Fetal iron deficiency alters the proteome of adult rat hippocampal synaptosomes

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iron deficiency, a highly prevalent micronutrient deficiency affecting more than 30% pregnancies worldwide (55), is a consequence of maternal iron deficiency anemia, hypertension, diabetes mellitus, or cigarette smoking during pregnancy (14, 22, 52, 65, 66). Fetal iron deficiency results in short- and long-term cognitive and behavioral abnormalities in humans including an increased risk of schizophrenia (27, 35). While iron treatment resolves some aspects of the acute developmental deficits including those in the sensory and motor domains, learning and affective behavior remain abnormal well beyond the period of deficiency (11, 15, 33, 35, 61, 64). These deficits have been replicated in rodent models of fetal-neonatal iron deficiency (35). Of relevance to the present study, hippocampal-dependent learning and memory as well as prepulse inhibition, an index of sensory-motor gating linked to the schizophrenia endophenotype, were abnormal in the rat model (10, 20, 35, 53, 67, 71).

The developing fetal brain is extremely susceptible to iron deficiency (44) in part because of the growth spurt that occurs during the late fetal-neonatal period (1, 54) and because fetal iron is prioritized over the brain for red blood cell production (21). Rodent models of maternal-fetal iron deficiency result in a 50% reduction in brain iron concentration by postnatal day 7 (57), a time point that approximates full-term human newborn in terms of brain development (1) and recapitulates the degree of iron deficiency found in newborns (52). Iron treatment starting at P7 resolves brain iron deficiency by P56 (56). The iron-deficient hippocampus has abnormal neuronal morphology (7, 30), glutamatergic neurotransmission (29), and reduced expression of genes critical for neural plasticity in the hippocampus (16, 68). Since an intact hippocampal structure is necessary for learning and memory (45, 59), these findings could underlie iron deficiency-induced learning, motor, and behavioral abnormalities seen in the model. More concerning, the abnormal gene expression, electrophysiology, and neuronal morphology persist in the adult rat hippocampus, potentially compromising synaptic structure and neurotransmission, and thus providing a partial explanation for the lasting learning disabilities found in the model (7, 29, 30, 60, 69). As such, it is important to determine whether the changes in gene expression seen in the whole hippocampus are localized to the synapses to provide more consistent proof of the putative long-term effect.

Quantitative proteomic analysis (isobaric tags for relative and absolute quantitation, iTRAQ) has been a useful approach to analyze global changes in expression of synaptic proteins in animal models of depression and with learning and memory deficits (32, 42, 74). Beyond the confirmation of previously reported iron deficiency-induced changes in suspected target proteins, the advantage of this approach is the discovery of novel molecules that allows a systematic analysis of protein networks. The present study employed the iTRAQ technique to quantify levels of proteins localized in the synapses derived from the iron-sufficient hippocampus of adult male rats that had been iron deficient as fetuses and compare them to an always iron-sufficient (IS) control group. The objective was to discover novel molecular markers and signaling pathways that potentially underlie the long-term learning and behavioral abnormalities reported in adult rats that experienced re-
covered from early iron deficiency. Whereas previous studies quantified long-term changes in expression of known substrates (e.g., BDNF, CXCL12, PSD-95) that are important for hippocampal plasticity (9, 69), the present study uniquely quantifies the changes in proteins expressed at the hippocampal synapses induced by early iron deficiency and following its resolution. As expected, the findings showed lower levels of postsynaptic proteins including NR2b and GluR1 that are important for synaptic long-term potentiation (LTP), a cellular basis of learning and memory (38, 40). Additionally, there were novel findings of higher levels of presynaptic proteins such as glutaminase and synapsin I; all are relevant for synthesis, delivery, and release of neurotransmitters (24, 47, 72). Pathway analysis uncovered novel pathways and neuropathologies that suggest ongoing recovery as well as an increased propensity for brain disorders. Finally, the parallel changes of protein expression in hippocampal lysates and synaptosomes suggest an overall defect of early iron deficiency in the programming of adult gene expression and not merely to a deficit in trafficking of synaptic proteins.

## MATERIALS AND METHODS

### Animals
The University of Minnesota Institutional Animal Care and Use Committee approved all experiments in this study. Gestational day 2 (G2) pregnant Sprague-Dawley rats were purchased from Charles Rivers (Wilmington, MA). Rats were kept in 12-h/12-h light/dark cycle with ad libitum food and water. Fetal-neonatal iron deficiency was induced by dietary manipulation as described previously (7, 9, 29, 30, 57, 60, 68–70). In brief, pregnant dams were given a purified iron-deficient (ID) diet (4 ppm Fe, TD 80396, Harlan Teklad, Madison, WI) from G2 to P7, at which time nursing dams were given a purified iron-sufficient (IS) diet (200 ppm Fe, TD 01583, Harlan Teklad) to generate ID pups. Control iron-sufficient (IS) pups (8 samples) were given a purified iron-sufficient (IS) diet (200 ppm Fe, TD 01583, Harlan Teklad) to which a contaminant database (thegpm.org/crap/index, 109 versions) database with canonical and isoform sequences (44394 proteins) was added. Control iron-deficient pups (IS) were generated from pregnant dams maintained on a purified iron-sufficient diet. Additionally, there was a duplicated group of IS dams that utilized the Paragon scoring algorithm to provide confidence levels for protein hits and the ProGroup algorithm tool (62) to group-related and homologous proteins. ProteinPilot searches were performed against the Paragon scoring algorithm to provide confidence levels for protein hits and the ProGroup algorithm tool (62) to group-related and homologous proteins. ProteinPilot searches were performed against the UniProt database (taxon 10114; April 21, 2012 version) database with canonical and isoform sequences (44394 proteins), to which a contaminant database (thegpm.org/crap/index, 109 proteins) was appended. Search parameters were cysteine MMTS, iTRAQ 8plex (Peptide Labeled), trypsin, and instrument Orbitrap MS (1–3 ppm) Orbitr MS/MS; biases corrections were applied to account for biological modifications including systematic errors in protein amount among samples, thorough search effort, and local 5% False Discovery Rate analysis (reversed database). Comparison of the results of protein search and identification are shown for the two datasets (Table 1).

### iTRAQ data treatment and selection
Two independent iTRAQ 8-plexes were performed using 6 IS and 6 FID hippocampal synaptosomal preparations. The first 8-plex was set up with following scheme: 113 (IS1), 114 (IS1), 115 (IS2), 116 (IS3), 117 (FID1), 118 (FID1), 119 (FID2), and 121 (FID3). The second 8-plex was similarly setup and used another set of IS(4, 5, and 6) and FID(4, 5, and 6)
biological replicates. In both data sets, iTRAQ label 113, 114, 117, and 118 served as technical replicates where proteins differing between 113 and 114 (or 117 and 118) with *P* < 0.05 were removed from further analysis. The *m/z* 113 of each dataset was used as denominators to generate ratio values (IS/IS and FID/IS) for each respective iTRAQ dataset. Proteins with <4 distinct peptides (95% confidence) or error factor (EF) >2.0 were eliminated from further analysis. These selection criteria resulted in 1,127 and 1,028 proteins from the first and second 8-plex data sets, respectively.

**Bioinformatics.** The knowledge-based Interactive Pathway Analysis (IPA, Ingenuity Systems, Redwood City, CA) was employed to identify networks, canonical pathways, molecular and cellular functions, and behavioral and neurological dysfunctions using proteins from the two iTRAQ data sets. IPA maps protein networks using algorithm based on molecular function, cellular function, and functional group. Proteins passed selection criteria from each data set were analyzed by the “Core” analysis specific to rat hippocampal tissue, an exclusion limit set at *P* = 0.05, and only direct relationship mapping. Ratio values were converted to fold change as follows: −1/x for 0.0 < *x* < 1.0 (e.g., 0.5 converts to −2.0, representing a twofold reduction), no conversion for *x* > 1.0. For FID/IS comparison (Supplemental Table S1), data set 1 (*m/z* 117, 119, 121 normalized by 113) and set 2 (*m/z* 118, 119, and 121 normalized by 113) were combined in IPA comparison analysis. Fisher’s exact test was used to calculate a *P* value determining the significance of the association between proteins in the data sets and analyzed pathways or functions.

**Western blot analysis.** To validate the iTRAQ findings, we used Western blot to quantify levels of selected proteins that function in the glutamate signaling pathway. These proteins are known to play critical role in mediating learning and memory in the rat hippocampus. Analyses were performed using a previously described protocol (70). In brief, 30 μg hippocampal protein lysate or 10 μg synaptosomal fraction were separated using a 4–20% SDS-PAGE gel (Novex, Life Technologies). Proteins were blotted onto a nitrocellulose membrane (Pierce, Rockford, IL), blocked with blocking buffer for fluorescent Western blotting (Rockland, Gilbertsville, PA) in a sealed plastic bag, and were analyzed by near infrared fluorescent using Odyssey Infrared Imaging System (Li-Cor Biosciences, Lincoln, NE). Integrated intensity of phosphor-protein normalized to total protein or protein of interested normalized to β-actin was determined using Photoshop CS5.1. Specific primary antibodies included P-GluR1 (1:1,000, Santa Cruz Biotechnology), GluR1 (1:1,000, Santa Cruz), NR2b (1:5,000, Santa Cruz), MAPK (1:5,000, Santa Cruz), GluR1 (1:1,000, Cell Signaling), MAPK (1:1,000, Cell Signaling), Synapsin-I (1:1,000, Chemicon), VAMP2 (1:1,000, Cell Signaling), and β-actin (1:2,000, Sigma). Secondary antibodies included Alexa-700 anti-mouse (1:12,500, Invitrogen) and IR Dye-800 anti-rabbit (1:12,500, Rockland). Unpaired *t*-test was used to calculate the mean difference between IS and FID groups.

## RESULTS

The present study utilized a quantitative proteomic approach to demonstrate differentially expressed proteins critical for intra- and intercellular signalings at the synapses of adult rat after recovery from early iron deficiency. The bioinformatics analysis uncovered novel pathways that could underlie the impaired synaptic transmission.

**Differential expression of proteins between FID and always IS control hippocampal synaptosomes.** Six biological replicates of IS and FID were used in two iTRAQ experiments, in which each identified over 1,900 unique proteins with a local false discovery rate of 5% (Table 1). Further analysis using exclusion criteria (see MATERIALS AND METHODS) resulted in 972 and 1,158 identified proteins in dataset 1 and 2, of which 836 and 1,015 proteins were functionally mapped by IPA, respectively (Fig. 1A). Comparison analysis of the IPA-mapped

### Table 1. Summary of iTRAQ results

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<tr>
<th>Data Level FDR Type</th>
<th>FDR</th>
<th>ID Yield</th>
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<td>Identification Yield at FDR Threshold</td>
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<tr>
<td></td>
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<tr>
<td>Corresponding Confidence in ProteinPilot Software</td>
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<tr>
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<td>Identification Yield at FDR Threshold</td>
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Qualities of protein, peptide and spectral identifications were compared between 5% local and 1% global false discovery rate (FDR) with corresponding confidence in ProteinPilot software.
FID/IS with P<0.05

**A**

<table>
<thead>
<tr>
<th>iTRAQ dataset</th>
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<tr>
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<tr>
<td>1 (Sample #1 – 3)</td>
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</tr>
<tr>
<td>2 (Sample #4 – 6)</td>
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</table>

**B**

Proteins analyzed by IPA

FID/IS with P<0.05

![Venn diagram](image_url)

**C**

<table>
<thead>
<tr>
<th>Protein</th>
<th>FID/IS</th>
<th>Total lysate</th>
<th>Synaptosome</th>
</tr>
</thead>
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<td>CamK2α/β-actin</td>
<td>0.91 (0.03)</td>
<td>0.64 (0.04)</td>
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<tr>
<td>NR2b/β-actin</td>
<td>0.62 (0.02)</td>
<td>0.83 (0.04)</td>
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<tr>
<td>Synapsin-I/β-actin</td>
<td>0.93 (0.78)</td>
<td>3.68 (&lt;0.01)</td>
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</tr>
<tr>
<td>Mbp/β-actin</td>
<td>1.05 (0.35)</td>
<td>0.60 (0.02)</td>
<td></td>
</tr>
<tr>
<td>VAMP2</td>
<td>0.68 (0.15)</td>
<td>0.65 (0.14)</td>
<td></td>
</tr>
<tr>
<td>MAPK/β-actin</td>
<td>1.22 (0.15)</td>
<td>1.18 (0.57)</td>
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</tr>
<tr>
<td>P-MAPK(T202)/MAPK</td>
<td>0.73 (0.07)</td>
<td>NC^a</td>
<td></td>
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<tr>
<td>P-GluR1(S831)/GluR1</td>
<td>0.53 (0.02)</td>
<td>0.60 (0.06)</td>
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</table>

Fig. 1. Validation analysis of isobaric tags for relative and absolute quantitation (iTRAQ) datasets. A: summary of identified proteins that were functionally mapped by Interactive Pathway Analysis (IPA). B: Venn diagrams showing protein overlap between two iTRAQ datasets with IPA-mapped proteins (right) and formerly iron deficient (FID)/iron sufficient (IS) comparison (left). C: Western blot quantified protein expressed in ratio of FID/IS with P value (in parentheses), unpaired t-test, n = 4–7/group. *Not computable due to negligible phosphoprotein level.

Proteins from the two datasets showed a greater than 69% overlap (Fig. 1B, left). With the use of an exclusion of criteria of P < 0.05, IPA analysis uncovered 331 differentially expressed protein between FID and IS synaptosomes (Supplemental Table S1) with at least 79% protein overlap between the two datasets (Fig. 1B, right). Western analysis confirmed the decreased levels of NR2b, CamK2a, Mbp, and increased level of presynaptic proteins Synapsin-I in the FID group (Fig. 1C). To provide biological meaning (e.g., molecule transport or trafficking) to these molecular changes, we also quantified selected proteins in total hippocampal protein lysates (Fig. 1C).

Except for Mbp and Synapsin-I, FID hippocampus showed lower levels of analyzed proteins in both total lysate and synaptosomal fraction. To determine further whether the reduced synaptic protein expression might lead to an increase in glutamate receptor activation and signaling, we analyzed levels of phosphor-GluR1 and phosphor-MAPK expressed as a ratio to their total protein level. Both showed lower levels of the phosphorylated protein in the synaptosomal fraction and hippocampal lysate, suggesting suppression of this signaling system in the FID hippocampus (Fig. 1C).

**Altered cellular and molecular properties in FID hippocampal synapses.** To reveal the biology associated with the changes of synaptic proteins identified by iTRAQ, we performed bioinformatic analysis using IPA, which clusters proteins based on molecular function, cellular function, and functional group. Comparison analysis identified important molecular and cellular functions that differed significantly between the groups (Fig. 2) including expected changes in cell-to-cell signaling, nervous system development and function, and cellular development and morphology. Novel altered functions included embryonic development and cellular assembly and organization. Unaffected functions included molecular transport, vitamin and mineral metabolism, and organ development (Fig. 2). Differentially expressed proteins with both molecular and cellular function typically were those involved in synaptogenesis (Supplemental Table S2). To further identify potential cellular dysfunction caused by early-life ID, iTRAQ data were organized into functional groups. The FID hippocampus showed decreased activity of mitochondrial machinery, glutamate receptor signaling, and cAMP response element-binding protein (CREB) signaling accompanied by increased glycolysis, gluconeogenesis, 14-3-3 signaling, and P70S6K signaling (Supplemental Table S3).

**Altered canonical pathways critical for LTP in FID hippocampus.** Proteins that are critical for efficient synaptic transmission were differentially expressed in FID and IS synaptosomes. We used IPA to categorically organize proteins into known functional pathways. When compared with IS, the FID hippocampus showed decreased glutamate receptor signaling and neuronal nitric oxide (NO) synthase (NOS) signaling (Fig. 3) concomitant with increased p70S6K signaling and 14-3-3-mediated signaling (Fig. 4). Unaffected pathways include CXCR4/RhoA, IGF1, HIF1α, thyroid hormone receptor, and VEGF (data not shown).

**Putative behavioral and neurological dysfunctions associated with FID hippocampus.** We used IPA to identify behavioral and neurological disorders associated with differentially expressed synaptic proteins in the FID hippocampus. Among these, the top behavioral and neurological disorders included learning, emotional and social behaviors, major depression, schizophrenia, and Alzheimer’s disease (Supplemental Table S4).

**DISCUSSION**

Previous studies in humans and animal models of fetal and early postnatal life iron deficiency demonstrate long-lasting impairments in learning and memory and socio-emotional behaviors (18, 20, 35, 60, 71), as well as increased risk for depression and schizophrenia (27, 37). The long-term behavioral deficits occur despite treatment with iron and have been ascribed in part to persistence of abnormalities in monoamine signaling, myelination, neural metabolism, and expression of neuroplasticity-associated proteins into adulthood (4, 17, 56, 69). The present study goes beyond previous studies by...
systematically analyzing the long-term effect of early-life iron deficiency specifically on proteins expressed in adult hippocampal synapses and addresses the persistent defect of learning and memory and sensory-motor gating. Importantly, it is an exploratory study that forms the bases for further targeted investigations. The study revealed reduced concentrations of multiple individual synaptic proteins, many of which are key to optimal synaptic efficacy. These findings are the first to provide molecular and cellular mechanisms at the level of the synapse to explicate the known persistent learning and behavioral abnormalities in adult rodent model following early iron deficiency (20, 60, 71). Pathway analysis allowed us to confirm and extend previously suspected and to uncover previously unsuspected negative effects of early-life iron deficiency on signaling pathways that play critical roles in adult cognitive function. Identification of these signaling pathways may allow more specific manipulations to alter their activity to demonstrate the causal effects of early iron deficiency on expression of synaptic proteins as well as to establish targets for therapeutic development. The findings validate a lasting repressive effect of early iron deficiency on specific synaptic gene expression. This provides insights into the susceptibility of brain disorders in the lifespan of subjects that experienced early-life iron deficiency.

A major finding of this study was the lasting negative impact of early-life iron deficiency on a wide array of molecules involved in glutamatergic neurotransmission at the hippocampal synapses. This is the first report of the long-term effect on
the expression of important mediators of the CREB signaling pathway, which is critical for LTP (40, 43). The new findings substantiate previous studies that showed 11% less hippocampal LTP (29), reduced hippocampal mRNA level of CamKIIa (−29%), PSD-95 (−28%), and VAMP1 (−19%) (9), reduced PKC-γ protein expression (23), 12% less hippocampal volume (56), and altered synaptic morphology (7) in this model. It is noteworthy that whereas all of the previous analyses were performed in whole hippocampus, the current study expands these findings by uncovering more proteins that are affected and localizes the defects to the synaptosome. For example, the new finding of reduced expression of the glutamate receptors (AMPAR and NMDAR) in both the synaptosomal fraction and hippocampal homogenate is in line with the long-term impaired synaptic transmission, given their important roles in mediating synaptic LTP (38, 40). While there is an ongoing debate regarding the specific recruitment of glutamate receptor subtypes that is critical for regulating synaptic activity underlying hippocampal LTP (26, 41), these findings implicate both AMPAR and NMDAR in a nondiscriminatory manner. The findings of lower levels of phosphor-GluR1 and phosphor-MAPK, which index glutamate receptor activity, are consistent with the hypothesis of reduced glutamatergic signaling. The greater magnitude of defects seen specifically in the synaptosome compared with the whole hippocampal lysate points to a site of action and implies functional significance.
Fig. 4. Canonical pathways with increased activity include p70S6K signaling (A) and 14-3-3 signaling (B). Data show proteins with decreased level (green) and increased level (red).
The iTRAQ methodology facilitated the discovery of novel components of impaired glutamatergic neurotransmission. For example, the reduced expression of presynaptic neuron (SLC1A1/4) and glial (SLC1A2/3) glutamate transporters may reflect a compensatory mechanism to keep additional glutamate in the synapse and increase the probability of binding the (decreased) numbers of glutamate receptors. This type of response in the reuptake mechanism has been postulated in the dopaminergic system with iron deficiency, where decreased levels of dopamine transporter (DAT) are thought to increase synaptic cleft dopamine in the nucleus accumbens (3). Upregulated glutaminase (GLS) (Fig. 3A) indicates that the presynaptic neuron may be attempting to compensate as well by synthesizing more glutamate. Although a previous report showed no change in intracellular level of glutamate by metabolomic analysis (56), in the present study, we were not able to elucidate the etiology of apparently less synaptic glutamate by this analysis, but the upregulation of GLS and synaptic vesicle fusion protein (Synapsin) suggest that fusion of the synaptic vesicle and release of the neurotransmitter from the presynaptic membrane may be impaired.

Another previously unidentified aspect of impaired glutamatergic signaling in FID hippocampus is the dysregulation of NOS activity. NO is an important signaling molecule for neural plasticity as well as iron uptake in the brain and is regulated by glutamate activation of the NMDA receptor (6, 12, 13, 49). Given that iron-containing heme is a cofactor for NOS, it is not surprising that iron deficiency affects NOS activity (25, 48, 51), which in turn could affect signaling pathways by which iron mediates synaptic plasticity (46). The present study utilized pathway analysis to reveal potentially reduced NOS activity even after the hippocampus was fully recovered from iron deficiency.

In addition, we discovered evidence of increased gluconeogenesis and glycolysis as well as cellular growth (p70S6K pathway). The increased activity of these particular pathways is rather surprising in light of evidence suggesting a reduced mitochondrial function as well as previous neurochemical findings that suggest a general suppression of energy metabolism in whole hippocampus of FID rats (56). While not necessarily contradictory, the finding suggests an increase in substrate (e.g., glucose) utilization and synthesis, and perhaps reflects the abnormal state of glutamine-glutamate cycling in FID hippocampus (39, 63). On the other hand, acute iron deficiency in the adult rat leads to increased glucose uptake by brain, suggesting dysregulated brain glucose homeostasis (5). Thus our findings demonstrate a long-term dysregulation of glucose homeostasis in early ID that is likely undergoing recovery to support cellular growth and maintenance. It is noteworthy that the 14-3-3 signaling pathway was also upregulated (Fig. 4B), which could serve to promote cellular survival as well as a braking mechanism by keeping cellular growth in check (19, 28, 31).

Ultimately, it is unclear whether these persistent abnormalities in young adulthood will remain throughout the lifespan. Human cohorts with early-life iron deficiency have now been studied into young adulthood and continue to show neurocognitive deficits (37). The P65 rat used in this study would be an appropriately aged model for these cohorts. However, the age (P56) at which iron was completely repleted in this model (56) occurs likely at a later developmental stage than in the human treated with iron shortly after birth. Thus our P65 findings may still be indexing recovery or possible compensatory responses from early-life iron deficiency. Additional study of hippocampal synapses at a later time point in adulthood will be necessary to resolve these possibilities. On the other hand, a number of proteins that are perturbed in the acutely ID hippocampus are no longer different from IS control at P65 and thus provide evidence for recovered pathways following iron treatment and repletion in our model. The unaltered signaling pathways include Cxcr4/Rh0A, IGF1, thyroid hormone receptor, and HIF1a (data not shown), all of which are altered during the period of iron deficiency (2, 7, 70).

Finally, the analysis of neurological diseases and behavioral dysfunctions associated with the altered synaptic proteins in FID hippocampus (Supplemental Table S4) suggests that early-life iron deficiency increases the propensity for a host of adult brain disorders that have been documented in human adult and animal models (18, 20, 60). The current study pinpoints the molecules or substrates that could underlie these abnormalities. For example, cognitive disorders that involve dysregulation of both glutamatergic and NOS pathways include Alzheimer’s disease, bipolar disorder, depression, and schizophrenia (Supplemental Table S4). The incidence of the latter is increased in a dose-dependent manner in offspring of iron-deficient mothers (27). By identifying these pathways, therapeutic targeting (besides iron treatment) may become evident. While it is likely that the long-term abnormalities are due to early-life iron deficiency anemia, an alternate possibility is that iron treatment may have led to the increased risk of these neuropathologies. A recent study utilized various doses of iron supplementation to treat early iron deficiency in the rat model suggests adverse effects associated with a high iron dose (58, 71). It is possible that the dose of iron supplementation used in this study could be too high and thus had negatively contributed to the risk of neuropathologies. Clearly, additional studies will be needed to resolve this concern.

Perspectives and Significance

Early-life iron deficiency is a major clinical condition with substantial economic loss associated with long-lasting learning, emotional, and behavioral disabilities despite prompt iron treatment. The discovery of long-term changes in synaptoposomal substrates and related molecular pathways in the present study may have important implications for the development of adjunctive therapies. Although prenatal and postnatal iron treatments show evidence of partial recovery of motor and mental development (11, 15, 33, 36, 61), the continued deficits in cognitive function and emotional behavior are concerning. Thus, there is a real need to identify biomarkers that allow timely iron status diagnosis for more effective intervention. In addition to providing putative molecular targets for the development of adjunctive remedial therapy, the quantitative proteomic approach in the present study corroborates and extends significantly the proteomic findings from cerebrospinal fluid and thus establishes a practical method augmenting the identification of biomarkers for iron status evaluation.

ACKNOWLEDGMENTS

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REFERENCES


