficient brain. J. Nutr. 129: 199–206, 1999.

KEY WORDS: • cytochrome c oxidase • hippocampus • hypoxia-ischemia • iron • rats

Iron levels are maintained within a very narrow range in the perinatal human, sheep and rat (Felt and Lozoff 1996, Guiang et al. 1997, Huebers 1990). Limited iron stores predispose the nonheme tissues, including the brain, to iron deficiency during periods of negative iron balance (Guiang et al. 1997, Huebers 1990). Iron in the form of cytochromes is a required component of cellular oxidative metabolism in the brain and is thus essential for normal neuronal function (Dallman 1985). Conversely, limited iron-binding capacity due to low levels of serum transferrin (Georgieff et al. 1989), highly saturated iron-binding capacity and poor antioxidant defenses (Palmer et al. 1990) predispose the perinate to free-iron exposure and to oxidative injury. Thus, relatively small perturbations in perinatal iron balance can have detrimental effects on the perinatal brain through the effects of either iron deficiency or iron overload.

Gestational conditions that increase fetal iron demand for erythropoiesis beyond placental iron transport capacity (diabetes mellitus) (Petry et al. 1994) or those that reduce transplacental iron delivery (intrauterine growth retardation with oligohydramnios) can result in a 30–40% reduction in neonatal brain iron (Georgieff et al. 1996, Petry et al. 1992). A higher prevalence of cognitive impairment has been documented in infants born to mothers with these gestational conditions (Rizzo et al. 1994, Winer and Tejani 1994), although the role of iron deficiency in such impairments has not been evaluated. In theory, iron deficiency could have a direct effect on brain development or could potentiate the effects of other adverse perinatal events. Decreased brain iron content due to postnatal (Yehuda 1990) or perinatal (Felt and Lozoff 1996) iron deficiency in young animals and postnatal dietary iron deficiency in children (Lozoff et al. 1991, Walter 1994) results in significant permanent impairments on cognitive tasks. These abnormalities tend to persist in spite of complete

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Nutrient Requirements and Interactions

The cognitive nature of the long-term impairments of infants of diabetic mothers and growth-retarded infants suggests that factors targeting the developing hippocampus may be particularly important. The hippocampus is necessary for normal cognitive function, especially for processing recognition memory and transferring short-term memory items into long-term storage (Nelson 1995, Zola-Morgan et al. 1986). In humans and in animal models, the hippocampus appears to be particularly vulnerable to perinatal hypoxia-ischemia (Ben-Ari 1992, Del Bigio and Becker 1994, Walsh and Emeric 1988), an event that occurs more commonly in gestations complicated by maternal diabetes mellitus or fetal growth retardation.

We hypothesized that perinatal brain iron deficiency increases the vulnerability of the hippocampus to hypoxic-ischemic (HI) injury. Iron deficiency of the heart, liver and skeletal muscle has been shown to affect cellular energy production and organ performance (Blayney et al. 1976, Mackler et al. 1984, Masini et al. 1994). Arguably, severe iron deficiency may lead to similar deficits in cellular energy metabolism and organ performance in the brain, resulting in a reduced ability to respond to restriction of oxygen and perfusion and in greater hippocampal damage. To test this hypothesis, we used cytochrome c oxidase (CytOx) activity and staining for Nissl substance as short- and long-term markers of neuronal injury, respectively. CytOx is the terminal enzyme involved in oxidative phosphorylation, and its level within neurons reflects their functional level of activity (Wong-Riley 1989). Loss of hippocampal CytOx activity has been shown to be an early and sensitive indicator of neuronal damage resulting from hypoxia-ischemia in the perinatal rat model (Nelson and Silverstein 1994). In addition, because free iron in ischemic and dying cells, through its catalytic action, has been postulated to potentiate free radical–induced neuronal injury (Kondo et al. 1995, Palmer et al. 1994 and 1998), we also studied iron staining in the region of the hippocampus.

**MATERIALS AND METHODS**

**Study design.** Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) were used for the study. The study was approved by the Animal Care Committee of the University of Minnesota and its guidelines were followed during the experiments. To induce perinatal iron deficiency in the pups, dams were fed a low iron diet ([Formula TD 80396, Harlan-Teklad, Madison, WI; composition reported by Felt and Lozoff (1996)] beginning on gestation d 1 and continuing until postnatal d (PND) 7. The formula contains 3–6 mg of elemental iron/kg diet. Pups of the same postnatal age born to dams fed a diet containing 198 mg iron/kg of nonpurified diet (Teklad 4% Mouse/Rat Diet 7001, Harlan-Teklad; composition reported in Table 1) formed the iron-sufficient control group. Rats were allowed free access to food and water and were maintained on a 12-h dark-light cycle at room temperature.

On PND 7, eight pups from each group were deeply anesthetized with sodium pentobarbital (60 mg/kg body weight intraperitoneally) and were killed by decapitation. The brain and liver were collected to measure nonheme iron concentrations. The remaining rats underwent right common carotid artery ligation followed by hypoxia to induce unilateral HI injury (Levine 1960, Rice et al. 1981). Because both ischemia and hypoxia are necessary to induce neuronal injury in this model (Nelson and Silverstein 1994), the unilateral side provides an internal control to the ligated side, allowing side-to-side comparison.

**CytOx histochemistry.** Serial 15-µm coronal frozen sections were obtained from the hippocampus of the experimental and control rats at the level of the posterior lateral thalamus using a cryostat at −20 to −25°C (Bright Instruments, Huntingdon, UK). Sections were mounted on poly-L-lysine coated slides and were stained for CytOx activity using the 3,3′-diaminobenzidine (DAB) method (Nelson and Silverstein 1994) at 37°C for 2 h in the dark. The reaction was terminated by immersing the slides three times for 5 min each in phosphate buffer at room temperature. The sections were then serially submerged in increasing concentrations of ethyl alcohol (5 min each in 70 and 80%, followed by 10 min each in 95% and absolute alcohol) before being immersed in a clearing agent (HEMO-DE, Fischer Scientific) for 10 min. They were then air dried and coverslipped using Permount (Fischer Scientific).

**CytOx histochemistry analysis.** Four brain sections per rat were visualized through a 4× objective in a light microscope (Model BH-2, 200 RAO ET AL.

![Table 1](https://academic.oup.com/jn/article-abstract/129/1/199/472244/1648)

**Proximate composition of iron sufficient diet**

<table>
<thead>
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<th>Substance</th>
<th>g/100 g</th>
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<tr>
<td>Protein</td>
<td>25.03</td>
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<tr>
<td>Fat</td>
<td>4.25</td>
</tr>
<tr>
<td>Fiber</td>
<td>4.67</td>
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<tr>
<td>Ash</td>
<td>10.09</td>
</tr>
<tr>
<td>Nitrogen-free extract</td>
<td>46.16</td>
</tr>
<tr>
<td>Linoel acid</td>
<td>0.91</td>
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<tr>
<td>Gross energy, kJ/g</td>
<td>16.48</td>
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</table>

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Abbreviations used: CytOx, cytochrome c oxidase; DAB,3,3′-diaminobenzidine; DG, dentate gyrus; HI, hypoxic-ischemic; PND, postnatal day.
Digital microscopic images were collected at 40X magnification using a Cohu 4915 CCD camera (Cohu, San Diego, CA), a Power Macintosh 7100 computer equipped with a model LG-3 frame grabber (Scion, Frederick, MD) and Scion Corporation’s version of the public domain NIH Image program (available on Internet by anonymous FTP from zippy. NIMH. NIH. Gov or on disk from the National Technical Information Service, Springfield, VA, part number PB95–500195GEI).

The intensity of background light was maintained at a constant level among the sections such that the histogram of the gray-scale values of the overall image showed a normal distribution and did not stack at either the black or white end of the spectrum. The intensity of CytOx reactions in the four principal hippocampal subareas of CA1, CA3a,b, CA3c and the lower blade of dentate gyrus (DG) were measured bilaterally for each brain from the image projected and frozen on the computer screen. Using the cursor, the specific subarea was outlined entirely to include all stained neurons of the subarea within the outline; the intensity of reaction of the entire subarea was measured by the computer-assisted program in gray-scale units (range: 1–254). With this particular program, dark areas are assigned a higher unit and lighter areas a lower unit (white 1; black 254). Hence, a lower gray-scale value indicated less staining, meaning a greater loss of CytOx activity. To obviate interobserver error, only one individual (R. R.) placed the outline of the subarea in all brain sections.

Staining for Nissl substance. Vogt’s method (Luna 1968) was used for staining for Nissl substance in the brain sections of rats maintained for 7 d post-HI injury to assess long-term neuronal injury. The brain sections were brought to room temperature for 10 min and then incubated in a working solution of cresyl violet acetate [625 mmol/L cresyl violet acetate (Dye content 70%, Sigma) in a buffer containing 15 mmol/L sodium acetate buffer and 3 mL/L glacial acetic acid] at room temperature for 60 min. Sections were rapidly differentiated in 95% ethyl alcohol, then dehydrated in absolute acetic acid at room temperature for 60 min. Sections were thoroughly rinsed in deionized water (twice for 5 min each) and were incubated in a freshly made solution of DAB (1 mol/L in PBS (pH 7.4) to which 30% H2O2 (0.8 mL/L) was added just before the incubation, for 25 min at room temperature. The reaction was stopped by rinsing the sections in PBS (three changes of 5 min each), and the sections were dehydrated, cleared and coverslipped as described for CytOx histochemistry above. Control sections were incubated in Perls’ solution with PBS substituted for potassium ferrocyanide. There was no positive staining in any of these control slides.

Iron staining histochemistry analysis. Brain sections were visualized under light microscope (Nikon Optiphot, Nippon Kogaku K. K., Tokyo, Japan) at 400 X and the hippocampal anatomy was defined as in Figure 1. A 1 mm × 1 mm calibrated grid was positioned over a hippocampal subarea and all of the iron-stained cells and tissue within the boundaries of the grid were counted. Caution was taken to place the grid on identical positions of the hippocampal subarea bilaterally for each animal. Six sections were quantified in each animal and the mean ± SD number of iron-stained cells per hippocampal subarea in the iron-deficient and iron-sufficient groups was determined.

Statistical analysis. The mean ± SD brain iron concentrations of the iron-deficient and the iron-sufficient groups were compared by a two-tailed unpaired Student t test to confirm brain tissue iron deficiency in the iron-deficient group. To determine the extent of losses of CytOx activity and Nissl staining after HI injury within each group, the mean ± SD gray-scale units of each of the four hippocampal subareas on the ligated right side were compared with the values of the corresponding hippocampal subareas on the unligated left side by paired t test. This analysis was done separately in the iron-deficient and the iron-sufficient groups because variabilities in tissue sectioning and staining could make direct gray-scale value comparisons of right-sided hippocampal subareas between rats unreliable. To compare iron-deficient to iron-sufficient rats directly, the percentage losses of right-sided CytOx activity and Nissl staining post-HI in each hippocampal subarea were calculated by comparing them to the control left side: % loss = [(left side gray level − right side gray level) × 100]/left side gray level. The overall percentage loss in the entire hippocampus was calculated from values obtained in each subarea and was compared between the iron-deficient group and iron-sufficient group by two-tailed unpaired Student t test. A greater percentage right-to-left difference in CytOx activity and staining for Nissl substance in the iron-deficient rats would be consistent with the hypothesis of greater vulnerability of the iron-deficient brains to HI insult. Potential iron deposition after HI injury was assessed by

![Photomicrograph of the brain section of an iron-deficient rat at the region of the mid hippocampus stained for cytochrome c oxidase (CytOx) activity. The subareas of the right hippocampus (right panel) are labeled as per Johansen (1993). In comparison to the internal control left side (left panel), the CytOx activity, measured in gray-scale units, was 9% (CA1), 11% (CA3a,b), 13% (CA3c) and 11% dentate gyrus (DG) lower on the ligated right side (X40).](https://academic.oup.com/jn/article-abstract/129/1/199/4723244/FIGURE-1)
comparing iron staining cells in the four hippocampal subareas on the ligated right side to the corresponding hippocampal subarea on the control left side by paired t test in each group of rats. A statistical analysis software package (SAS, Version 6.12, SAS Institute, Cary, NC) was used for analysis. Data are presented as mean ± SD. A P-value <0.05 was considered significant.

RESULTS

All rats survived carotid artery ligation and exposure to hypoxia. Confirmation that the experimental dietary manipulation of the dam was successful was inferred from the fact that brain iron concentration of the iron-deficient pups was 55% that of the iron-sufficient group, whereas the nonheme liver iron concentration was 22% of control (Table 2). Hemoglobin concentrations were not measured, but the iron-deficient rats were presumed to be anemic on the basis of the degree of organ iron loss. Iron-deficient pups had lower body and brain weights than the iron-sufficient group, although mild brain-sparing relative to the body was seen in the iron-deficient group.

Twenty-four hours after the HI injury, 100% of the rats in the iron-deficient group and 93% of the iron-sufficient group showed detectable loss of CytOx activity on the ligated right side in at least one of the four hippocampal subareas. An example of a section at the region of the midhippocampus of an iron-deficient rat brain stained for Nissl substance is shown in Figure 1.

The iron-deficient group demonstrated significant reductions in mean right-sided CytOx activity in subareas CA1, CA3c and the DG (Fig. 2). In the iron-sufficient group, significant loss of CytOx activity on the right side was present only in CA1 (Fig. 3). The overall percentage of CytOx activity loss per rat in the right hippocampus was 6.3% greater in the iron-deficient group than in the iron-sufficient group (P < 0.001).

Figure 4 is a composite photomicrograph of hippocampal subareas CA1, CA3a,b and the DG of a 14-d-old iron-deficient rat brain at the region of midhippocampus stained for Nissl substance. There was significant loss of Nissl substance on the ligated right side (compared with the control left side) in subareas CA1, CA3a,b and the DG (Fig. 5) in the iron-deficient group. Such loss was present only in subarea CA1 in the iron-sufficient group (Fig. 6). The overall percentage Nissl substance loss per rat on the right hippocampus was 8.3% (P = 0.001) greater in the iron-deficient group compared with the iron-sufficient group.

There was no difference in iron-staining cells between the ligated right side and the control left side in any of the four hippocampal subareas in either group (Table 3).

### DISCUSSION

This study demonstrates that iron deficiency increases the vulnerability of certain areas of the perinatal rat hippocampus to HI insult. There was greater disruption of neuronal metabolic function at 24 h and greater neuronal damage 7 d after the insult in the iron-deficient rats compared with the iron-sufficient controls. These findings may have clinical implications regarding the greater propensity for HI injury and poorer neurocognitive outcome of groups of infants born after gestational conditions complicated by perinatal iron deficiency.

Iron in the form of cytochromes is a required component of cellular oxidative metabolism in the brain and is thus essential for normal neuronal cellular function (Dallman 1985). The high rate of oxidative metabolism in the brain coupled with its intolerance to even minimal degrees of hypoxia require that iron be available as cytochromes in sufficient amounts and in sufficient concentrations in the iron-deficient group.

Table 2: Body and brain weights and liver and brain iron concentrations of the iron-deficient and the iron-sufficient rat pups

<table>
<thead>
<tr>
<th></th>
<th>Iron-deficient</th>
<th>Iron-sufficient</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>9.0 ± 1.5</td>
<td>14.4 ± 2.0</td>
</tr>
<tr>
<td>Brain dry weight, mg</td>
<td>73.3 ± 11.9</td>
<td>95.8 ± 17.1</td>
</tr>
<tr>
<td>Brain iron μmol/g dry tissue</td>
<td>0.50 ± 0.12</td>
<td>0.91 ± 0.14</td>
</tr>
<tr>
<td>Liver iron μmol/g dry tissue</td>
<td>1.14 ± 0.11</td>
<td>5.153 ± 1.43</td>
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</table>

1 Values are means ± sd, n = 4 for liver, 8 for brain and 53 for body weight. All values differ significantly, P < 0.01.
In rats, nonheme iron, the principal form of intracerebral iron, begins to accumulate from d 14 of gestation (Moos 1995) and its concentration is highest at birth (Connor 1994). Dysregulation of the iron delivery system during the perinatal period permanently alters the brain iron content and its distribution (Connor 1994).

Iron deficiency may cause neuronal dysfunction by several mechanisms. Reduced DNA synthesis as a result of reductions in the iron-containing enzyme ribonuclease reductase has been postulated as a reason why the hippocampus is dysfunctional in iron deficiency (Connor 1994). The lower brain weight (and smaller size of the hippocampus noted on histology) of the iron-deficient group in our study is consistent with this mechanism. Reduced membrane fluidity due to an increase in the amount of cholesterol in the plasma membrane of the hippocampus has also been described in iron deficiency (Larkin and Rao 1990).

Our laboratory has shown that cytochrome c concentrations decrease proportionately to organ iron content, including brain, in perinatal animals with iron deficiency (Georgieff et al. 1992, Guiang et al. 1997). Others have shown a decrease in the amount of CytOx activity in brains of post-suckling rats with severe dietary iron deficiency (Dallman 1985, Tanaka et al. 1995).

The hippocampus, especially the DG, is one of the iron-rich areas of the rat brain (Erikson et al. 1997, Hill and Switzer 1984). Although the effect of perinatal iron deficiency on hippocampal iron concentrations has not been assessed, postnatal dietary iron deficiency results in 43% lower hippocampal iron levels, which do not fully recover in spite of complete iron rehabilitation (Erikson et al. 1997). Moreover, iron deficiency during the vulnerable period of perinatal neurodevelopment has been shown to have a significant, permanent adverse effect on behaviors mediated by the hippocampus, such as the ability to navigate the Morris maze (Felt and Lozoff 1996). The learning disabilities present in that study occurred in spite of no supervening perinatal insult such as hypoxia-ischemia. These findings suggest that the integrity of the perinatal hippocampus and its cholinergic input are important for normal development of memory and learning (Felt and Lozoff 1996, Morris et al. 1982, Walsh and Emerich 1988).

The hippocampus is preferentially susceptible to a wide variety of toxic insults and disease processes, including hypoxia-ischemia and hypoglycemia (Ben-Ari 1992, Johansen 1993, Walsh and Emerich 1988, Zola-Morgan et al. 1986). This area of the brain (particularly the dentate region) is also vulnerable to damage when glucocorticoids are elevated as occurs, for example, when an organism is stressed (Sapolsky 1996). The susceptibility of this structure to a broad spectrum of insults and its involvement in higher order cognitive processes make it an important focal point for studies of behavioral and neurologic toxicity (Walsh and Emerich 1988).

In this study, we focused on whether iron deficiency further increases the vulnerability of the hippocampus to hypoxia-ischemia, a common perinatal insult. Because severe tissue
iron deficiency affects the recoverability of non-brain organs such as skeletal muscle, liver and heart after physiologic stressors (Blayney et al. 1976, Mackler et al. 1984, Masini et al. 1994), we hypothesized that a similar adverse effect might occur in severe perinatal brain iron deficiency after perinatal hypoxia-ischemia.

Histologically discernible hippocampal damage on the ligated side is a predictable finding in the immature Levine model, occurring in more than 80% of presumably iron-sufficient animals (Rice et al. 1981). In our study, the iron-sufficient rats had a damage rate >90% 24 h after the HI insult, although, in general, neuronal injury was not as severe as reported by others (Nelson and Silverstein 1994). Rats of the same strain may vary in their response to hypoxia-ischemia (Pulsinelli and Buchan 1988). Subtle variations in the experimental conditions also can affect the findings (Nelson and Silverstein 1994, Palmer et al. 1994).

The distribution of hippocampal damage after HI injury in the iron-deficient group in our study was similar to earlier reports in presumably iron-sufficient animals, at both 24 h and 7 d post-HI insult (Johansen 1993, Kandel 1991, Nelson and Silverstein 1994, Towfighi et al. 1991). The DG and CA3c had the highest CytOx loss, at 24 h after the HI injury. CA1 had slightly less damage, whereas CA3a,b had no loss of CytOx activity. Predilection of the DG and CA3c involvement in HI brain injury has been consistently reported in non-iron-deficient perinatal rat models (Johansen 1993, Nelson and Silverstein 1994) and also in human infants (Del Bigio and Becker 1994). The distribution of neuronal injury within the hippocampus is age related and is thought to be due to the immaturity of synaptic connections in the perinatal period (Nelson and Silverstein 1994, Rice et al. 1981). The damage to the DG is of particular concern because projections via the mossy fibers extend from the granular cells of this subarea to CA3, from which they continue via the Schaffer collaterals to CA1 (Ben-Ari 1992, Johansen 1993). These pathways are central to the hippocampal role in memory processing, specifically long-term potentiation, during both associative and nonassociative learning in mammals (Bliss and Lomo 1973, Kandel 1991). Although CA3a,b was relatively spared initially, it was involved at 7 d post-HI injury, suggesting possible deafferented degeneration over time as a result of the involvement of subareas DG and CA3c proximally and CA1 distally in the neural pathway. In a perinatal HI experiment on presumably iron-sufficient rats, damage to subareas CA3a,b and the peripheral portion of CA3c, with relative sparing of the proximal portion of CA3c, was seen at 30 d of life (Towfighi et al. 1991). A similar distribution of neuronal damage seen in our rats at 14 d of life suggests that perinatal iron deficiency results in more rapid neuronal damage.

The distribution and severity of Nissl substance loss on d 14 closely paralleled the distribution of hippocampal CytOx activity loss at 24 h post-HI injury, suggesting that the CytOx loss did not represent a merely transient reduction in neuronal metabolic activity but was indeed a harbinger of permanent neuronal damage. The severity of neuronal damage in the iron-deficient group may also have been exacerbated by reduced cardiac contractility resulting from the effect of iron deficiency on myocardial myoglobin and cytochrome c concentrations (Guiang et al. 1998), and the inevitable iron-deficiency anemia that occurs with the dietary manipulation in our study.

![FIGURE 5](image-url) Hippocampal Nissl staining in the iron-deficient rat pups 7 d after right-sided hypoxic-ischemic injury. The intensity of Nissl staining on the ligated right side is expressed as a percentage of the staining on the internal control left side. Values are means ± SD, n = 15. DG, dentate gyrus. *P = 0.01; **P < 0.001; and ***P = 0.008.

![FIGURE 6](image-url) Hippocampal Nissl staining in the iron-sufficient rat pups 7 d after right-sided hypoxic-ischemic injury. The intensity of Nissl staining on the ligated right side is expressed as a percentage of the staining on the internal control left side. Values are means ± SD, n = 15. DG, dentate gyrus. *P = 0.004.

### TABLE 3

Comparison of iron staining cells in the four hippocampal subareas 7 d after right-sided hypoxic-ischemic injury in iron-deficient and iron-sufficient rats.

<table>
<thead>
<tr>
<th>Hippocampus Subarea</th>
<th>Iron-deficient</th>
<th>Iron-sufficient</th>
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<tr>
<td></td>
<td>Left</td>
<td>Right</td>
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<tr>
<td>CA1</td>
<td>0.5 ± 0.8</td>
<td>0.6 ± 0.6</td>
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<tr>
<td>CA3a,b</td>
<td>0.2 ± 0.4</td>
<td>0.9 ± 1.1</td>
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<tr>
<td>CA3c</td>
<td>0.3 ± 0.5</td>
<td>0.9 ± 1.1</td>
</tr>
<tr>
<td>Dentate gyrus</td>
<td>0.8 ± 1.4</td>
<td>1.3 ± 1.5</td>
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1. All values are means ± SD iron-staining cells within a 1 mm × 1 mm calibrated grid at X400 magnification, n = 15 in each group.
2. There were no significant differences between right and left in any of the four hippocampal subareas in either of the groups.
The role of iron in free radical–induced neuronal damage in perinatal HI insult is also a subject of intense research (MacMillan et al. 1993, Palmer et al. 1998, Vannucci 1990). Iron-stained cells increase in the region of the hippocampus (Kondo et al. 1995) and in the cortex (Palmer et al. 1998) after HI insult in adult and neonatal rats, typically as a late effect. As in adult rats, the number of iron-positive cells in the region of the hippocampus is quite low in neonatal rats. Iron deficiency in this study resulted in a significant reduction of these cells in all four hippocampal subareas. There was no increase in the number of iron-positive cells or free-iron staining on the ligated right side in either of the groups 7 d after HI insult. Whether the number of these iron-positive cells would be different between the groups at an older age was not studied. Because it can be argued that iron deficiency should have protected the rats from the oxidative aspects of HI injury, the increased loss of metabolic activity and neuronal integrity seen in the iron-deficient rats suggests causes other than oxidative processes in the increased vulnerability of this group.

The findings in this study may have relevance to human newborns because infants of diabetic mothers and growth-retarded infants have an increased incidence of neonatal iron deficiency (Choquette et al. 1987, Georgieff et al. 1990) as well as perinatal asphyxia and its sequelae (Rizzo et al. 1994, Winer and Tejani 1994). The brain nonheme iron concentration of a cohort of newborn infants of diabetic mothers at autopsy was 60% of normal (Petry et al. 1992), a value that approximates the percentage reduction in brain iron concentration achieved in the model used in this study. A similar degree of brain iron deficiency is also present in pregnancies complicated by severe chronic placental insufficiency (Georgieff et al. 1996) in which restriction of placental nutrient transport occurs. Given the role of the hippocampus in infant memory and the present finding that the hippocampus is made more vulnerable by iron deficiency, the role of perinatal iron deficiency in the genesis of the neurologic delays in these populations must be established.

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