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Research report

Growth restriction, leptin, and the programming of adult behavior in mice

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HIGHLIGHTS

- Perinatal growth restriction increases the risk of adult behavioral problems.
- Growth restriction decreases circulating leptin, a neurotrophic hormone.
- Neonatal leptin deficiency decreases frontal lobe volume.
- Leptin deficiency programs impaired socialization, learning, and fear response.
- Neonatal leptin supplementation ameliorates observed behavioral changes in adult mice.

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ABSTRACT

Prematurity and neonatal growth restriction (GR) are risk factors for autism and attention deficit hyperactivity disorder (ADHD). Leptin production is suppressed during periods of undernutrition, and we have shown that isolated neonatal leptin deficiency leads to adult hyperactivity while neonatal leptin supplementation normalizes the brain morphology of GR mice. We hypothesized that neonatal leptin would prevent the development of GR-associated behavioral abnormalities. From postnatal day 4–14, C57BL/6 mice were randomized to daily injections of saline or leptin (80 ng/g), and GR was identified by a weanling weight below the tenth percentile. The behavioral phenotypes of GR and control mice were assessed beginning at 4 months. Within the tripartite chamber, GR mice had significantly impaired social interaction. Baseline escape times from the Barnes maze were faster for GR mice (65+/−6 s vs 87+/−7 s for controls, $p < 0.05$), but GR mice exhibited regression in their escape times on days 2 and 3 (56% regressed vs 22% of control saline mice, $p < 0.05$). Compared to controls, GR mice entered the open arms of the elevated plus maze more often and stayed there longer (72+/−10 s vs 36+/−5 s, $p < 0.01$). Neonatal leptin supplementation normalized the behavior of GR mice across all behavioral assays. In conclusion, GR alters the social interactions, learning and activity of mice, and supplementation with the neurotrophic hormone leptin mitigates these effects. We speculate neonatal leptin deficiency may contribute to the adverse neurodevelopmental outcomes associated with postnatal growth restriction, and postnatal leptin therapy may be protective.

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1. Introduction

Leptin has well described roles in the regulation of adult body composition and metabolism. Classically, increased food intake leads to increased adipocyte leptin production, which in turn suppresses appetite and stimulates metabolism, completing a negative feedback cycle. Unfortunately, the fetus lacks control over their own nutritional intake during a critical developmental window in which leptin exerts important neurotrophic effects. While transplacental delivery and endogenous leptin production typically support perinatal brain development, this system fails in the presence of maternal–fetal undernutrition or premature delivery, and both intrauterine growth restricted and premature infants have critically low circulating leptin levels [1–3].

With advances in healthcare facilitating cardiopulmonary support at earlier gestational ages and lower birth weights, the survival of low birth weight and preterm infants has improved significantly over the past 30 years [4]. As a consequence, there is a growing population that may be vulnerable to the long-term effects of preterm

Abbreviations: GR, growth restriction; ADHD, attention deficit hyperactivity disorder.

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birth. Despite advances in neonatal nutrition including the early provision of protein supplementation, postnatal growth restriction (GR) develops in a majority of premature infants, and together, prematurity and neonatal GR increase the risk of neurodevelopmental impairment, autism and attention-deficit hyperactivity disorder (ADHD) [5–12].

Rodents are born with neurodevelopmental immaturity. The first two postnatal weeks of life of a mouse approximates the third trimester of brain development of humans. This correlation allows modeling of the effects of prematurity associated neonatal GR on the developing brain. Our previous studies have shown that neonatal GR mice experience cardiovascular and metabolic sequelae, reminiscent of the phenotypes described in premature and otherwise GR populations [13]. We have further shown that GR mice have alterations in brain morphology that are mitigated by leptin supplementation, but classic tests for learning, autism-like and ADHD-like behavior were not performed [13]. Our most recent investigations revealed that otherwise well-nourished mice with isolated neonatal leptin deficiency have reduced adult brain volumes and increased adult locomotor activity [25]. We hypothesized that neonatal GR alters adult behavior in mice, and that these behavioral disturbances can be prevented with neonatal leptin supplementation.

2. Methods

2.1. Animal model

All animal procedures were approved by the University of Iowa Animal Care and Use Committee. Utilizing an established model of neonatal GR [13], C57BL/6J mice were bred from initial stock (Jackson Laboratories, Bar Harbor, ME). Pups with appropriate intrauterine growth (birth weight > 10th percentile) were cross fostered into litters of 6 or 12 from day of life 1 to 21 to obtain control and GR mice respectively. GR pups were randomized to daily intraperitoneal injections of leptin (80 ng/g) or vehicle alone (10 ml/kg normal saline), while control mice received daily normal saline injections (10 ml/kg). The injections encompassed the phase of leptin-dependent neurodevelopment extending from postnatal day 4–14. We previously demonstrated this leptin dose normalizes circulating leptin levels and brain morphology in GR mice [13]. Upon weaning on day of life 21, neonatal GR was confirmed by a weight < 10th percentile (<7.1 g in males, <6.75 g in females); mice from litters of 12 pups that exceeded this cut-off were excluded from further investigation. Behavior testing was performed beginning at 4 months. Overall, 194 out of 225 mice (86%) underwent a single behavioral test, and no mouse underwent all three behavioral tests.

2.2. Social interaction

Social interaction was assessed using a tripartite chamber using previously described methods [14], [15], and [16]. The apparatus is constructed of Plexiglas with dimensions 40 cm wide × 22 cm high × 22 cm deep with three identical size chambers connected by 5 cm × 5 cm doors. Each test mouse was placed in the center chamber without access to the lateral chambers for a 5 min habituation period. Then, a stranger mouse of the same sex and strain as the test mouse was placed under a wire enclosure in one lateral chamber and an identical empty wire enclosure was placed in the opposite lateral chamber. The test mouse was then allowed to freely explore for 10 min. The amount of time spent in each chamber was measured, along with the amount of time spent interacting with (sniffing) the stranger mouse versus empty enclosure. As a screen for impaired social interaction, the percentage of sniffing time directed towards the stranger mouse was determined, and poor social interaction was identified whenever this value was more than 1 standard deviation below the colony mean. Videos were scored by three independent investigators who were blinded to group assignment.

2.3. Spatial learning

Spatial learning was assessed by Barnes maze using established methods [17]. The apparatus is a circular platform of 125 cm diameter with 40 potential escape holes of 5 cm in diameter. The escape hatch is a black enclosure 8 cm × 6 cm × 20 cm in dimensions that attaches below the target escape hole. Colored shapes were placed on the four walls of the secluded test room to allow orientation to the environment. Test mice underwent 3 min trials in triplicate on 5 consecutive days and the amount of time taken to find the escape hole was measured using video tracking software (ViewPoint Live Sciences, Inc. Montreal, Canada). If a mouse did not find the escape hole during a particular trial, the mouse was placed next to the escape hole and allowed to escape spontaneously; latency to escape was then assigned a maximum value of 3 min. Trials were performed at 30 min intervals.

2.4. Anxiety/fear response

Anxiety and fear were assessed using an elevated plus maze apparatus using established methods [18]. The maze had dimensions of 35 cm long by 5 cm wide for the open and closed arms, 5 cm by 5 cm center platform, and 10 cm height of the closed arm walls. The entire apparatus was elevated 50 cm above the ground. Testing was performed in a darkened room with a red spectrum light the only light source. The mice underwent a testing trial of 5 min by being placed on the central platform of the plus maze, and allowed to explore all arms of the maze freely. The amount of time spent in the open and closed arms was recorded using video tracking software (ANY Maze version 4.98, Stoelting Co.).

2.5. Statistical analysis

One way ANOVA was used for between-group comparisons. Fisher's exact test was used to compare categorical variables. Analysis was performed using Sigma Plot 12.0. A p value of <0.05 was considered significant.

3. Results

3.1. Animal model

Control mice came from 26 litters, GR mice from 19 litters and GR-leptin mice from 23 litters. Weanling weight was not altered by neonatal leptin administration (GR-saline: 6.17+/−0.08 g, $N = 73$, GR-leptin: 6.05+/−0.10, $N = 72$, P = 0.33). By definition, both groups of GR mice had weanling weights significantly below control mice (control: 9.43+/−0.13, $N = 80$, $P < 0.001$). Both male and female mice were included in each of the behavioral assays, and no sex-specific effects were observed.

3.2. Social interaction

There were no significant between group differences in the number of times the test mice entered either side of the tripartite chamber (data not shown). Likewise, the total time spent in each lateral chamber and the overall interaction time (sniff time) was not significantly altered by neonatal GR (Fig. 1A). For each test mouse, interest in social interaction with the stranger mouse was corrected for differences in general activity by dividing the time sniffing

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