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SARAH NEWBOLDS

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THE PATTERN ELECTORETINOGRAM AS AN INDIRECT MEASURE OF DOPAMINERGIC STATUS IN IRON DEFICIENCY

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BY THE COMMITTEE CONSISTING OF

Dr. Michael Wenger, Chair

Dr. Lauren Ethridge

Dr. Michael Markham

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Abstract

Animal studies have suggested that iron deficiency negatively affects dopamine (DA) synthesis and reuptake, which in turn negatively affects memory and cognition. However, the literature on the possible relationships among iron deficiency, DA dysregulation, and deficits in memory and cognition in humans is sparse. This study was intended to assess whether the pattern electroretinogram (pattern ERG) could be used as an indirect measure of DA in college-age women and to assess the extent to which the features of the pattern ERG were related to measures of iron levels and measures of cognition. For the pattern ERG to be useful as an indirect measure of dopamine in the context of iron deficiency, at least two things need to be true. First, the features of the pattern ERG should be sensitive to variations in blood measures of iron. Second, the features of the pattern ERG need to be related to other measures that have been suggested as indirect measures of DA, such as blink rate. It is known that DA is present in the retina, and because the pattern ERG measures retinal activity, it has been used in a variety of contexts to assess dopaminergic functioning. The pattern ERG was measured in a total of 21 iron deficient non-anemic (IDNA) and 21 iron sufficient (IS) women, who also performed a contrast detection and probabilistic selection task, both with concurrent electroencephalography (EEG). In addition, both their spontaneous and task-related blink rates were measured. The implicit times of the two features of the pattern ERG—the A- and B- waves –were significantly longer for the IDNA than for the IS women. Both the amplitudes and implicit times of the A- and B-waves were significantly correlated with levels of serum ferritin (sFt). However, only the amplitude of the A-wave was correlated with spontaneous blink rate. IDNA women had higher contrast detection thresholds and lower levels of accuracy in the probabilistic selection task than did the IS women. Finally, there was evidence that the implicit times of the ERG features, as proxy measures of DA, mediated the

relationship between iron levels and accuracy levels. The results suggest the utility of the pattern ERG in testing the hypothesis that iron deficiency affects DA levels in humans and that this may be on the mechanisms by which iron deficiency negatively affects cognition.

The Pattern Electroretinogram as an Indirect Measure of Dopamine in Iron Deficiency

Iron deficiency is the world's most prevalent nutritional problem, affecting billions of people worldwide, especially women of reproductive age and children (Beard 2003; McClung & Murray-Kolb, 2013; Stevens et al., 2013). For comparison, anemia, a condition which is most often caused by severe iron deficiency, affects an estimated 29.9% of women of reproductive age worldwide (World Health Organization, 2023). This may be an underestimate for college-aged women, as our own studies have indicated that 40-60% of college age women suffer from iron deficiency, anemia, or both, and are unaware of their conditions (Rhoten, 2019, unpublished data). Iron deficiency tends to disproportionately affect women of reproductive age, due to regular blood loss from menstruation, iron requirements during pregnancy, as well as a low intake of iron through diet (McClung & Murray-Kolb, 2013).

These findings are relevant as studies have shown that iron deficiency contributes to negative effects on behavior, emotion, and cognition, including problems with attention, memory, intelligence, and perception that are replaced with iron repletion (Ferreira et al., 2019; Jáuregui-Lobera, 2014). For example, Wenger et al. (2017a) found that iron deficient (ID) Indian women of reproductive age performed significantly better at tasks on attention, perception, and memory after consuming double-fortified salt (containing ferrous fumarate) for 10 months, as compared to a group consuming control salt. It was found that low-level attentional functioning and perceptual sensitivity had the greatest improvements with iron repletion.

As another example, a study was done on Indian male and female adolescents, with a high proportion of them being either ID or anemic (Scott et al., 2018). This study found that the adolescents who consumed a diet that included an iron-biofortified pearl millet for six months performed better on tasks of memory, attention, and reaction time (RT) compared to a control

group. It was found that, when compared to the control group, the adolescents in the iron-biofortified group had shorter RTs and larger improvements in attentional control, and their RTs in a cued recognition task were more positively responsive to increasing cues after repletion relative to the control group.

In a study of college-age women in Rwanda, Wenger et al. (2019) found that women with ID showed positive changes on memory and attentional tasks after consuming iron bio-fortified beans for 128 days relative to those who consumed a control bean. The participants in the iron-biofortified bean group had shorter RTs, higher scores on measures of spatial selective attention, and higher sensitivity and capacity measures in a cued recognition task.

Other studies corroborate these findings on iron deficiency and cognition. Bruner et al. (1996) found that iron deficient non-anemic (IDNA) adolescent girls taking an iron supplement performed better on a test of memory and learning than those taking a placebo. Importantly, it has been found that cognition can be improved with iron repletion. In a critical study, Murray-Kolb and Beard (2007) found that iron repletion in iron deficient anemic (IDA) and IDNA women was associated with a five to seven-fold improvement in cognitive performance, including on tasks of attention, memory, and learning.

Research on IDNA women of reproductive age demonstrated that ID is associated with deficits in executive functioning, including slower reaction times, less inhibitory control, and slower planning capabilities (Scott & Murray-Kolb, 2016). Further, another study suggested that ID, combined with low aerobic fitness, was responsible for a reduction of GPA in college-age women, with a mean difference of .34 GPA units between IS high-fitness women and ID low-fitness women (Scott et al., 2017). Additionally, long-term effects of ID on cognition have been

observed, with ID in infancy affecting school performance and cognitive functioning test scores a decade later (Lozoff et al., 2000).

The relationships among ID, cognition, and brain dynamics have been studied with electroencephalography (EEG; e.g., Otero et al., 1999; Otero et al., 2019; Tucker et al., 1984). In a comparison between IDNA and IS women, Wenger et al. (2017b) examined the relationships between behavioral performance on a visual short-term memory task and measures of α- and θ-band power in EEG, where greater changes are associated with better responses to task difficulty, and the γ-band power, where greater changes are associated with more attentional function. It was found that the ID participants demonstrated larger reductions in α-band power and smaller increases in θ- and γ-band power during the task than their IS matches. Additionally, the researchers found that the IS group demonstrated an increase in N1 amplitude (the peak of the first negative event-related potential), known to indicate object-level representation.

Similarly, a secondary analysis (Wenger et al., 2022) performed on the data from a study involving adolescents' consumption of either an iron biofortified or a comparison pearl millet found that improvements in iron status in those that consumed the biofortified grain were associated with changes in the α- and γ-band power and N1 amplitude while performing a set of cognitive tasks. Taken altogether, the current literature suggests that ID is responsible for substantially negative effects on cognition, including perception, attention, and memory, as well as on related measures of brain dynamics.

Dopamine and Iron Deficiency

It has been suggested that a potential reason for iron deficiency's (ID) negative effects on cognition is the negative relationship between ID and dopaminergic functioning (Beard, 2003; Beard & Connor, 2003; Lozoff, 2011; Lukowski et al., 2010). Dopamine (DA) is a

neurotransmitter and hormone that is associated with basal ganglia pathways relating to a range of cognitive functions, including attention, memory, and learning (e.g., Nieoullon, 2002; Lozoff, 2011).

Animal studies have shown that ID produces changes in dopaminergic systems in basal ganglia structures, especially the striatum: ID leads to reductions in dopamine D1 and D2 receptors in the caudate putamen and nucleus accumbens, leading to a significant decrease of central DA neurotransmission (Beard, 2003; Erikson et al., 2001; Pino et al., 2017; Youdim et al., 1989). Additionally, studies have shown that rats made iron deficient by diet exhibit higher levels of extracellular dopamine in the striatum than rats that are iron sufficient, providing evidence that iron deficiency negatively affects the striatal DA reuptake system (Erikson et al., 2000; Nelson et al., 1997). This is bolstered by the finding that ID decreases the density of dopamine transporters (DAT, a protein involved in DA regulation by taking DA into the cell) in the caudate putamen and nucleus accumbens (Erikson et al., 2000). Additionally, Pino et al. (2017) found that ID is associated with a reduction in the DA metabolites homovanillic acid (HVA) and 3,4-Dihydroxyphenylacetic acid (DOPAC) in the striatum. Further research on the substantia nigra (SN) in rats revealed that increasing iron in the ventral midbrain in ID rats impacts striatal DA levels, returning extracellular DA levels to normal and increasing intracellular DA levels (Unger et al., 2014). Taken together, the research with animal models suggests that iron deficiency is responsible for significant dysregulation of dopaminergic functioning.

As the basal ganglia are associated with many different functions and networks, widespread downstream effects can be expected with DA dysfunction (Lozoff, 2011). An area of special note is the prefrontal cortex (PFC), associated with higher-order cognition, which is

negatively affected by dopaminergic dysfunction caused by iron deficiency (Algarín et al., 2013; Lozoff, 2011; Lukowski et al., 2010). A study by Li et al. (2011) determined that ID is associated with reduced extracellular dopamine in the PFC. Pino et al. (2017), in work with an animal model of ID, found that iron deficiency in the PFC was associated with an increase of HVA in the PFC; the researchers suggested that this increase was due to the increased monoamine oxidase A (MAO-A) activity in the iron deficient group. MAO-A is involved in DA catabolism, and an increase in MAO-A is known to be related to mood disorders (Parr et al., 2023; Pino et al., 2017). Overall, iron deficiency's negative effects on DA in the PFC are notable and could provide more context for the significant negative effects of iron deficiency on aspects of attention.

As mentioned, iron has a role in DA synthesis and regulation. Iron is a known cofactor for tyrosine hydroxylase (TH), an enzyme which transforms tyrosine to DA (Dichtl et al., 2018; Li et al., 2011; Pino et al., 2017). As such, it has been speculated that lower iron levels lead to less production of TH, further lowering DA production (Li et al., 2011; Pino et al., 2017; Yien & Paw, 2016). Additionally, iron is a cofactor for monoamine oxidase (MAO), an enzyme which is responsible for breaking down neurotransmitters such as DA, norepinephrine, and serotonin (Parr et al., 2023; Sub Laban & Saadabadi, 2022). Inhibition of MAO is known to increase levels of DA in the human brain (Youdim, 2018). As Pino et al. (2017) demonstrated, ID can lead to an increase of MAO-A activity in the PFC; the researchers noted that the mechanism behind this particular association is unclear, but it provides evidence that ID is responsible for modulation of MAO activity, which can in turn influence DA functioning.

Beyond having a relationship with DA, iron is also known to affect the functioning of other neurotransmitters. Beard et al. (1994) found that iron deficient anemic rats had

significantly increased extracellular norepinephrine in the caudate and putamen as compared to controls, indicating ID also affects norepinephrine reuptake. Norepinephrine is a known contributor to a range of cognitive processes including working memory, mnemonic processing, and attention (Borodovitsyna et al., 2017). ID also has effects on serotonin. Morse et al. (1999) demonstrated that striatal serotonin transporters are decreased in ID in mice. A study by Jellen et al. (2021) also demonstrated that in Parkinson's disease patients, higher levels of iron in the substantia nigra pars compacta were associated with lower levels of plasma serotonin. Reduced serotonin functioning is also associated with cognitive deficits (Švob Štrac et al., 2016). Considering this evidence, it is possible ID affects cognition through the modulation of neurotransmitters other than, or in addition to, DA. However, most of the animal literature has focused on the effects of ID on DA, and studies of this relationship in humans is rather sparse, being limited to studies that have examined indirect measures of DA, such as blink rate in iron deficient anemic infants (Elsworth et al., 1991; Karson, 1988; Lawrence et al., 1991; Lozoff et al., 2010). It should be noted, however, that at least three recent papers (Dang et al., 2017; Sescousse et al., 2018; van den Bosch et al., 2023) have seriously questioned the relationship between DA levels and blink rates.

Indirect Measures of Central Dopamine and the PERG

Direct measures of DA in vivo in humans require invasive or risky techniques, such as a spinal tap to measure cerebrospinal fluid, or exposure to radiation with positron emission tomography (PET, Goldstein et al., 2012; Parr et al., 2023). However, there is literature that suggests that a measure of visual function—the electroretinogram (ERG)—may be a good indirect measure of central DA (e.g., Bodis-Wollner & Tzelepi, 1998; Langheinrich et al., 2000; Lavoie et al., 2014).

The ERG is a non-invasive, low-risk electrophysiological test that has been used to measure DA levels indirectly through retinal activity; it has been used especially in research on disorders impacted by DA dysfunction, such as Parkinson's disease and schizophrenia (Asanad & Karanjia, 2022; Bodis-Wollner, 1990; Bodis-Wollner & Tzelepi, 1998; Lavoie et al., 2014; Witkovsky, 2004). The ERG is considered an indirect measure of DA because of the presence of DA throughout the retina: the bipolar, horizontal, amacrine, and ganglion cells have D1 receptors, the photoreceptors have D2 receptors, and the retinal pigment epithelium has D5 receptors (Lavoie et al., 2014; Witkovsky, 2004).

Importantly, research has found that there is a positive relationship between the levels of retinal and central DA in Parkinson's disease, which is associated with lower levels of central DA (Karson, 1983). Harnois and Di Paolo (1990) examined the retinas of Parkinson's patients post-mortem and found that those who had received levodopa (L-Dopa) therapy 2-15 hours before death had greater concentrations of DA in the retina than those who had received L-Dopa treatment at least five days before death. In a recent study, Ortuño-Lizarán et al. (2020) found similar results; examining the retinas of human donors, they found that dopaminergic amacrine cells in those with Parkinson's disease were reduced by 26-58%, and the remaining cell dendrites were shorter and thicker. Additionally, they found that the plexus of dopaminergic amacrine cells in the retina had lost their typical ring-like structure, resulting in damage of the network of dopaminergic cells. The results of these studies suggest that levels of retinal DA are a reliable indicator of central dopamine levels.

The pattern electroretinogram (PERG) is a version of ERG that looks at retinal functioning in response to rapidly alternating high-contrast patterned stimuli, specifically tracking the inner retinal neuron activity, including the amacrine and ganglion cells (Asanad &

Karanjia, 2022; Witkvosky, 2004). The PERG is measured using electrodes that are either inserted below the lower lid of the eye in contact with the cornea or affixed to the skin just below the lower lid of the eye. There are two characteristics of the PERG (see Figure 1): an initial negative deflection that peaks in less than 50 ms, followed by a positive deflection that peaks in less than 100 ms. The negative deflection is referred to as the A-wave and the positive deflection is referred to as the B-wave. The peak amplitudes of these two waves are small, normally measured at 1–8 μV (Cupp et al., 2021). The two PERG waveforms are considered in terms of their amplitude (the maximum stimulus-evoked electrical response) and their implicit time (the period from stimulus onset to the peak amplitudes, Asanad & Karanjia, 2022).

Research from a variety of studies suggest that the amplitude and implicit time of the B-wave is correlated with dopaminergic status, with lower central dopamine levels associated with reduced amplitudes and increased implicit times of the B-waves (Brandies & Yehuda, 2008; Ikeda et al., 1994; Langheinrich et al., 2000). We (Cupp et al., 2021) previously reviewed studies that used the PERG in human participants to look at the strength of the evidence supporting the ability to use the PERG as an indirect measure of DA. The studies reviewed involved human participants with varying levels of DA due to a variety of clinical conditions, including Parkinson's disease, schizophrenia, and ADHD, or studies where a pharmacological manipulation was used, and where there was a comparison to a control group, allowing for a low and a high DA condition to be defined. Overall, the standardized difference in the means between the high DA and low DA conditions for the amplitude of the B-wave was 1.029, and the standardized mean difference between the two groups for the implicit time of the B-wave was .789, both considered as large effect sizes, suggesting that the PERG is a viable indirect measure of central DA levels.

Blink rates have been suggested as another indirect measure of DA although, as noted earlier, there is recent evidence suggesting that blink rates may not be systematically related to DA (Dang et al., 2017; Sescousse et al., 2018; van den Bosch et al., 2023). A variety of studies have suggested that both spontaneous and task-related blink rates are correlated with dopaminergic levels; specifically, higher blink rates are associated with higher levels of central DA, whereas lower blink rates are associated with lower levels of central DA (Jongkees & Colzato, 2016; Karson, 1983; Slagter et al., 2015). The studies that have provided evidence for the use of blink rates as an indirect measure of DA include both studies of hypo- and hyper-dopaminergic states (e.g., Parkinson's disease and schizophrenia) and studies that have used pharmacological manipulations and include work in both humans and animal models.

A study by Groman et al. (2014) found that spontaneous blink rate in monkeys had a strong positive correlation with striatal D2-like receptor availability; further, the researchers found that a D2-like receptor agonist resulted in heightened blink rate, and positively correlated with D2-like receptor availability in the striatum. In a study on Parkinson's disease patients, those with L-dopa-induced dyskinesia (a condition associated with higher levels of central DA) demonstrated twice the mean blink rate as patients who had not received L-dopa (Karson, 1983). Karson et al. (1983) demonstrated that patients with schizophrenia (a hyperdopaminergic state) had significantly higher blink rates on average compared to those without schizophrenia. Additionally, the researchers found that when given DA antagonists, the schizophrenic patients' blink rates were significantly reduced. Blin et al. (1990) found that in a group of young men, apomorphine, a dopaminergic agonist, significantly increased blink rate as compared to a control group. Chronic use of cocaine, known to reduce D2 receptors, has also been associated with lower spontaneous blink rates in young adults (Colzato et al., 2008).

There is a precedent for considering blink rates as an indicator of DA status in the context of ID, although to our knowledge there is only one published study that has used this approach. Lozoff et al. (2010) found that infants who were ID had significantly lower blink rates than those that were IS. After receiving iron treatment for three months, the researchers found that the blink rates increased in infants that were initially ID, whereas the blinks did not increase in infants that were IS. The researchers suggested that this finding was due to the changes in DA levels associated with iron repletion.

Another indirect measure of DA status, used specifically in studies on Parkinson's disease, is the contrast detection threshold, or the level of contrast (quantified as the Michelson contrast, Peli, 1990). at which a person can reliably detect the presence of differences in light and dark regions of an image. Research on Parkinson's patients found that, for a large proportion of the patients, the ratio of contrast sensitivity at peak spatial frequency compared to spatial frequency at .5 cpd was smaller for Parkinson's patients than controls (Bodis-Wollner et al., 1987; Bodis-Wollner, 1990). Langheinrich et al. (2000) found similar results: patients with Parkinson's disease demonstrated significantly higher contrast detection thresholds (less sensitivity to contrast) than the control patients. Other studies have found that contrast detection thresholds can be modulated by pharmacological means. For example, Hutton et al. (1993) demonstrated that contrast sensitivity can be improved in Parkinson's disease with L-dopa treatment. Contrast detection thresholds have also been studied in the context of ID. Wenger et al. (2017a) found that contrast detection thresholds for ID women were reduced after iron repletion.

A cognitive task that has been suggested to be sensitive to levels of DA is the probabilistic selection task (PST, e.g., Frank et al., 2004). In this task, participants are first

presented with a set of characters in a script foreign to them (to minimize verbal encoding) and are asked to select the character that they believe will be the "rewarded" choice (Frank et al., 2004). Rewards for choosing one of the two characters are delivered in a probabilistic manner, and the ratio of rewarded to non-rewarded is varied. Participants practice with these choices until they develop a set of preferences for the rewarded characters. Frank et al. (2004) demonstrated that Parkinson's patients on dopaminergic medication learned from positive feedback but had difficulty learning from negative feedback. The opposite was seen in patients not taking DA medication: they learned better from negative feedback but did not learn as well from positive feedback. Cools et al. (2001) found comparable results in Parkinson's patients on a similar probabilistic task. Additionally, the PST has been examined in terms of feedback-related negativity (FRN), an electrophysiologic marker that is elicited by feedback during uncertain situations (Cavanagh et al., 2010; Frank et al., 2005). Research has also suggested that the FRN can be an indirect marker for DA, with FRNs associated with dips in DA release (Holroyd & Coles, 2002).

Hypotheses

The purpose of this study was to assess the potential utility of using the PERG as an indirect measure of DA in the context of ID, allowing an assessment of the extent to which, as has been indicated by the animal literature, ID disrupts DA. For the PERG to be useful in this pursuit, at least two things need to be true. First, the features of the PERG—the amplitudes and implicit times of the A- and B-waves—should be sensitive to differences in iron status. Second, the features of the PERG should be related to other measures that have been suggested as indirect indicators of DA, specifically blink rates, contrast detection thresholds, and performance on the PST. These were the primary hypotheses that were tested.

Additionally, we tested the secondary hypothesis that the features of the PERG, functioning as markers of central DA, would allow us to distinguish ID from IS women. Finally we assessed the related hypothesis that the features of the PERG, acting as indirect measures of DA, mediate the relationship between iron levels and cognitive performance. If we can show that these things are true, then it suggests that the PERG may be useful in exploring the potential mechanism by which ID produces negative effects on cognition and allows the human literature on the effects of ID to be related to the animal literature.

Methods

Participants

The sample was comprised of 42 female students at the University of Oklahoma, aged 19-29. The sample was 71% White and 29% Asian. To qualify, participants could not be pregnant or lactating and were required to have a normal menstrual cycle. Additionally, participants could not be under treatment for depression, could not have a history of cardiovascular conditions or physical injury, and were required to not be on any prescription medications, except for contraceptives. All participants reported normal or corrected-to-normal vision and fell within a BMI range of 18-30. Participants were identified as iron deficient non-anemic (IDNA) if their serum ferritin (sFt) < 12 ng/mL and their hemoglobin (Hb) > 12 g/dL. Participants were identified as iron sufficient (IS) if their sFt > 20 ng/mL and their Hb > 12 g/dL. Participants were matched on age and ethnicity, with each IDNA participant matched to an IS participant (see Figure 2). Subjects were recruited via mass emailing sent to all students at the university, and participants received $75 in gift cards for their participation.

Apparatus and Materials

Stimulus presentation and behavioral response recording were done using a Mac Mini, with all experimental programs written using the Psychophysics Toolbox (Brainard, 1997; Pelli, 1997; Kleiner et al., 2007) for MATLAB version 9.6 (Mathworks, Natick MA). Stimuli were presented on a 61 cm (diagonal) monitor running at a resolution of 1920 x 1080 pixels with a gray-to-gray time of 1 ms. Responses were made using the computer keyboard and were timed to ±1 ms.

The stimulus for the measurement of spontaneous blink rate was a single character that changed orientation by 90° every 15 seconds (see Figure 3).

The stimuli for the measurement of the PERG were high contrast checkerboard and square wave gratings (see Figure 4). The stimulus image size was set at 400 pixels. The width of the individual checks was 0.8° of visual angle at a fixed viewing distance of 72 cm, and the width of the individual bars of the square wave were 0.5°. The width of the total pattern was 15°.

Contrast thresholds were assessed using greyscale Gabor patches which were fixed in orientation and spatial frequency and differed only in contrast (see Figure 5). Orientation was fixed at 60° and spatial frequency was fixed at 0.7 cycles per 100 pixels, and each pattern subtended 4.3°. A total of 800 stimuli were created, differing in contrast by 0.1% Michelson contrast each. The minimum level of contrast in the stimuli was 0.1% and the maximum level of contrast in the stimuli was 80%

The probabilistic selection task (PST) used six different black and white characters from the Nepalese Devanagari script, chosen to be unrecognizable to our participants (see Figure 6). Each stimulus subtended 3.2° and, presented in pairs, each stimulus offset 75 pixels from the center of the screen.

EEG data were collected using high-density (128 channel) electrode nets (Magstim EGI, Eugene, OR). Data were acquired using a 128-channel Net Amps 300 amplifier and were digitized at a sampling rate of 1 kHz. Impedances were kept at or below 50 kΩ during testing. Data were collected with online filters set at 1 Hz (highpass), 70 Hz (lowpass), and 60 Hz (notch). ERG data were acquired using RETeval sensor strip electrodes (LKC Technologies, Gaithersburg, MD). These electrodes were connected to the EEG amplifier, allowing the ERG signals to be recorded synchronously with the EEG signals.

Procedure

Laboratory Assays and Anthropometry

Blood samples were taken by trained phlebotomists at the University of Oklahoma's Health Sciences Center (OUHSC) in Oklahoma City and were analyzed in the clinical laboratories of OUHSC. Standard clinical assays were done for sFt (ng/mL), Hb (g/dL), C-reactive protein (CRP, mg/L), red blood cell count (RBC, M/mm3), hematocrit (HCT, %), mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), mean corpuscular hemoglobin concentration (MCHC, g/dL), and red blood cell distribution width (RDW, %). Body mass index (BMI, kg/m2) was determined by measuring height on a stadiometer and measuring weight on a digital scale. Body composition was assessed by measuring mid-upper-arm circumference (MUAC, cm).

Visual and Cognitive Testing with Concurrent EEG

The 90-minute experimental testing session was run in a dimly lit sound-attenuated and electrically shielded chamber on the University of Oklahoma campus in Norman, Oklahoma. Participants were seated in an adjustable height chair. The session began with the fitting of the electrode net and the application of the ERG sensors. During all tasks, participants placed their

chins on a chinrest located 72 cm away from the computer display. The keyboard was placed on the table on which the chinrest was mounted.

EEG data was collected throughout the entirety of the session. Participants then performed four tasks, with task order decided by a balanced Latin square.

Participants were first assessed for their spontaneous blink rate during a five-minute period. Participants were asked to focus on a small character (see Figure 3) on the center of the computer screen that changed orientation every 15 sec.

The procedure for the PERG followed that specified by Bach et al. (2013). During this task, participants were asked to try to refrain from blinking while the stimuli were presented. Participants self-initialized each sequence, which consisted of either the checkerboards of the square wave grating (see Figure 4) which reversed in contrast four times per sec. There were six sequences of each pattern, with 50 reversals in each sequence. Half of the participants saw the checkboard patterns first, and half saw the square wave gratings first.

Prior to the contrast detection threshold task, the lights in the testing chamber were extinguished and participants were given 5 min to adapt their eyes to the dark. They then viewed a sequence of 150 greyscale Gabor patches on the computer screen (see Figure 5). Participants self-initiated each trial, each of which began with a fixation cross whose duration was determined by a sample from an exponential distribution with a mean of 750 ms and which was left censored at 500 ms and right censored at 1250 ms. This was followed by a noise pre-mask, the test stimulus, and a noise post-mask. Presentation durations for the masks and the test stimulus were 50 ms, and the masks and the test stimulus were presented at the same level of contrast. Participants responded after the presentation of the second mask by indicating whether or not they could detect any contrast in the test image, using the index finger of their dominant

hand for the positive response. If no response was made, the trial timed out after 2 sec, and the next trial began.

Contrast was determined on each trial using a three-down/one-up adaptive staircase procedure, such that contrast was decreased by 20% after every 3 correct responses and increased by 20% after each incorrect response (following Wenger & Rhoten, 2020).

The procedure for the PST followed on Frank et al. (2004) and Frank et al. (2007). There were two phases of this task. The first part of the task was the learning phase. Participants self-initialized each trial. First a fixation cross appeared with the exposure duration being exponentially distributed, with a mean of 750 ms, and left- and right- censored at 500 and 1000 ms, respectively. Participants were then presented with a pair of black and white characters from Devanagari script, (see Figure 6). The two characters appeared for a maximum of 3 sec, and participants were asked to press a key for the character they believed was the "rewarded" choice. One character in each pair was rewarded at a higher rate than the other in the learning phase, with three levels of reward differential, AB trials at 80% (A) vs. 20% (B), CD trials at 65% (C) vs. 35% (D), and EF trials at 55% (E) vs. 45% (F). Participants were given feedback on their choice for 1.5 sec. Feedback given was probabilistic, based on the reward differentials. Response rates were tracked throughout this phase, and the response rates for each pair had to exceed a threshold in order to end the training phase. Specifically, A had to be chosen in AB pairs at least 65% of the time, C had to be chosen in CD pairs at least 60% of the time, and E had to be chosen in EF pairs at least 50% of the time. Participants worked with these pairings until their response rates exceeded these thresholds or until they completed three rounds of presentations.

The second phase of the task was the trial phase. This phase ran the same way as the training phase, but participants were presented with all possible combinations of the stimuli and were not provided with feedback. Each pairing was repeated 6 times.

ERG and EEG Data Preprocessing

Prior to analysis, the EEG and ERG data were preprocessed using MATLAB version 9.8 with the EEGLab toolbox (version 2021.1) (Delorme & Makeig, 2004). Spontaneous and task-related blink rates were determined using the BLINKER plugin for EEGLab (Kleifges et al., 2017).

EEG preprocessing was done through a series of several steps. In the first step, data were filtered at .5 Hz at highpass and 30 Hz at lowpass. Channels were visually examined, and noisy channels were removed. Bad data were then rejected by visual examination. The data was then re-referenced to the mean. The second step involved running an independent component analysis on the data and fitting dipoles to the independent components. In the third step, the data was epoched for viewing the independent components, and the independent components were examined and those associated with artifacts (e.g., blinks, muscle activity, cardiac activity) were removed from the continuous data, following the methodology for retaining independent components outlined by Pion-Tonachini (2023).

The final step extracted different features based on the task. For the PERG, the grand average ERG, averaged across the left and right eye and across both types of stimuli, was computed in the final step. The PST final step extracted the feedback-related negativity (FRN) for the training phase of the task. The FRN was estimated by first low-pass filtering the data at 20 Hz, then averaging across 6 electrodes located around the standard FCz location. Trial data were epoched with reference to the onset of the feedback from 200 ms prior to feedback to 800

ms after the feedback. The amplitude of the FRN was then determined as the difference between the first positive peak and following negative peak occurring between 175 and 325 ms after the feedback. For the contrast threshold task, the final step extracted pre-stimulus α-band power, based on research (Bays et al., 2015) that suggests that high pre-stimulus α power is associated with stimulus awareness. Pre-stimulus α-power was estimated using a segment of 1000 ms prior to the onset of the stimulus and was averaged across 9 occipital/parietal electrodes centered around the standard OCz location.

Results

Group Differences for the Iron Biomarkers and Participant Characteristics

Table 1 presents the means and standard deviations for each of the blood measures, as well as for age and MUAC for the IDNA and IS subjects (n = 21 for each group). The distribution of sFt was found to be non-normal. Consequently, sFt values were log transformed and the distribution of the transformed values did not differ from normality. For completeness, both sFt and log10(sFt) values are presented. Differences between groups were assessed with t-tests using SAS 9.4 for Linux and the results of those tests are also presented in Table 1. An α-level of .05 was used for these and all analyses that follow. Significant differences between the groups were found for six measures: sFt and log10(sFt), HCT, MCV, MCH, and MCHC; for all these variables, the values for the IS participants were greater than those for the IDNA participants. Critically, and consistent with our inclusion criteria, the two groups did not differ with respect to Hb levels.

Group Differences for the Electrophysiological and Behavioral Variables

Table 2 presents the means and the standard deviations for the IDNA and IS subjects for all of the electrophysiological and behavioral variables, including the features of the PERG.

Differences in groups were analyzed with *t*-tests and (where appropriate) repeated-measures analyses of variance (ANOVAs); the results of the *t*-tests are included in Table 2 and the results of the repeated-measures ANOVAs are presented in Table 3. Due to a variety of computer problems, there were cases of missing data for many of the variables, consequently for each effect noted we include the number of participants who contributed data to the analyses.

For the features of the PERG, there were significant group differences for both the A- and B-wave implicit times (n = 19 IS, n = 20 IDNA), with both implicit times longer for the IDNA than for the IS participants. No group differences were found for the A- and B-wave amplitudes. There were also no significant group differences for either spontaneous or task-related blink rates. There was a significant difference for contrast threshold (n = 21 IS, n = 21 IDNA), with the contrast threshold significantly greater for the IDNA group than the IS group. There were no significant group differences for pre-stimulus α-band power.

The RT data (n = 21 IS, n = 20 IDNA) from testing phase of the PST need to be considered with respect to the level of conflict in the tested pairing. Following Frank et al. (2007), low conflict trials involved the pairing of stimuli with large differences in reward rates (A with D or F, B with C or E) while high conflict trials involved the pairing of stimuli with similar reward rates (A with C or E, B with D or F, C with E, D with F). Consequently, the RT data for trials with correct responses were analyzed using a 2 (Group: IDNA, IS) x 2 (Conflict: low, high) repeated-measures ANOVA with Group being a between-subjects factor and Conflict being a within-subjects factor. The results of that ANOVA are presented in Table 3. There was a main effect for Conflict, with high conflict trials having longer RTs (M = 994, SD = 236) than low conflict trials (M = 838, SD = 182). Neither the main effect for Group nor the interaction of Group and Conflict were significant.

The accuracy data (n = 21 IS, n = 20 IDNA) from the testing phase of the PST need to be considered with respect to the extent to which participants chose the most-highly rewarded element (choosing A when paired with C, D, E, or F) and avoided the least-highly rewarded element (choosing C, D, E, or F when paired with B). Consequently, the accuracy data were analyzed using a 2 (Group: IDNA, IS) x 2 (Choice Type: choose A, avoid B) repeated-measures ANOVA, with Group as a between-subjects factor and Choice Type being a within-subjects factor. The results of that ANOVA are also presented in Table 3. There was a main effect for Group, with the IS participants being more accurate (M = 0.85, SD = 0.16) than the IDNA participants (M = 0.74, SD = 0.20). Neither the main effect for Choice Type or the interaction of Group and Choice Type were significant.

The FRN amplitudes (n = 21 IS, n = 19 IDNA) from the testing phase of the PST need to be considered with respect to the accuracy of the trial for which participants were receiving feedback (correct vs. incorrect). Example waveforms for the correct and incorrect trials for the IDNA and IS participants are presented in the two panels of Figure 7. The FRN amplitudes were analyzed using a 2 (Group: IDNA, IS) x 2 (Trial Type: correct, incorrect) repeated-measures ANOVA, with Group being a between-subjects factor and Trial Type being a within-subjects factor. The results of this analysis are also presented in Table 3. Neither of the main effects or their interaction were significant. This is in contrast to the results of the t-tests (Table 2) which found significant differences between the IDNA and IS participants for the FRN amplitudes for both correct and incorrect trials.

Correlations Among the Measures

Table 4 displays the correlation coefficients for the relationships between the features of the PERG and the blood iron biomarkers. All four features of the PERG were significantly

correlated with sFt, with the correlation coefficients ranging from 0.36 to 0.48. When the log-transformed sFt data were considered, the only correlations that were significant were those involving the implicit times of the A- and B-waves. The implicit times of the A- and B-waves were also significantly correlated with MCHC. There were two other correlations that were unusual, relating B-wave amplitude to HCT and MCV, with both of these being negative, and there being no obvious explanation (e.g., no unusual outliers) for these results. All of the other correlations were either marginal ($p < .10$) or non-significant.

Table 5 displays the correlation coefficients for the relationships between the features of the PERG and variables that have been suggested as other indirect measures of DA. Spontaneous blink rate was significantly correlated with the peak amplitude of the A-wave but was not correlated with any of the other features. Neither of the task-related blink rates (from the contrast detection task and the PST) showed significant correlations with any of the features of the PERG. Among the remaining variables, the amplitude of the FRN on incorrect trials was significantly correlated with the amplitudes of both the A- and the B-waves.

Table 6 displays the correlation coefficients for the relationships between the blink rates and the measures of blood iron. While spontaneous blink rate was significantly correlated with sFt, there were no other significant correlations.

Table 7 displays the correlation coefficients for the relationships between the blink rates and variables that have been suggested as other indirect measures of DA. There were significant correlations with pre-stimulus α-band power for the contrast threshold task and all three measures of blink rates. There was also a significant correlation between choice accuracy on avoid B trials in the PST and spontaneous blink rate. None of the other correlations achieved significance.

Predicting Iron Status from the Indirect Measures of DA

Another way of considering the hypothesis that ID results in dysregulation of DA is to examine the extent to which knowing the indirect measures of DA allows us to identify a woman as IS or IDNA. To do this, we created four models using logistic regression to predict iron status using the features of the ERG, spontaneous blink rate, and task-related blink rate. The first model was estimated using all four features of the PERG, with stepwise model selection to choose the smallest set of predictors that provided the highest classification accuracy (area under the curve, AUC). The second, third, and fourth models used as predictors the spontaneous and task-related blink rates. A summary of the performance of the four models is presented in Table 8. As can be seen in Table 8, only the model involving the implicit time of the B-wave had an AUC that was significantly different than 0.50 and had an estimated slope parameter that was significantly different from 0, with this model accounting for 21% of the variance.

Mediation Models

A final way of considering the hypothesis that ID results in dysregulation of DA is to test the hypothesis that DA, measured indirectly, is a mediator between iron status and cognitive performance. We estimated four mediation models using the PROCESS macro for SAS (Hayes, 2022). This approach offers several advantages to traditional mediation models (e.g., Baron & Kenny, 1986), including the ability to specify multiple variables as mediators and the ability to include covariates. Figure 8 illustrates the basic form for the models that we tested. As the indicator of iron status, we considered both raw and log10 transformed sFt values. As mediators representing DA, we considered the implicit times of the A- and the B-waves. As the outcome variable representing cognitive performance, we constructed a composite score from the average accuracies for the choose A and avoid B trials of the PST. Finally, we considered models both

with and without covariates. For the covariates, we constructed a composite score from the values of HCT, MCV, MCH, and MCHC, which can be interpreted as an index of oxygen carrying capacity. All of the variables were transformed to Z-scores before fitting the models.

Table 9 presents the parameter estimates of the path coefficients and measures of quality of fit for the model that provided the best account of the data (in terms of R^2 values for all of the outcomes). In addition, the path coefficients for this model are included in Figure 8. In this model, raw sFt reliably predicted the implicit times of both the A- and the B-waves. The implicit time of the B-wave was the only mediator to reliably predict the cognitive outcome. When the two features of the PERG were considered as mediators, the direct relationship between iron status and the cognitive outcomes failed to reach significance. Finally, this model did include the composite variable representing oxygen carrying capacity as a covariate. With respect to the three variables considered as outcome in estimating the regressions, this model accounted for between 21% and 29% of the variance.

Discussion

The purpose of this study was to investigate the potential use of the PERG as an indirect measure of DA in the context of ID, which could allow for an assessment of the extent to which ID disrupts DA functioning and allow the human literature on the effects of ID to be related to the animal literature. For the PERG to be useful this this context, our results needed to show at least two things to be true. First, the features of the PERG—the amplitudes and implicit times of the A- and B- waves—had to be sensitive to differences in iron status. Second, the features of the PERG should be related to other measures that have been suggested as indirect measures of DA status. Additionally, we hypothesized that the PERG features would allow us to distinguish IS

from IDNA women, and that the indirect measures of DA would act as a mediator between iron status and cognitive performance. We found the evidence to support most of these hypotheses.

Critically, we found evidence that features of the PERG are sensitive to iron status. The IS and IDNA groups differed significantly on A- and B- wave implicit times, with both implicit times longer for the IDNA than for the IS participants. This finding corroborates the literature that has suggested that the implicit time of the B-wave is negatively correlated with dopaminergic status (Brandies & Yehuda, 2008; Cupp et al., 2021; Langheinrich et al., 2000). Additionally, all four features of the PERG were significantly correlated with sFt, and the log-transformed sFt values were correlated with the implicit times of the A- and B-waves. The implicit times of the A- and B-waves were also significantly correlated with MCHC. The directionality of these correlations is in line with what we expect; namely, iron status is negatively correlated with A- and B- wave implicit times, negatively correlated with the A-wave amplitude, and positively correlated with the B-wave amplitude.

Contrary to our hypothesis, we did not find differences between IS or IDNA groups for A- or B- wave amplitude. This was unexpected, given that the literature has suggested that both implicit times and amplitudes of the PERG features are correlated with dopaminergic status; specifically, B-wave amplitudes are positively correlated with dopaminergic status (Brandies & Yehuda, 2008; Cupp et al., 2021; Langheinrich et al., 2000). The exact reasons behind this finding are uncertain. One possible reason for this finding may be that we used a full-field presentation rather than one in which the eyes are either completely covered or the head is isolated in a visual Ganzfeld (Sen et al., 2021). This is a possibility that needs to be considered in future work.

In addition to finding that features of the PERG were sensitive to differences in iron status, there is partial evidence to suggest that there was a relationship between the PERG and other measures that have been suggested as indirect measures of DA status. There was a significant correlation between spontaneous blink rate and the peak amplitude of the A-wave, although this was the only correlation involving any of the blink rate measures to reach significance. Additionally, there were significant correlations for the FRN amplitude for incorrect trials and the amplitudes of the A- and B- waves. The literature suggests that FRN is elicited in trials with uncertain outcomes, and it has been looked at in the context of PST and has been suggested as an indirect marker of DA levels. Some studies suggest a larger FRN would indicate better awareness of errors and better performance monitoring (Cavanagh et al., 2010; Frank et al., 2005; Holroyd & Coles, 2002). Additionally, we found that the IS and IDNA groups differed significantly on contrast threshold, with the threshold level significantly lower for the IS group than the IDNA group; this is consistent with literature that suggests that contrast detection thresholds can be used as an indirect indicator of DA status (Bodis-Wollner et al., 1987; Hutton et al., 1993; Langheinrich et al., 2000). Additionally, the IS and IDNA groups differed significantly on accuracy levels for the PST for both choose A and avoid B trials, which is consistent with previous research suggesting that the outcomes of the PST are sensitive to DA status (Frank et al., 2004).

The other correlations between the features of PERG and indirect measures of DA, as well as comparisons between IS and IDNA groups on indirect measures of DA, were either marginally significant or non-significant. This was unexpected, given the literature supporting the use of these measures as indirect measures of central DA status. It is difficult to say if the

lack of findings were due to the indirect measures of DA not being as robust as thought or because of other factors in the study.

An unexpected finding was the lack of relationships among blink rates and features of the PERG as well as measures of blood iron. The only significant finding involving blink rates and blood iron measures was a significant correlation between sFt and spontaneous blink rate. There were no significant differences between the IS and IDNA groups in spontaneous or task-related blink rates. This finding was surprising, especially considering that the only study that has looked at the relationship between DA and ID in humans suggested that infants with ID had lower blink rates compared to IS infants (Lozoff et al., 2010). It is possible that the reason for this discrepancy was that Lozoff et al. (2010) considered a sample of infants, whereas our study relied on a sample of women aged 19-29, although this possibility is difficult to assess, given that blink rates increase from infancy to the middle adult years (Bacher, 2010). Additionally, several recent studies have questioned the use of blink rates as an indirect indicator of DA levels (Dang et al., 2017; Sescousse et al., 2018; van den Bosch et al., 2023). Due to these conflicting findings, the use of blink rates as an indirect measure of DA should be considered cautiously and examined more closely in future research.

Two other results, along with the evidence that PERG is sensitive to variations in iron status, suggest that the PERG is a possible tool for indirectly measuring DA in the context of ID. First, the results of our logistic regression analysis demonstrated that B-wave implicit time can be used to distinguish between IDNA versus IS women. Second, our mediation model supported the hypothesis that ID results in dysregulation of DA, as measured indirectly by the PERG, and serves as a mediator between iron status and cognitive performance.

In closing, we have demonstrated that features of the PERG appear to be promising as indirect measures of DA in the context of ID. The results of our study suggest that the use of the PERG may be a more reliable indirect measure of DA in the context of ID than the use of blink rates. Critically, the ability of the PERG to be used as an indicator of central DA levels in this study suggests that it is possible to consider the role of dysregulation of DA levels as a mechanism by which ID extracts costs in cognition in humans, as has been previously suggested by the animal literature.

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Tables & Figures

Table 1

Group Differences for Iron Biomarkers and Participant Characteristics

Biomarker	IS	IDNA	*t*-score
	M(SD)	*M(SD)*	
Serum ferritin (sFt), ng/mL	49.9 (37.4)	10.3 (3.4)	4.83***
log10 (sFt), log10(ng/mL)	1.6 (0.3)	1.0 (0.2)	9.37***
Hemoglobin (Hb), g/dL	12.9 (2.4)	12.6 (0.7)	0.41
C-reactive protein (CRP), mg/L	3.4 (2.1)	3.1 (.6)	0.68
Red blood cell count (RBC), M/mm^3	4.5 (0.3)	4.5 (.4)	0.17
Hematocrit (HCT), %	40.6 (2.4)	38.9 (2.9)	2.02*
Mean corpuscular volume (MCV), fL	89.5 (5.4)	86.3 (4.0)	2.19*
Mean corpuscular hemoglobin (MCH), pg	29.3 (2.1)	27.6 (1.9)	2.81**
Mean corpuscular hemoglobin concentration (MCHC), g/dL	32.8 (0.8)	31.9 (1.0)	2.86**
Red blood cell distribution width (RDW), %	12.8 (0.8)	13.4 (1.0)	-1.9
Body mass index (BMI), kg/m^2	21.6 (4.0)	22.8 (3.4)	-1.07
Mid-upper arm circumference (MUAC), cm	26.3 (3.6)	27.7 (3.0)	-1.33
Age, y	21.2 (2.2)	21.1 (2.6)	0.13

Note. $n = 21$ for both IS and IDNA groups; IS = Iron sufficient, IDNA = Iron deficient non-anemic; *M* = mean, *SD* = standard deviation; * = $p < .05$, ** = $p < .01$, *** = $p < .001$.

Table 2

Group Differences for Electrophysiological and Behavioral Variables

Measure	IS	IDNA	*t*-score
	M(SD)	*M(SD)*	
A-wave peak amplitude, μV	-0.33 (0.53)	-0.31 (0.35)	-0.09
A-wave implicit time, ms	66.75 (23.67)	81.40 (15.06)	-2.29*
B-wave peak amplitude, μV	0.67 (0.45)	0.65 (.51)	0.11
B-wave implicit time, ms	91.67 (18.01)	108.6 (15.63)	-3.13**
Spontaneous blink rate, bpm	12.58 (7.31)	11.40 (6.40)	0.56
Task-related blink rate, contrast detection, bpm	10.09 (4.50)	9.81 (4.28)	0.2
Task-related blink rate, PST, bpm	10.94 (6.31)	12.21 (4.53)	-0.75
Contrast threshold, %	2.73 (1.19)	15.28 (22.40)	-2.56**
Pre-stimulus-α power, contrast detection, dB	-30.94 (4.87)	-29.93 (7.65)	-0.51
RT, low conflict trials, PST, ms	815 (161)	862 (204)	-0.82
RT, high conflict trials, PST, ms	1001 (238)	986 (241)	0.21
Accuracy, choose A trials, PST, proportion	0.85 (0.16)	0.74 (.20)	2.16*
Accuracy, avoid B trials, PST, proportion	0.89 (0.09)	0.79 (.18)	2.38*
FRN amplitude, incorrect trials, PST, μV	2.83 (2.30)	3.07 (1.92)	-0.36
FRN amplitude, correct trials, PST, μV	2.01 (1.75)	3.03 (1.98)	-1.72

Note. IS = Iron sufficient, IDNA = Iron deficient non-anemic; *M* = mean, *SD* = standard deviation; BPM = blinks per minute, PST = probabilistic selection task, RT = reaction time, FRN = feedback-related negativity. * = $p < .05$, ** = $p < .01$, *** = $p < .001$.

Table 3

Results of the repeated-measures ANOVAs on RT and accuracy from the PST

Variable	Factor	df	MSE	F
RT	Group (G)	1	5107	0.80
	Conflict (C)	1	13565	36.37***
	G x C	1		1.46
Accuracy	G	1	0.024	9.66**
	Choice type (C)	1	0.032	1.53
	G x C	1		0.01
FRN amplitude	G	1	6.56	1.20
	Trial type (T)	1	1.47	2.51
	G x T	1		2.03

Note: ** = $p < .01$, *** = $p < .001$.

Table 4

Correlations Among PERG Features and Measures of Blood Iron

Biomarker	A-wave peak amplitude	A-wave implicit time	B-wave peak amplitude	B-wave implicit time
Hemoglobin (Hb), g/dL	0.02	-0.18	-.26+	-0.15
Serum ferritin (sFt), ng/mL	-0.36*	-0.40**	0.38**	-0.48***
log10(sFt), log10(ng/mL)	-0.18	-0.40*	0.20	-0.48***
Hematocrit (HCT), %	0.07	-0.04	-0.36*	0.01
Mean corpuscular volume (MCV), fL	0.03	-0.09	-.31*	-0.08
Mean corpuscular hemoglobin (MCH), pg	0.02	-0.26+	-0.24	-.26+
Mean corpuscular hemoglobin concentration (MCHC), g/dL	0	-.48**	-0.05	-.49***
Red blood cell distribution width (RDW), %	0.11	0.19	-0.02	0.03

Note. Values are correlation coefficients. + = $p < .10$, * = $p < .05$, ** = $p < .01$, *** = $p < .001$.

Table 5

Correlations Among PERG Features and Indirect Measures of DA

Measure	A-wave peak amplitude	A-wave implicit time	B-wave peak amplitude	B-wave implicit time
Spontaneous blink rate	-0.47**	-0.09	0.05	-0.05
Task-related blink rate, contrast detection	-0.06	-0.08	0.28+	-0.09
Task-related blink rate, PST	-0.04	-0.05	0.08	-0.06
Contrast threshold	0.01	0.19	0.01	0.2
Pre-stimulus alpha power, contrast detection	0.18	-0.14	0.09	-0.15
RT, low conflict trials, PST	0.11	0.26	0.01	0.05
RT, high conflict trials, PST	.27+	0.15	-0.1	-0.02
Accuracy, choose A trials, PST	0.02	-0.21	0.11	-0.17
Accuracy, avoid B trials, PST	0.1	0.13	-0.23	-0.12
FRN amplitude, incorrect trials, PST, uV	-.36*	-0.17	.62***	-0.02
FRN amplitude, correct trials, PST, uV	-0.02	0.10	0.28+	0.29+

Note. Values are correlation coefficients. + = $p < .10$, * = $p < .05$, ** = $p < .01$, *** = $p < .001$.

Table 6

Correlations Among Blink Rates and Measures of Blood Iron

Biomarker	Spontaneous blink rate	Task-related blink rate, contrast detection	Task-related blink rate, PST
Hemoglobin (Hb), g/dL	-0.09	-0.16	-0.11
Serum ferritin (sFt), ng/mL	0.32*	0.26	0.18
log10(sFt), log10(ng/mL)	0.21	0.17	-0.01
Hematocrit (HCT), %	-0.17	-0.27	-0.24
Mean corpuscular volume (MCV), fL	-0.07	-0.04	-0.3
Mean corpuscular hemoglobin (MCH), pg	0.01	0.01	-0.2
Mean corpuscular hemoglobin concentration (MCHC), g/dL	0.15	0.1	0.03
Red blood cell distribution width (RDW), %	0.06	-0.17	0.06

Note. Values are correlation coefficients. + = $p < .10$, * = $p < .05$, ** = $p < .01$, *** = $p < .001$.

Table 7

Correlations Among Blink Rates and Other Indirect Measures of DA

Measure	Spontaneous blink rate	Task-related blink rate, contrast detection	Task-related blink rate, PST
Contrast threshold	0.15	0.12	-0.01
Pre-stimulus alpha power, contrast detection	-0.32*	-0.35*	-0.30+
RT, low conflict trials, PST	-0.07	0.11	0.06
RT, high conflict trials, PST	0.00	0.16	-0.03
Accuracy, choose A trials, PST	0.09	-0.22	-0.17
Accuracy, avoid B trials, PST	-0.33*	-0.06	-0.06
FRN amplitude, incorrect trials, PST, uV	-0.20	-0.13	-0.15
FRN amplitude, correct trials, PST, uV	-0.07	-0.23	-0.21

Note. Values are correlation coefficients. + = $p < .10$, * = $p < .05$, ** = $p < .01$, *** = $p < .001$.

Table 8

Results of Using Logistic Regression to Predict Iron Status from the Indirect Measures of DA

Predictor	β	Wald Chi-Squared Test	R^2	AUC
B-wave implicit time	-0.067	6.18*	0.214	0.725*
Spontaneous blink rate	0.026	0.32	0.008	0.537
Task-related blink rate, contrast detection	0.047	0.37	0.008	0.567
Task-related blink rate, PST	-0.041	0.52	0.011	0.534

Note. For the Wald Chi-Square, * = $p < .05$, chi-square $\neq 0$. For the AUC, * = AUC $\neq .50$

Table 9

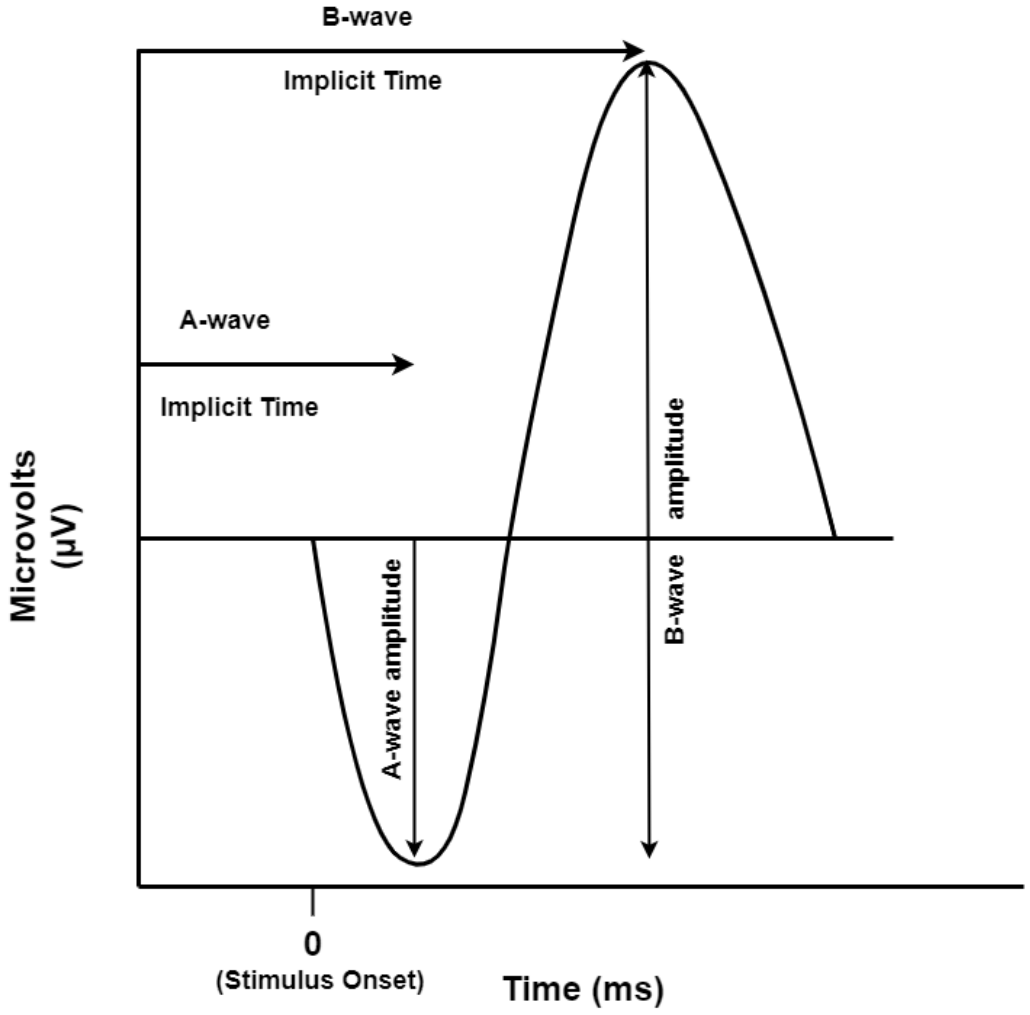
Parameters and Measures of Fit for the Model Testing the Mediating Role of the Indirect Measures of DA in the Relationship Between Iron Status and Cognitive Performance

X	Y	Mediators	Covariate	Outcome			Overall Predictors		
					R^2	*F*	Effect	β	*t*
sFt	PST accuracy	M1: A-wave implicit time	Composite: HCT, MCV, MCH, MCHC	A-wave implicit time	0.21	4.77*	X -> M1	-0.36	-2.43*
		M2: B-wave implicit time		B-wave implicit time	0.29	7.19**	X -> M2	-0.45	-3.23**
				PST accuracy	0.25	2.57*	M1 -> Y	0.26	1.24
							M2 -> Y	-0.44	2.54*
							X -> Y	0.16	1.26

Note. PST accuracy is the mean of the Z-scores for accuracy of choose A and avoid B trials. The covariate was the composite of the Z-scores for the variables listed. + = $p < .10$, * = $p < .05$, ** = $p < .01$, *** = $p < .00$

Figure 1

Schematic Representation of the Features of the PERG



Note. Modeled after Sen et al. (2021).

Figure 2

CONSORT Diagram for Participant Recruitment

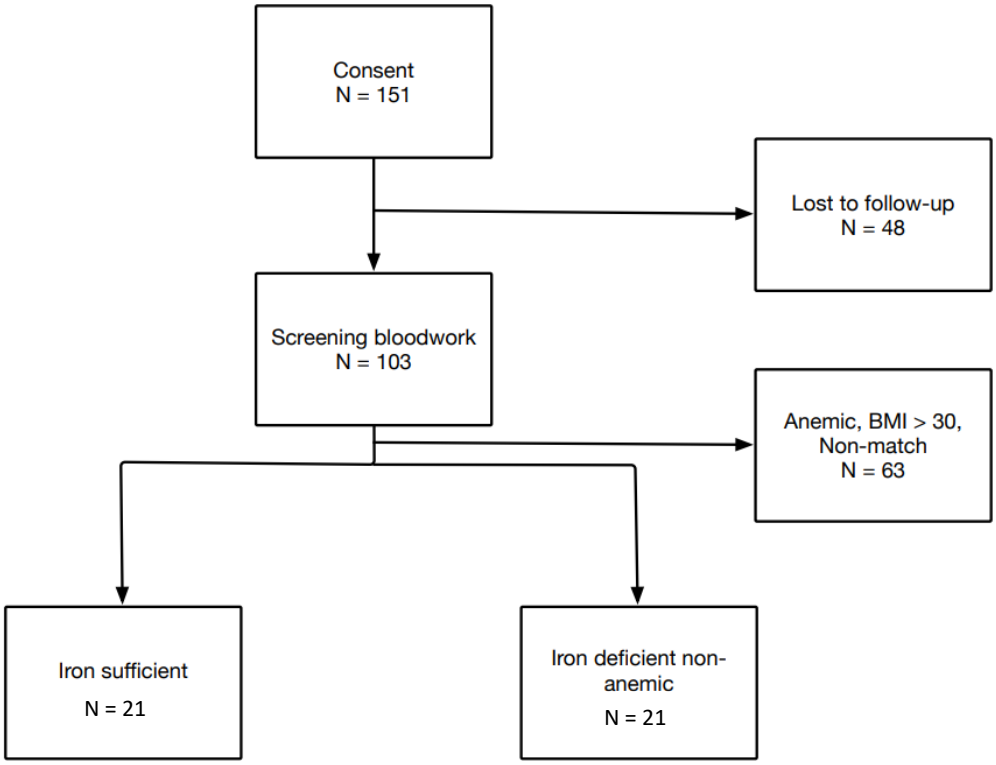


Figure 3

Stimulus Used in Spontaneous Blink Rate Task

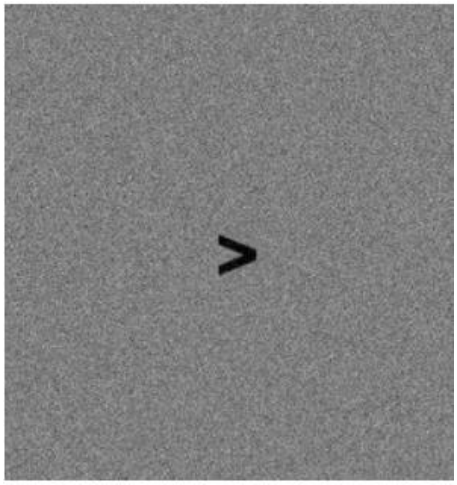


Figure 4

Stimuli Used in PERG Task

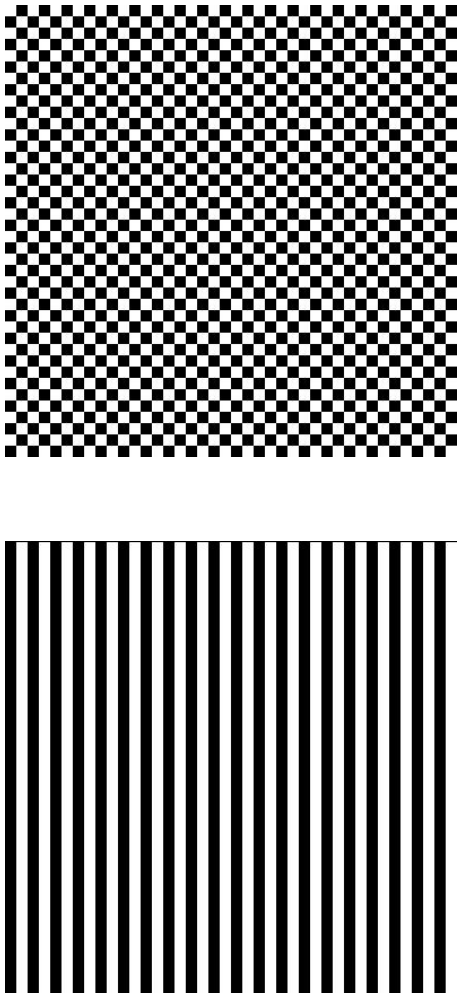


Figure 5

Stimuli Used in Contrast Threshold Task

(a)

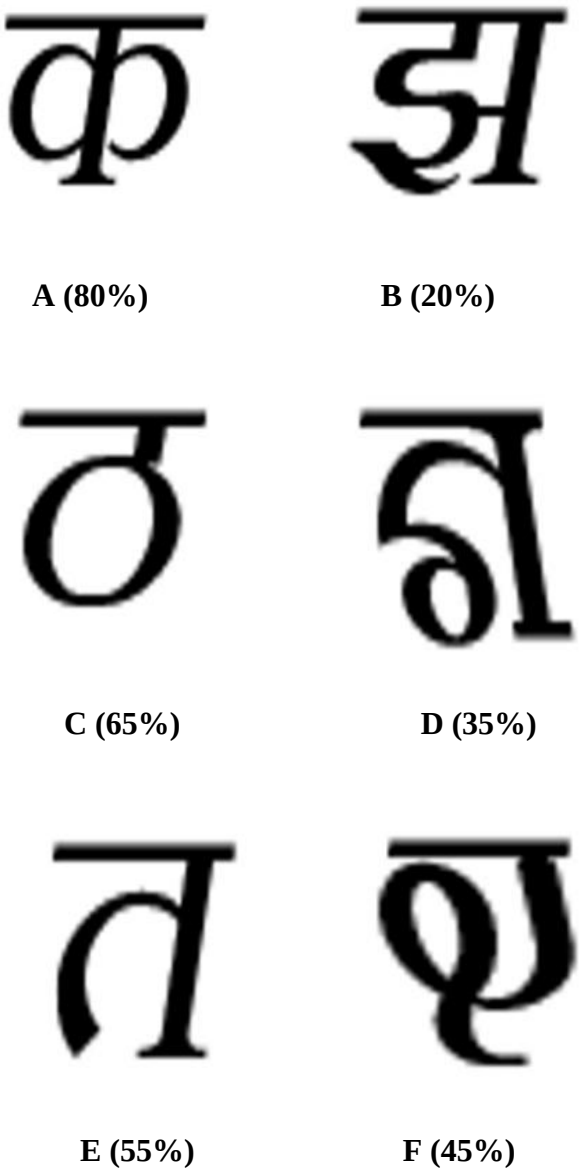
(b)

(c)

Note. (a) Represents a Gabor patch at 0.1% contrast (b) Represents Gabor patch at 40% contrast (c) Represents a Gabor patch at 80% contrast

Figure 6

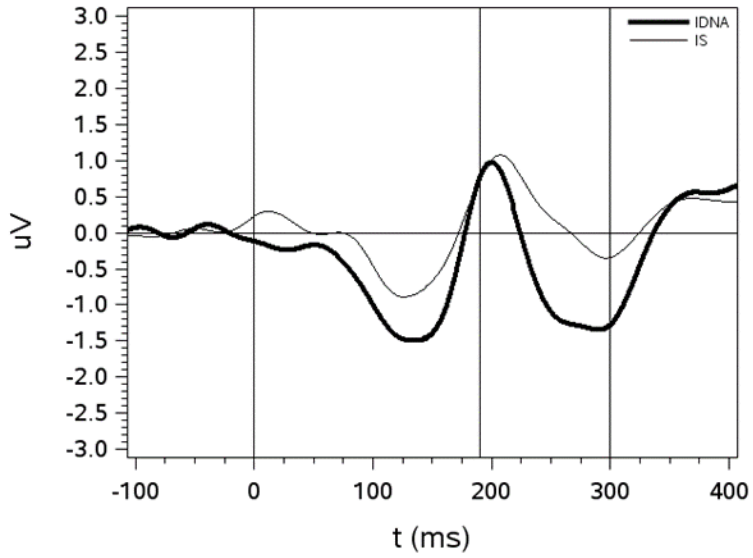
Stimuli Used in PST



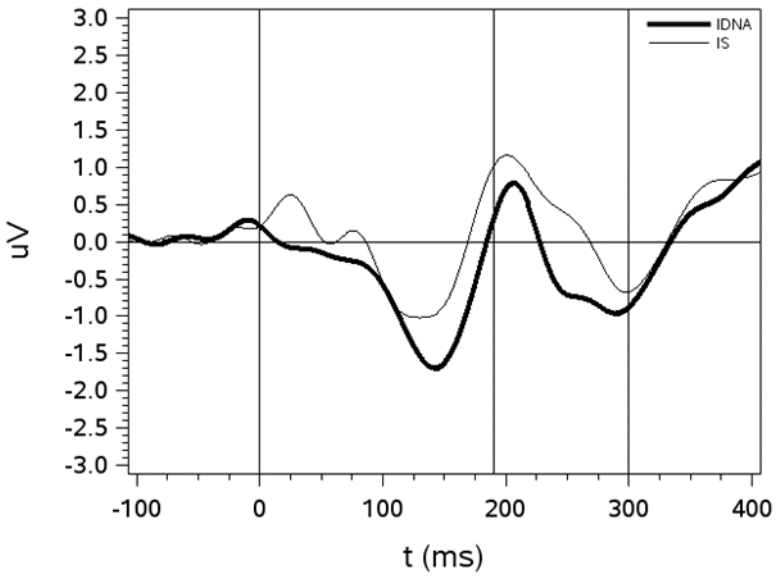
Note. Reward differentials were as follows: AB trials at 80% (A) vs. 20% (B), CD trials at 65% (C) vs. 35% (D), and EF trials at 55% (E) vs. 45% (F).

Figure 7

Example FRN Waveforms for PST



(a)

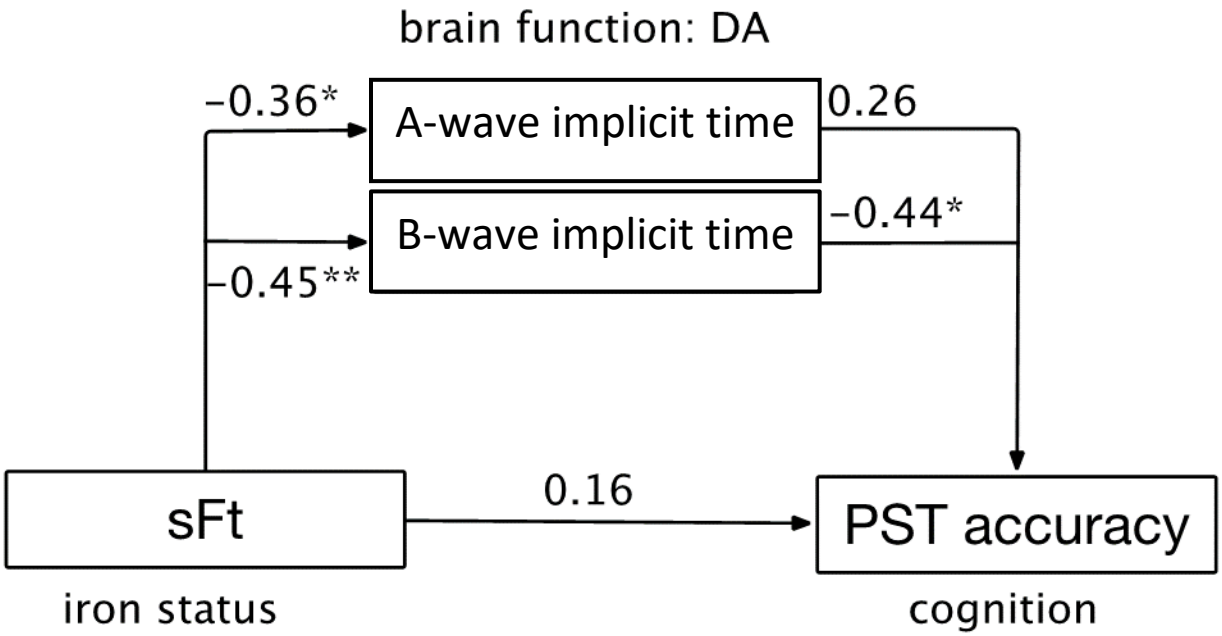


(b)

Note. Example waveforms for the FRN for (a) correct and (b) incorrect trials in the PST. Reference lines in each panel indicate the time limits for which the FRN was estimated.

Figure 8

General Form of Four Mediation Models



Note. General form of the four mediation models that were considered. Path coefficients are shown for the model that provided the best overall fit. Note: * = p < .05, ** = p < .01.