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Michael J. Wenger[1,2], Diane M. DellaValle [2,3], Laura E. Murray-Kolb[4], Jere D. Haas [2]

[1]Center for Applied Social Research, The University of Oklahoma, 201 Stephenson Parkway, Suite 4100, Norman, OK 73019, USA, [2]Division of Nutritional Sciences, Cornell University, Ithaca, NY, USA, [3]Department of Nutrition and Dietetics, Marywood University, Scranton, PA, USA, [4]Department of Nutritional Sciences, The Pennsylvania State University, State College, PA, USA

Objectives: Iron deficiency (ID) – the highly prevalent nutritional deficiency – has been shown to have deleterious effects on measures of cognitive performance and brain activity. Many of these results are suggestive of the impact of ID on neurotransmitter regulation and myelination. A third critical potential effect of ID on brain function is at the level of brain energy expenditure; however, to date there has not been any method for indirectly estimating the impact of ID on energy expenditure in humans in the context of cognitive work.
Methods: We report here a study comparing ID and iron sufficient (IS) college students in which simultaneous behavioral, encephelographic (EEG), and metabolic data were collected in a task designed as a cognitive analog to standard physical exertion tasks.
Results: We show that increases in cognitive demands produced decrements in behavioral measures of performance, and increases in EEG and metabolic measures of work. Critically, we found that the magnitudes of those changes were directly related to iron levels.
Discussion: We find support for the idea that brain activity mediates the relationship between cognitive demands and energy expenditure, with ferritin and hemoglobin moderating those relationships in distinct ways. Finally, we show that levels of energy expenditure can be indirectly estimated by measures of EEG spectral power.

Keywords: Iron, Iron deficiency, Cognition, Memory, Attention, Energy expenditure, Energetic efficiency, Electroencephelography

Introduction

There is increasing evidence that iron deficiency (ID) and iron deficiency anemia (IDA) – among the most prevalent nutritional deficiencies in the world[1] – are related to deficits in perceptual and cognitive performance.[2–4] Iron is transported across the blood–brain barrier,[5,6] and is distributed in a non-uniform manner throughout the brain.[7] There are four general classes of mechanisms by which iron can affect brain function:[7–10] myelination (as the oligodendrocytes are sites of iron storage), neurotransmitter synthesis and regulation (particularly the monoamines), synaptogenesis and neurogenesis, and energy expenditure. In humans, there have been a variety of experimental methods that allow for indirect estimates of the effect of iron status on myelination[11] and neurotransmitters,[3] but to date there have been no methods for obtaining indirect estimates for the effect of variations in iron on brain energy expenditure.

We describe here a study in which we observed ID-related decrements in levels of energy expenditure and cognitive performance, using a novel approach to estimating brain energy expended based on body energy expended during the performance of cognitive work. Our approach combines behavioral, metabolic, and electroencephelographic (EEG) measures acquired during the performance of a cognitive task that increases in difficulty across the experimental session: a cognitive analog to a treadmill test. The potential for using EEG as a basis for this type of indirect calorimetry comes from the fact that brain glucose use is

Correspondence to: Michael J. Wenger, Center for Applied Social Research, The University of Oklahoma, 201 Stephenson Parkway, Suite 4100, Norman, OK 73019, USA. Email: michael.j.wenger@ou.edu

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directly related to the intensity of neural activation, in particular the generation and propagation of action and generator potentials and the release, uptake, and re-uptake of neurotransmitters.[12–16] Activation of neural tissue produces increases in oxygen consumption and glucose metabolism that are nearly linearly related to spiking frequency.[12,15,17–19] In addition, there is evidence that increases in task difficulty are regularly related to changes in EEG spectral power.[20–22] Given that activity at the synapses – the apical dendrites in particular – and overall spike frequency are among the determinants of the major features of EEG,[23] along with the ability to measure EEG at time-scales that are comparable to the durations of cortical processing, suggests that EEG may be potentially sensitive to task-dependent onset and duration of neural activity.

We recruited matched samples of ID and iron-sufficient (control, CN) college-aged women, who performed a cognitive task designed as an analog to the physical exertion tasks that are commonly used to assess energy expenditure.[24,25] The cognitive task was a visual short-term memory task, performed concurrently with a math task. The cognitive task increased in difficulty as the experimental session proceeded. Along with standard behavioral measures of performance, simultaneous measures of metabolic activity and EEG were also obtained. We tested the hypotheses that (a) increases in the difficulty of a mental task would result in measurable changes in behavior, EEG, and measures of energy expenditure; (b) changes in each of these measures would be related to an individual's iron status; and (c) measures of EEG spectral power could be used to estimate total body energy expenditure and thus infer brain energy expenditure.

Methods

Design

The visual short-term memory task was a version of the classic memory scanning task of Sternberg.[26] The task was implemented as a 2 (group: ID, CN) ×4 (number of segments: 1, 2, 3, 5) ×4 (set size: 1, 2, 3, 4) ×2 (trial type: positive, negative) factorial, with all factors except group being manipulated within subjects. The concurrent math task was implemented as a 2 (group: ID, CN) ×4 (number of operations per trial: 1, 2, 3, 4) factorial, with number of operations (determined by the set size on any given trial) manipulated within subjects.

Subjects

A total of 210 subjects recruited from the Cornell University community were initially screened for eligibility. Exclusion criteria included self-reported current acute or chronic illness, hemolytic anemia, impaired hepatic or renal function, or use of prescription medications (excepting contraceptives). Women were classified as either iron sufficient (CN) or iron deficient without anemia (ID). ID was defined as hemoglobin (Hb) concentrations ≥ 12 g/dl and serum ferritin (sFt) $\leq 16\,\mu$g/l.[27] Subjects in the control condition were matched to those in the ID group on the basis of age, education, and activity level (as reported on initial questionnaires). Of the initial 210 subjects, a total of 20 women were identified for each of the groups. A variety of equipment problems resulted in the final number of subjects providing usable data being 19 in the ID group (A total of four of these subjects, all in the ID group, had Hb levels that were between 11.9 and 12.0 g/dl. All of the analyses reported below were repeated with and without the data for these subjects, and there were no qualitative differences.) and 20 in the CN group.

Materials

The stimuli for the short-term visual memory task were based on those of Blaha *et al*.[28] Examples of the stimuli at each level of complexity (number of segments), along with the dimensions of the stimuli, are presented in Fig. 1. The stimuli were created by having segments of four deviations from a baseline, with those deviations differing in sign and amplitude (including 0), under the constraint that no two adjacent deviations could have the same sign and amplitude. A total of 250 stimuli were created at each level of complexity, and for each of those, a second stimulus, identical except for one deviation, was also created. This second stimulus served as the potential test stimulus on negative trials. The stimuli for the concurrent math task were the digits 1–9, presented in a 24-point bold sans serif font (Arial). All stimuli were presented on a 53 cm (diagonal) VGA CRT monitor positioned 72 cm from the subject, at a resolution of 1024×768 pixels and a refresh rate of 60 Hz. Response choices and latencies were recorded using the standard computer keyboard, with a temporal resolution of ±1 ms. EEG data were acquired using a 64-channel system (Brain Products, Munich, Germany). Impedances were kept at or below 5 kΩ, channels were referenced to electrode Cz, and digitized at 1K Hz with a 12-bit analog-to-digital converter. Metabolic variables were acquired using a computerized metabolic cart (TrueOne 2400; ParvoMedics, Salt Lake City, UT , USA), using a mask attached to the face and a heart rate monitor attached to the chest. Concentrations of O_2 and CO_2 in expired air were analyzed with gas analyzers, which were calibrated with gases of known concentration at the start of each testing session. Respiratory volume was measured with a respiratory pneumotachograph (Fitness Instrument Technologies, Farmingdale, NY,

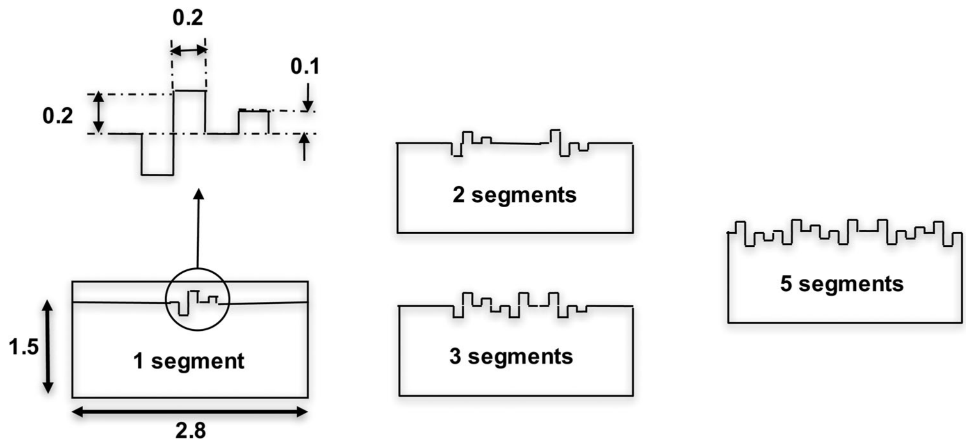

Figure 1 Example stimuli at each level of complexity from the visual memory task. Dimensions are given in degrees of visual angle at a 72 cm viewing distance.

USA) through a two-way breathing valve (Hans Rudolph, Kansas City, MO, USA).

Procedure

All data were collected in the Human Metabolic Research Unit (HMRU) on the campus of Cornell University. At the time of the initial screening, subjects were given three questionnaires to complete: (a) a questionnaire for demographics and basic health status, (b) a questionnaire assessing menstrual status, and (c) a physical activity questionnaire. The questionnaires were administered by trained research assistants. Blood samples were acquired by a trained phlebotomist, and the samples were transferred to the laboratory of the HMRU for analysis.

Subjects who were selected to participate in the testing returned to the HMRU within 2 weeks of their initial screening. All visits were scheduled during standard business hours. The session (lasting approximately 90 min) began with the collection of anthropometric measures, using standard procedures and equipment:[29] (a) height, to the nearest 0.1 cm; (b) weight, to the nearest 0.1 kg; and (c) mid-upper-arm circumference, to the nearest 0.1 cm. Following this, the EEG electrode cap, heart-rate monitor, and face mask for the metabolic cart were attached. The room lights were then dimmed, and subjects were instructed to rest for 2 min with their eyes closed. Following this, the visual short-term memory task and along with the concurrent math task were explained to the subject, who practiced each task separately and then together before beginning the test trials. For the visual short-term memory task, subjects were instructed to remember the set of items. For the concurrent math task, subjects were instructed to keep a running sum of the digits. Note that the concurrent math task was included in order to tax attention, with the memory task being of primary interest.

The events on each trial of the concurrent tasks were as follows. Subjects initiated each trial by pressing a key (space-bar) on the computer keyboard. A fixation cross was then presented at the center of the screen; its duration was set on each trial by generating a value from an exponential distribution with a mean of 500 ms, censored at 300 and 1000 ms. The screen was then blanked, and the duration of this period was set on each trial by generating a second value from an exponential distribution with a mean of 500 ms, censored at 300 and 1000 ms. Following this, each of the to-be-remembered (TBR) stimuli for the current level of number of segments and set size were presented at a 1 s rate. At the onset of each TBR stimulus, a single digit was presented immediately to the left of that stimulus, with the particular value (1–9) being determined at random, with the constraint that no two sequential digits could be identical. Following the presentation of the last TBR item and digit (determined on each trial by the set size), there was a 2 s retention interval, during which the screen was blank. This was followed by the onset of a test stimulus, which was either identical to one of the presented TBR items, or its complementary negative item. Subjects indicated whether the item was old (presented as a TBR item on that trial) or new (the complimentary negative item) using the z and / keys on the computer keyboard, with the index finger of the dominant hand assigned to the 'old' response. The test item was cleared after the subject's response or after 2 s had elapsed, in which case the trial was coded as an error; such errors were extremely rare (< 8 total across all subjects), and data from these trials were excluded from the analyses. Finally, a test sum, either correct or incorrect by either 1 or 2, was presented, and subjects had to verify the correctness of the test sum using the same two keys, with the index finger of the dominant hand assigned to the 'correct' judgment. A total of 30 positive and 30 negative visual memory trials were run at each level of number of segments and set size. Trials were blocked by a number of segments, and subjects were allowed a very brief break between blocks. The order of presentation of trials within a block was randomized for each subject. The status of the test sum (correct, incorrect) on each trial was determined at random. No feedback was provided for either task to maintain a constant level of task engagement.

Results

An initial examination of the blood, behavioral, EEG, and metabolic measures revealed no reliable relationships with menstrual status or any of the anthropometric variables. As such, they were not included as covariates in any of the analyses.

Iron biomarkers

Table 1 presents the blood measures for the two groups of subjects. As expected, the ID and CN samples differed reliably on measures of Hb , sFt, and body iron (BdFe), with the CN subjects having higher values than the ID subjects. There were no reliable group differences for soluble transferrin receptor (sTfR), or for either of the two measures of inflammation (C-reactive protein and α-1 acid glycoprotein).

Behavioral measures

The behavioral, EEG, and metabolic variables were all analyzed in two ways. First, reflecting the experimental design, they were analyzed using a 2 (group: ID, CN) ×4 (number of segments: 1, 2, 3, 5) ×4 (set size: 1, 2, 3, 4) repeated-measures analysis of variance (ANOVA). Second, assessing the amount of change in the dependent variables as a function of iron levels, slopes as a function of the two task difficulty variables (number of segments, set size) were estimated separately for each subject. These slopes were then regressed onto the iron status variables, alone and in combination, with the best model (highest proportion of variance accounted for with the smallest number of predictors) selected on the basis of comparison first to a null model (intercept only) and to the other possible models, with the constraint that the selected model had to offer a superior fit than the null model and had to account for at least 10% of the variance.

Table 1 Means (*M*), standard errors (*SE*), and group comparisons for each of the blood measures

	ID		CN		
Variable	*M*	*SE*	*M*	*SE*	*t*
Hemoglobin (Hb, g/dl)	12.1	0.2	13.3	0.2	$4.48^{\ddagger}$
Serum ferritin (sFt µg/ml)	10.8	4.2	40.0	11.1	$10.62^{\ddagger}$
Soluble transferrin receptor (sTfR ug/ml)	6.2	1.7	5.4	1.7	−1.39
Body iron (BdFe)	0.3	2.5	5.8	1.9	$7.58^{\ddagger}$
C-reactive protein (CRP mg/l)	4.9	7.8	1.9	5.4	−1.38
α-1 acid glycoprotein (AGP mg/dl)	70.0	18.9	70.3	15.5	0.05

$^{\ddagger}P < 0.001$.

We restrict our presentation of the behavioral data to those from the visual short-term memory task. Performance on the concurrent math task was uniformly high (error rates < 10%), and RTs were significantly correlated with iron status; details are available on request. A summary of the ANOVA results for the behavioral data is presented in Table 2 and the means for each group as a function of the two difficulty variables are presented in Fig. 2. ID subjects, relative to CN subjects, had lower hit and false alarm rates, with these differences indicating lower levels of

Table 2 ANOVA results for the behavioral variables in the visual short-term memory task

Variable	Effect	df	*F*	MSE
False alarm rates	Group (G)	1	4.24^{*}	0.22
	Number of segments (N)	3	$16.97^{\ddagger}$	0.04
	Set size (S)	3	$6.71^{\ddagger}$	0.05
	G×N	3	0.93	0.04
	G×S	3	1.33	0.05
	N×S	9	1.59	0.03
	G×N×S	333	0.76	0.03
Hit rates	G	1	$8.66^{\dagger}$	0.33
	N	3	$23.68^{\ddagger}$	0.04
	S	3	$5.02^{\dagger}$	0.05
	G×N	3	0.78	0.05
	G×S	3	1.00	0.05
	N×S	9	2.20^{*}	0.03
	G×N×S	333	1.12	0.03
Sensitivity (d')	G	1	5.22^{*}	1.16
	N	3	$60.01^{\ddagger}$	0.64
	S	3	$11.78^{\ddagger}$	0.70
	G×N	3	1.73	0.64
	G×S	3	1.68	0.70
	N×S	9	$4.28^{\ddagger}$	0.65
	G×N×S	333	0.91	0.65
Bias (c)	G	1	6.88^{*}	2.82
	N	3	0.59	0.33
	S	3	2.81^{*}	0.34
	G×N	3	0.67	0.33
	G×S	3	0.70	0.34
	N×S	9	0.35	0.19
	G×N×S	333	1.40	0.19
RT, positive trials	G	1	$24.83^{\ddagger}$	276162
	N	3	$63.51^{\ddagger}$	50211
	S	3	0.17	45632
	G×N	3	1.57	50211
	G×S	3	0.22	45632
	N×S	9	1.23	26617
	G×N×S	333	0.75	26617
RT, negative trials	G	1	$68.97^{\ddagger}$	199401
	N	3	$85.92^{\ddagger}$	59943
	S	3	$20.77^{\ddagger}$	40773
	G×N	3	2.17^{+}	59943
	G×S	3	0.70	40773
	N×S	9	2.17^{*}	28660
	G×N×S	333	0.75	28660

df = degrees of freedom, MSE = mean square error; $^{+}P < 0.10$, $^{*}P < 0.05$, $^{\dagger}P < 0.01$, $^{\ddagger}P < 0.001$.

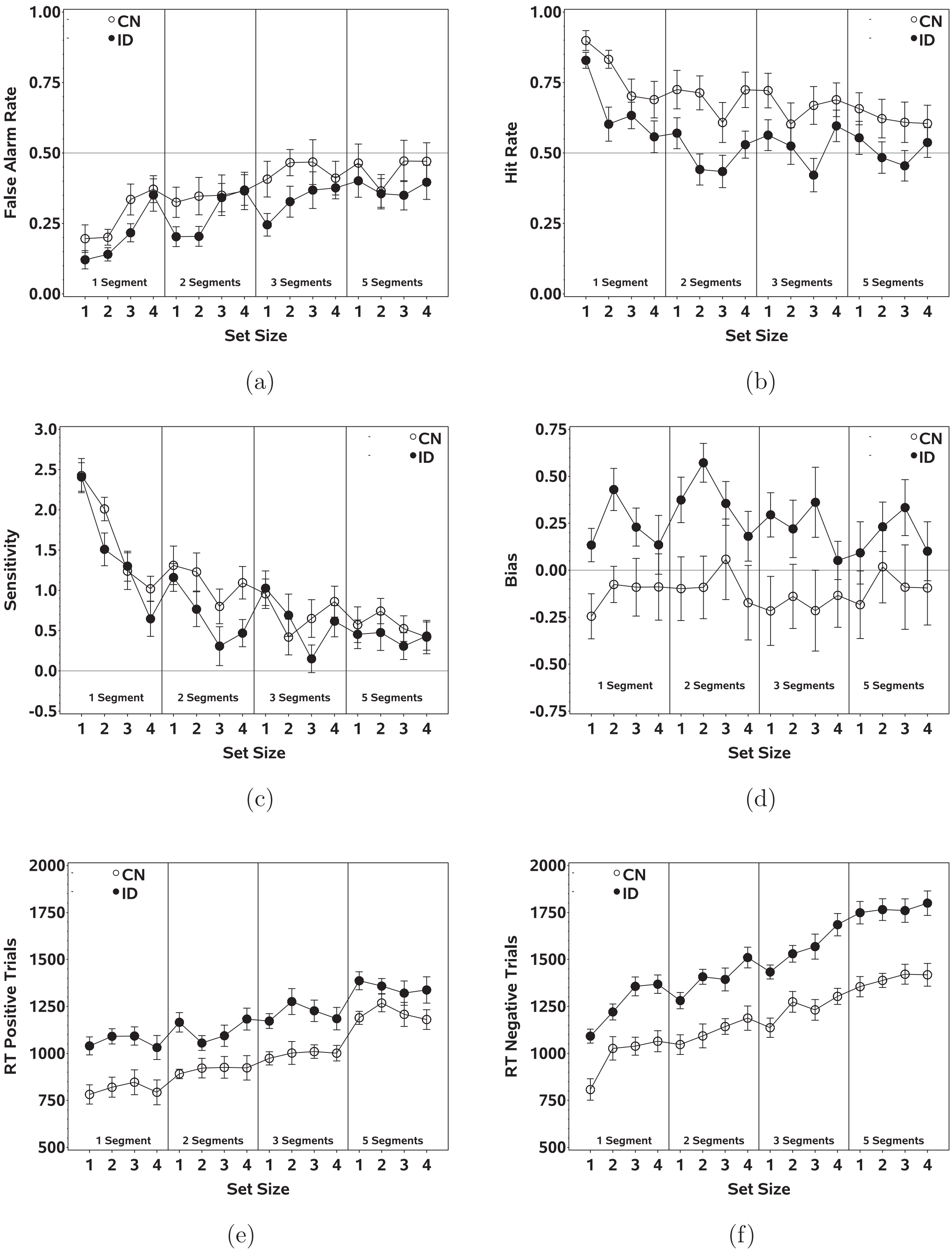

Figure 2 Means (±1 standard error) for the behavioral data for the ID and CN subjects in the visual short-term memory task: (A) false alarm rates, (B) hit rates, (C) sensitivity (*d′*), (D) bias (*c*), (E) RT for positive trials, (F) RT for negative trials.
Note: Segments refers to the complexity of any one TBR item (Fig. 1), while set size refers to the total number of TBR items on a trial.

sensitivity and a more conservative response bias. (A hit was defined as correctly identifying a presented item as having been seen in the set, and a false alarm was defined as incorrectly identifying a new item as having been seen in the set. Sensitivity was calculated as $d' = Z^{-1}(HR) - Z^{-1}(FR)$, and response bias was calculated as $c = -\frac{1}{2}[Z^{-1}(HR) + Z^{-1}(FR)]$, where HR = hit rate, FR = false alarm rate, and Z^{-1} is the inverse normal transformation of a proportion.[30,31]) In addition,IDsubjects were slower than CN subjects on both positive and negative trials. Both of the difficultymanipulations produced reliable decrements in performance,with a number of segments affecting all of the variables except response bias, and set size affecting all of the variables except mean RT on positive trials. The joint effect of the two difficulty variables was reliable for hit rates, sensitivity, and RT on negative trials. None of the other interactions were reliable for any of the variables, including any two-way or three-way interactions involving group. Thus, although the performance of ID subjects was less sensitive, more conservative, and slower, the difficulty manipulations did not impact ID subjects more so than for the CN subjects.

The results of the regression analyses on the behavioral variables are presented in Table 3. With respect to the effect of increasing number of segments, lower levels of sFt predicted less of an increase in false alarms, larger decreases in hit rates and sensitivity, and larger increases in RTs on both positive and negative trials. With respect to the effect of increasing set size, lower levels of sFt predicted larger decreases in hit rates and sensitivity, and greater increases in RTs on negative trials. This more limited effect of set size can be seen in the diminishing slopes as a function of set size as the number of segments increased, with the set size effect becoming asymptotic at either three or five segments. Finally, lower levels of sFt predicted more conservative response bias overall (collapsed across both difficulty variables).

EEG measures

For the EEG data, we focused on change from baseline in normalized spectral power in the α, θ, and γ bands, as changes in α- and θ-band power have been associated with effects of increases in task difficulty and changes in γ-band power have been associated with effortful attention.[20,21,32,33] Results of the ANOVA on these data are presented in Table 4 and the means for these variables are presented in Fig. 3. ID subjects, relative to the CN subjects, had reliably larger reductions in α-band power and reliably smaller increases in θ- and γ-band power. Increasing task difficulty by way of increasing the number of segments resulted in reliable reductions in α- and increases in θ- and γ-band power. In contrast, increasing set size had no reliable effect on changes in any of the three ranges of spectral power. Finally, the increases in θ- and γ-band power were significantly larger for the ID relative to the CN subjects.

The results of the regression analyses on the EEG variables are presented in Table 3. With respect to increasing difficulty by increasing the number of segments, lower levels of sFt predicted smaller increases in both θ- and γ-band power. There were no models identified for effects related to increasing set size or for either number of segments or set size for changes in α-band power.

Metabolic measures

For the metabolic data, we focused on three variables: heart rate, respiratory rate, and energy expended. Energy expended was estimated[34] as

$$EE(Mj/\min) = VO_2 \times [3.90 \times (1.10 \times RER)] \times 4.19$$

where VO_2 is the volume of O_2 consumed and RER is the respiratory exchange ratio. Results of the ANOVA on these variables are presented in Table 4 and the means are presented in Fig. 4. Subjects who had ID expended less energy, while having lower heart rates. Increasing the number of segments and the set size each resulted in overall higher values for all three

Table 3 Results of the regression analyses for all of the variables (behavioral, EEG, and metabolic)

Type	Variable	Effect	Predictor	Intercept	$\hat{\beta}$	R^2
Behavioral	FA rates	Number of segments (N)	sFt	0.0351	0.0036	0.34
		Set size (S)	–	–	–	–
	Hit rates	N	sFt	−0.2304	0.0036	0.33
		S	sFt	−0.1232	0.0019	0.25
	Sensitivity (d')	N	sFt	−0.5548	0.0089	0.16
		S	sFt	−0.2974	0.0077	0.34
	Bias (c)	Collapsed across N and S	sFt	0.3916	−0.0126	0.19
	RT, positive trials	N	sFt	91	−0.9129	0.10
		S	–	–	–	–
	RT negative trials	N	sFt	169	−2.6379	0.25
		S	sFt	101	−2.3210	0.33
EEG	% change in α	N	–	–	–	–
		S	–	–	–	–
	% change in θ	N	sFt	1.6022	0.1561	0.27
		S	–	–	–	–
	% change in γ	N	sFt	2.7547	0.2766	0.59
		S	–	–	–	–
Metabolic	Energy expended	N	sFt	−0.009	0.009	0.43
		S	sFt	0.102	0.006	0.46
	Heart rate	N	sFt	0.012	0.086	0.26
		S	–	–	–	–
	Respiratory rate	N	sFt	0.880	0.017	0.25
		S	–	–	–	–

Note: Dashes indicate that an acceptable model was not identified for that variable and effect.

Table 4 ANOVA results for the EEG and metabolic variables

Variable	Effect	df	*F*	*MSE*
	EEG variables			
% change in α	Group (G)	1	8.84†	2215
	Number of segments (N)	3	212.47‡	100
	Set size (S)	3	0.69	54
	G × N	3	2.04	100
	G × S	3	0.57	54
	N × S	9	0.94	82
	G × N × S	333	0.48	82
% change in θ	G	1	16.26‡	706
	N	3	260.35‡	34
	S	3	0.92	16
	G × N	3	20.13‡	34
	G × S	3	0.96	16
	N × S	9	1.54	15
	G × N × S	333	0.79	15
% change in γ	G	1	5.89*	1113
	N	3	129.44‡	108
	S	3	1.96	75
	G × N	3	16.49‡	108
	G × S	3	1.76	75
	N × S	9	0.93	68
	G × N × S	333	0.81	68
	Metabolic variables			
Energy expended	G	1	3.64*	7.62
	N	3	60.32‡	0.19
	S	3	38.03‡	0.29
	G × N	3	19.43‡	0.19
	G × S	3	1.85	0.29
	N × S	9	1.11	0.15
	G × N × S	333	0.34	0.15
Heart rate	G	1	9.53†	1846.93
	N	3	14.95‡	138.56
	S	3	18.56‡	53.00
	G × N	3	10.97‡	138.56
	G × S	3	0.91	53.00
	N × S	9	1.56	28.30
	G × N × S	333	0.90	28.30
Respiratory rate	G	1	1.38	122.30
	N	3	28.06‡	2.78
	S	3	27.39‡	2.10
	G × N	3	16.05‡	2.78
	G × S	3	5.85†	2.10
	N × S	9	1.56	1.09
	G × N × S	333	0.49	1.09

df=degrees of freedom, MSE=mean square error; *P < 0.05, †P < 0.01, ‡ = P < 0.001.

metabolic variables. The number of segments, as a difficulty factor, produced higher heart and respiratory rates for the CN relative to the ID subjects. Set size, as a difficulty factor, produced higher respiratory rates for the CN relative to the ID subjects. None of the other interactions were reliable for any of the variables.

Results of the regression analyses for the metabolic variables are presented in Table 3. Lower levels of sFt predicted smaller increases in respiratory and heart rate and energy expended as a function of number of segments. In addition, lower levels of sFt predicted smaller increases in energy expended as a function of set size.

Relating behavior, brain, and metabolism

Having shown that our task difficulty manipulations produced significant effects on the behavioral, EEG, and metabolic variables, we then considered the manner in which all of these variables might be related. We did this by fitting a set of regression-based moderated mediation models.[35] We formed a 16-level ordinal task difficulty variable by recoding the combination of number of segments and set size. As mediating variables, we restricted consideration to percent change in power in the θ- and γ-bands, given the lack of consistent results for the α-band. We considered all three metabolic variables as dependent variables . We considered a set of competing models, including models in which there was no mediating effect of brain activity on the relationship between cognitive demands and energy, models in which only the number of segments or set size was the difficulty variable, and models in which iron status played no role. To be selected, a model had to account for more of the variance than any of its competitors, and all of the parameter estimates and indicators of mediation, moderation, and moderated mediation needed to be reliably different from 0. We were able to identify a model for energy expended (see Fig. 5). In this model, brain activity mediates the relationship between cognitive demands and energy, sFt moderates the relationship between cognitive demands and brain activity, and Hb, even at normal (non-anemic) levels, moderates the relationship between brain activity and energy. Note that inclusion of Hb as a moderating variable significantly improved the fit of the model, even though Hb was never selected as a reliable predictor in the regression analyses. This is most likely because the moderated mediation models are considering a more complex set of relationships than those considered in the regression analyses.

Finally, we sought to determine the extent to which the EEG activity in concert with iron status could be used as an estimator for energy expenditure. We restricted consideration to energy expended, and fit a set of competing linear regression models using stepwise selection. To be selected, a model had to account for at least 10% of the variance and had to be superior to a null model (intercept only). Using these criteria, we were able to identify a model for energy expenditure:

$$EE = 3.64 + 0.14 BdFe + 0.04\theta + 0.01\gamma,$$

where BdFe is body iron and θ and γ are percent change from baseline for θ- and γ-band power, respectively. This model accounted for 38% of the variance. Note that BdFe is calculated[36] as a ratio of sTfR and sFt. This is important in that (a) sFt was

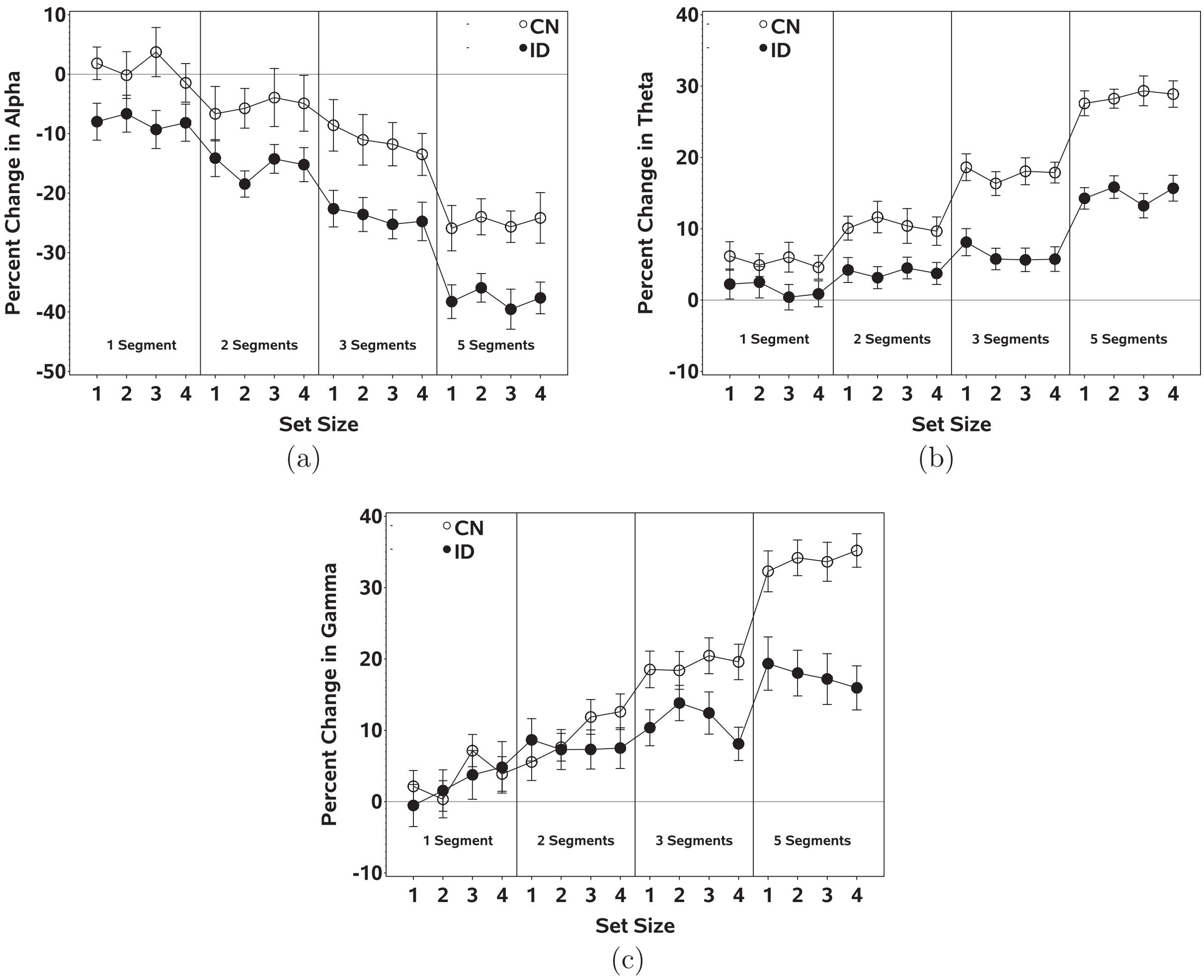

Figure 3 Means (± 1 standard error) for the EEG data (percent change baseline from baseline in spectral power) for the ID and CN subjects: (A) α-band, (B) θ-band, (C) γ-band.
Note: Segments refers to the complexity of any one TBR item (Fig. 1), while set size refers to the total number of TBR items on a trial.

consistently identified as the best predictor of the behavioral, metabolic, and EEG data (see Table 3); and (b) a model for energy expenditure using sFt and sTfR as predictors accounted for 39% of the variance, with this increase in R^2 not being sufficiently large enough to justify the increase in the number of model parameters.

Discussion

Using three simultaneous streams of data – behavioral, electrophysiological, and metabolic – in a task designed to be a cognitive analog to tests of physical endurance, we demonstrated reliable effects of increases in cognitive demands on all three classes of dependent variables, and were able to do this in a manner that allowed performance to remain above chance until the very highest levels of difficulty were reached.

Critically, we found that ID significantly impaired the extent to which participants were able to respond to increases in task difficulty, in terms of behavior, brain dynamics, and energy expended. This indicates that the effects of ID on the ability to respond to increases in mental workload are similar in many respects to the effects of ID on the ability to respond to increases in physical workload.[24,25,37–39] One potential difference between our findings and those on ID-related impairments in physical work is with respect to heart rate. Our data show reliably lower heart rates for ID relative to CN participants; however, both this ordering[38,39] and the reverse[25,40] have been observed, although in all cases these orderings were not reliable.

We found support for the idea that brain activity mediates the relationship between cognitive demands and energy expenditure. Of potentially greater interest is that we found support for the idea that iron status moderates the relationships between task difficulty and brain activity, and between brain activity and energy expenditure. Critically, two different measures of iron status played these moderating roles, with sFt moderating the relationship between task difficulty and brain activity, and Hb moderating the relationship between brain activity and energy expenditure. We

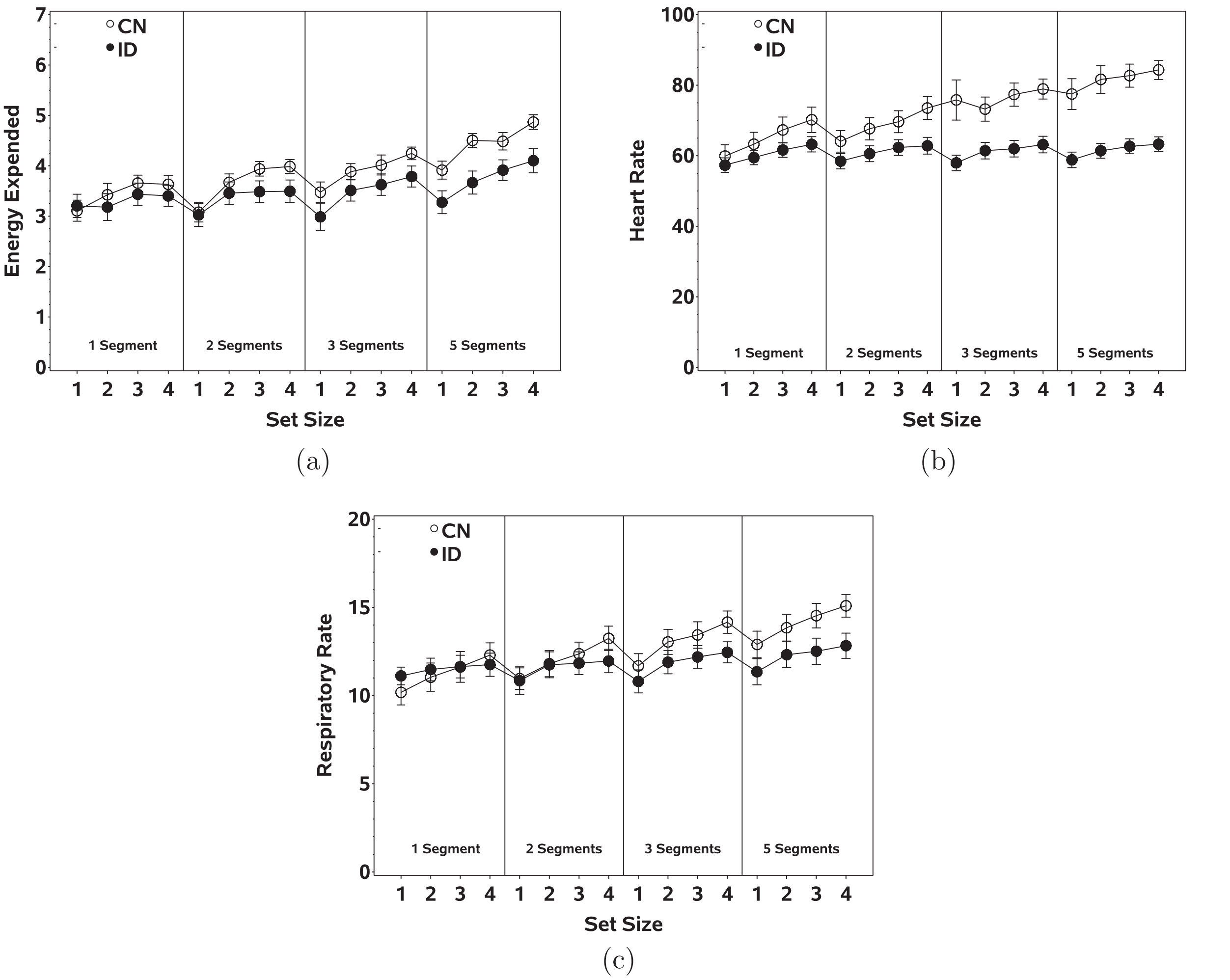

Figure 4 Means (± 1 standard error) for the metabolic data for the ID and CN subjects: (A) energy expended, (B) respiratory rate, (C) heart rate.
Note: Segments refers to the complexity of any one TBR item (Fig. 1), while set size refers to the total number of TBR items on a trial.

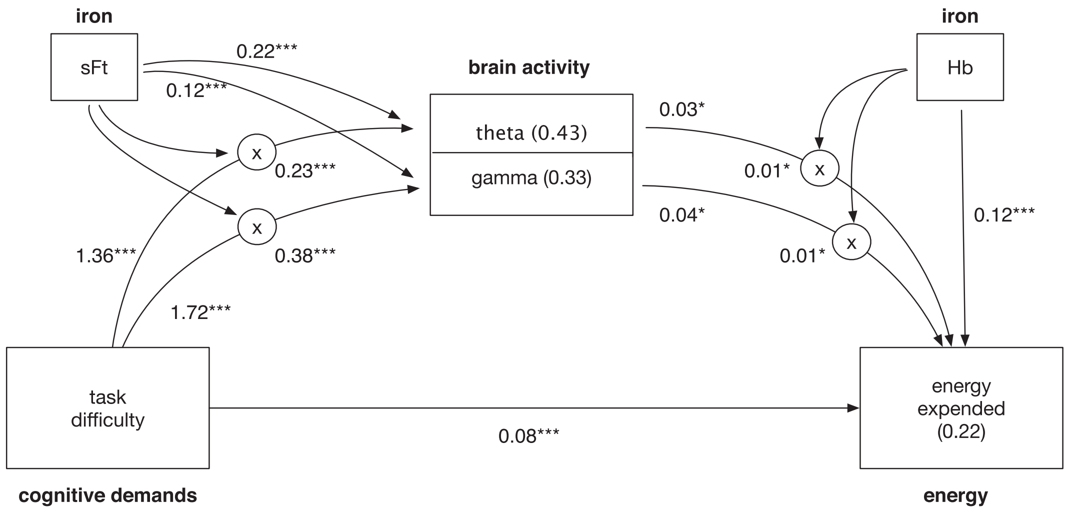

Figure 5 Model for the manner in which brain activity mediates the relationship between cognitive demands and energy expended, with iron moderating the relationships between cognitive demands and brain activity, and between brain activity and energy expended.
Note: numbers in parentheses indicate the proportion of variance accounted for in the prediction of the associated variable; numbers next to lines indicate estimated parameter values; numbers next to an 'x' enclosed in a circle indicate estimated parameter values for an interaction; $* = P < 0.05$, $** = P < 0.01$, $*** = P < 0.001$; sFt = serum ferritin, Hb=hemoglobin.

suggest that the effects due to sFt are indicative of the status of neurotransmitter regulation, potentially affecting the quality and efficiency of the neural computations whose overall execution was indexed by changes in power in the θ- and γ-bands. We suggest that the effects due to Hb are indicative of the status of an individual's energetic reserves by way, e.g. of capability for O_2 transport, and that this is indicative of the level of energetic resources needed to support increases in brain activity due to increased cognitive demands. Note that this is true for a sample of individuals whose Hb levels were in the normal (non-anemic) range. Although these possibilities require further investigation, the ability to potentially distinguish separable roles for these two aspect of iron status is a critical advance.

We also demonstrated that it is possible to obtain indirect estimates of brain energy use by simultaneous EEG and metabolic measures, combined with measures of an individual's iron status. This was accomplished in a situation in which the increases in cognitive demands produced changes in all of our metabolic variables. Given that there were no physical demands on subjects, it is reasonable to assume that the majority of the effects were due to increased demands on the brain, even with increases in heart rate. It is known that, even at rest, the brain is responsible for a much larger proportion of the total energetic requirements of the body, including the heart.[12,14,15] Consequently, having a way of indirectly estimating energy expenditure, using low-cost technologies, allows for increased precision in characterizing the neural effects of variations in iron status in a variety of populations. However, it should be noted that it would be useful to refine the approach used here to allow for a partialing-out of non-task-related increases in energy expenditure, such as any that might be attributable to sympathetic arousal.

There are a natural set of steps to be pursued, given these results. First, the measures of iron status obtained here are systemic; although they can be assumed to be proportional to measures of brain iron, the magnitude of that proportionality is not well understood, nor is the temporal relationship between changes in blood iron and changes in brain iron.[41] This suggests that combining these measures with estimates of brain iron from structural magnetic resonance imaging (MRI),[42,43] and measures of brain iron metabolism from MRI spectroscopy[44] could be quite informative. Further, measures of overall O_2 consumption are not specific to the brain, suggesting that a further refinement could be the addition of measures of cerebral oxygenation, such as might be possible with simultaneous EEG and functional near-infrared spectroscopy. Finally, the behavioral measures of performance used here can be augmented with more precise measures,[45] including those that directly index changes in performance to accuracy.[46] For any of these possible directions, we believe that a critical methodological detail is that the levels of cognitive demand need to span a range from those that produce moderate decreases in response accuracy and increases in response latency to those that reduce performance to chance. Much of the literature on the relationships between cognitive demands and metabolic measures of work has been hampered by manipulations that produce rather small changes in performance.[47–49]

In closing, we have shown that it is possible to characterize the relationships among cognitive demands, neural activity, and energy expended in the context of variations in iron status. Critically, we have shown that two distinct aspects of iron status – as indexed by sFt and Hb – play two distinct roles in these relationships. Finally, we have shown that it is possible to use measures on neural activity to assess variations in brain energy expenditure as a function of iron levels. This opens the door for questions regarding the role of iron in brain health and cognition to be asked with increased precision.

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Geolocation

The corresponding author is located at 35° 10' 58.03" N, 97° 26' 9.52" W. The work was performed at 42° 26' 59.02" N,

ORCID

Diane M. DellaValle http://orcid.org/0000-0002-3855-432X

Jere D. Haas http://orcid.org/0000-0002-4624-8488

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