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Eye-Blinking Rates Are Slower in Infants with Iron-Deficiency Anemia than in Nonanemic Iron-Deficient or Iron-Sufficient Infants[1,2]

Betsy Lozoff,[3,4]* Rinat Armony-Sivan,[5] Niko Kaciroti,[3] Yuezhou Jing,[3] Mari Golub,[6] and Sandra W. Jacobson[7]

[3]Center for Human Growth and Development and [4]Department of Pediatrics and Communicable Diseases, University of Michigan, Ann Arbor, MI 48109; [5]Department of Psychology, Ashkelon Academic College, Ashkelon 78109, Israel; [6]California National Primate Research Center, University of California, Davis, CA 95616; and [7]Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI 48207

Abstract

Iron deficiency has been shown to impair dopamine functioning in rodent models, but it is challenging to obtain evidence of such effects in human infants. Because spontaneous eye-blink rate may provide a noninvasive assessment of dopamine functioning, we hypothesized that eye-blink rate would be lower in infants with iron-deficiency anemia and would increase with iron therapy. A 4-min eye-blink assessment was conducted for quiet, alert infants sitting on their mother's lap. Data were available for 61 9- to 10-mo-old infants from inner-city Detroit (19 iron-deficient anemic, 23 nonanemic iron-deficient, and 19 nonanemic iron-sufficient). Iron-deficient and iron-sufficient nonanemic groups had similar eye-blink rates ($P = 0.90$) and were therefore combined. We used Poisson regression based on generalized estimation equation methodology to test for differences between iron-deficient anemic and nonanemic infants in blinks/min and change after 3 mo of iron therapy. Iron-deficient anemic infants had a lower initial eye-blink rate than nonanemic infants (mean ± SD) (4.0 ± 1.9 vs. 5.3 ± 2.8 blinks/min; $P = 0.02$; effect size = 0.6 SD). At 12 mo, eye-blink rate increased by 2.1 blinks/min in the iron-deficient anemic group ($P = 0.008$); there was no change in the nonanemic group ($P = 0.96$). These results are consistent with reduced dopamine function in iron-deficient anemic infants. The clinical importance of a lower eye-blink rate is unclear, but impaired dopamine functioning is likely to have broader impact, given the role dopamine plays in regulating movement, motivation, cognition, and hormone release. J. Nutr. 140: 1057–1061, 2010.

Introduction

An estimated 22–33% of infants and young children in developing countries experience iron-deficiency anemia (1,2) and poor and/or minority children are at increased risk everywhere (3,4). Iron deficiency has been shown to alter brain dopamine systems in animal models, including dopamine D1 and D2 receptors and striatal dopamine transporter levels [(5–7); see (8,9) for review]. However, finding evidence of impaired dopamine functioning in iron-deficient human infants is challenging. We used the rate of spontaneous eye-blink to do so.

Eye-blinking has been studied in relation to ocular disorders, eye lubrication, attention, response to salient or novel stimuli, and cognitive processes, among other functions (10–14). However, the aspect of interest for iron-deficiency research relates to the role of dopamine. Research with human (11,13,15,16) and nonhuman primates (17–21) involving drug challenges and clinical conditions has documented the important role of dopaminergic neurotransmission in the rate of spontaneous eye-blinking. For instance, spontaneous eye-blink rate can be increased by drugs that stimulate dopamine (dopamine agonists) (17,21) and decreased by drugs that inhibit dopamine (dopamine antagonists) (17,18). The nigrostriatal system, which connects the substantia nigra and the striatum, seems to be especially important (18,22), as further evidenced by reduced eye-blink rate in Parkinson's disease (17), a condition characterized by loss of dopamine neurons in the substantia nigra. In addition, a series of nonhuman primate studies involving pharmacologic lesions specific to the substatia nigra and its dopamine projections (18) and dopamine agonists/antagonists (17–21) show that dopamine appears to modulate spontaneous eye-blink via D1 and D2 receptors independently (19). Based on such findings, spontaneous eye-blink rate has been proposed as a noninvasive in vivo measure of dopamine functioning. Eye-blink rate, which can reliably be observed using simple techniques and a sampling period of a few minutes (23–25), increases almost linearly from

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* To whom correspondence should be addressed. E-mail: blozoff@umich.edu.

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birth through adolescence (23). It shows inter-individual variation but considerable stability within a given individual (14,23).

If iron deficiency substantially impairs dopamine functioning in infants, we predicted that eye-blink rate would be lower with iron-deficiency anemia and iron therapy would raise the rate.

Materials and Methods

Participants

Data on eye-blink rate were obtained in the course of a study on brain and behavioral effects of iron deficiency in infancy (26). Eye-blink data for 3–4 min were available for 61 otherwise healthy, normal birth weight, African-American 9- to 10-mo-old infants with venous iron measures sufficient for a final classification of iron status (see below). The sample was restricted to African-Americans because the inner-city clinic population where the study was conducted was >90% African-American and the remaining 10% varied widely in race and ethnicity. After the initial assessment, infants were provided with iron drops (22 mg elemental iron/d) for 3 mo. All infants were given iron, because the time interval corresponded to that of introducing unmodified cow milk for many infants.

The age of infants at initial testing was (means ± SD) 9.8 ± 0.3 mo; 57% were male. The birth weight and gestational age were 3.25 ± 0.37 kg and 39.8 ± 1.0 wk, respectively. Almost all families were under considerable economic stress, as indicated by Medicaid insurance and participation in the Women, Infants, and Children program.

Signed informed consent was obtained from the infant's primary caregiver. The protocol was approved by the Institutional Review Boards at Wayne State University School of Medicine and the University of Michigan.

Measurements

Iron status. As previously reported (26), initial venous blood tests included a complete blood count, lead, and zinc protoporphyrin:heme ratio. Remaining blood was separated and frozen for subsequent determination of serum iron, total iron binding capacity, transferrin saturation, and ferritin. Infants with lead ≥ 4.83 μmol/L (10.0 μg/dL) were excluded. Anemia was defined as hemoglobin < 110 g/L (27) and iron deficiency as ≥2 abnormal values among mean corpuscular volume < 74 fL (28), red cell distribution width > 14.0% (27), zinc protoporphyrin > 69 μmol/mol heme (29), transferrin saturation < 12% (30), and ferritin < 12 μg/L. The latter was between suggested cutoffs (27,29). Iron sufficiency was defined as the clear absence of anemia (hemoglobin ≥ 115 g/L) and no more than 1 abnormal iron measure. Of the 61 infants with eye-blink data and sufficient blood work to classify iron status by these criteria, 19 had iron-deficiency anemia, 23 were nonanemic iron-deficient, and 19 were nonanemic iron-sufficient. Forty-one (67%) had iron status measures after 3 mo.

Eye-blink. For the eye-blink assessment, infants in the quiet alert state were seated on their mother's lap. To provide a reasonably consistent context for a 4-min observation period, an experimenter gently blew soap bubbles across the infant's line of sight at a distance of 1.5 m, following a protocol used in infant neurophysiologic studies (31,32). The bubbles offered quiet visual stimuli that were neither particularly exciting nor particularly dull. The procedure was videotaped with the camera focused on the infant's face. The number of eye-blinks in each minute of observation was counted from videotape by a single coder who was unaware of infant iron status. To be counted as a blink, the lids of both eyes had to close completely and at the same time. Eye-blink counts were analyzed for the 61 infants who remained quiet and alert. Eye-blink data for 16 other infants were considered unusable: drowsiness in 4 nonanemic and 4 iron-deficient anemic infants, <3 min of data for 3 infants, crying or feeding for 2 infants, and technical difficulties for 3 tapes. Forty-six of the 61 infants (75%) with usable data at 9–10 mo had repeat eye-blink data after 3 mo.

Data analysis

Statistical analyses were performed with SAS version 9.2 (SAS Institute). We used ANOVA to test for differences in background and hematology variables by iron status group. Pairwise comparisons (least square means with $\alpha = 0.05$) were performed if the overall ANOVA P-value was < 0.10.

As a count variable, the number of eye-blinks followed a Poisson distribution. Therefore, we used Poisson regression based on generalized estimation equation methodology (33) to test for group differences in eye-blinks per minute, with $\alpha = 0.05$ in 2-tailed tests of significance. (The test statistic for comparisons using Poisson regression is χ^2, which is distinct from that for 2 × 2 tables but shares the same distribution.) To eliminate the initial attention response, eye-blink data for the first minute was not analyzed, in keeping with other research (34). The number of eye-blinks in min 2, 3, and 4 was substantially correlated (r values = 0.6–0.7; P-values < 0.001; intra-class correlation = 0.63). Therefore, the number of eye-blinks in the available min 2–4 was summed and divided by the number of minutes of usable data for a given infant to produce the outcome variable of eye-blinks/min. We also used Poisson regression with repeated measures implemented through generalized estimation equation methodology to test for changes in eye-blinks/min from initial to repeat assessment and to determine whether any such change differed between iron status groups.

Background factors, including gender, birth weight, gestational age, anthropometry, and age at testing, were considered as covariates. Any factor even weakly related to eye-blink rate ($P < 0.10$) was considered as a potential covariate in statistical analyses of eye-blinks/min. Those that were significant ($P < 0.05$) in the regression models were retained as covariates. Values in the text are means ± SD.

Results

The iron status groups were similar in gender, birth weight, length, and gestational age (**Table 1**). However, infants with iron-deficiency anemia had lower weight-for-age Z-scores than iron-sufficient infants initially and after 3 mo. By definition, the groups differed on several iron status measures (**Table 2**). The groups differed from each other in hemoglobin concentration. Iron-deficient infants with and without anemia had lower mean corpuscular volume, higher red cell distribution width, and a higher zinc protoporphyrin:heme ratio than the iron-sufficient group. Iron-deficiency anemia was generally mild, as would be expected in a sample that received Women, Infants, and Children program benefits. Mean hemoglobin increased after 3 mo in the iron-deficient anemic group (7.2 g/L; $P = 0.03$), whereas it was unchanged in the nonanemic iron-deficient group and decreased in the iron-sufficient group (Table 2). Infants with and without hematology data after 3 mo were similar with

TABLE 1 Background characteristics of the infants[1]

	Iron-deficient, anemic	Iron-deficient, nonanemic	Iron-sufficient, nonanemic
n	19	23	19
Age at initial testing, *mo*	9.7 ± 0.4	9.8 ± 0.2	9.8 ± 0.3
Age at repeat testing, *mo*	12.7 ± 0.5	12.7 ± 0.7	12.8 ± 0.7
Gender, *% male* (*n*)	47.4 (9)	65.2 (15)	57.9 (11)
Birth weight, *kg*	3.23 ± 0.30	3.20 ± 0.37	3.32 ± 0.43
Gestational age, *wk*	39.6 ± 0.8	40.0 ± 1.3	39.7 ± 0.9
Breast-fed, *% yes* (*n*)	42.1 (8)	47.8 (11)	47.4 (9)
Initial height-for-age Z-score	−0.6 ± 1.9	−0.4 ± 1.1	−0.4 ± 1.3
After 3 mo[2]	−0.4 ± 0.9	−0.3 ± 1.1	0.1 ± 1.0
Initial weight-for-age Z-score[3]	−0.6 ± 1.0[b]	0.0 ± 1.4[a,b]	0.2 ± 0.9[a]
After 3 mo[2]	−0.9 ± 1.7[b]	0.0 ± 1.1[a,b]	0.1 ± 1.1[a]

[1] Values are means ± SD and % (*n*) for categorical variables.
[2] *n* = 14, except nonanemic, iron-deficient, *n* = 13.
[3] ANOVA *P*-values = 0.08–0.09. Means in a row with superscripts without a common letter differ, $P < 0.05$.

TABLE 2 Iron status of iron-deficient anemic, iron-deficient, nonanemic, and iron-sufficient, nonanemic infants at 9–10 mo of age[1]

	Iron-deficient, anemic	Iron-deficient, nonanemic	Iron-sufficient, nonanemic
n	19	23	19
Hemoglobin,[2] *g/L*	101.7 ± 5.0[c]	118.9 ± 5.5[b]	123.3 ± 5.3[a]
Change after 3 mo,[2] *g/L*	7.2 ± 13.2[a]	0.5 ± 6.9[a]	−6.9 ± 10.5[b]
Mean corpuscular volume,[2] *fL*	71.9 ± 5.2	73.8 ± 4.5	78.8 ± 3.3
Red cell distribution width,[2] *%*	14.9 ± 1.7[a]	14.4 ± 0.9[a]	12.9 ± 0.8[b]
Zinc protoporphryin:heme,[2] *μmol/mol*	113.1 ± 37.3[a]	94.0 ± 55.9[a]	64.3 ± 15.1[b]
Transferrin saturation, *%*	19.6 ± 9.9	25.6 ± 12.7	24.3 ± 9.1
Ferritin, *μg/L*	58.0 ± 63.2	34.4 ± 30.5	33.4 ± 22.1
Lead, *μmol/L*	0.11 ± 0.08	0.14 ± 0.13	0.12 ± 0.07

[1] Values are means ± SD, *n* = 14, except nonanemic, iron-deficient, *n* = 13.
[2] ANOVA *P*-values = < 0.001–0.05. Means in a row with superscripts without a common letter differ, $P < 0.05$.

respect to all variables in Tables 1 and 2, with the exception of breast-feeding; more of those with follow-up data had been breast-fed (59 vs. 20% among those without; $P < 0.01$).

Gender was the only background factor associated with eye-blink rate. Girls had higher eye-blink rates than boys at 9–10 mo (5.7 ± 2.7 vs. 4.2 ± 2.4 blinks/min; $\chi^2 = 3.73$; $P = 0.05$). Analyses relating iron status to eye-blink rate included gender as a covariate.

Nonanemic iron-deficient and iron-sufficient groups were similar in eye-blink rate (**Fig. 1**) and therefore combined to form a nonanemic group for comparison to infants with iron-deficiency anemia. The mean number of minutes of analyzable eye-blink data was comparable for the iron-deficient anemic and nonanemic groups (**Table 3**).

Infants with iron-deficiency anemia had fewer eye-blinks/min than nonanemic infants. The magnitude of the difference was 0.6 SD, a large effect size. After 3 mo, the change in eye-blinks/min from the initial assessment differed between iron-deficient anemic and nonanemic groups ($\chi^2 = 4.69$; $P = 0.03$). Eye-blink rate increased 2.1 blinks/min in infants with iron-deficiency anemia ($\chi^2 = 7.15$; $P = 0.008$), whereas there was no change in nonanemic infants ($\chi^2 = 0.01$; $P = 0.96$). Thus, there was no group difference in eye-blink rate after 3 mo. Nonanemic iron-deficient and iron-sufficient infants again had similar eye-blink rates, averaging 5.2–5.6 blinks/min.

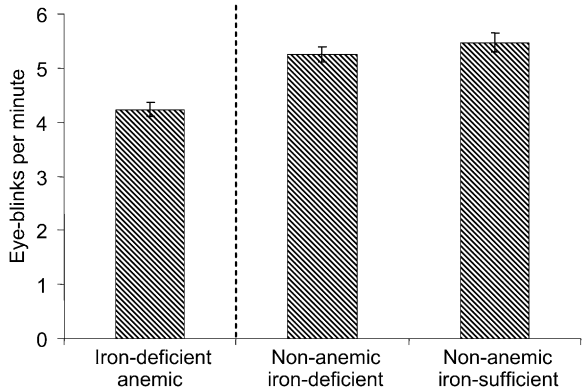

FIGURE 1 Eye-blinks by iron-deficient, anemic, iron-deficient, nonanemic, and iron-sufficient, nonanemic infants at 9–10 mo of age. Values are means (adjusted for gender) ± SE, *n* = 19–23. The dotted line indicates that the threshold of effects was iron-deficiency anemia vs. no anemia, based on Poisson regression using generalized estimating equation methodology. Nonanemic, iron-deficient and iron-sufficient groups did not differ in eye-blink rate (5.3 ± 0.6 SE vs. 5.4 ± 0.6 SE blinks/min; $\chi^2 = 0.02$; $P = 0.90$).

Discussion

The functional significance of a lower eye-blink rate is unclear. In adults (other than in ocular pathologies), it has been related to less visuomotor binding (13) and reduced cognitive flexibility (16), but more cognitive stability (15). Recent studies have also explored the role of genetic polymorphisms related to dopamine metabolism (15,16). Such research has been interpreted with respect to altered central dopaminergic activity, rather than eye-blink rate per se. In keeping with this approach, we propose that a lower eye-blink rate in infants with iron-deficiency anemia is consistent with reduced dopamine function in humans and the dopaminergic effects observed in rodent models of iron deficiency [see (8) and (9) for review].

Our finding that eye-blink rate was lower in infants with iron-deficiency anemia but not in those who were iron-deficient without anemia leads us to hypothesize that iron deficiency must be severe and prolonged enough to cause iron-deficiency anemia

TABLE 3 Eye-blink data of iron-deficient anemic and nonanemic infants

	Anemic	Nonanemic
Infants at 9–10 mo, *n*	19	42
Analyzable data, *n*		
Min 2 only	0	0
Min 2 and 3	5	16
Min 2, 3, and 4	14	26
Blinks/min[1,2]	4.0 ± 1.9	5.3 ± 2.8
Infants after 3 mo, *n*	16	30
Analyzable data, *n*		
Min 2 only	1	0
Min 2 and 3	5	6
Min 2, 3, and 4	10	24
Blinks/min[1]	6.3 ± 3.7	5.4 ± 3.0

[1] Values are means (adjusted for gender) ± SD.
[2] Significant group difference based on Poisson regression using generalized estimating equation methodology ($\chi^2 = 5.10$; $P = 0.02$), indicating fewer blinks/minute for the anemic group.

(and associated impairment of dopaminergic functioning in brain circuits regulating spontaneous eye-blink) before eye-blink rate is affected. This hypothesis might best be tested in nonhuman primate models where iron deficiency can be induced experimentally and the rate of spontaneous eye-blinking determined repeatedly.

The observed increase in eye-blink rate following iron therapy seems to provide evidence for recovery of a dopamine-related behavior with treatment. However, this interpretation is offered with caution. The study had incomplete data on iron status at 12 mo, because over one-third of the infants did not have repeat blood testing and we were not able to monitor infant iron intake personally. The hemoglobin increase in the iron-deficient anemic group, although significant, was not as pronounced as often observed. Nonetheless, any impact on eye-blink of increasing hemoglobin cannot be separated from the postulated improvement in dopaminergic function due to improved iron status. Examining eye-blink rate in anemias that are not due to iron deficiency could determine whether anemia itself is associated with a lower rate of spontaneous eye-blink.

The gender difference we observed cannot be directly compared with previous research with young children, because most such studies have not reported results separately for boys and girls and we were unable to find any study of infants of a comparable age. In older children and adults, however, findings about gender differences are conflicting. Some studies report higher rates in females (15,16), one reports higher rates in males (12), and still others report no significant differences (10,23,24). Whether these discrepant findings are due to differences in testing conditions, recording methods, age of participants, or other factor(s) is unknown.

This study is limited in several ways. Our sample size was relatively small. The transitory nature of infant attention meant that the eye-blink observation was short, although most infants had at least 3 min of data, as has been recommended (25). Our methods were simple, a count of eye-blinks from routine videotape, whereas some studies record eye-blinks with more sophisticated techniques, such as electro-oculogram (12,13, 15,35), specialized cameras (10), or software (36). Because all infants were observed in the daytime, the study cannot determine whether eye-blink rate would increase in the evening in infants, as in adults (35).

We would like to emphasize that eye-blink rate should not be used as a clinical tool or a substitute for appropriate blood testing. Rather, the clinical relevance of our findings is that impaired dopamine functioning, indexed by less eye-blinking, might have broader impacts given the role dopamine plays in regulating movement, motivation, cognition, and hormone release (37). Differences in eye-blink rate appeared to resolve after iron therapy in this study, but long-lasting alterations in these more complex domains are consistently observed with lack of iron in infancy [reviewed in (9)].

This study provides evidence that iron-deficiency anemia in human infants is associated with alterations in a dopamine-related behavior that seems to recover after iron treatment. Because iron deficiency is considered the most common single nutrient deficiency in the world and infants are at particular risk (1,2), the impact of iron deficiency effects on central nervous system functioning could be substantial at the individual and societal levels.

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