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Title: Supplementation with iron syrup or iron-containing multiple micronutrient powders alters resting brain activity in Bangladeshi children

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Abbreviations: CI: confidence interval; CRP: C-reactive protein; EEG: electroencephalography; FDR: false discovery rate; IQR: interquartile range; MNP: multiple micronutrient powder; RCT: randomized controlled trial; SD: standard deviation; WHO: World Health Organization.

ABSTRACT

Background: Anemia and iron deficiency have been associated with poor child cognitive development. A key rationale for the prevention of anemia using supplementation with iron has been the benefits to neurodevelopment. Yet, little causal evidence exists for these gains.

Objective: We aimed to examine effects of supplementation with iron or MNPs on brain activity measures using resting electroencephalography (EEG).

Methods: Children included in this neurocognitive substudy were randomly selected from the Benefits and Risks of Iron Supplementation in Children study, a double-blind, double-dummy, individually randomized, parallel-group trial in Bangladesh, in which children, starting at 8 months of age, received 3 months of daily iron syrup, multiple micronutrient powders (MNPs), or placebo. Resting brain activity was recorded using EEG immediately post-intervention (month 3), and after a further 9-month follow-up (month 12). We derived EEG band power measures for delta, theta, alpha, and beta frequency bands. Linear regression models were used to compare the effect of each intervention group to placebo on the outcomes.

Results: Data were analyzed from 412 children at month 3 and 374 at month 12. At baseline, 43.9% were anemic and 26.7% were iron deficient. Immediately post-intervention, iron syrup, but not MNPs, increased mu alpha-band power, a measure that is associated with maturity and the production of motor actions (Iron vs Placebo mean difference=0.30, 95% CI: 0.11, 0.50 $\mu V^{2,}$ P=0.003, false discovery rate adjusted P=0.015). Despite effects on hemoglobin and iron status, effects were not observed on the posterior alpha, beta, delta, and theta bands, nor were effects sustained at the 9-month follow-up.

Conclusions: The effect size for immediate effects on mu alpha-band power is comparable in magnitude to psychosocial stimulation interventions and poverty reduction strategies. However, overall, we did not find evidence for long-lasting changes in resting EEG power spectra from iron interventions in young Bangladeshi children.

Trial registration: ACTRN12617000660381

Keywords: iron, anemia, multiple micronutrient powders, neurodevelopment, electroencephalography, resting state, brain activity, child

INTRODUCTION

Anemia is an important public health problem that affects populations worldwide (1). Anemia and iron deficiency has been associated with poor child development with possible repercussions into adulthood (2-4); therefore, a key rationale for the prevention of anemia in young children has been the benefits to cognitive development. Micronutrients, especially iron, play a critical role in the developing infant brain through effects on myelination (5-8), neurotransmission (9), dendritogenesis (10), and synaptogenesis (10). The World Health Organization recommends that all children under 2 years of age living where the prevalence of anemia exceeds 20% receive treatment with iron interventions: either universal home fortification with iron-containing multiple micronutrient powders (MNPs) (11), or iron supplements (e.g., iron syrup) (12). However, the neurophysiologic effects of iron interventions in a population of children facing a high prevalence of iron deficiency and anemia have not been defined.

In large public health evaluations, sensitive measures of neurocognitive functioning have seldom been used (13, 14). In high-income countries, measures derived from electroencephalographic (EEG) recordings while children are at rest, such as spectral power in frequency bands known to be relevant to cognitive functioning, have been used to assess neurocognitive development (15-17). This technique has rarely been used in resource-limited settings, however, where children face significant insults in their physical and psychosocial environments.

Childhood EEG-derived brain activity has been shown to be predictive of later cognitive development and sensitive to iron status. Measures related to the periodic contributions to the EEG (also called oscillations), have been shown to correlate with behavioral indices of cognitive and motor functioning in adults (e.g., alpha peak frequency (18-20)) and children (e.g., alpha-band power (15); theta-band power (16, 17)). In addition, certain aspects of the aperiodic component, which reflects EEG activity spread across a broad frequency range, has recently been found to correlate with markers of cognitive function (21, 22) and varies with maturation from childhood to adulthood (23). Observational studies have shown associations between iron status and resting EEG measures in children (24-26). However, due to their cross-sectional or pre-experimental designs, these studies do not demonstrate a causal relationship. Finally, previous studies did not attempt to isolate periodic activity from the contribution of aperiodic signals to Fourier power spectra, meaning that it is difficult to determine whether changes in periodic activity (i.e., neural oscillations) actually underlie these delta, theta and alpha-band effects (27).

Despite the lack of rigorous evidence (28, 29), the proposed benefits of iron supplementation on cognitive development in children have been used to guide iron intervention programs and recommendations across the world. This study addressed the need to objectively determine the effects of iron interventions on brain activity in young children. Our study sought to evaluate the effects of supplementation with iron syrup and iron-containing multiple micronutrient powders (MNPs) compared to double-dummy placebo on brain functioning using resting EEG recorded from Bangladeshi children. We used computational modelling of EEG power spectra (30) to isolate and quantify key components of the resting EEG signal, including periodic activity in cognition-relevant frequency bands (alpha, theta, beta) and aperiodic broadband activity that systematically changes with maturation from birth until adulthood (23, 31).

METHODS

This neurocognitive substudy was nested within the Benefits and Risks of Iron Supplementation in Children (BRISC) trial (www.anzctr.org.au; ACTRN12617000660381). The BRISC trial was a collaboration between the International Center for Diarrheal Disease Research, Bangladesh (icddr,b), the University of Melbourne, Australia, and the Walter and Eliza Hall Institute. It was approved by the Ethical Review Committees of icddr,b and Melbourne Health.

BRISC Study Design

The BRISC study was a three-arm, double-blind, double-dummy, individual randomized, superiority trial conducted in the Rupganj Upazila of Narayanganj district, Bangladesh. A detailed explanation of the study characteristics is provided elsewhere (32-34). Briefly, at 8 months of age +/-14 days, 3300 children were randomized to receive 3 months of 1) 12.5mg elemental iron (ferrous sulfate) syrup + placebo MNPs, or 2) MNPs (containing iron 12.5mg as ferrous fumarate, retinol 200ug, zinc 5mg, and Vitamin C) + placebo iron syrup, or 3) placebo iron syrup and placebo MNPs. Exclusion criteria included severe anemia (hemoglobin <80g/L measured on HemoCue 301 (HemoCue) using single drop capillary blood sample), drinking water iron content > 1mg/L, mid-upper-arm circumference < 11.5cm, current illness with fever, or previously known inherited red cell disorders. Children were randomized to one of the three arms using a 1:1:1 allocation, stratified by union (local area) of residence and sex. The study team and participants were blinded to the intervention. Assessments were completed in children at baseline, 3 months post-intervention (month 3, when children were 11 months of age), and after a further 9 months follow-up (month 12, when children were 20 months of age). The study began recruitment in July 2017 and completed follow-up visits in February 2020. A detailed description of outcomes measures for this study is presented elsewhere (34). Hemoglobin concentration, as an outcome, was assessed using venous blood on HemoCue 301 (HemoCue), serum ferritin was measured by electrochemiluminescence immunoassay using the Cobas e601 analyzer (Roche Diagnostics), and C-reactive protein was measured by particle enhanced immunoturbidimetric assay using the Cobas c311 analyzer (Roche Diagnostics). The full BRISC study will henceforth be referred to as the parent study.

Neurocognitive substudy design

A community sensitization campaign was undertaken prior to this substudy. Village leaders and community members were educated on the EEG technique and were shown an instructional video in Bangla, which included a description of the substudy rationale, the measurement technique, and the risks and benefits of participation. Prior to consenting, caregivers watched an instructional video. Caregivers were shown all the equipment and given a full explanation of the testing procedures. Written informed consent was obtained from all caregivers of children participating in this substudy. Refusal to participate in the neurocognition substudy did not exclude participation in the parent study. Participants were reimbursed for their travel and compensated for their time.

Recruitment to the substudy was based on recruitment of up to 5 participants per day, capped at the upper limit of the workload for the EEG testing team. Starting in December 2018, using a random number generator, we randomly selected children enrolled in the parent trial to also be included in the neurocognitive substudy starting at the 3-month post-intervention visit. Children who completed the 3-month post-intervention EEG measurement were asked to return for the same measurement at the 9-month follow-up visit. Additional children were enrolled using random selection to obtain resting EEG measurements at the 9-month follow-up visit (**Figure 1)**.

Measurements for the neurocognition substudy were conducted within 7 days following the measurements from the parent study. EEG recordings were conducted in a dedicated, air-conditioned testing room. During testing, children were seated on their caregiver's lap; caregivers were instructed not to talk to or distract the child. Children watched a video display consisting of 350 randomly-generated Gabor patches superimposed on a green background, generated using a modified version of the 'Gaborium' function in PsychToolbox v3.0.10 (35, 36) running in MATLAB 2018b (The Mathworks, Natick, MA, USA). Stimuli were presented on a 24-inch LED ZOWIE RL-55 Benq monitor that subtended approximately 48° × 27° of visual angle, with a refresh rate of 60 Hz. If children looked away from the display or were distracted, their attention was drawn back toward the screen by an experimenter. Testing continued until the experimenters judged they had collected at least two minutes of clean EEG data.

EEG data acquisition and processing

EEG data were recorded from 32 active scalp electrodes, placed according to the international 10-20 system, using a Brainvision LiveAmp system (Brain Vision, LLC) according to standard procedures. Data pre-processing was conducted using EEGLab v13.4.4 (37) running in

MATLAB (2018b, The MathWorks, Natick, MA). EEG data processing is detailed in **Supplemental Methods**.

Measures Derived From Fourier Power Spectra

We derived three sets of EEG measures for use in our analyses. We first derived band power measures in the delta, theta, alpha (posterior and mu) and beta bands from the original power spectra (stage 1) to be comparable to existing work, followed by analyses of flattened spectra with the aperiodic component removed (stage 2) to isolate effects on periodic (or oscillatory) EEG activity. We then analyzed measures from parameterized spectra (stage 3) including the aperiodic exponent, theta and alpha peak power measures, and theta and alpha peak frequencies.

Original Power Spectra

We first derived EEG band power measures from the original power spectra (Stage 1). Based on visual inspection of the group-averaged Fourier power spectra from the month 3 data we identified delta (1-3 Hz), theta (3-6 Hz), alpha (6-9 Hz) and Beta (9-30 Hz) frequency bands, which correspond to the ranges of these frequency bands in children of a similar age range (15, 38-45). The same frequency band ranges were also identified in the month 12 data.

Following identification of each frequency band, we then identified regions of interest (ROIs) that corresponded to relatively localized sources of frequency band-limited activity on the scalp for the month 3 data. These included electrode Fz (frontal ROI) for delta, electrodes Fz, F3, F4, FC1, FC2, Cz, C3, C4 (frontocentral ROI) for theta, and two distinct ROIs for alpha: Oz, O1, O2 (posterior alpha ROI) and Cz, C3, C4 (central/Mu alpha ROI). Clear localization could not be determined for beta activity, and so the average of electrodes Fz, F3, F4, FC1, FC2, Cz, C3, C4, CP1, CP2, Pz, P3, P4, Oz, O1, O2 was used for measures of activity in this frequency band. The mean power across each frequency band, averaged across channels in each ROI was calculated, obtaining one value per participant for each frequency band (except for alpha band power, which was measured at both posterior and central ROIs). As we used a Pz reference electrode for the month 12 data, we did not include this electrode in our ROIs for measuring beta-band activity. ROIs at month 12 were otherwise equivalent to those used at month 3.

Flattened Power Spectra

We also calculated the same frequency band power measures using ‘flattened’ spectra, whereby the aperiodic component of the power spectra was estimated and subtracted using the FOOOF algorithm (Stage 2). This allowed us to control for effects of aperiodic activity that contribute to the Fourier power spectra, where this aperiodic activity does not necessarily index periodic (or oscillatory) neural activity (30). This approach can help to disentangle effects that are specific to sources of periodic and aperiodic activity (27, 30).

Computational Modelling of Resting EEG Power Spectra

We also included measures derived from modelling the shape of the Fourier power spectra using the FOOOF algorithm (version 0.1.3) (stage 3) (46). This algorithm partitions Fourier power spectra into components that reflect aperiodic and periodic activity, each of which can be described by distinct sets of model parameters. From these parameterisations, we could measure the aperiodic exponent, as well as peak power and peak frequency values in the theta and alpha bands. By explicitly modelling neural activity associated with periodic activity (which can vary in both power and frequency independently), this allowed us to further control for, and measure, variability in the frequency at which periodic activity occurs in each individual. Settings for the algorithm were set as: peak width limit (0.5, 12.0); maximum number of peaks: 3; minimum peak height: 0.0; peak threshold: 2 standard deviations; and aperiodic mode: 'fixed'. Power spectra were parameterized across the frequency range of 1-30 Hz, for each channel separately. Parameter estimates were then averaged across channels within each ROI for each participant.

Several parameters of the fitted models were used as outcome variables (Stage 3). The aperiodic exponents were computed from the aperiodic component of the model. The FOOOF algorithm fits exponential functions to the power spectra, whereby the exponent is equivalent to the slope of a linear fit in log-log space. The peak center frequency and power at the peak frequency were computed from the periodic component of the model. FOOOF fits Gaussian functions to the data not accounted for by the aperiodic activity in order to identify sources of periodic activity. The center frequencies are the frequencies that correspond to the peaks of each Gaussian, and power at the peak frequencies correspond to the height of each Gaussian at its peak. We further subdivided all identified peaks based on different frequency bands. Peaks within theta range (3 – 6 Hz) were labelled as the theta peak and peaks within the alpha range (6 – 9 Hz) were labelled as the alpha peak. The largest peaks within each frequency band were selected as the outcome variables for each child. Center frequency and peak power measures were not obtained for the delta and beta bands, as clear peaks were not observable (and were not identified by the algorithm) in a substantial portion of datasets.

For measuring aperiodic exponents we used the same channels as for the beta band activity ROI, as aperiodic signals do not have a clear focal source (31). For modelling of theta and alpha band peak frequencies and peak power estimates, we used the same ROIs as for the band-power measures.

Sample size planning

A sample size of 120 in each treatment group allows to detect a standardized difference of 0.4 between each intervention arm and placebo, with 80% power at a 2-sided 5% alpha level. Based on previous literature of associations between iron deficiency and resting state EEG measures in children, this effect size was considered appropriate (24, 25). EEG data loss for reasons such as movement and child fussiness of about 20% has been reported (47, 48) and therefore, we aimed to enrol a total of 450 children of those who were planned to attend the month 3 visit of the parent study after initiation of the substudy.

An additional opportunistic sample of 257 children was randomly selected for EEG measurements at month 12 only. The sample included children who were not assessed as part of the neurocognitive substudy at month 3, but had recently completed month 12 measurements for the parent study. We anticipated some participant drop-out between months 3 and 12 and therefore, we assessed these additional children to allow us to examine longer term effects after a further 9 months of follow up.

Statistical analyses

A modified intention-to-treat sample consisting of children with sufficient numbers of artefact-free epochs (i.e., ≥58 epochs, also termed evaluable EEG data) was analyzed at month 3 and 12 separately according to their randomized treatment group. Baseline characteristics were compared between children who were enrolled in the neurocognitive substudy and those not included in the substudy, children with sufficient artefact-free epochs and those with insufficient artefact-free epochs, and between treatment groups.

We performed analyses in a staged approach, with each stage using a different set of Fourier power spectra measures. Each stage included 5 measures and two between-group comparisons, resulting in a total of 10 tests per stage. We first analysed delta, theta, alpha (at central and posterior ROIs), and beta band power measures using the original spectra (comprising 5 different measures). We then performed analyses using the flattened spectra (which controls for influences of aperiodic activity) using the same set of band power measures. Finally, we analysed model parameters derived using the FOOOF algorithm, including the aperiodic exponent, theta and alpha peak power measures, and theta and alpha peak frequencies (comprising 5 measures). The following additional analyses were conducted, adjusting for potential confounders, in addition to adjusting for union and sex. Model 1: adjusting for baseline Family Care Indicator score (continuous measure) (49) and maternal education (no education, 1-8, 9-12, >12 years of schooling); Model 2: adjusting for other baseline variables which were not comparable across treatment groups at baseline; Model 3: adjusting for baseline Family Care Indicator score, maternal education, and any baseline variables which were not comparable across treatment groups at baseline; Model 4: analysis restricted to those considered compliant with the intervention. Overall compliance across the 3-month intervention period was derived as the total number of days the child was reported taking both the iron syrup and the MNPs divided by the child's participation duration during the intervention period, with 'complier' defined as those with overall compliance ≥70%.

Pre-planned subgroup analyses were run to examine whether treatment effects differed between subgroup categories, by adding subgroup and subgroup-by-treatment interaction term in the model. The following subgroups were examined: a) sex (female/male), b) baseline anemia status (yes/no, anemia defined as hemoglobin <11g/dL), c) baseline iron deficiency status (yes/no, iron deficiency defined as ferritin<12μg/L or <30μg/L if CRP>5mg/L), d) baseline iron deficiency anemia status (yes/no, iron deficiency anemia defined as concurrent anemia and iron deficiency), e) baseline Family Care Indicator scores (using the median, <13/≥13), f) baseline food insecurity status (yes/no), g) baseline wealth status (using the median, <0.49/≥0.49), and h) union (Bhulta, Golakandail, Rupganj).

Resting EEG data were analyzed in Stata/SE 16.1 (StataCorp LLC, College Station, TX). Across the sets of measures within each visit-comparison-analysis stage combination we applied a false discovery rate (FDR) correction procedure (q=0.05) (50). An FDR correction was used given the exploratory nature of the study (51). We compared iron and MNP intervention groups to the placebo group. We did not compare EEG measures across the iron and MNP groups. Underlying model assumptions of normality and homoscedasticity were examined using residual plots.

RESULTS

Recruitment to the substudy started during the second half of the parent study when about two-third of children had completed their month 3 assessment. Of the 440 children enrolled at month 3 in the EEG substudy, 412 (93.6%) provided sufficient amounts of artefact free data. At month 12, 594 children were measured, including 257 newly recruited children, of whom 374 (63.0%) provided sufficient artefact-free EEG data (**Figure 1**). A total of 103 participants who underwent assessment at month 3 did not receive a repeated EEG measurement at month 12 due to loss to follow-up (N=13) or parents declining follow-up EEG measurement (N=90).

Analyses of Resting EEG Measures

Qualitative Patterns of Resting EEG Data

Group averaged resting EEG spectra at month 3 were typical of those observed in recordings of infants within a similar age range (**Supplemental Figure 1**). EEG spectra showed a typical pattern whereby amplitudes decreased with increasing frequency, which can be approximated by an exponentially-decreasing function. Prominent peaks of activity were also observed in the infant theta (3-6 Hz) and alpha (6-9 Hz) bands (**Figure 2**). Scalp maps show that amplitudes in the delta band were most prominent around electrode Fz, and within the theta band were most prominent around a group of fronto-central electrodes (**Supplemental Figure 1**). There were two visible sources of alpha: one over central electrodes (commonly called the mu alpha rhythm) and one over more posterior occipital channels (commonly called posterior alpha). Beta band activity was broadly spread across the scalp and did not show any focal patterns. Low amplitudes around bilateral occipito-parietal sites were likely due the use of TP9 and TP10 as reference electrodes for the month 3 data.

Month 12 data using a Pz reference were similar to patterns seen at month 3 (**Supplemental Figure 2**). Amplitudes were overall lower in the month 12 data, likely due to the proximity of the Pz reference electrode to the electrodes that made up each ROI. Scalp maps of amplitudes averaged over all infants at month 12 also look different due to the Pz reference, resulting in lower amplitudes at Pz and proximal electrodes. In the month 12 data there was a prominent peak in the alpha band, but not in the theta band.

Baseline comparison between intervention arms

Children enrolled in the neurocognitive substudy at month 3 were similar to those enrolled at month 12 in their prevalence of baseline anemia (43.4% of children enrolled at month 3; 46.8% at month 12), iron deficiency (26.9% at month 3; 28.6% at month 12), and iron deficiency anemia (17.6% at month 3; 20.3% at month 12). Baseline stunting, an indicator of a deprived environment, was identified in 26.9% of children enrolled at month 3 and 23.4% of children enrolled at month 12. Among children with sufficient artefact-free EEG data at month 3 and month 12, there were differences between intervention groups in terms of iron deficiency (**Table 1, Supplemental Table 1**). Minor, non-clinically relevant differences in baseline characteristics were observed between children enrolled and not enrolled in the neurocognitive substudy and between children with and without sufficient amounts of artefact-free EEG data (**Supplemental Table 2, Supplemental Table 3**). Baseline characteristics were similar between children in the full neurocognitive substudy and those within the analysis restricted to children with compliance ≥ 70% (**Supplemental Table 4**).

Treatment effects

We first verified that iron syrup and MNPs improved iron status in children enrolled in the neurocognitive substudy. Daily iron syrup improved hemoglobin concentration (mean difference Iron vs Placebo = 4.5 g/L (95% CI: 2.3, 6.7), p <0.001 at month 3; 2.8 g/L (95% CI: 0.3, 5.4), p =0.031 at month 12) and ferritin concentration (geometric mean ratio Iron vs Placebo = 1.7 ng/mL (95% CI: 1.4, 2.1), p <0.001 at month 3; 1.5 ng/mL (95% CI: 1.2, 1.8), p <0.001 at month 12). Daily MNPs improved hemoglobin concentration at month 3, but effects were not sustained at month 12 (mean difference MNPs vs Placebo = 4.2 g/L (95% CI: 2.0, 6.5), p <0.001 at month 3; 2.3 g/L (95% CI: -0.4, 5.0), p =0.096 at month 12). Effects of MNPs on ferritin concentration were observed at both timepoints (geometric mean ratio MNPs vs Placebo = 1.5 ng/mL (95% CI: 1.2, 1.8), p <0.001 at month 3; 1.6 ng/mL (95% CI: 1.3, 2.0), p <0.001 at month 12).

Table 2 and **Figure 3** show the treatment effect estimates immediately post-intervention (month 3) and at follow-up (month 12), with bolded estimates and p-values as those that reach significance after applying a false discovery rate (FDR) correction procedure across sets of measures within each visit-comparison-analysis stage combination (q=0.05) (50). An FDR correction was used given the exploratory nature of the study (50, 51). Compared to placebo, children who received daily iron syrup had higher mu alpha-band power at month 3. Results were consistent across the original spectra and flattened spectra, but did not reach statistical significance for the peak power measures after applying the FDR correction (mean difference estimate for original spectra=0.30 μV^2 (95% CI: 0.11, 0.50); flattened spectra=0.28 μV^2 (95% CI: 0.10, 0.46)) (**Table 2, Figure 3**). Treatment effects on mu alpha band power measures were not sustained at month 12 (**Table 2**). For analyses of original, flattened, and parameterized spectra, no other comparisons between iron and placebo groups at either timepoint were statistically significant. Children who received MNPs did not differ from those who received placebo at month 3 and month 12 on any of the measures across the original, flattened, and parameterized spectra.

Estimates were similar after adjusting for pre-identified potential confounders, baseline characteristics which were not comparable across treatment groups at baseline (i.e., iron deficiency), or when analysis was restricted to children in the intervention arm with compliance ≥70% (**Supplemental Table 5).** There was no evidence of differences in treatment effects between the subgroups defined by sex of child, anemia, iron deficiency, iron deficiency anemia, family care indicator (a measure of home environment, score above vs below median), wealth index (above vs below median), and food security for any of the outcomes at month 3 or month 12 (**Supplemental Table 6**).

DISCUSSION

Associations between iron status and cognitive development in children have been reported in observational studies (2-4); yet, the causal relationship between iron supplementation, in accordance with the WHO recommendations (11, 12), and brain function in young children is not well understood. This trial sought to determine how improvements in iron status following supplementation with iron and iron-containing MNPs, compared to placebo, in populations with anemia above 40% affect child brain function. We examined the efficacy of universal iron supplementation on resting EEG measures in young children living in a low-income setting with prevalence estimates of 28% for iron deficiency and 45% for anemia. We found evidence of higher mu alpha-band power over central electrodes in children who received iron syrup compared to placebo 3 months after starting the intervention, even after FDR correction. The effect size for effects of iron syrup on mu alpha-band power (Cohen's d=0.38, 95% CI: 0.14, 0,62) was larger in magnitude than for other nutrition interventions' effects on child cognition (52), but comparable to psychosocial stimulation (53) and poverty reduction (54) interventions. However, effects were not sustained at follow-up 9 months later. We did not find statistically significant differences for the other, broad-ranging set of resting EEG measures, including posterior alpha, beta, delta, and theta bands. Overall, we did not find consistent evidence for long-lasting changes in resting EEG power spectra associated with iron or micronutrient interventions in this sample of Bangladeshi children.

Effects of iron syrup on mu alpha power (at central electrodes over motor cortex) immediately after the intervention were consistent across measures that included and excluded contributions from aperiodic activity to resting EEG power spectra. This indicates that treatment group differences at least partially index changes in periodic (also called oscillatory) EEG activity. Mu alpha band neural activity has been closely linked with the perception and production of motor actions. Mu alpha power in resting state recordings has been found to gradually increase in magnitude with maturation over 10-24 months of age, a period over which there is substantial development of motor skills (38). Reductions in mu alpha power are reliably time-locked to the production of motor movements in infants (55, 56), and also occurs with observation of others' actions (38, 55, 57, 58). Our resting EEG design did not allow us to identify links with specific motor behaviors, and the role of iron in infant motor development should be further investigated using paradigms designed to systematically assess covariation between mu alpha activity and motor behavior (56, 58).

Notably, iron-containing MNPs did not result in significant differences in any of the resting EEG measures, despite similar effects to iron syrup on hemoglobin and ferritin concentrations. However, we are cautious to overinterpret these findings given treatment effects from MNPs were not significantly different to those from iron syrup. The MNPs contained micronutrients besides iron, including vitamin A, zinc, and vitamin C, which may have resulted in interactions between nutrients and subsequent reductions in absorption (59-62).

Previous studies of iron supplementation in young children in resource-limited settings have shown mixed, predominantly null, effects on child development and behavior (28, 29). However, many were limited by small sample size, lack of a placebo control, and importantly, the use of broad child development assessment measures. Resting EEG measures, on the other hand, provide insights into brain function which are predictive of later cognitive achievement (63-68). Our findings indicate that any effects on dynamic patterns of resting brain activity are restricted to a narrow subset of EEG measures and are not sustained over a 9-month post intervention follow-up period. Existing work has identified lower power in the alpha band for iron deficient children 3-15 months of age (25, 26), complementing our findings that indicate causal effects of iron supplementation. However, we did not replicate previous observations of higher theta- and delta-band power in iron deficient children (24-26). These discrepancies could be due to previous studies not utilizing measures that separate the contributions of aperiodic and periodic EEG activity, and using cross-sectional study designs, meaning that differences between groups in characteristics that co-occur with iron status could have contributed to observed group differences. While our data show high variability for theta and delta band power, increased noise is typical for low frequencies as compared to higher frequency ranges, including alpha band power.

Our randomized trial was sufficiently powered to detect effects on EEG outcomes and was conducted in a population with the potential to benefit from iron supplementation. The 3-month intervention duration was in accordance with the WHO guidelines for iron and MNP supplementation in children (11, 12), and was enough to improve hemoglobin and ferritin concentrations. Therefore, any functional effects on resting EEG measures due to improvements in hemoglobin or iron status would have been captured within this time frame. It is worth noting that statistical power to detect treatment differences between subgroups is low, including subgroups defined by anemia and iron deficiency. Furthermore, it is possible that effects on resting state brain activity in the general population may be different than those found in this trial given our exclusion criteria (including hemoglobin <80g/L, current infection, high groundwater iron, and inherited red cell disorders) (34). However, of those screened, only 12.8% of children were ineligible to participate (34).

One additional important difference between our study and existing work is that the environmental, nutritional, and psychosocial conditions faced by the children included in our study are likely meaningfully different than those experienced by children in high-income countries, where the vast majority of EEG studies in relation to iron status have been conducted. While children in our study faced substantial iron deficiency and anemia, they may have been less likely to respond to iron supplementation because they faced other important constraints on their brain development, such as repeated infection or inadequate psychosocial stimulation. There are reports of correlations between resting EEG measures and different measures of a child's environment, such as low household income (54, 69) and maternal stress (69), conditions which are both prevalent in Bangladesh (69, 70). Interventions to alleviate poverty and improve responsive caregiving and early learning opportunities have been shown to have effect sizes 4-5 times larger than nutrition interventions (52). That said, other meaningful electrophysiological and behavioral outcomes not measured in this study, such as attention and executive function (71, 72), may be responsive to iron supplementation, and future research would benefit from exploring these using rigorous designs.

The results of our rigorously designed study demonstrate that iron syrup, but not MNP, interventions can increase mu-alpha brain activity. However, despite observed links between iron availability and multiple facets of neurophysiological development (24-26), our study also shows that increased iron intake does not necessarily lead to large, measurable changes in other frequency bands. In addition, changes in mu-alpha were not sustained after a 9-month follow-up period. Our findings suggest that other forms of adversity that children in this region may face, in particular sub-optimal early learning opportunities (34), may be critical to consider when designing public health interventions, and there is a need for evidence on the effects of addressing these conditions in combination with iron deficiency.

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Statement of authors' contributions to manuscript

LML, DF, SB, KAJ, JDH, SBo, and SRP designed the study. LML, DF, SBr, and JJ designed the analysis plan. LML, DF, MIH, JDH, and SRP led the data collection for this study. LML, DF, and JJ provided data management. LML, DF, SBr, and JJ processed and analyzed the data. LML, DF, BAB, JDH, KAJ, SBo, and SRP provided scientific oversight and study supervision. LML and DF prepared the first draft of the manuscript. All authors critically reviewed and edited the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Competing interests: None declared.

Data availability: De-identified individual participant data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. WHO. Global Health Observatory. Accessed January 6 2022 from https://www.who.int/data/gho/data/themes/topics/anaemia_in_women_and_children; 2021.
2. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. The Journal of nutrition. 2001;131:649S-68S.
3. Stoltzfus RJ, Mullany L, Black RE. Iron Deficiency Anaemia. In: Ezzati M, Lopez AD, Rodgers A, Murray CJL, editors. Comparative quantification of health risks: sexual and reproductive health. Switzerland: World Health Organization; 2004.
4. Walker SP, Wachs TD, Gardner JM, Lozoff B, Wasserman GA, Pollitt E, Carter JA, Group ICDS. Child development: risk factors for adverse outcomes in developing countries. The lancet. 2007;369:145-57.
5. Ortiz E, Pasquini JM, Thompson K, Felt B, Butkus G, Beard J, Connor JR. Effect of manipulation of iron storage, transport, or availability on myelin composition and brain iron content in three different animal models. J Neurosci Res. 2004 Sep 01;77:681-9.
6. Rao R, Tkac I, Townsend EL, Gruetter R, Georgieff MK. Perinatal iron deficiency alters the neurochemical profile of the developing rat hippocampus. The Journal of nutrition. 2003 Oct;133:3215-21.
7. Roncagliolo M, Benitez J, Eguibar JR. Progressive deterioration of central components of auditory brainstem responses during postnatal development of the myelin mutant taiep rat. Audiol Neurootol. 2000 Sep-Oct;5:267-75.
8. Roncagliolo M, Schlageter C, Leon C, Couve E, Bonansco C, Eguibar JR. Developmental impairment of compound action potential in the optic nerve of myelin mutant taiep rats. Brain Res. 2006 Jan 05;1067:78-84.
9. Beard JL, Felt B, Schallert T, Burhans M, Connor JR, Georgieff MK. Moderate iron deficiency in infancy: biology and behavior in young rats. Behav Brain Res. 2006 Jun 30;170:224-32.
10. Jorgenson LA, Wobken JD, Georgieff MK. Perinatal iron deficiency alters apical dendritic growth in hippocampal CA1 pyramidal neurons. Dev Neurosci. 2003 Nov-Dec;25:412-20.
11. WHO. WHO guideline: Use of multiple micronutrient powders for point-of-use fortication of foods consumed by infants and young children aged 6–23 months and children aged 2–12 years. World Health Organization: Geneva, Switzerland. 2016.
12. WHO. Guideline: daily iron supplementation in infants and children. Geneva. http://apps.who.int/iris/bitstream/handle/10665/204712/?sequence=1. Accessed Jan 2018: World Health Organization; 2016. Report No.: 9241549521.
13. Wenger MJ, Murray Kolb LE, Scott SP, Boy E, Haas JD. Modeling relationships between iron status, behavior, and brain electrophysiology: evidence from a randomized study involving a biofortified grain in Indian adolescents. BMC public health. 2022;22:1-14.
14. Wenger MJ, Rhoten SE, Murray-Kolb LE, Scott SP, Boy E, Gahutu J-B, Haas JD. Changes in iron status are related to changes in brain activity and behavior in Rwandan female university students: results from a randomized controlled efficacy trial involving iron-biofortified beans. The Journal of nutrition. 2019;149:687-97.
15. Xiao R, Qi X, Patino A, Fagg AH, Kolobe TH, Miller DP, Ding L. Characterization of infant mu rhythm immediately before crawling: A high-resolution EEG study. NeuroImage. 2017;146:47-57.

16. Braithwaite EK, Jones EJ, Johnson MH, Holmboe K. Dynamic modulation of frontal theta power predicts cognitive ability in infancy. Developmental Cognitive Neuroscience. 2020;45:100818.

17. Jones EJ, Goodwin A, Orekhova E, Charman T, Dawson G, Webb S, Johnson MH. Infant EEG theta modulation predicts childhood intelligence. Scientific reports. 2020;10:1-10.

18. Klimesch W. EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. Brain research reviews. 1999;29:169-95.

19. Grandy TH, Werkle-Bergner M, Chicherio C, Schmiedek F, Lövdén M, Lindenberger U. Peak individual alpha frequency qualifies as a stable neurophysiological trait marker in healthy younger and older adults. Psychophysiology. 2013;50:570-82.

20. Grandy TH, Werkle-Bergner M, Chicherio C, Lövdén M, Schmiedek F, Lindenberger U. Individual alpha peak frequency is related to latent factors of general cognitive abilities. Neuroimage. 2013;79:10-8.

21. Voytek B, Kramer MA, Case J, Lepage KQ, Tempesta ZR, Knight RT, Gazzaley A. Age-related changes in 1/f neural electrophysiological noise. Journal of Neuroscience. 2015;35:13257-65.

22. Ouyang G, Hildebrandt A, Schmitz F, Herrmann CS. Decomposing alpha and 1/f brain activities reveals their differential associations with cognitive processing speed. NeuroImage. 2020;205:116304.

23. He W, Donoghue T, Sowman PF, Seymour RA, Brock J, Crain S, Voytek B, Hillebrand A. Co-increasing neuronal noise and beta power in the developing brain. BioRxiv. 2019:839258.

24. Otero GA, Aguirre DM, Porcayo R, Fernandez T. Psychological and electroencephalographic study in school children with iron deficiency. International journal of neuroscience. 1999;99:113-21.

25. Otero GA, Fernández T, Pliego-Rivero FB, Mendieta GG. Iron therapy substantially restores qEEG maturational lag among iron-deficient anemic infants. Nutritional Neuroscience. 2019;22:363-72.

26. Ayala R, Otero GA, Porcayo Mercado R, Pliego-Rivero FB. Delayed CNS maturation in iron-deficient anaemic infants. Nutritional neuroscience. 2008;11:61-8.

27. Ostlund B, Donoghue T, Anaya B, Gunther KE, Karalunas SL, Voytek B, Pérez-Edgar KE. Spectral parameterization for studying neurodevelopment: How and why. Developmental cognitive neuroscience. 2022;54:101073.

28. Pasricha S-R, Hayes E, Kalumba K, Biggs B-A. Effect of daily iron supplementation on health in children aged 4–23 months: a systematic review and meta-analysis of randomised controlled trials. The Lancet Global Health. 2013;1:e77-e86.

29. Thompson J, Biggs BA, Pasricha SR. Effects of daily iron supplementation in 2- to 5-year-old children: systematic review and meta-analysis. Pediatrics. 2013 Apr;131:739-53.

30. Donoghue T, Dominguez J, Voytek B. Electrophysiological frequency band ratio measures conflate periodic and aperiodic neural activity. Eneuro. 2020;7.

31. Schaworonkow N, Voytek B. Longitudinal changes in aperiodic and periodic activity in electrophysiological recordings in the first seven months of life. Developmental cognitive neuroscience. 2021;47:100895.

32. Braat S, Larson L, Simpson JA, Hasan MI, Hamadani JD, Hossain SJ, Shiraji S, Bhuiyan MSA, Biggs B-A, Pasricha S-R. The Benefits and Risks of Iron interventionS in Children (BRISC) trial: Statistical analysis plan. F1000Research. 2020;9:427.

33. Hasan MI, Hossain SJ, Braat S, Dibley MJ, Fisher J, Grantham-McGregor S, Tofail F, Simpson JA, Arifeen SE, et al. Benefits and risks of Iron interventions in children (BRISC): protocol for a three-arm parallel-group randomised controlled field trial in Bangladesh. BMJ open. 2017 Nov 15;7:e018325.
34. Pasricha S-R, Hasan MI, Braat S, Larson LM, Tipu SMM-U, Hossain SJ, Shiraji S, Baldi A, Bhuiyan MSA, et al. Benefits and Risks of Iron Interventions in Infants in Rural Bangladesh. New England Journal of Medicine. 2021;385:982-95.
35. Brainard DH, Vision S. The psychophysics toolbox. Spatial vision. 1997;10:433-6.
36. Kleiner M, Brainard D, Pelli D. What's new in Psychtoolbox-3? 2007.
37. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. Journal of neuroscience methods. 2004;134:9-21.
38. Marshall PJ, Bar-Haim Y, Fox NA. Development of the EEG from 5 months to 4 years of age. Clinical neurophysiology. 2002;113:1199-208.
39. Dreyfus-Brisac C, Curzi-Dascalova L. The EEG during the first year of life. Handbook of electroencephalography and clinical neurophysiology. 1975;6:6B-24.
40. Stroganova TA, Orekhova EV, Posikera IN. EEG alpha rhythm in infants. Clinical neurophysiology. 1999;110:997-1012.
41. Orekhova EV, Stroganova TA, Posikera IN. Alpha activity as an index of cortical inhibition during sustained internally controlled attention in infants. Clinical Neurophysiology. 2001;112:740-9.
42. Köster M, Langeloh M, Kliesch C, Kanngiesser P, Hoehl S. Motor cortex activity during action observation predicts subsequent action imitation in human infants. NeuroImage. 2020;218:116958.
43. Futagi Y, Ishihara T, Tsuda K, Suzuki Y, Goto M. Theta rhythms associated with sucking, crying, gazing and handling in infants. Electroencephalography and clinical neurophysiology. 1998;106:392-9.
44. Saby JN, Marshall PJ. The utility of EEG band power analysis in the study of infancy and early childhood. Developmental neuropsychology. 2012;37:253-73.
45. Begus K, Bonawitz E. The rhythm of learning: Theta oscillations as an index of active learning in infancy. Developmental Cognitive Neuroscience. 2020;45:100810.
46. Donoghue T, Haller M, Peterson EJ, Varma P, Sebastian P, Gao R, Noto T, Lara AH, Wallis JD, Knight RT. Parameterizing neural power spectra into periodic and aperiodic components. Nature neuroscience. 2020;23:1655-65.
47. DeBoer T, Scott LS, Nelson CA. Methods for acquiring and analyzing infant event-related potentials. Infant EEG and event-related potentials. 2007;500:5-37.
48. deRegnier RA, Nelson CA, Thomas KM, Wewerka S, Georgieff MK. Neurophysiologic evaluation of auditory recognition memory in healthy newborn infants and infants of diabetic mothers. The Journal of pediatrics. 2000 Dec;137:777-84.
49. Hamadani JD, Tofail F, Hilaly A, Huda SN, Engle P, Grantham-McGregor SM. Use of family care indicators and their relationship with child development in Bangladesh. Journal of health, population, and nutrition. 2010;28:23.
50. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological). 1995;57:289-300.

51. Glickman ME, Rao SR, Schultz MR. False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. Journal of clinical epidemiology. 2014;67:850-7.

52. Prado EL, Larson LM, Cox K, Bettencourt K, Kubes JN, Shankar AH. Do effects of early life interventions on linear growth correspond to effects on neurobehavioural development? A systematic review and meta-analysis. The Lancet Global health. 2019 Oct;7:e1398-e413.

53. Aboud FE, Yousafzai AK. Global health and development in early childhood. Annu Rev Psychol. 2015 Jan 3;66:433-57.

54. Troller-Renfree SV, Costanzo MA, Duncan GJ, Magnuson K, Gennetian LA, Yoshikawa H, Halpern-Meekin S, Fox NA, Noble KG. The impact of a poverty reduction intervention on infant brain activity. Proceedings of the National Academy of Sciences. 2022;119:e2115649119.

55. Southgate V, Johnson MH, Osborne T, Csibra G. Predictive motor activation during action observation in human infants. Biology Letters. 2009;5:769-72.

56. Meyer M, Chung H, Debnath R, Fox N, Woodward AL. Social context shapes neural processing of others' actions in 9-month-old infants. Journal of Experimental Child Psychology. 2022;213:105260.

57. Saby JN, Meltzoff AN, Marshall PJ. Infants' somatotopic neural responses to seeing human actions: I've got you under my skin. PloS one. 2013;8:e77905.

58. Cuevas K, Cannon EN, Yoo K, Fox NA. The infant EEG mu rhythm: methodological considerations and best practices. Developmental Review. 2014;34:26-43.

59. Festa MD, Anderson HL, Dowdy RP, Ellersieck MR. Effect of zinc intake on copper excretion and retention in men. The American journal of clinical nutrition. 1985;41:285-92.

60. Hamadani JD, Fuchs GJ, Osendarp SJ, Huda SN, Grantham-McGregor SM. Zinc supplementation during pregnancy and effects on mental development and behaviour of infants: a follow-up study. Lancet (London, England). 2002 Jul 27;360:290-4.

61. Hamadani JD, Fuchs GJ, Osendarp SJ, Khatun F, Huda SN, Grantham-McGregor SM. Randomized controlled trial of the effect of zinc supplementation on the mental development of Bangladeshi infants. The American journal of clinical nutrition. 2001 Sep;74:381-6.

62. Solomons NW. Competitive interaction of iron and zinc in the diet: consequences for human nutrition. The Journal of nutrition. 1986;116:927-35.

63. Benasich AA, Gou Z, Choudhury N, Harris KD. Early cognitive and language skills are linked to resting frontal gamma power across the first 3 years. Behavioural brain research. 2008;195:215-22.

64. Gou Z, Choudhury N, Benasich AA. Resting frontal gamma power at 16, 24 and 36 months predicts individual differences in language and cognition at 4 and 5 years. Behavioural brain research. 2011;220:263-70.

65. Maguire MJ, Schneider JM. Socioeconomic status related differences in resting state EEG activity correspond to differences in vocabulary and working memory in grade school. Brain and cognition. 2019;137:103619.

66. Williams IA, Tarullo AR, Grieve PG, Wilpers A, Vignola EF, Myers MM, Fifer WP. Fetal cerebrovascular resistance and neonatal EEG predict 18-month neurodevelopmental outcome in infants with congenital heart disease. Ultrasound in obstetrics & gynecology. 2012;40:304-9.

67. Brito NH, Elliott AJ, Isler JR, Rodriguez C, Friedrich C, Shuffrey LC, Fifer WP. Neonatal EEG linked to individual differences in socioemotional outcomes and autism risk in toddlers. Developmental psychobiology. 2019;61:1110-9.

68. Harmony T, Hinojosa G, Marosi E, Becker J, Rodriguez M, Reyes A, Rocha C. Correlation between EEG spectral parameters and an educational evaluation. International Journal of Neuroscience. 1990;54:147-55.

69. Jensen SK, Xie W, Kumar S, Haque R, Petri WA, Nelson III CA. Associations of socioeconomic and other environmental factors with early brain development in Bangladeshi infants and children. Developmental cognitive neuroscience. 2021;50:100981.

70. Research NIoP, Training - NIPORT, Health Mo, Family Welfare, ICF. Bangladesh Demographic and Health Survey 2017-18. Dhaka, Bangladesh: NIPORT/ICF; 2020.

71. Soewondo S, Husaini M, Pollitt E. Effects of iron deficiency on attention and learning processes in preschool children: Bandung, Indonesia. The American journal of clinical nutrition. 1989;50:667-74.

72. Jáuregui-Lobera I. Iron deficiency and cognitive functions. Neuropsychiatric disease and treatment. 2014;10:2087.

73. Coates J, Swindale A, Bilinsky P. Food and Nutrition Technical Assistance Project (FANTA): Household Food Insecurity Access Scale (HFIAS) for Measurement of Food Access: Indicator Guide (v. 3). Washington, DC: Food and Nutrition Technical Assistance Project. 2007.

74. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva, Switzerland: World Health Organization; 2011. Geneva. http://apps.who.int/iris/bitstream/handle/10665/85839/WHO_NMH_NHD_MNM_11.1_eng.pdf?sequence=3. Accessed on January 2014.: World Health Organization; 2014.

75. WHO. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Geneva: Vitamin and Mineral Nutrition Information System, 2011 (WHO/NMH/NHD/MNM/11.2). Geneva. http://www.who.int/vmnis/indicators/serum_ferritin.pdf. Accessed on March 2014.: World Health Organization; 2011.

76. WHO. C-reactive protein concentrations as a marker of inflammation or infection for interpreting biomarkers of micronutrient status. Geneva. http://apps.who.int/iris/bitstream/10665/133708/1/WHO_NMH_NHD_EPG_14.7_eng.pdf?ua=1, Accessed November 2015: World Health Organization; 2014.

77. Bayley N. Bayley Scales of Infant Development Manual. Third ed. Antonio, TX: The Psychological Corporation; 2006.

Table 1. Participant baseline characteristics for children with sufficient artefact-free EEG data mmediately post-intervention (month 3) and the further 9-month follow-up (month 12), by treatment group[1]

	Children with sufficient artefact-free EEG data at month 3 (N=412)			**Children with sufficient artefact-free EEG data at month 12 (N=374)**		
	Iron	**MNPs**	**Placebo**	**Iron**	**MNPs**	**Placebo**
	N=138	**N=139**	**N=135**	**N=127**	**N=119**	**N=128**
Household characteristics						
Maternal education (years)	2 (2-3)	2 (2-3)	2 (2-3)	2 (2-3)	2 (2-3)	2 (2-3)
Paternal education (years)	2 (2-3)	2 (2-3)	2 (2-3)	2 (2-3)	2 (2-3)	2 (2-3)
Wealth index						
Quintile 1 (relative poorest)	31/138 (22.5%)	34/139 (24.5%)	27/135 (20.0%)	34/127 (26.8%)	32/119 (26.9%)	30/128 (23.4%)
Quintile 2	27/138 (19.6%)	20/139 (14.4%)	29/135 (21.5%)	30/127 (23.6%)	25/119 (21.0%)	28/128 (21.9%)
Quintile 3 (relative middle)	32/138 (23.2%)	33/139 (23.7%)	34/135 (25.2%)	25/127 (19.7%)	21/119 (17.6%)	27/128 (21.1%)
Quintile 4	23/138 (16.7%)	19/139 (13.7%)	24/135 (17.8%)	18/127 (14.2%)	21/119 (17.6%)	20/128 (15.6%)
Quintile 5 (relative wealthiest)	25/138 (18.1%)	33/139 (23.7%)	21/135 (15.6%)	20/127 (15.7%)	20/119 (16.8%)	23/128 (18.0%)
Household Food Secure status[2]	112/138 (81.2%)	106/136 (77.9%)	110/134 (82.1%)	106/127 (83.5%)	92/115 (80.0%)	108/127 (85.0%)
Child characteristics						
General						
Female sex	75/138 (54.3%)	64/139 (46.0%)	68/135 (50.4%)	72/127 (56.7%)	56/119 (47.1%)	67/128 (52.3%)
Age (months)	8.0 (0.3)	8.0 (0.3)	7.9 (0.3)	8.0 (0.3)	7.9 (0.3)	8.0 (0.3)
Family Care Indicator total score[3]	12.3 (7.3)	12.9 (6.5)	14.6 (7.3)	13.0 (7.8)	13.1 (6.4)	13.3 (7.1)
Laboratory indices						
Hemoglobin concentration (g/L) venous	109.5 (11.0)	109.2 (9.2)	111.2 (8.4)	108.9 (9.8)	109.5 (8.3)	109.4 (8.9)
Anemia venous[4]	64/138 (46.4%)	58/132 (43.9%)	53/129 (41.1%)	61/125 (48.8%)	53/112 (47.3%)	61/123 (49.6%)
Serum ferritin (µg/L)	22.8 (11.4-37.1)	23.9 (13.1-37.6)	23.4 (15.4-40.2)	22.7 (10.5-41.0)	24.7 (14.5-37.2)	21.6 (11.8-38.5)
Iron deficient[5]	46/137 (33.6%)	35/129 (27.1%)	24/127 (18.9%)	44/121 (36.4%)	28/109 (25.7%)	35/121 (28.9%)
Iron deficiency anemia[6]	34/137 (24.8%)	18/129 (14.0%)	18/127 (14.2%)	32/121 (26.4%)	19/109 (17.4%)	24/121 (19.8%)
Serum C-reactive protein (mg/L)	0.80 (0.39-2.91)	1.03 (0.36-3.36)	0.93 (0.31-2.37)	0.75 (0.34-2.13)	0.86 (0.36-2.65)	0.88 (0.35-2.47)

Inflammation[7]	21/137 (15.3%)	25/129 (19.4%)	19/127 (15.0%)	16/121 (13.2%)	18/109 (16.5%)	12/121 (9.9%)
Child growth						
Length/height-for-age z-score	-1.35 (1.04)	-1.39 (1.04)	-1.34 (1.03)	-1.26 (1.07)	-1.36 (1.05)	-1.25 (0.95)
Weight-for-age z-score	-0.56 (1.08)	-0.68 (1.03)	-0.49 (1.01)	-0.56 (1.00)	-0.75 (1.03)	-0.50 (0.94)
Weight-for-length/height z-score	0.37 (1.02)	0.24 (0.95)	0.45 (1.07)	0.30 (0.96)	0.12 (1.05)	0.34 (1.03)
Child development						
Bayley score[8]						
Cognitive composite	96.6 (8.3)	96.7 (7.6)	97.0 (7.7)	95.9 (8.0)	95.7 (7.3)	96.3 (7.9)
Language composite	89.4 (6.4)	89.6 (6.4)	90.8 (7.3)	88.2 (7.3)	89.7 (6.6)	88.7 (8.0)
Motor composite	93.5 (10.1)	93.8 (10.4)	94.5 (9.9)	93.8 (10.1)	94.0 (9.7)	94.0 (10.6)

[1] Data are presented as mean (SD) or median (IQR) for continuous measures, and n/total (%) for categorical measures. Percentages may not total 100 because of rounding. Complete participant characteristics at baseline by treatment group are presented in Supplementary Table 1. At month 3, children were 11 months of age; at month 12, children were 20 months of age. EEG, electroencephalography.

[2] Food secure was defined 'no' or 'rarely' to question 1 and 'no' to questions 2-9 on the Household Food Insecurity Assess Scale (73).

[3] Scores on family care indicator total score range from 0 to 42, with higher scores indicating more activities/use of play or reading material (49).

[4] Anemia was defined as hemoglobin <110g/L (74).

[5] Iron deficiency was defined ferritin<12μg/L or <30μg/L if C-reactive protein >5 mg/L (75).

[6] Iron deficiency anemia was defined as anemia and iron deficiency.

[7] Inflammation was defined as C-reactive protein >5 mg/L (76).

[8] Scores on Bayley were derived as described in Bayley N. Bayley Scales of Infant and Toddler Development: Motor Scale. Psychological Corporation; 2006 (77). Higher scores indicating better development.

Table 2. Effects of iron syrup and MNP supplementation vs placebo on EEG outcomes immediately post-intervention (month 3) and the further 9-month follow-up (month 12) among children with sufficient artefact-free EEG data[1]

			Iron	MNPs	Placebo	Iron vs Placebo		MNPs vs Placebo	
Visit	**Analysis stage**	**Outcome**[2] (all units are μV^2, expect for peak frequency Hz)	**Mean(SD)**	**Mean(SD)**	**Mean(SD)**	**Mean difference (95% CI)**[3]	**P Value**[3]	**Mean difference (95% CI)**[3]	**P Value**[3]
Month 3	Stage 1	Posterior alpha (original spectra)	1.08 (0.62)	1.02 (0.55)	1.06 (0.66)	0.01 (-0.13, 0.15)	0.91	-0.03 (-0.18, 0.11)	0.67
		Mu alpha (original spectra)	1.47 (0.93)	1.31 (0.85)	1.16 (0.70)	0.30 (0.11, 0.50)	0.003[4]	0.15 (-0.05, 0.35)	0.15
		Beta (original spectra)	0.31 (0.23)	0.34 (0.25)	0.34 (0.33)	-0.03 (-0.10, 0.03)	0.33	0 (-0.07, 0.06)	0.89
		Delta (original spectra)	15.12 (4.91)	14.84 (4.70)	14.64 (4.76)	0.47 (-0.67, 1.61)	0.42	0.16 (-0.98, 1.29)	0.79
		Theta (original spectra)	3.54 (2.12)	3.75 (2.28)	3.23 (2.50)	0.32 (-0.23, 0.87)	0.25	0.51 (-0.04, 1.06)	0.07
	Stage 2	Posterior alpha (flattened spectra)	0.37 (0.48)	0.28 (0.46)	0.33 (0.55)	0.03 (-0.08, 0.15)	0.58	-0.05 (-0.16, 0.07)	0.44
		Mu alpha (flattened spectra)	0.73 (0.85)	0.54 (0.74)	0.45 (0.66)	0.28 (0.10, 0.46)	0.002[5]	0.10 (-0.08, 0.28)	0.29
		Beta (flattened spectra)	0.04 (0.07)	0.04 (0.07)	0.05 (0.12)	-0.01 (-0.04, 0.01)	0.19	-0.01 (-0.04, 0.01)	0.19
		Delta (flattened spectra)	6.45 (4.57)	7.28 (4.76)	7.14 (4.42)	-0.68 (-1.77, 0.41)	0.22	0.08 (-1.01, 1.17)	0.88
		Theta (flattened spectra)	1.95 (1.98)	2.19 (2.10)	1.74 (2.36)	0.22 (-0.29, 0.73)	0.40	0.44 (-0.08, 0.95)	0.10
	Stage 3	Posterior alpha (peak frequency)	7.41 (0.61)	7.29 (0.56)	7.22 (0.56)	0.19 (0.00, 0.38)	0.049	0.07 (-0.13, 0.27)	0.48
		Mu alpha (peak frequency)	7.46 (0.46)	7.42 (0.47)	7.45 (0.47)	0 (-0.12, 0.12)	0.97	-0.03 (-0.16, 0.09)	0.59
		Theta (peak frequency)	4.58 (0.51)	4.58 (0.63)	4.43 (0.60)	0.16 (0.00, 0.31)	0.043	0.15 (-0.00, 0.30)	0.06
		Posterior alpha (peak frequency power)	0.32 (0.15)	0.34 (0.16)	0.36 (0.23)	-0.04 (-0.10, 0.02)	0.21	-0.02 (-0.09, 0.04)	0.50
		Mu alpha (peak frequency power)	0.52 (0.21)	0.48 (0.18)	0.46 (0.21)	0.06 (0.01, 0.12)	0.016	0.02 (-0.03, 0.07)	0.43
		Theta (peak frequency power)	0.50 (0.21)	0.56 (0.23)	0.48 (0.22)	0.02 (-0.04, 0.08)	0.47	0.07 (0.01, 0.13)	0.014
		Aperiodic exponent	1.40 (0.34)	1.31 (0.37)	1.32 (0.37)	0.08 (-0.01, 0.17)	0.07	-0.01 (-0.09, 0.08)	0.85
Month 12	Stage 1	Posterior alpha (original spectra)	1.00 (0.30)	0.98 (0.28)	1.01 (0.31)	-0.01 (-0.12, 0.10)	0.81	-0.03 (-0.14, 0.08)	0.58
		Mu alpha (original spectra)	1.03 (0.37)	0.99 (0.34)	0.99 (0.30)	0.04 (-0.05, 0.12)	0.39	0 (-0.09, 0.09)	0.99
		Beta (original spectra)	0.35 (0.15)	0.35 (0.13)	0.36 (0.15)	-0.01 (-0.05, 0.02)	0.52	-0.01 (-0.05, 0.02)	0.52
		Delta (original spectra)	3.25 (0.63)	3.23 (0.49)	3.25 (0.54)	-0.01 (-0.15, 0.13)	0.88	-0.03 (-0.17, 0.11)	0.71
		Theta (original spectra)	1.47 (0.38)	1.47 (0.37)	1.45 (0.36)	0.01 (-0.09, 0.10)	0.90	0.01 (-0.08, 0.10)	0.83

Stage 2	Posterior alpha (flattened spectra)	0.37 (0.27)	0.36 (0.23)	0.37 (0.28)	0 (-0.10, 0.10)	0.99	-0.01 (-0.11, 0.08)	0.80
	Mu alpha (flattened spectra)	0.46 (0.32)	0.43 (0.30)	0.42 (0.26)	0.04 (-0.03, 0.11)	0.29	0.01 (-0.07, 0.08)	0.81
	Beta (flattened spectra)	0.02 (0.02)	0.01 (0.01)	0.02 (0.02)	0 (-0.01, 0.00)	0.27	-0.01 (-0.01, -0.00)	0.033
	Delta (flattened spectra)	0.29 (0.28)	0.35 (0.41)	0.33 (0.34)	-0.05 (-0.13, 0.04)	0.26	0.02 (-0.07, 0.11)	0.68
	Theta (flattened spectra)	0.40 (0.30)	0.41 (0.32)	0.39 (0.31)	0.01 (-0.06, 0.09)	0.73	0.02 (-0.06, 0.10)	0.59
Stage 3	Posterior alpha (peak frequency)	7.84 (0.64)	7.76 (0.54)	7.76 (0.59)	0.04 (-0.19, 0.27)	0.71	-0.02 (-0.25, 0.20)	0.83
	Mu alpha (peak frequency)	8.03 (0.46)	7.97 (0.46)	8.03 (0.39)	0.01 (-0.10, 0.12)	0.81	-0.05 (-0.16, 0.06)	0.376
	Theta (peak frequency)	4.62 (0.59)	4.55 (0.64)	4.53 (0.55)	0.09 (-0.06, 0.24)	0.25	0.01 (-0.14, 0.17)	0.86
	Posterior alpha (peak frequency power)	0.28 (0.12)	0.26 (0.12)	0.27 (0.14)	0.01 (-0.04, 0.05)	0.83	-0.01 (-0.05, 0.04)	0.83
	Mu alpha (peak frequency power)	0.32 (0.15)	0.32 (0.13)	0.32 (0.13)	0.01 (-0.03, 0.04)	0.65	0 (-0.04, 0.03)	0.97
	Theta (peak frequency power)	0.17 (0.09)	0.18 (0.10)	0.17 (0.09)	0 (-0.02, 0.02)	0.92	0.01 (-0.02, 0.03)	0.61
	Aperiodic exponent	0.83 (0.15)	0.83 (0.13)	0.82 (0.15)	0 (-0.03, 0.04)	0.82	0 (-0.03, 0.04)	0.84

[1] The analysis sample includes all randomized participants with sufficient artefact-free EEG data at the specified visit. The estimate, CI, and P Value were obtained from a linear regression model adjusted for union and sex of the child. Stages refer to how band power measured were derived, using the original power spectra (stage 1), flattened spectra with the aperiodic component removed (stage 2), and from parameterized spectra (stage 3). At month 3, children were 11 months of age; at month 12, children were 20 months of age. CI denotes Confidence Interval, MNPs denotes multiple micronutrient powders. SD denotes Standard Deviation.

[2] Posterior and Mu refer to electrode location. Posterior includes Oz, O1, and O2. Mu includes Cz, C3, and C4. Aperiodic exponent refers to the rate at which the aperiodic (background) activity decreases as a function of frequency in Hz.

[3] All CIs and P values are presented unadjusted for multiple testing.

[4] Q =0.015. Q refers to the false discovery rate adjusted P value.

[5] Q=0.01

Figure 1 CONSORT diagram

Figure 2. Fourier amplitude spectra at central electrodes for the iron, MNP and placebo treatment groups immediately post-intervention (month 3).

The grey shaded area spans the range of the infant mu alpha band (6-9 Hz). Shaded error regions represent standard errors. The scalp map depicts alpha band amplitudes for each electrode, averaged across all infant datasets at month 3. The grey outlined area in the scalp map shows the positions of electrodes Cz, C3 and C4 used to measure mu alpha activity. MNP denotes multiple micronutrient powders.

Figure 3. Treatment effects of iron syrup and MNP supplementation vs placebo on EEG outcomes immediately post-intervention (month 3)[1]

[1] Iron N=138, MNPs N=139, Placebo N=135. EEG denotes electroencephalography. MNP denotes multiple micronutrient powders.