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OBESITY/HYPERLEPTINEMIC PHENOTYPE ADVERSELY AFFECTS HIPPOCAMPAL PLASTICITY: EFFECTS OF DIETARY RESTRICTION

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Abstract

Epidemiological studies estimate that greater than 60% of the adult US population may be categorized as either overweight or obese and there is a growing appreciation that obesity affects the functional integrity of the central nervous system (CNS). We recently developed a lentivirus (LV) vector that produces an insulin receptor (IR) antisense RNA sequence (IRAS) that when injected into the hypothalamus selectively decreases IR signaling in hypothalamus, resulting in increased body weight, peripheral adiposity and plasma leptin levels. To test the hypothesis that this obesity/hyperleptinemic phenotype would impair hippocampal synaptic transmission, we examined short term potentiation (STP) and long term potentiation (LTP) in the hippocampus of rats that received the LV-IRAS construct or the LV-Control construct in the hypothalamus (hypo-IRAS and hypo-Con, respectively). Stimulation of the Schaffer collaterals elicits STP that develops into LTP in the CA1 region of hypo-Con rats; conversely, hypo-IRAS rats exhibit STP that fails to develop into LTP. To more closely examine the potential role of hyperleptinemia in these electrophysiological deficits, hypo-IRAS were subjected to mild food restriction paradigms that would either: 1) prevent the development of the obesity phenotype; or 2) reverse an established obesity phenotype in hypo-IRAS rats. Both of these paradigms restored LTP in the CA1 region and reversed the decreases in the phosphorylated/total ratio of GluA1 Ser845 AMPA receptor subunit expression observed in the hippocampus of hypo-IRAS rats. Collectively, these data support the hypothesis that obesity impairs hippocampal synaptic transmission and support the hypothesis that these deficits are mediated through impairment of hippocampal leptin activity.

Keywords

triglycerides; leptin; adiposity; long term potentiation; GluA1 receptor; lentivirus

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1. Introduction

Ongoing epidemiological studies by the Centers for Disease Control estimate that greater than 60% of the adult US population may be categorized as either overweight or obese [1]. In addition to peripheral complications, there is a growing appreciation that the complications of obesity extend to the central nervous system (CNS). While the vast majority of these studies have focused upon the hypothalamus, more recent studies suggest that the complications of obesity may also affect the functional integrity of the hippocampus. For example, hippocampal-dependent behaviors are impaired in experimental models of obesity [2–14] and obesity/increased body mass index (BMI) is associated with decreased cognitive function in humans [15–18]. Unfortunately, the underlying molecular and cellular mechanisms responsible for obesity-induced cognitive deficits remain unclear. One potential mediator of hippocampal plasticity deficits in obesity may be hyperleptinemia.

Leptin is synthesized and secreted by adipocytes and is transported across the blood-brain barrier (BBB) via a saturable transport system [19]. In the hypothalamus, leptin is recognized as an important integration factor for the coordination of peripheral and central signals to regulate food intake, metabolism, body weight and body composition (For review see, [20]). In addition, there is a growing literature to support a role for leptin in the facilitation of hippocampal structure and function [21]. For example, leptin regulates hippocampal plasticity by converting short term potentiation (STP) into long term potentiation (LTP) [22], a functional enhancement that may contribute to leptin's ability to improve hippocampal-dependent behavioral performance [23;24]. In this regard, intrahippocampal [23], as well as peripheral administration of leptin [24], enhances behavioral performance in a dose-dependent manner. However, leptin transport across the blood-brain barrier is impaired in obesity phenotypes [19;25;26], which has led to the hypothesis that decreases in hippocampal leptin signaling and/or leptin transport are associated with decreases in synaptic plasticity.

We recently developed a model of obesity using a lentiviral vector that produces an antisense RNA selective for the insulin receptor (IRAS) [27]. When injected into the third ventricle to target IRs expressed in the arcuate nucleus, hypo-IRAS rats exhibit significant decreases in IR expression and signaling when compared to rats treated with the control virus (hypo-Con). Additionally, hypo-IRAS rats exhibit increases in body weight, body adiposity and plasma leptin levels in the absence of other metabolic and neuroendocrine abnormalities or deficits in hippocampal IR expression and signaling. As such, the hypo-IRAS rat provides a unique model system to more selectively examine the mechanisms through which impaired hippocampal leptin activity contributes to or is responsible for hippocampal plasticity deficits in obesity/hyperleptinemic phenotypes. Accordingly, the aim of the current studies was to examine hippocampal synaptic transmission in hypo-IRAS rats, as well as examine the ability of food restriction paradigms to reverse this obesity/hyperleptinemic phenotype and thereby restore hippocampal synaptic plasticity.

2. Materials and Methods

2.1. Animal protocols

Adult male Sprague Dawley rats (CD strain, Charles River) weighing 200–250 g were housed in groups of three with *ad libitum* access to food and water, in accordance with all guidelines and regulations of The University of South Carolina Animal Care and Use Committee. Prior to administration of lentiviral vectors, tail bleeds were performed for analysis of baseline plasma leptin levels. Hypothalamic IRAS administration was performed as described in our previous studies [27]; rats received either the LV-Control construct (hypo-Con) or the LV-IRAS construct (hypo-IRAS). After surgery, hypo-IRAS and hypo-

Con rats were housed individually in a BSL2 facility for at least four weeks. Body weight was monitored daily. In *experiment 1*, hypo-IRAS and hypo-Con rats were sacrificed and used for electrophysiological analyses as described below. Prior to electrophysiology, the hypothalamus was dissected and membrane homogenates were prepared for analysis of IR expression. Additionally, trunk blood was collected and the bodies were saved and frozen at -20°C for subsequent body composition analysis. In *experiment 2*, hypo-IRAS rats were divided into three experimental groups: 1) rats provided *ad libitum* access to food, 2) rats subjected to food restriction at day 5 (Prevention group), and 3) rats subjected to food restriction at day 21 (Reversal group). The hypo-Con group was treated as described above. The rationale of the day 5 time point was that hypo-IRAS rats have recovered from surgery but not yet developed the obesity/hyperleptinemia phenotype and food restriction would 'prevent' the development of the obesity/hyperleptinemic phenotype. In the Reversal group, the obesity/hyperleptinemic phenotype was allowed to develop in the hypo-IRAS rats and the goal of the food restriction paradigm was to return body weight, body adiposity and plasma leptin levels to those observed in hypo-Con rats. In order to achieve these outcomes, the prevention and reversal groups were provided approximately 80% of their normal food intake; food intake was determined during the 5 days following lentivirus administration. Food restriction was maintained in the Prevention group and the Reversal group until the completion of the study.

2.2. Acute restraint stress test

Hypo-Con and hypo-IRAS rats were subjected to an acute restraint stress session as described in our previous studies [28]. Briefly, rats were subjected to restraint stress in wire mesh restrainers secured at the head and tail ends with clips. Immediately after the rat was secured in the restrainer, a tail bleed was performed to determine baseline corticosterone (CORT) values. Thirty minutes after the initiation of stress, the tail was gently massaged to recover blood for peak stress-mediated increases in plasma CORT. Upon completion of this tail bleed, rats were released from the restrainers and returned to their home cage. One hour later, the tail was gently massaged to recover blood for post-stress measurement of plasma CORT levels. Plasma CORT levels were determined by enzyme-linked immunosorbent assay.

2.3. Enzyme-linked immunosorbent assay (ELISA)

ELISA analysis was performed for leptin (Linco Research, Billerica, MA) and CORT (Assay Designs, Ann Arbor, Michigan) in plasma isolated from hypo-Con and hypo-IRAS rats [27]. ELISA plates were analyzed according to the manufacturer's instructions using a Tecan SPECTRAFluor plate reader (Tecan U.S., Inc., Durham, NC). Statistical analysis was performed using an unpaired t-test with $P < 0.05$ as the criterion for statistical significance.

2.4. Immunoblot analysis

Immunoblot analysis was performed as described in our previous studies (5). Briefly, 20 μg of total membrane fractions were separated by SDS/PAGE (10%), transferred to nitrocellulose (NC) membranes and blocked in TBS plus 10% non fat dry milk for 60 min. NC membranes were incubated with primary antisera in TBS/5% non fat dry milk. After overnight incubation at 4°C , blots were washed with TBS plus 0.05% Tween 20 (TBST) and incubated with peroxidase-labeled species-specific secondary antibodies. NC membranes were then washed with TBST and developed using enhanced chemiluminescence reagents (ECL, Amersham) as described by the manufacturer. Normalization for protein loading was performed using a mouse monoclonal primary antibody selective for actin (Sigma Chemical Company).

2.5. In vitro phosphorylation assays

In vitro phosphorylation of the insulin receptor was performed as described in our previous study [27] based upon protocols developed by Alkon and co-workers [29;30]. Briefly, 50 µg of hypothalamic total membrane fractions were incubated with reaction buffer (50 mM Tris-HCl, pH 7.4; 1 mM MgCl₂; 2 mM EGTA; 1× protease inhibitor cocktail [Sigma Chemical Company]; 1× phosphatase inhibitor cocktail [Sigma Chemical Company]). In vitro phosphorylation was stimulated by addition of 1 µM insulin and 5 mM ATP. Following addition of insulin/ATP, samples were incubated for 3 minutes at 37°C. SDS/PAGE sample buffer was quickly added, the samples were boiled for 10 minutes and added to a precast 4–20% SDS/PAGE gel (Bio-Rad Laboratories).

2.5. Hippocampal slice electrophysiology

Hippocampal slices were prepared from isoflurane anesthetized rats and maintained as previously described [31;32]. Briefly, brain slices were prepared in cold (4°C), oxygenated (95% O₂ / % CO₂) sucrose-based 'cutting' artificial cerebrospinal fluid (aCSF) that contained (in mM): 248 sucrose, 2.7 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 10 glucose, 0.5 CaCl₂ and 7 MgSO₄ (350mOsm). Slices were incubated for approximately one hour at room temperature in oxygenated (95% O₂ / 5% CO₂) aCSF containing (in mM): 125 NaCl, 2.7 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 10 glucose, 0.5 CaCl₂ and 7 MgSO₄, 0.02 D-APV and 1 kynurenic acid (pH 7.4; 305–312mOsm). For field potential recording, slices were transferred to a recording chamber maintained at 32 – 34° C and continuously perfused (2–3 ml/min) with oxygenated recording ACSF containing (in mM): 125 NaCl, 2.7 KCl, 1.25 NaH₂PO₄, 25 NaHCO₃, 10 glucose, 2.0 CaCl₂ and 1.0 MgSO₄ (pH 7.4; 305–312 mOsm). Stimuli were 0.1 ms, monophasic, cathodal, rectangular, constant current pulses (10 – 200 µA) delivered to CA3 axons in stratum radiatum of CA1c through a monopolar platinum-iridium electrode. For recording, a glass pipette (resistance 2–3 MΩ) filled with recording ACSF was placed in stratum radiatum of CA1b. Responses were recorded using an AxoProbe 1A amplifier (Molecular Devices, Sunnyvale, CA), and analyzed using pClamp software (Molecular Devices, Sunnyvale, CA). Baseline test stimuli were delivered every 30 sec at a stimulus intensity that evoked a field excitatory postsynaptic potential (fEPSP) that was 30% of maximal slope. After at least 10 min of stable baseline responses, LTP was induced with a high frequency stimulus train (100 Hz, 1 sec) delivered at the test stimulus intensity. Paired pulse facilitation was assessed by delivering identical stimuli 50 msec apart. Statistical analysis was performed using a two-way ANOVA followed by Bonferroni post hoc tests, with P < 0.05 as the criterion for statistical significance.

3. Results

In agreement with our previous studies [27], IR expression was significantly decreased in the hypothalamus of hypo-IRAS-treated rats when compared with hypo-Con rats; hippocampal IR levels were unaffected by virus administration (Table 1). Prior to virus administration, all rats exhibited similar plasma leptin levels (Table 1). However, approximately three weeks after hypothalamic lentivirus administration, rats that received the LV-IRAS construct exhibited significant increases in plasma leptin levels when compared to rats treated with the LV-Con construct. Hypo-IRAS rats also exhibited significant increases in body weight gain and adiposity (Table 1). As described in our previous studies [27], hypo-IRAS rats subjected to an oral glucose tolerance test (OGTT) cleared glucose as effectively and exhibited similar changes in plasma insulin levels as hypo-Con rats. These results indicate that hypo-IRAS rats do not develop insulin resistance. Plasma corticosterone (CORT) levels in response to an acute stress challenge were also examined to assess hypothalamic-pituitary-adrenal (HPA) axis function in hypo-Con and hypo-IRAS rats. In agreement with our previous studies [27], basal plasma CORT levels

were unaffected by LV-IRAS administration; in addition, peak plasma CORT levels in response to an acute restraint stress challenge, as well as post-stress plasma CORT levels, were similar in hypo-IRAS rats and hypo-Con rats (Table 1). Such results suggest that HPA axis activity, at least in response to an acute restraint stress challenge, is unaffected by downregulation of hypothalamic IR expression. Collectively, these data demonstrate that third ventricular administration of the LV-IRAS construct decreases hypothalamic IR expression and signaling without affecting these parameters in the hippocampus and elicits increases in body weight, body adiposity and plasma leptin levels without affecting peripheral glucose metabolism or neuroendocrine function.

Downregulation of hypothalamic IRs impairs hippocampal long term potentiation

Long term potentiation (LTP), a cellular correlate of learning and memory in the mammalian brain, is reduced in the hippocampus of obese/hyperleptinemic rodents, such as the *db/db* mouse and the *fa/fa* Zucker rat [3;33;34] and in rats fed a high-fat diet [9;35]. In order to determine whether hippocampal synaptic transmission is adversely affected by the obesity phenotype elicited by the LV-IRAS construct, we examined LTP in the hippocampus of hypo-Con and hypo-IRAS rats. In hypo-Con rats, high-frequency stimulation (HFS) of the Schaffer collaterals elicited LTP of field excitatory postsynaptic potentials (fEPSPs) in the CA1 region (Figure 1). Conversely, HFS of the Schaffer collaterals elicited STP that failed to develop into LTP in the CA1 region of hypo-IRAS rats. Paired-pulse facilitation (PPF) in the CA1 region of the hippocampus was unaffected by downregulation of hypothalamic IRs (hypo-Con, $125 \pm 2\%$, $n = 7$; hypo-IRAS, $125 \pm 4\%$, $n = 5$). The lack of a change in both STP and PPF suggest that the deficits in hippocampal LTP likely result from postsynaptic impairments in excitatory signaling.

3.2. Food restriction of hypo-IRAS rats returns body weight, adiposity and plasma leptin levels to those observed in hypo-Con rats

One way to assess the impact of the obesity/hyperleptinemia phenotype upon hippocampal synaptic transmission would be to food restrict hypo-IRAS to either prevent the increases in body weight, body adiposity and plasma leptin levels or to attempt to reverse an established obesity/hyperleptinemia phenotype; we examined both of these possibilities. The LV-Con or LV-IRAS construct was administered as described above and all rats were provided *ad libitum* access to food. Five days after virus administration, a subset of hypo-IRAS rats were subjected to a mild food restriction paradigm in order to match their body weights to hypo-Con rats ('Prevention' group). At this time point, body weights (Figure 2) did not differ in hypo-Con rats and the hypo-IRAS rats, demonstrating that the Prevention paradigm was initiated prior to the development of the obesity phenotype.

In agreement with our previous studies [27], approximately three weeks following lentivirus administration hypo-IRAS rats provided *ad libitum* access to food exhibited the expected increases in body weight (Figure 2) and plasma leptin levels (Figure 3, Day 21) when compared to hypo-Con rats. Hypo-IRAS rats in the Prevention group exhibited body weights (Figure 2) and plasma leptin levels (Figure 3, Day 21) that were similar to hypo-Con rats. At this time, a separate group of hypo-IRAS rats were subjected to a mild food restriction paradigm ('Reversal' group). This food restriction paradigm effectively reversed the increases in body weight (Figure 2) and plasma leptin levels (Figure 3, Day 45) in hypo-IRAS rats. At the time of sacrifice, plasma leptin levels were elevated exclusively in the *ad libitum* hypo-IRAS group (Figure 3, Day 45). Since increases in plasma triglyceride levels are proposed to impair leptin transport across the BBB [36], we also examined plasma triglyceride levels in hypo-Con rats, hypo-IRAS *ad libitum* rats, hypo-IRAS Prevention rats and hypo-IRAS Reversal rats. As shown in Figure 4, plasma triglyceride levels were significantly increased in the hypo-IRAS group compared to hypo-Con rats, hypo-IRAS

Prevention rats and the hypo-IRAS Reversal rats; not unexpectedly, hypo-IRAS Prevention and Reversal rats also exhibited decreases in plasma triglyceride levels compared to hypo-Con rats. Body carcass analysis revealed that body adiposity was elevated exclusively in hypo-IRAS rats provided *ad libitum* access to food (Figure 5). These data demonstrate that these food restriction paradigms effectively prevent or reverse the development of an obesity/hyperleptinemic phenotype in hypo-IRAS rats.

To further confirm the efficacy of LV-IRAS administration, insulin-stimulated *in vitro* phosphorylation assays were performed in hypothalamic extracts isolated from hypo-Con, hypo-IRAS rats provided *ad libitum* access to food, the hypo-IRAS Prevention group and the hypo-IRAS Reversal group. Western blotting analysis revealed that insulin-stimulated phosphorylation of the IR (pIR) was reduced in LV-IRAS-treated rats irrespective of food access (Figure 6). This functional measure of IR activity clearly demonstrates that the restoration of metabolic and endocrine parameters in hypo-IRAS-treated rats is not the result of a 'recovery' of hypothalamic IR expression in the Prevention or Reversal groups.

3.3. Food restriction of hypo-IRAS rats restores hippocampal synaptic transmission

In order to examine the ability of the food restriction paradigms to restore hippocampal synaptic transmission, we examined STP and LTP in the CA1 region of the hippocampus of hypo-Con rats and the hypo-IRAS rats (i.e. *ad libitum*, Prevention and Reversal groups). As described above, HFS of the Schaffer collaterals elicited LTP in the CA1 region of hippocampus of hypo-Con rats, while this stimulation paradigm did not elicit LTP in the CA1 region of hypo-IRAS rats provided *ad libitum* access to chow. The food restriction paradigms that prevent or reverse the IRAS-induced increases in plasma leptin levels completely restored the ability of HFS of the Schaffer collaterals to elicit LTP in the CA1 region of hypo-IRAS rats (Figure 7). As described above, paired-pulse facilitation was unaffected, suggesting that deficits in pre-synaptic mechanisms are not responsible for the inability of HFS to elicit LTP in the CA1 region of hypo-IRAS rats.

3.4. GluA1 phosphorylation in hypo-IRAS rats

The ability of food restriction to reverse the obesity/hyperleptinemic phenotype and thereby restore hippocampal synaptic transmission is consistent with our hypothesis that leptin signaling is impaired in the hippocampus of hypo-IRAS rats. Since the actions of leptin upon synaptic transmission are proposed to be mediated through the glutamate receptor subunits [37], we examined the expression and phosphorylation state of NMDA and AMPA receptor subunits in the hippocampus of hypo-Con and hypo-IRAS rats. Western blotting analysis was performed in hippocampal membrane extracts prepared from hippocampal slices of hypo-Con and hypo-IRAS rats that were not used in the electrophysiological studies described above. The expression of NR1, NR2A, NR2B and GluA2 receptor subunits did not change in hypo-IRAS rats compared to hypo-Con rats, irrespective of whether rats had *ad libitum* access to food or were subjected to one of the food restriction paradigms (data not shown). Similarly, total GluA1 levels were unchanged among the groups; however, the ratio of phosphorylated GluA1 Ser845 to total GluA1 was significantly reduced in the hippocampus of hypo-IRAS rats provided *ad libitum* access to food compared to hypo-Con rats, decreases that were not observed in the Prevention paradigm group and the Reversal paradigm group (Figure 8). Since the actions of leptin upon synaptic transmission are proposed to be mediated in part through the GluA1 subunit, these results provide a mechanism through which a hyperleptinemia phenotype could impair hippocampal synaptic transmission.

4. Discussion

The results of the current study demonstrate that region-specific decreases in hypothalamic IR expression and activity adversely affect functional plasticity in the hippocampus. Specifically, selective downregulation of hypothalamic IRs impairs hippocampal synaptic plasticity, as evidenced by deficits in LTP in the CA1 region of the hippocampus of hypo-IRAS-treated rats provided *ad libitum* access to food. Food restriction paradigms that prevent or reverse the development of the obesity/hyperleptinemia phenotype in hypo-IRAS rats restore hippocampal synaptic transmission as well as the phosphorylation state of hippocampal GluA1 receptor subunits. Collectively, these data suggest that downregulation of hypothalamic IRs impairs functional and pharmacological plasticity in the rat hippocampus and suggest that hyperleptinemia may be a mechanistic mediator of impaired hippocampal plasticity in obesity phenotypes.

We found that Ser845 phosphorylation of GluA1 was significantly reduced in hypo-IRAS rats. Phosphorylation of this residue on GluA1 is important in the activity-dependent trafficking of AMPA receptors [38]. Phosphorylation of Ser845 serves as a “priming” step for LTP by promoting surface expression of AMPA receptors at extrasynaptic sites [39]. According to this two-step model for LTP [39;40], these extrasynaptic AMPA receptors can then be incorporated into synapses by synaptic activity during LTP. We suggest that the reduction in Ser845 phosphorylation produced by hypo-IRAS treatment suppressed LTP by reducing this extrasynaptic pool of AMPA receptors. Indeed, dephosphorylation of Ser845 through chemical long term depression (Chem-LTD) has previously been demonstrated to reduce the surface pool of GluA1 and suppress LTP [39]. Both insulin [40] and leptin [41] regulate surface trafficking of GluA1-containing AMPA receptors. However, hypo-IRAS treatment does not affect hippocampal insulin receptor expression, but causes animals to develop hyperleptinemia. This finding suggests that the suppression of LTP observed in the present study was caused by impaired hippocampal leptin signaling. Food restriction paradigms reversed this hyperleptinemia and restored LTP, suggesting that leptin signaling modulates the dynamic range of LTP amplitude by regulating the amount of extrasynaptic AMPARs available for synaptic incorporation.

Several other molecular approaches have examined the functional role of hypothalamic insulin receptors, including neuronal IR knockout mice (NIRKO) [42] and antisense oligonucleotide approaches [43]. While these various molecular approaches yielded similar findings related to body weight and composition, there are some provocative differences. For example, hyperinsulinemia and insulin resistance are observed in female NIRKO mice [42], while hepatic insulin resistance is observed following downregulation of hypothalamic IRs using antisense approaches [43]. Our previous studies revealed that lentivirus-mediated downregulation of hypothalamic IRs does not modulate fasting plasma glucose or insulin levels and that hypo-IRAS rats exhibit similar responses to an oral glucose tolerance test when compared to hypo-Con rats [27]. Since these observations cannot rule out the possibility that hypo-IRAS rats exhibit hepatic insulin resistance, examination of how hepatic insulin resistance and/or deficits in peripheral metabolism may contribute to impairments in hippocampal synaptic plasticity represents an important area of future investigation. In this regard, our studies to date indicate that hippocampal IR signaling is unaffected in hypo-IRAS rats [27], suggesting that other metabolic or endocrine parameters are responsible for the hippocampal plasticity deficits we observe. Based upon our findings, leptin represents a likely candidate.

The actions and interactions of leptin and insulin in the hypothalamus are well described, especially in relation to normal metabolism and in pathophysiological settings such as diabetes and obesity phenotypes [44]. In addition to the literature describing the effects of

insulin upon hippocampal plasticity [45], there is a growing literature to support a role for leptin in the facilitation of hippocampal function. For example, studies by Harvey and co-workers determined that leptin enhances hippocampal excitability via NMDA receptor-mediated mechanisms [21]. Additionally, leptin further regulates hippocampal plasticity by converting STP into LTP [46] and dose-dependently modulates excitability of hippocampal CA1 neurons [24]. Confocal immunofluorescence analyses also determined that leptin regulates the morphological features of primary hippocampal cultures, including the motility of dendritic filopodia and hippocampal synaptic density [47]. These structural and functional enhancements of hippocampal plasticity may contribute to leptin's ability to improve hippocampal-dependent behavioral performance [23;24].

Conversely, genetic mutations that result in disrupted leptin signaling such as in the *db/db* mouse and the Zucker *fa/fa* rat are associated with reductions in LTP [3;33;34]. Deficits in hippocampal LTP are also observed in diet-induced obesity models [9;35]. These deficits in hippocampal synaptic transmission may contribute to the impairments in hippocampal-dependent behaviors in rodent models of obesity [2–14]. Some of these studies have investigated hippocampal insulin resistance as a potential mechanistic mediator of these behavioral deficits, including our previous studies that demonstrated that insulin signaling is reduced in obese Zucker rats [12]. However, these studies have overlooked a potential role for leptin. Indeed, it is important to note that while *db/db* mice and Zucker rats are insulin resistant and exhibit deficits in insulin receptor signaling, these rodent models of obesity are also hyperleptinemic and lack leptin receptors. The results of the current study support the hypothesis that hyperleptinemia contributes to or is responsible for deficits in hippocampal synaptic plasticity in models of obesity. To begin, hypo-IRAS rats exhibit normal insulin sensitivity to an oral glucose tolerance test and also exhibit no deficits in hippocampal insulin signaling. While elevated glucocorticoids may contribute to plasticity deficits in obesity phenotypes [48], hypo-IRAS rats exhibit similar basal levels of CORT and normal HPA axis activity in response to an acute stressor. When combined with our previous observations [27], it would appear that the primary endocrine parameter that is affected in hypo-IRAS rats is leptin, what allows us to dissect the effects of this hormone upon hippocampal plasticity in an *in vivo* model. Lastly, the ability of the food restriction paradigms to return body weight, body adiposity and plasma leptin levels to control values, as well as restore hippocampal plasticity and phosphorylation state of GluA1 provides compelling evidence that hyperleptinemia is a key component of obesity-induced deficits in hippocampal synaptic plasticity.

Beyond leptin, a metabolic parameter that is affected following downregulation of hypothalamic IRs is plasma triglycerides. Previous studies by Banks and co-workers have determined that triglycerides impair BBB transport of leptin [36]. Moreover, triglycerides also impair hippocampal LTP and performance in hippocampal-dependent behavioral tasks [2]. From a mechanistic perspective, these previous studies suggest that increases in plasma triglycerides to levels observed in hypo-IRAS may directly impair hippocampal LTP and/or indirectly impair hippocampal LTP by reducing BBB transport of leptin. In support of this hypothesis, triglyceride-induced impairments in BBB transport of leptin are reversed by gemfibrozil [36], a dyslipidemia drug that more selectively reduces plasma triglyceride levels. In the current study, the food restriction paradigms that reduce plasma triglycerides levels may re-establish leptin transport across the BBB, as well as eliminate the deleterious consequences of elevated triglycerides in the hippocampus. Re-establishment of hippocampal leptin signaling may restore leptin regulation of the glutamate receptor subunit activity [37], including the phosphorylation state of GluA1 that is critical to hippocampal synaptic plasticity. From a clinical perspective the current results emphasize the importance of reducing body adiposity as a practical approach to attenuating obesity-induced CNS complications, including decreased cognitive function [15–18] and increased risk for the

development of mild cognitive impairment and Alzheimer's disease (For reviews, see [15;49]).

In summary, the current results identify potential mechanisms through which obesity adversely affects hippocampal synaptic plasticity and importantly, how these deficits may be reversed. Future studies will be required to further investigate how these interactions between leptin and triglycerides in obesity phenotypes impacts hippocampal synaptic plasticity at the cellular, pharmacological and molecular levels.

Research highlights

- Decreasing hypothalamic insulin receptors (hypo-IRAS) elicits hyperleptinemia
- Long-term potentiation (LTP) is impaired in CA1 pyramidal neurons of hypo-IRAS rats
- Food restriction prevents or reverses hyperleptinemia in hypo-IRAS rats
- Food restriction paradigms restore hippocampal LTP in hypo-IRAS rats
- Food restriction restores phosphorylation of GluA1 Ser845 in hypo-IRAS rats

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Figure 1.

Hypo-IRAS-treated rats fail to develop LTP. Panel A. Twenty-one days after virus administration hypo-Con-treated rats developed LTP in response to high frequency (100 Hz, 1 sec) stimulation of the Schaffer collaterals (arrow, $n = 8$). In contrast, LTP, but not STP, was blocked in hypo-IRAS-treated rats provided *ad libitum* access to food ($n = 7$). Inset shows sample fEPSPs collected at the times indicated on the graph. Panel B. Paired pulse facilitation (PPF) of the fEPSP (50 ms interstimulus interval) was not different in hypo-Con ($n = 8$) or hypo-IRAS ($n = 7$) treated rats given *ad libitum* access to food. Paired pulse facilitation of fEPSPs from a hypo-IRAS-treated rat is shown.

Figure 2.

Hypo-IRAS-treated rats exhibit significant increases in body weight that are prevented or reversed by mild food restriction. All groups exhibit similar post-operative decreases in body weight. Beginning 7 days following administration, LV-IRAS rats provided *ad libitum* access to food exhibit significant increases in body weight, increases that were prevented when a subset of hypo-IRAS rats were subjected to mild food restriction beginning at day 5 (Prevention Group). Food restriction of an additional subset of hypo-IRAS rats 21 days post virus administration (Reversal Group) effectively returned body weight to levels observed in hypo-Con rats. [* = $p \leq 0.05$ compared to hypo-Con and Prevention groups; # = $p \leq 0.05$ compared to hypo-Con, Prevention and Restriction groups. Data based upon at least 10 rats/group]

Figure 3.

Food restriction paradigms prevent and reverse hypo-IRAS mediated increases in plasma leptin levels. Panel A. Twenty-one days after virus administration, blood was collected for plasma leptin analysis from hypo-Con rats, hypo-IRAS *ad libitum* rats, hypo-IRAS Prevention rats and the hypo-IRAS rats that were designated for the Reversal food restriction paradigm. ELISA analyses determined that plasma leptin levels were elevated in the hypo-IRAS rats provided *ad libitum* access to food and the hypo-IRAS rats that had not yet been subjected to the food restriction paradigm. Conversely, hypo-IRAS Prevention rats exhibited plasma leptin levels that were indistinguishable from hypo-Con rats. Panel B. At the time of sacrifice, plasma leptin levels were elevated exclusively in hypo-IRAS rats provided *ad libitum* access to food. [* = $p \leq 0.001$ compared to hypo-Control; # = $p \leq 0.001$ compared to hypo-IRAS Prevention group; % = $p \leq 0.001$ compared to hypo-IRAS Reversal group. Data based upon at least 10 rats/group]

Figure 4.

Hypo-IRAS administration increases plasma triglyceride levels, increases that are inhibited by food restriction paradigms. Hypo-IRAS rats provided *ad libitum* access to food exhibit significant increases in plasma triglyceride levels compared to hypo-Con rats and hypo-IRAS rats subjected to the Prevention and Restriction food paradigms. Additionally, food restriction significantly decreased plasma triglyceride levels in the hypo-IRAS Prevention group and hypo-IRAS Reversal group compared to hypo-Con rats. [$* = p \leq 0.001$ compared to hypo-Control; $\# = p \leq 0.001$ compared to hypo-IRAS. Data based upon at least 10 rats/group]

Figure 5.

Food restriction paradigms inhibit the increases in peripheral adiposity in hypo-IRAS rats. Approximately six weeks after virus administration, total body adiposity is significantly increased in hypo-IRAS rats provided *ad libitum* access to food compared to hypo-Con rats, hypo-IRAS Prevention rats and hypo-IRAS Reversal rats. [* = $p \leq 0.001$; Data based upon at least 10 rats/group]

Figure 6.

In vitro insulin-stimulated phosphorylation of the insulin receptor (pIR) is reduced in the hypothalamus of hypo-IRAS rats irrespective of access to food. Panel A. Representative immunoblot of pIR levels in 50 g of hypothalamic total membrane extracts from hypo-Con rats (Con), hypo-IRAS rats provided *ad libitum* access to food (Ad lib), hypo-IRAS Prevention rats (Prev) and hypo-IRAS Reversal rats (Rev) that were treated with 1 M insulin and 5 mM ATP, subjected to SDS/PAGE and probed with pIR antisera (Cell Signaling # 3021). Panel B: Autoradiographic analysis determined that pIR levels are reduced in hypo-IRAS rats irrespective of access to food. [* = $p \leq 0.01$ compared to hypo-Con; data based upon at least 10 rats/group]

Figure 7.

Food restriction restores LTP in the CA1 region of hypo-IRAS rats. High frequency stimulation (100 Hz, 1 sec, arrow) of Schaffer collaterals evoked LTP in CA1 of hypo-Con ($n = 6$), but not hypo-IRAS rats provided *ad libitum* access to food ($n = 4$). In contrast, hypo-IRAS rats in both the Prevention ($n = 6$) and Reversal ($n = 6$) food restriction groups exhibited LTP that did not differ from hypo-Con rats. In all animals LTP was assessed six to eight weeks after virus administration.

Figure 8.

Decreases in phosphorylated GluA1 Ser845 levels in the hippocampus of hypo-IRAS are inhibited by food restriction paradigms. Panel A: Representative immunoblot of phosphorylated GluA1 Ser845 levels in the hippocampus of hypo-Con rats (Control), hypo-IRAS rats provided *ad libitum* access to food (ad lib), hypo-IRAS Prevention rats (Prevention) and hypo-IRAS Reversal (Reversal) rats. Panel B. Autoradiographic analysis revealed that phosphorylation of GluA1 at serine 845 is significantly reduced in the hippocampus of hypo-IRAS rats provided *ad libitum* access to food. Hypo-IRAS Prevention and hypo-IRAS Reversal rats exhibited pGluA1 Ser 845 levels that were similar to levels observed in hypo-Con rats. [$* = p \leq 0.001$ compared to hypo-Con, Prevention and Reversal groups; data based upon at least 10 rats/group. GluA1 Ser845 antisera from Cell Signaling # 5849; data are expressed as the ratio of phosphorylated GluA1 Ser 845/total GluA1]

Table 1

Physiological and endocrine parameters in hypo-IRAS and hypo-Con rats.

	hypo-Con	hypo-IRAS
Plasma leptin, baseline (ng/ml)	3.15 ± 0.35	3.50 ± 0.43
Plasma leptin, post-injection (ng/ml)	3.83 ± 0.63	6.52 ± 0.71 [*]
Body weight gain, post-injection (g)	57.4 ± 5.5	79.9 ± 5.7 [*]
Hypothalamic IR levels (% of hypo-Con)	100.0 ± 9.2	61.4 ± 6.1 [#]
Hippocampal IR levels (% of hypo-Con)	100.0 ± 5.1	104.5 ± 5.6
Stress challenge: plasma CORT AUC	15.6 ± 1.4	14.2 ± .0.89
OGTT: plasma Glucose AUC	167.3 ± 6.0	165.2 ± 5.1
OGTT: plasma insulin AUC	132.8 ± 14.4	129.1 ± 14.98

Data based upon at least 10 rats/group. Plasma analyses: Glucosetrinder (glucose); Enzyme-linked immunosorbent assay (corticosterone, insulin, leptin). AUC: area under the curve. Units for CORT AUC: mg min/L. Units for glucose AUC: g min/L. Units for insulin AUC: ug min/L

^{*} P < 0.05

[#] P < 0.001.