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Iron Deficiency with or without Anemia Impairs Prepulse Inhibition of the Startle Reflex

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Abstract

Iron deficiency (ID) during early life causes long-lasting detrimental cognitive sequelae, many of which are linked to alterations in hippocampus function, dopamine synthesis, and the modulation of dopaminergic circuitry by the hippocampus. These same features have been implicated in the origins of schizophrenia, a neuropsychiatric disorder with significant cognitive impairments. Deficits in sensorimotor gating represent a reliable endophenotype of schizophrenia that can be measured by prepulse inhibition (PPI) of the acoustic startle reflex. Using two rodent model systems, we investigated the influence of early-life ID on PPI in adulthood. To isolate the role of hippocampal iron in PPI, our mouse model utilized a timed (embryonic day 18.5), hippocampus-specific knockout of *Slc11a2*, a gene coding an important regulator of cellular iron uptake, the divalent metal transport type 1 protein (DMT-1). Our second model used a classic rat dietary-based global ID during gestation, a condition that closely mimics human gestational ID anemia (IDA). Both models exhibited impaired PPI in adulthood. Furthermore, our DMT-1 knockout model displayed reduced long-term potentiation (LTP) and elevated paired pulse facilitation (PPF), electrophysiological results consistent with previous findings in the IDA rat model. These results, in combination with previous findings demonstrating impaired hippocampus functioning and altered dopaminergic and glutamatergic neurotransmission, suggest that iron availability within the hippocampus is critical for the neurodevelopmental processes underlying sensorimotor gating. Ultimately, evidence of reduced PPI in both of our models may offer insights into the roles of fetal ID and the hippocampus in the pathophysiology of schizophrenia.

Keywords

Hippocampus; long term potentiation; murine; schizophrenia; dopamine

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INTRODUCTION

Iron deficiency (ID) represents one of the most prevalent nutrient deficiencies, affecting roughly two billion people worldwide (Benoist et al., 2008). Early-life ID, whether present at birth (Siddappa et al., 2007) or acquired during early infancy (Lozoff et al., 2006), exerts a panoply of detrimental effects on brain development, including alterations in neuronal metabolism (deUngria et al., 2000), monoamine synthesis and neurotransmission (Kwik-Uribe et al., 2000; Beard et al., 2003), and myelination (Yu et al., 1986). Such neuropathologies contribute to cognitive, sensory, and affective deficits that persist regardless of and well beyond the period of therapeutic iron repletion (Algarín et al., 2003; Lukowski et al., 2010; Fretham, 2011).

Several lines of convergent evidence now suggest that early-life ID anemia (IDA) may influence the development of many of the underlying neural systems implicated in schizophrenia, a neuropsychiatric disorder in part characterized by cognitive, sensory, and affective impairments. For one, experimental studies have documented that synthesis and neurotransmission of dopamine, a key monoamine in schizophrenia pathophysiology, is altered in IDA rats (Ben-Shachar et al., 1986; Youdim et al., 1989). Fetal/neonatal IDA in the rat has also been shown to preferentially target the hippocampus (Georgieff, 2008; Brunette et al., 2010; Rao et al., 2013), a region highlighted in schizophrenia neuropathology (Saykin et al., 1991; Harrison, 1999). Disruption of hippocampal processes may in turn impair glutamatergic signaling, including between the ventral subiculum of the hippocampus and nucleus accumbens (NAc) and associated mesolimbic circuitry (Legault and Wise, 1999; Floresco et al., 2001; Lodge and Grace, 2008). Thus, in addition to direct effects on dopamine synthesis, IDA may influence dopaminergic neurotransmission via hippocampus-specific dysfunction. This same circuitry has been posited to underlie the pathophysiology of schizophrenia (Bogerts et al., 1985; Luchins, 1990; Lodge and Grace, 2008), particularly its positive symptoms (i.e., disorganized thoughts, hallucinations, and delusions) (Saykin et al., 1991; Kriekhaus et al., 1992; Venables, 1992).

The relationship of this circuitry to the positive symptoms of schizophrenia has been investigated through the behavioral phenomenon of prepulse inhibition (PPI) (Wan and Swerdlow, 1996; Swerdlow et al., 2008). PPI provides an operational measure of sensorimotor gating, in which a relatively weak stimulus (the prepulse) inhibits the motor response to a stronger, startle-eliciting stimulus (Graham, 1975). PPI deficits represent a reliable endophenotype of schizophrenia; that is, both individuals with schizophrenia and – to a lesser extent – relatives of individuals with schizophrenia exhibit PPI deficits (Braff and Light, 2005; Quednow et al., 2008). Mechanistically, PPI is regulated by subcortical regions comprising the cortico-striato-pallido-pontine circuit (Koch and Schnitzler, 1997; Swerdlow et al., 2001a). Integral to this regulation are glutamatergic projections from the ventral subiculum of the hippocampus to the NAc (Wan et al., 1996; Bakshi and Geyer, 1998; Swerdlow et al., 2001a) and frontal cortex (Saint Marie et al., 2010). As noted already, these same circuitry components are postulated to be dysregulated by IDA. Therefore, employing PPI to assess deficits in sensorimotor gating may lend insights into common, underlying pathophysiology of IDA and the positive symptoms of schizophrenia.

This study aimed to further elucidate the effects of generalized IDA and isolate the effects of hippocampus-specific ID on PPI. Two previously published rodent model systems were employed for this purpose. The first was a mouse hippocampal-specific knockout of the *Slc11a2* gene coding the divalent metal transport type 1 protein (DMT-1) (Carlson et al., 2009). Along with transferrin receptor-1, DMT-1 regulates the transport of iron across the blood-brain barrier and its uptake into neurons and glial cells (Burdo and Connor, 2001). This model exhibits shorter hippocampal CA1 apical dendrites, disorganized apical dendritic

architecture, and impaired spatial memory (Carlson et al., 2009). The second model was the extensively characterized and traditionally utilized rat gestational-early lactational dietary IDA model (Felt and Lozoff, 1996; Jorgenson et al., 2003; Rao et al., 2011). This dietary model was chosen to complement the genetic model because it closely recapitulates the human condition where ID results in total brain ID and anemia. The IDA rat exhibits impaired memory (Felt and Lozoff, 1996; Schmidt and Waldow, 2007) as well as abnormal sensorimotor development via greater dopaminergic activity within the striatum (Unger et al., 2007). Given the collective evidence of the commonalities between the neuropathology of ID and the positive symptoms of schizophrenia, it was predicted that PPI would be disrupted in both models with the mouse providing specific evidence for the importance of hippocampal iron in the process. Additionally, electrophysiological measurements were collected in the hippocampal-specific mouse model to provide a potential neural correlate of the predicted effects of ID on PPI. It was hypothesized that these measurements would mirror previous electrophysiological findings in the rat IDA model, which include reduced adult LTP and PPF (Jorgenson et al., 2005; McEchron et al., 2010).

MATERIALS AND METHODS

Animals—All experiments were performed in accordance with the NRC Guide for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee of the University of Minnesota. Animals were housed in a humidity-controlled (50 $\pm$ 10%) room at 23 $\pm$ 1°C on a 12 hour light/dark cycle. All animals were transferred to a conventional housing area for testing. Experimental animals were allowed access to food and water *ad libitum* between testing. Only male animals were analyzed.

Mice—*Slc11a2^{hipp/hipp}* (DMT-1 KO) and *Slc11a2^{WT/WT}* (WT) mice were bred as described previously (Carlson et al., 2009). Briefly, *Slc11a2^{flox/flox}* mice (Gunshin et al., 2005) were crossed with *CaMKIIa-Cre* (L7ag#13 line (Dragatsis and Zeitlin, 2000)) transgenic mice to generate hippocampal pyramidal cell-neuron specific knockout of *Slc11a2* induced at E18.5. Genotypes were confirmed by PCR of individual mouse-tail DNA using previously published parameters (Carlson et al., 2009). DMT-1 KO mice have a 40–50% reduction of hippocampus-specific iron concentration at P90 (Carlson et al., 2009). Separate cohorts of mice were used in PPI and behavioral (i.e., open field and elevated plus-maze) experiments.

Rats—Sprague-Dawley dams from Charles River Laboratories (Wilmington, MA) were obtained within 1–2 days of plug-positive evidence of pregnancy in order to generate the classic dietary model of gestational-early lactational IDA in the rat (Rao et al., 2003; Brunette et al., 2010; Schmidt et al., 2010). At gestational day two, half of the dams were given an iron-sufficient diet (198 mg of elemental Fe/kg chow; Harlan Teklad; Madison, Wisconsin); the remaining dams were given a low-iron diet (3–6 mg of elemental Fe/kg chow; Harlan Teklad). Litters were culled to eight pups two or three days after birth. Dams given a low-iron diet were returned to a standard, iron-sufficient diet on postnatal day (P) 7. Pups of ID dams display significantly reduced iron levels at P7, as indicated by a 53% reduction in hematocrit and a 61% reduction in brain iron concentration (Schmidt et al., 2010). This dietary model induces a similar degree of brain ID as found in newborn iron-deficient humans (Petry et al., 1992). All rats were tested at adulthood (P100–120), a time point at which iron has returned to normal levels (Jorgenson et al., 2003; Schmidt et al., 2010; Rao et al., 2011).

Prepulse Inhibition Mice

Mice—Four identical Plexiglas startle cages (8.6cm $\times$ 7.6cm $\times$ 5.1cm; Med Associates, St. Albans, VT) situated within individual sound-attenuating chambers were used. Each startle

chamber was affixed to a Plexiglas frame attached to a load cell transducer. Force was measured and transformed into electrical signal, which was amplified and recorded by a computer and interface ensemble (Med Associates Inc., St. Albans, VT).

PPI experiments were performed as previously described (Sun et al., 2010). Each mouse was placed in a startle chamber and allowed 5 minutes acclimation. A single block of startle stimuli was then presented, followed by ten blocks of sixteen balanced and pseudorandomized trial types – prepulse alone, prepulse with startle, startle alone, and no stimulus. Three different pulse intensities were used (100, 110, and 120dB) in conjunction with four prepulse intensities (68, 71, 77, and 83dB). The average startle amplitude was calculated across all startle alone trials.

The inter-trial intervals averaged 25s (range 20–30s). All stimuli had a rise time of 3ms. Data were recorded over a 500-ms time bin occurring 300ms prior to pulse onset to 200ms after pulse onset. Movement produces a sinusoidal motion pattern, so startle amplitudes were taken from maximum peak to minimum peak of the sinusoidal response. Background noise was set to 65dB.

Rats—Four identical, custom-built Plexiglas cages (17cm × 8.5cm × 11cm) resting on compression springs and located within individually ventilated, sound-attenuating chambers were used. Cage displacement was recorded by a piezoelectric accelerometer (Model ACH-01, Measurement Specialties, Valley Forge, PA). Voltage output was filtered and amplified by a custom-built signal processor, digitized on a scale of arbitrary units ranging from 0–1000 (National Instruments SCB100 and PCI-6071E boards), and recorded using MATLAB (The MathWorks, Inc., Natick, MA).

Baseline acoustic startle was tested on two consecutive days before PPI testing. For each baseline session, rats were placed in the startle chambers for a 5-min acclimation period, and then presented with 40 startle stimuli (20 each at 95 or 105dB in a pseudorandomized order) with a 30s inter-stimulus interval. PPI experiments were performed as described elsewhere (Ellenbroek et al., 1996). Each rat was placed in the startle chamber and allowed five minutes acclimation. Five baseline startle trials were then presented, along with prepulse alone blocks of five trials each. Then, five blocks of each prepulse level (70, 74, 76, 78dB) with startle, startle alone (105dB), and no stimulus were presented pseudo-randomly. Finally, a single block of five startle stimuli was presented. The average startle amplitude was calculated across all startle alone trials.

Startle amplitude was defined as the maximum peak-to-peak accelerometer voltage during the first 200ms after onset of the startle stimulus. High-frequency speakers (Radio Shack Supertweeters, range = 5–40kHz) located 10cm beside each cage delivered the startle stimuli, which were 50ms bursts of filtered white noise (low pass: 22 kHz, rise-decay of 5ms). Ventilating fans elevated background noise to 60dB. For all animals, percent PPI was calculated using the following formula:

$$|(\text{pulse alone trial} - (\text{pulse with prepulse trial}) / (\text{pulse alone trial}))| \times 100$$

Electrophysiology

Hippocampal slices were prepared from mice of 8–10 weeks of age, following standard procedures (Zhao et al., 2011). Briefly, mice were anesthetized by a lethal dose of a ketamine and xylazine mixture and perfused through the heart with ice-cold solution containing the following (in mM): 240 sucrose, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 0.5 CaCl₂, and 7 MgCl₂, saturated with 95% O₂/5% CO₂ before freezing. Both hemispheres were then removed quickly and 350µm slices were prepared with a vibratome. After incubation in a holding chamber for at least 1 hour at room temperature, slices were

transferred into the recording chamber. In the recording chamber, slices were constantly perfused at near-physiological temperature (30–31°C) with a CSF solution containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 2.0 CaCl₂, 1.0 MgCl₂, and 10 dextrose. For field excitatory postsynaptic potential (fEPSP) recordings, a CSF-filled glass pipette with approximate resistance of 1–2 Mohm was placed in the striatum radiatum of CA1, and a tungsten bipolar electrode was positioned to stimulate the Schaffer collaterals. Signals were recorded using a Dagan amplifier. Input/output (I/O) curves were constructed by plotting fEPSP amplitude as a function of stimulus intensity. To measure paired-pulse facilitation, a series of paired pulses were delivered, separated by intervals of 20, 40, 80, 160, 300, and 500ms. LTP was induced using a theta burst protocol in which 5 EPSPs were evoked at 100Hz to form a burst and repeated 10 times at 5Hz. Stimulus current was adjusted so the fEPSP slope was 40–50% of the maximum and test pulses delivered every 30s.

Behavior

Open Field Task—Open field experimentation and analysis was conducted as described previously (Sun et al., 2010). The open field consisted of a Plexiglas box (50cm × 50cm × 40cm) into which each mouse was carefully introduced at one of the corners and allowed to explore for 15 minutes. The box contained a novel object (a bottle cap adhered to the ground with an odorless adhesive), such that responses to novelty could also be assessed. Average velocity, distance traveled, percent time spent in field center (using an imaginary square that made up 50% of total area of the box), and time spent sniffing the novel object were analyzed using the TopScan software system (Clever Systems Inc., Reston, VA).

Elevated Plus-Maze—Elevated plus-maze experimentation and analysis was conducted as described previously (Walf and Frye, 2007). The apparatus was composed of two opposing open arms (30×5cm) and two opposing closed arms (30×5cm), each extending from a central square platform (5×5cm). The apparatus was elevated 75cm above the floor. Mice were placed on the center platform and allowed to explore the maze for 5 minutes before returning to their home cage. The following parameters were analyzed using the TopScan software system (Clever Systems Inc., Reston, VA): number of entries into each arm, time in each arm, and risk assessment of the open arms from the protected area. Risk assessment was defined as when the mouse stopped at the entryway into one of the arms.

Statistical Analysis

For all startle experiments, residual analysis was completed to detect potential outliers. In each case, a studentized deviate test indicated values >2SD from means to be significant, and individual subjects corresponding to significant residual values were removed from analysis. No outliers were removed in mice experiments; one outlier from both IDA and iron sufficient groups were removed in rat experiments. Unpaired t-tests were used to compare treatment effects for all behavioral tests and extracellular field recording with a Type I error rate of $\alpha = 0.05$ (two-tailed). Two and three-way ANOVAs were also conducted to analyze interactions between treatment effects, prepulse levels, and – in mice experiments – pulse levels. Iron status acted as the between group variable; prepulse or pulse intensity acted as the within group, repeated measure.

RESULTS

DMT-1 KO mice display impaired PPI

The effect of early-life ID on PPI has not been reported in the DMT-1 KO model of hippocampus-specific ID. The basal startle reflex averaged across all pulse levels did not differ between DMT-1 KO and WT groups (DMT-1 KO, 8.09 ± 0.742 units, n=15; WT,

8.33 $\pm$ 0.605 units, $n=13$; ns), nor was there a main effect of pulse level on PPI or interaction effect between pulse and prepulse levels (data not shown). Thus, all pulse levels were averaged together in order to compute PPI. However, as has been documented previously (Yee, 2005), we observed substantially elevated startle at the 83dB prepulse level alone compared to baseline activity ($t(31)=4.47$; $p<0.0001$), as well as compared to 77dB($t(31)=2.99$; $p=0.005$), 71dB($t(31)=4.12$; $p=0.0002$), and 68dB($t(31)=2.86$; $p=0.007$) levels. This prepulse level was therefore removed from further analyses. As expected, intensity of the prepulse level significantly affected PPI, with higher intensity prepulses producing greater PPI ($F_{2,250}=97.36$; $p<0.0001$) (Fig. 1). There was an overall significant reduction in PPI in the DMT-1 KO group compared to the WT mice group ($F_{1,251}=12.02$; $p=0.0006$). Studentized t-tests designed to analyze treatment effects at each prepulse level demonstrated significantly reduced PPI in the DMT-1 KO group at the 71dB ($t(81)=2.77$; $p=0.007$) and 77dB ($t(81)=3.17$; $p=0.002$) levels.

DMT-1 KO mice show anxiety-like features in the open field test and elevated plus-maze

Open field and elevated plus-maze tasks were completed on the DMT-1 KO mouse to investigate motor functioning and anxiety. In the open field test, no differences in total velocity (DMT-1 KO, 49.93 $\pm$ 3.39mm/s, $n=15$; WT, 52.34 $\pm$ 4.04mm/s, $n=15$; ns) or total distance (DMT-1 KO, 44.89 $\pm$ 3.05m, $n=15$; WT, 47.05 $\pm$ 3.63m, $n=15$; ns) were observed, indicating that DMT-1 KO mice do not exhibit disparate motor activity. Furthermore, both groups displayed velocities that remained nearly constant throughout individual trials, providing further evidence that both groups exhibit similar motor adaptation. When exposed to a novel object, DMT-1 KO mice trended toward a reduced number of times investigating the object compared with WT mice (DMT-1 KO, 8.73 $\pm$ 1.39, $n=15$; WT, 12.33 $\pm$ 1.60, $n=15$; $p=0.10$). Interestingly, the DMT-1 KO mice spent more time in the center of the arena ($t(29)=2.63$; $p=0.014$) demonstrating a lower level of anxiety-like behavior than their WT counterparts (Fig. 2A).

In the elevated plus-maze test, DMT-1 KO mice spent a nearly equivalent percentage of total time within the open arms (DMT-1 KO, 36.27 $\pm$ 5.66%, $n=12$; WT, 38.83 $\pm$ 8.78%, $n=9$; ns), as compared to WT mice. However, when the number of open and closed arm entries were quantified, DMT-1 KO mice showed a significantly greater percentage of entries into the open arms (DMT-1 KO, 45.18 $\pm$ 2.54%, $n=12$; WT, 35.68 $\pm$ 1.99%, $n=9$; $p=0.01$) (Fig. 2B). Together with data collected from the open field test, these results suggest that DMT-1 KO mice display reduced anxiety-like behavior.

DMT-1 KO mice exhibit alterations in electrophysiological measures of synaptic plasticity

Long-term potentiation (LTP) was tested in the DMT-1 KO mice in order to assess the effect of hippocampus-specific ID on synaptic plasticity. At P65, in response to a single train of theta burst stimulation (TBS), the magnitude of potentiation 60min post-induction was significantly reduced in DMT-1 KO mice compared with WT (DMT-1 KO, 125 $\pm$ 8.5%, slice $n=8$, animal $N=5$; WT, 166 $\pm$ 9.6%, slice $n=10$, animal $N=6$; $p=0.006$; Fig. 3A,B). This deficit in synaptic plasticity was not due to impairment of basal synaptic transmission as the input-output curves were similar between genotypes (Fig. 3C). Paired-pulse facilitation (PPF), a form of short-term plasticity dependent on pre-synaptic functioning, was also significantly elevated at time intervals 20ms ($p=0.02$), 40ms ($p=0.04$), and 80ms ($p=0.045$; Fig. 3D) in DMT-1 KO mice compared with WT (DMT-1 KO, slice $n=7$, animal $N=6$; WT, slice $n=10$, animal $N=8$).

Gestational IDA rats display reduced PPI

PPI experiments were conducted to ascertain whether IDA in the rat gestational-early lactational model could contribute to similar deficits as observed in DMT-1 KO mice. No

significant differences were observed in baseline startle responses between IDA and iron sufficient (IS) rats (IDA, 0.328 $\pm$ 0.027, n=23; IS, 0.324 $\pm$ 0.024, n=18; ns), as reported previously (Gewirtz et al., 2008). As expected, there was a significant effect of prepulse level on PPI ($F_{3,160}=3.86$; $p=0.01$), with larger prepulse levels producing greater PPI (Fig. 4). There was also a significant overall effect of iron status on PPI ($F_{1,161}=9.73$; $p=0.002$). Studentized t-tests designed to analyze treatment effects at each prepulse level demonstrated significantly reduced PPI in the IDA group at the 70dB level ($t(40)=2.45$; $p=0.02$).

DISCUSSION

Early-life ID is known to exert a range of independent, deleterious effects on the rapidly developing hippocampus and dopamine systems, resulting in residual cognitive impairments that persist after iron repletion. The goal of this study was to assess the effects of ID on PPI, an operational measure of sensorimotor gating, using two separate rodent models – the hippocampus-specific ID DMT-1 KO mouse, and the classic diet-based gestational-early lactational IDA rat that induces whole brain ID. In both models, deficits in PPI were observed during adulthood. Similar PPI deficits have been documented after manipulations that lesion the hippocampus (Swerdlow et al., 1995, 2001b; Caine et al., 2001; Daenen et al., 2003) or alter hippocampal functioning temporarily (Wan et al., 1996). Furthermore, in the DMT-1 KO mouse, deficits in hippocampus-specific measures of synaptic plasticity were observed. This finding agrees with previous investigations of the DMT-1 KO mouse demonstrating shorter apical dendrites, disorganized apical dendrite architecture, and reductions in cellular metabolites within the hippocampus (Carlson et al., 2009). Thus, it is likely that the observed deficits in hippocampus-specific plasticity explain, at least in part, previous findings of spatial memory deficits (Carlson et al., 2009, 2010) and current findings of PPI deficits in the DMT-1 KO mouse model.

Reduced synaptic plasticity in the DMT-1 KO mouse parallels reports of morphological (Jorgenson et al., 2003) and electrophysiological (Jorgenson et al., 2005; McEchron et al., 2010) alterations in the hippocampus of the IDA rat model and strongly suggests that the findings in the rat were indeed due to tissue level ID in the hippocampus rather than anemia-induced hypoxia. This finding is important because non-anemic ID is three times more common than IDA (WHO, 2001). The IDA rat model also shows reduced expression of hippocampal BDNF and BDNF-regulated genes involved in synaptic formation, an effect that persists despite early-life (P7) iron repletion (Tran and Carlson, 2008; Tran et al., 2009). While these rodent models are dissimilar in both temporal and spatial specificity of ID, it is possible that the synaptic plasticity deficits observed in the DMT-1 KO model may similarly be due to improper development of synapses, resulting from lower BDNF expression both early in development and into adulthood (Tran et al., 2009). This prediction is bolstered by recent evidence that hippocampus-specific ID in gestationally-induced dominant negative transferrin receptor-1 mice results in suppressed BDNF expression in adulthood (Fretham et al., 2012). However, because BDNF increases cellular metabolism (Markham et al., 2004), and levels of BDNF can be decreased in ID, synaptic alterations may, in fact, be a secondary result of impaired metabolism.

We posit that the ID-induced PPI deficits are related, either directly or indirectly, to the observed diminution of synaptic plasticity within the hippocampus. However, there exist few studies that empirically support the link between hippocampal measures of plasticity and PPI. A pair of studies demonstrated reduced LTP in socially isolated rats previously shown to have reduced PPI (Greene et al., 2001; Roberts, 2003). Similarly, our own group has previously documented reduced LTP and PPI in a developmentally disrupted Smad4 knockout mouse model (Sun et al., 2010). Even so, the causal link between these findings remains speculative. One potential underlying mechanism explaining the concomitant

deficits in PPI and LTP is that they are both secondary effects of hippocampal hypometabolism on glutamatergic processes. Notably, perinatal ID has been shown to down-regulate glutamatergic neurotransmission (Rao et al., 2003), expression of the NR2b subunit of the NMDA receptor (Jorgenson et al., 2003), as well as synaptic binding of glutamate (Agarwal, 2007). Since the majority of hippocampal projections are glutamatergic (Bast and Feldon, 2003), reduced glutamatergic output to the NAc and associated mesolimbic circuitry could impact PPI (Swerdlow et al., 2001a). Reduced intrinsic hippocampal glutamatergic signaling could also impact PPF and LTP, both established measures of glutamatergic neurotransmission (Malenka and Bear, 2004).

Interestingly, reduced hippocampus-specific glutamatergic activity also represents one hypothesis of schizophrenia pathophysiology (Greene, 2001). Such reduced glutamatergic activity has been postulated to result from NMDA receptor dysfunction (Moghaddam, 2003; Tang et al., 2009). In humans and non-human primate models, NMDA receptor antagonists (e.g., MK801, ketamine, phencyclidine) induce positive, psychotic-like symptoms (Newcomer and Krystal, 2001). PPI, a putative marker of positive symptoms in schizophrenia, is also reduced in mouse models lacking mGluR5 receptors (Brody et al., 2004) or in rat models following administration of ketamine (Sabbagh et al., 2012). Moreover, genetic manipulation of the NMDA receptor in mouse models of schizophrenia causes a reduction in LTP (Sakimura et al., 1995; Tang et al., 2009). These findings suggest a common role of NMDA receptors in the modulation of LTP and PPI, as well as link these respective electrophysiological and behavioral measures to both ID and schizophrenia.

We hypothesized that ID would reduce PPI, a validated endophenotype of schizophrenia and measure of positive symptoms (Swerdlow et al., 2000). Even so, the pathophysiological commonalities between ID and schizophrenia remain unclear and speculative, but nevertheless intriguing. One current hypothesis for the etiology of schizophrenia posits abnormal neurodevelopmental processes (Brown and Susser, 2002; Lewis and Levitt, 2002; Fatemi and Folsom, 2009), which can arise, in part, from environmental factors (Kendler, 1995; Tsuang, 2001). Indeed, an emerging epidemiological literature has highlighted the relationship of neonatal hypoxic-ischemic injury (resulting from maternal diabetes or ID during pregnancy) with elevated risk for developing schizophrenia (Georgieff et al., 1990; Cannon et al., 2002; Rao and Georgieff, 2007; Lieshout and Voruganti, 2008). That is, early-life ID may predispose an individual to develop schizophrenia in a dose-dependent manner (Insel et al., 2008). Another, albeit indirect, line of evidence is the similarity of motor, cognitive, and behavioral alterations observed in children with ID with those who go on to develop schizophrenia (Erlenmeyer-Kimling, 2000). Taken together, these human studies support the speculation that both ID and schizophrenia involve similar, albeit still not fully characterized, underlying neural mechanisms.

Human studies have documented alterations in motor functioning (Shafir et al., 2006) and increased anxiety in adulthood of formerly ID children (Lozoff et al., 2000). In contrast, we observed no differences in motoric behavior in the DMT-1 KO mouse, a finding consistent with the adult gestational IDA rat model (Unger et al., 2007). Moreover, findings of increased anxiety have not been replicated in the gestational rat model following recovery from IDA – either using the open field testing (Felt et al., 2006) or elevated plus-maze (Eseh and Zimmerberg, 2005). Interestingly, our hippocampus-specific DMT-1 KO mice showed reduced anxiety-like behavior in both the open field test and elevated plus-maze, albeit with modest effect sizes. The source for the variability in human and rodent studies of ID is unclear. One possibility is that anxiety-like behavior in formerly ID rodents is restricted to hippocampus-dependent forms of fear conditioning, whereas levels of fear or anxiety evoked in other tasks are unaffected or even reduced. This is suggested by the observation that rats exhibit exaggerated “trace” fear conditioning and inhibitory avoidance – two

hippocampus-dependent forms of fear – after recovery from gestational IDA (Findlay et al., 1981; McEchron et al., 2005; Gewirtz et al., 2008). Since the DMT-1 mutation is hippocampus-specific, it is likely that these animals will show similarly exaggerated fear responses in “trace” or contextual Pavlovian fear conditioning and inhibitory avoidance. Such a dissociation between different forms of fear and anxiety, which has already been reported in the formerly IDA rat (Gewirtz et al., 2008), would be consistent with the functional differentiation of dorsal and ventral regions of the hippocampus (Bannerman et al., 2004; Burman et al., 2006).

While the similar hippocampus-specific synaptic effects observed in both models of ID suggest that PPI disruptions are related to ID in the hippocampus itself, it remains important to consider the possible involvement of accessory changes in associated brain structures. For example, it is known already that the striatum of the DMT-1 KO mouse, although iron sufficient, is hypometabolic, and these mice perform poorly in a striatum-dependent cued navigation task (Carlson et al., 2010). We have also found changes in performance on a prefrontal cortex (PFC)-dependent delayed alternation task in the gestational IDA rat, an effect that may result from changes in hippocampal-PFC connectivity (Schmidt et al., 2010). Since both the dorsal striatum and PFC are important in modulating PPI (Kodsi and Swerdlow, 1995; Swerdlow et al., 1995; Bakshi and Geyer, 1998) it will be important to evaluate whether the reduction in PPI seen in this study results from a functional disruption of areas closely connected to the hippocampus, rather than from disruption of the hippocampus itself.

Other compensatory responses to DMT-1 deletion are possible at the cellular level. Previously our group reported that DMT-1 KO mice showed increased hippocampal mRNA expression of transferrin receptor-1, which transports extracellular iron into the cell through an endosomal process (Carlson et al., 2009). Nevertheless, for the iron to be bioavailable intracellularly, DMT-1 is needed to extrude ferrous iron from the endosome. Thus, in spite of the upregulation of transferrin receptors, iron levels remain significantly and selectively depleted within the hippocampal CA1 of DMT-1 KO mice (Carlson et al., 2009), suggesting that increased expression of the transferrin-1 receptor does not compensate for DMT-1 deletion. Another caveat is that DMT-1 transports other divalent cations intracellularly, including Mn^{2+} , Co^{2+} , Cu^{2+} , and Zn^{2+} (Garrick et al., 2006). Although all of these metals have their own specific transporters, altered trafficking of one or more of these substrates could be the causal agent of the observed effects. The trafficking would differ between our two models. In the IDA rat, DMT-1 is upregulated and thus there would be risk of toxicity from overload by the other divalent metals (e.g., Mn^{2+}) (Siddappa et al., 2003). Conversely, the DMT-1 KO would restrict uptake of essential divalent metals (e.g., Zn^{2+} and Cu^{2+}). While the concentrations of these metals has not been measured in the DMT-1 KO mouse, Mn^{2+} superoxide dismutase and Cu^{2+}/Zn^{2+} superoxide dismutase gene expression is not different from WT controls (Carlson et al., 2009), suggesting that the specific transporters for these metals compensate for the lack of DMT-1. Among the divalent ions, iron appears to be the most physiologically important substrate and has the highest binding affinity for DMT-1 (Gunshin et al., 2005). Hence, the lack of neuronal iron is likely to be the source of behavioral and electrophysiological findings observed in the current investigation.

Previous studies investigating PPI effects in the IDA rat model have observed decreases in the acoustic startle reflex without changes in PPI (Burhans et al., 2006; Unger et al., 2006). Importantly, these studies initiated ID during adolescence. In contrast, our IDA rat model received iron-restricted diets during gestation/infancy and our DMT-1 KO mouse exhibited ID during late embryonic development and throughout its lifespan. Therefore, we reason that the critical period of brain ID responsible for producing deficits in PPI occurs prior to adolescence, concurrently with significant hippocampal development. Indeed, late-

embryonic and early-life hippocampus development is characterized by substantial neurogenesis and synaptogenesis, processes that can be irreversibly disrupted by ID (Kempermann et al., 2004; Fretham, 2011; Mihaila et al., 2011). Such processes peak at P10–15 in rats (Pokorný and Yamamoto, 1981; Steward and Falk, 2004), a period during which our rat IDA model exhibits significantly reduced iron hematocrit levels (Schmidt et al., 2010). This same period also coincides with initial rapid development of the dopamine system (Tarazi and Baldessarini, 2000). These overlapping critical periods of hippocampus and dopamine system development may both be particularly vulnerable to the effects of ID. Interestingly, one model of schizophrenia dependent on hyper-dopaminergic activity required hippocampal dysfunction to be induced prenatally (Lodge and Grace, 2007). In addition, a transient disruption of neural activity in the ventral hippocampus during the neonatal period results in several endophenotypic features of schizophrenia in adulthood (Lipska, 2004). Despite these suggestive findings, it remains to be seen whether the deficits in hippocampal synaptic plasticity seen in the DMT-1 KO mouse in this study and in the IDA rat previously (Jorgenson et al., 2003, 2005) are similarly sensitive to the critical period during which ID occurs.

In conclusion, we have shown that two different rodent models of ID can produce similar deficits in sensorimotor gating. Our DMT-1 mouse model may be valuable for understanding the hippocampus' role in the etiology of schizophrenia since in these animals the hippocampus also shows abnormal synaptic functioning as a result of ID. Reduction of LTP and elevation of PPF in the hippocampus mirrors previous findings from the IDA rat model. This suggests that, despite the ubiquity of brain ID and concomitant functional abnormalities in other brain regions, ID in the hippocampus itself is sufficient to produce some of the behavioral abnormalities seen in the IDA rat model. This is consistent with the fact that early-life ID disproportionately compromises the development of the hippocampus (Fretham, 2011). Overall, the current findings of reduced PPI and altered hippocampus-specific synaptic functioning further substantiate the growing literature that highlights the deleterious effects of ID on hippocampal function.

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Figure 1. DMT-1 KO mice exhibited significantly reduced prepulse inhibition. Values represent mean $\pm$ SEM for each group. ** $p < 0.01$, compared with WT group.

Figure 2.

DMT-1 KO mice performance on the open field (A) and elevated plus-maze (B) tests. A) Total amount of time spent in the center of the testing arena was significantly increased in DMT-1 KO mice. B) Percentage of open arm entries was significantly increased in DMT-1 KO mice. Values represent means $\pm$ SEM for each group. * $p < 0.05$

Figure 3.

DMT-1 KO mice displayed altered synaptic signaling in the Schaffer-collateral pathway in the CA1 region of hippocampus. A) Time course of LTP in response to a single train of theta burst stimulation (1xTBS). B) The overall magnitude of LTP was significantly reduced in DMT-1 KO mice ($p < 0.01$). C) Input-output curves showed no significant difference between WT and DMT-1 KO mice. D) PPF was significantly elevated at shorter paired pulse intervals (20ms, 40ms, and 80ms) in DMT-1 KO mice. * $p < 0.05$; ** $p < 0.01$.

Figure 4. Gestational IDA rats displayed significantly reduced prepulse inhibition. Values represent means $\pm$ SEM for each group. IDA, iron deficient anemia; IS, iron sufficient. * $p < 0.05$, compared with IS group.