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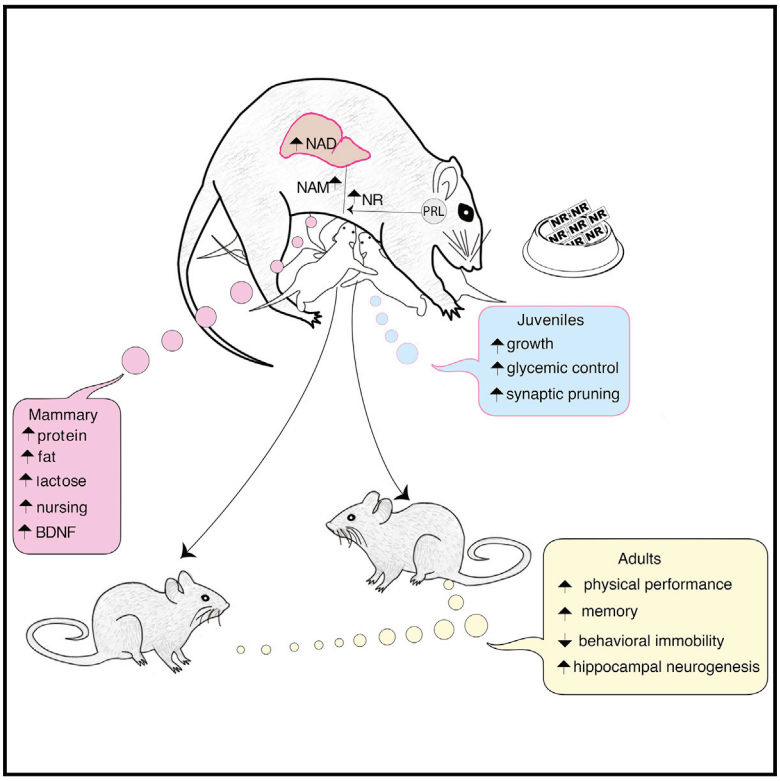
Maternal Nicotinamide Riboside Enhances Postpartum Weight Loss, Juvenile Offspring Development, and Neurogenesis of Adult Offspring

Graphical Abstract



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In Brief

Postpartum constitutes a profound episode of metabolic stress. Ear et al. show that nicotinamide riboside supplementation of rodent mothers relieves postpartum metabolic stress and increases lactation, nursing behavior, and transmission of brain-derived neurotrophic factor into milk, resulting in improved physical and neurobehavioral development of offspring, whose advantages persist into adulthood.

Highlights

- Postpartum liver circulates NAD metabolites to increase mammary NAD^+ and $NADP^+$ >20-fold
- NR supplementation superinduces prolactin, mammary biosynthetic programs, and lactation
- Weanlings of NR-fed mothers are hypoglycemia resistant and advanced in motor learning
- Adult offspring of NR-fed mothers retain striking physical and behavioral advantages

Ear et al., 2019, Cell Reports *26*, 969–983
January 22, 2019 © 2019 The Author(s).
https://doi.org/10.1016/j.celrep.2019.01.007

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Maternal Nicotinamide Riboside Enhances Postpartum Weight Loss, Juvenile Offspring Development, and Neurogenesis of Adult Offspring

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https://doi.org/10.1016/j.celrep.2019.01.007

SUMMARY

Conditions of metabolic stress dysregulate the NAD metabolome. By restoring NAD, nicotinamide riboside (NR) provides resistance to such conditions. We tested the hypotheses that postpartum might dysregulate maternal NAD and that increasing systemic NAD with NR might benefit mothers and offspring. In postpartum mothers, the liver NAD metabolome is depressed while blood increases circulation of NAD metabolites to enable a >20-fold increase in mammary NAD^+ and $NADP^+$. Lactation and NR synergize in stimulating prolactin synthesis and mammary biosynthetic programs. NR supplementation of new mothers increases lactation and nursing behaviors and stimulates maternal transmission of macronutrients, micronutrients, and BDNF into milk. Pups of NR-supplemented mothers are advantaged in glycemic control, size at weaning, and synaptic pruning. Adult offspring of mothers supplemented during nursing retain advantages in physical performance, anti-anxiety, spatial memory, delayed onset of behavioral immobility, and promotion of adult hippocampal neurogenesis. Thus, postgestational maternal micronutrition confers lasting advantages to offspring.

INTRODUCTION

Nicotinamide adenine dinucleotide (NAD) and related co-enzymes are the central mediators of virtually all metabolic processes (Belenky et al., 2007; Bogan and Brenner, 2008). Despite the importance of maintaining sufficient levels and redox ratios of NAD^+, NADH, $NADP^+$, and NADPH, a variety of metabolic stresses disturb one or more NAD metabolites. In the liver, alcohol depresses the NAD^+/NADH ratio (Cederbaum, 2012), while overnutrition and type 2 diabetes depress $NADP^+$ and NADPH (Trammell et al., 2016b). When DNA is damaged, tissue NAD^+ is consumed by poly(ADPribose) polymerase (Bouchard et al., 2003), and NADPH can be depleted by reactive oxygen species (Pollak et al., 2007). Target tissue NAD^+ is depressed in noise-induced hearing loss (Brown et al., 2014), central brain injury (Vaur et al., 2017), and heart failure (Diguet et al., 2018). In addition, liver NAD^+ is dysregulated by circadian disruption (Sato et al., 2017), and tissue NAD^+ reportedly declines as vertebrates age (Braidy et al., 2014). In many of these diseases and conditions, the ability to salvage nicotinamide (NAM) is dampened, while the nicotinamide riboside (NR) kinase 1 and/or 2 genes (Bieganowski and Brenner, 2004) are transcriptionally induced (Diguet et al., 2018; Vaur et al., 2017). By repletion of the stress-challenged NAD metabolome, NR is an orally active agent that ameliorates diseases and conditions of metabolic stress in rodents (Brown et al., 2014; Diguet et al., 2018; Hou et al., 2018; Trammell et al., 2016b; Vaur et al., 2017; Zhang et al., 2016). Consequently, NR is also being tested in human diseases and conditions (Dollerup et al., 2018) and is in human use as a nutritional supplement (Martens et al., 2018; Trammell et al., 2016a).

Postpartum constitutes an understudied episode of metabolic stress in which a new mother experiences a storm of hormonal alterations and sleep disruption, while her pituitary, liver, and mammary become programmed for lactation, and she undertakes the physical and emotional caretaking of her offspring (Brown et al., 2006). Maternal health influences milk quantity, milk quality, and neonatal health, which affect brain development and somatic health through mechanisms that are incompletely defined (Innis, 2014).

We initially hypothesized that increasing systemic NAD metabolism during gestation and postpartum might support postpartum weight loss and allow neonates to resist the effects of maternal obesity. In the course of doing these experiments, we discovered that adding NR to normal chow (NC) solely in the 21 days in which new mouse and rat mothers are co-housed with pups produces a series of profound effects on maternal metabolism and juvenile development, which result in persistent physical, neurobehavioral, and neurodevelopmental advantages to the adult offspring of NR-supplemented mothers.

Lactation is known to ramp up macronutrient mobilization to and macronutrient synthesis by the mammary (Barber et al.,

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1992) for the benefit of neonates in a process termed homeorhesis (Bauman and Currie, 1980). Here we discovered that the hepatic and mammary NAD systems are profoundly altered during the postpartum period, with the liver sacrificing and distributing its NAD metabolome to provide a strikingly elevated set of NAD co-enzymes in mammary. We found that oral NR relieves the depressed liver NAD metabolome and superinduces mobilization of NAD precursors by liver and mammary. Furthermore, NR boosts prolactin (PRL) expression by the pituitary and results in increased milk production and increased time of arched-back nursing. Milk produced by NR-supplemented mothers is as rich in protein, fat, and carbohydrate as milk from nonsupplemented mothers but is more plentiful and contains higher levels of brain-derived neurotrophic factor (BDNF), a growth factor that enhances juvenile brain development (Egan et al., 2003; Huang and Reichardt, 2001; Huang et al., 1999; Martinowich et al., 2007). Moreover, offspring of NR-supplemented mothers have better glycemic control and are larger at weaning. Offspring of NR-supplemented mothers have multiple behavioral advantages, which include decreased anxiety-like behavior, resistance to the onset of behavioral immobility, improved spatial memory, and enhanced motor learning and performance. These advantages were apparent in 15-day-old pups and persisted into adulthood, despite the fact that the intervention was to the mother and only lasted from parturition to weaning at 21 days. Finally, we showed that the adult offspring of NR-supplemented mothers have increased hippocampal neurogenesis in the dentate gyrus. These data show that in rodents, a new mother's metabolism and maternal functions can be enhanced by increasing her systemic NAD synthesis, thereby conferring long-lasting advantages to her offspring.

RESULTS

NR Promotes Postpartum Weight Loss in Mice and Increases Lactation in Mice and Rats

In male mice, NR protects against high-fat diet-induced weight gain (Cantó et al., 2012) and hepatic steatosis (Trammell et al., 2016b). By performing quantitative targeted NAD metabolomics, we discovered that obesity and type 2 diabetes modestly depress hepatic NAD^+ and greatly depress hepatic $NADP^+$ and NADPH (Trammell et al., 2016b). One previous study examined the effect of NR on female rodents, that is, prevention and reversal of chemotherapeutic neuropathy in female rats (Hamity et al., 2017). We considered that the female life cycle has unique developmental features that might be addressed by increasing NAD^+-dependent fuel oxidation programs and NADPH-dependent biosynthetic and detoxification pathways. Thus, we hypothesized that pregnancy and postpartum constitute metabolic stresses that might be addressed by oral supplementation with NR and that the metabolic stresses of pregnancy and postpartum might be aggravated by obesity.

As shown in Figure S1A, we used two male C57BL/6NJ males to impregnate 16 18-week-old females of the same strain. Four females were given NC; four, NC supplemented with 3 g/kg NR chloride (NR); four, a 60% fat high-fat diet (HFD); and four, HFD supplemented with 3 g/kg NR (HFDNR). This degree of high-fat feeding produced a high proportion of stillbirths—there were only 2 live births to 1 HFDNR-fed mother and no live births to any HFD mother—rendering it impossible to assess advantages of offspring of NR-supplemented mothers in the obese cohorts. Because fecundity was normal in the NC and NR cohorts, we continued NC-fed mothers on NC and NR-fed mothers on NR and made three surprising observations. First, 22-day-old pups of NR-fed mothers were protected against fasting-induced hypoglycemia (Figure S1B). Second, we noticed that 15- to 21-day-old pups of NR-fed mothers were more active than pups of nonsupplemented mothers. Third, NR-supplemented mothers lost 3 g of weight in the 21-day postpartum period compared with 1.5 g lost by NC-fed mothers in the same period (Figure S1C).

We hypothesized that NR-supplemented mothers might lose weight by virtue of increased lactation. To test this hypothesis and to determine whether supplementing mothers solely in the postpartum period is sufficient for increased weight loss, we generated a new cohort of mice. In these and subsequent experiments (Figure 1A), dams were 12 weeks old when mated and were exclusively fed NC prior to parturition. Thus, the NR intervention was only for the 21 days in which pups were co-housed with their mothers. At 21 days, pups were weaned, and whether the mother was fed NC or NR, all cages were provided with NC. As shown in Figure 1B, mothers fed NR lost an average of 0.95 g of body weight during these 3 weeks, whereas mothers on NC gained 1.88 g during the 3 weeks. We note that because 12-week-old mice have not reached maximal size, the younger age of this and subsequent maternal cohorts precludes the weight loss by NC-fed mothers observed in Figure S1C. As shown in Figure 1C, despite their weight loss, NR-supplemented mothers tend to eat more than NC-fed mothers. As shown in Figures S1D and S1E, NR-supplemented mothers lose body weight with a loss of fat mass and retention of lean mass.

In principle, mothers with more pups might be expected to be advantaged in weight loss, while pups from smaller litters might be advantaged in weight gain (Habbout et al., 2013). Cross-fostering is an approach to normalize litter size, particularly after a gestational intervention. However, because cross-fostering introduces unwanted behavioral and physical effects (Bartolomucci et al., 2004) and because our intervention was post-parturition, we avoided this intervention. As shown in Figure 1D, the mean litter size of mothers who were given NC (6.4) and NR (7.1) was nearly identical ($n = 8$, $p = 0.48$).

On days 7, 14, and 21 postpartum, mothers were separated from their pups for 4 h, given a 2 IU/kg dose of oxytocin, and milked for 15 min. As shown in Figure 1E, NR supplementation results in increased lactation, particularly at day 14. To test whether NR promotes increased lactation in another species, we performed the same experiment in Fisher 344 rats with an oxytocin dose of 4 IU/kg and confirmed that oral NR increases lactation (Figure S1F). As shown in Figures S1G–S1L, milk from NR-supplemented mouse and rat mothers has a normal density of total fat, carbohydrate, and protein. Thus, increased milk production by NR-supplemented mothers indicates that provision of oral NR increases maternal metabolism and transmission of macronutrients to offspring.

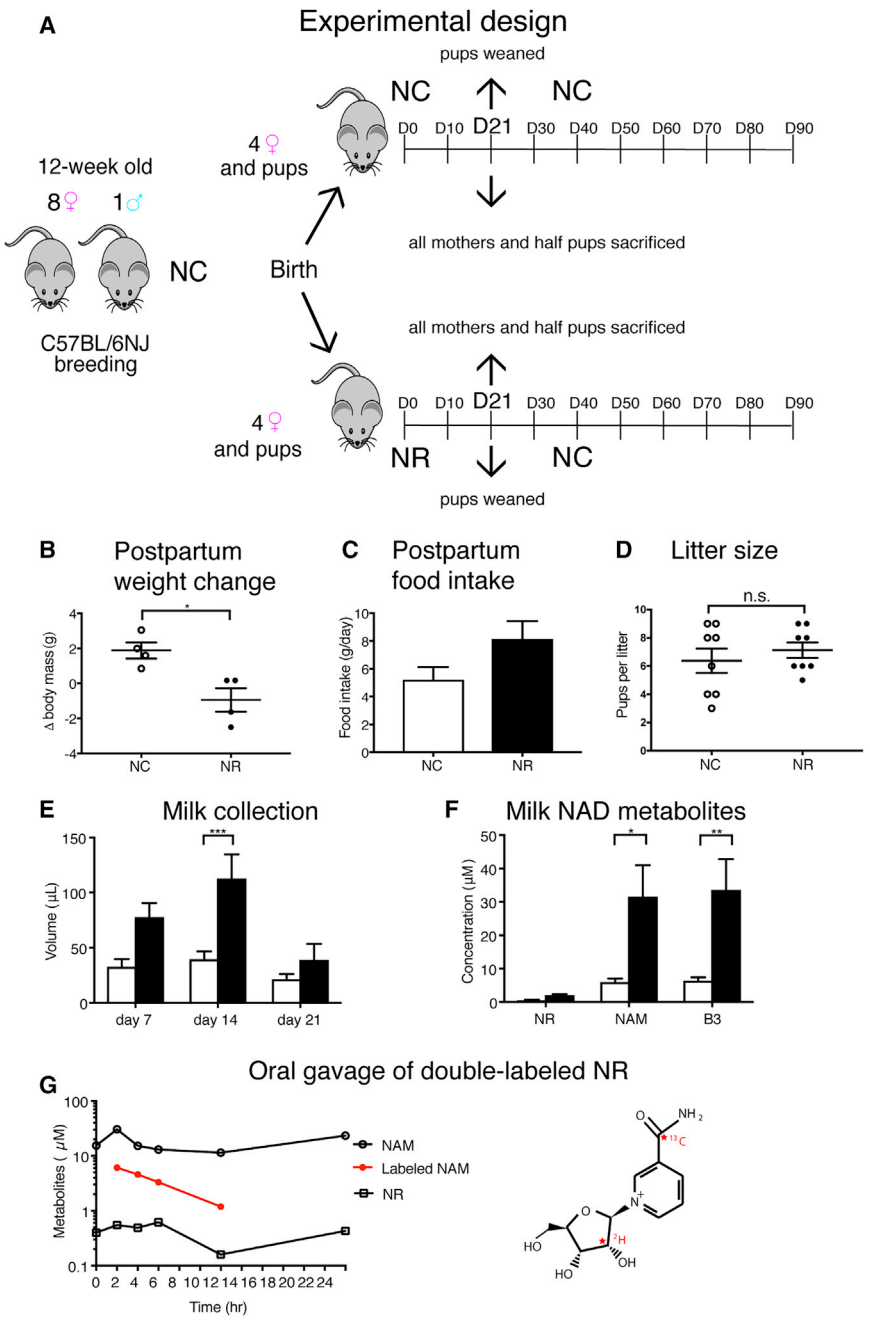

Figure 1. Postpartum NR Promotes Maternal Weight Loss and Lactation

(A) Experimental design for a 21 day NR intervention for postpartum mothers. Prior to parturition and upon weaning, all mice were on normal chow (NC).

(B) Postpartum (21 day) weight change shows that nicotinamide riboside (NR) protects mothers from weight gain (n = 4 in each group).

(C) Average daily food consumption during the 21 day intervention (n = 4 in each group).

(D) Litter size of NC and NR-supplemented mothers (n = 8 in each group).

(E) Oxytocin-induced milk collected from NC and NR mothers on days 7, 14, and 21 post-parturition (n = 8 in each group) shows that NR boosts lactation.

(F) Concentration of NAD metabolites (n = 4 in each group) shows that maternal NR increases the concentration of milk NAD metabolites. B3 is the sum of NR and nicotinamide (NAM).

(G) Time course of oral gavage of double-labeled NR (one mouse at each of six time points). The concentration of nonlabeled metabolites and single-labeled NAM is reported by quantitative targeted metabolomics at each time point. Double-labeled NR was never detected.

Data were analyzed using unpaired Student's t test and two-way ANOVA with the Holm-Sidak multiple-comparisons test. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Data are represented as individuals with mean or mean ± SEM. Open symbols and plungers are data from NC-fed mothers; filled symbols and plungers are data from NR-fed mothers.

Oral NR Increases NR Transmission to Milk but Is Not Directly Transmitted to Milk

NR is a natural product that is found in milk and is highly stable in milk (Bieganowski and Brenner, 2004; Trammell et al., 2016c). Thus, we aimed to test what effect oral NR would have on the milk NAD metabolome and whether the mother's oral NR is directly transmitted to milk or has targets in the mother's tissues. As shown in Figure 1F, the two NAD precursor vitamins that were found in bovine milk by liquid chromatography-mass spectrometry (LC-MS) (Trammell et al., 2016c), namely NAM and NR, are present in mouse milk at higher concentrations when mothers were NR supplemented. As with macronutrients, with a greater degree of lactation, mothers would transmit more micronutrients by transmitting greater volumes of milk. To test whether oral NR is directly transmitted to offspring, we used a double-labeled NR in which the NAM and ribose moieties each incorporate a heavy atom (Ratajczak et al., 2016). The chloride salt of this compound (185 mg/kg) was introduced by gavage to lactating mice at day 13 post-parturition. At 2–24 h post-gavage, mice were milked. As shown in Figure 1G, these mice transmitted nonlabeled NAM and NR at the expected concentrations. Single-labeled NAM was detected over the time course, but double-labeled NR was never found in milk. These data suggest that NR has targets in maternal organs that serve to mobilize macronutrients and micronutrients for transmission to neonates.

Postpartum Drives an NAD Metabolome Circulation Program in Liver that Is Stimulated by NR

Liver is one of the primary sites of NR metabolism (Liu et al., 2018; Trammell et al., 2016a). Because obesity and type 2 diabetes moderately depress hepatic NAD^+ and greatly depress

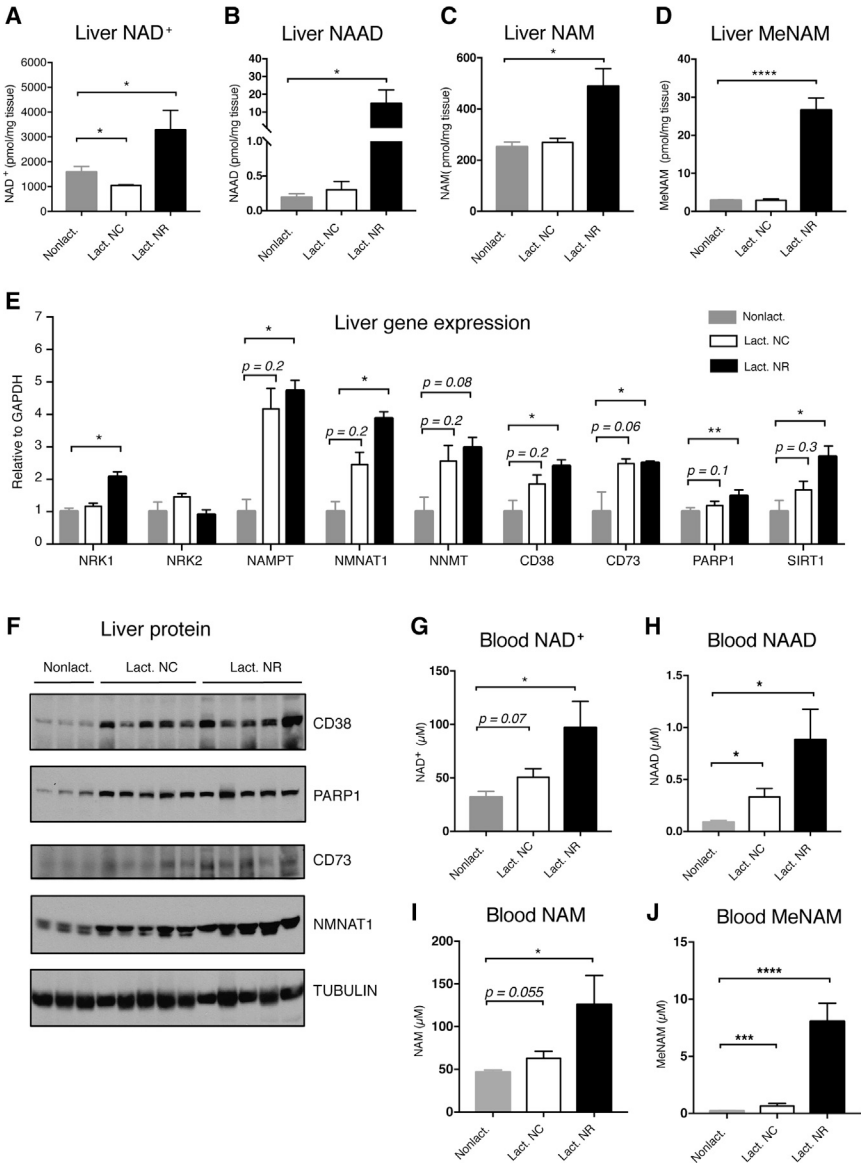

Figure 2. Postpartum Programs the Liver to Circulate NAD Metabolites

Analysis of liver and blood of lactating dams (n = 5 on NC, n = 5 on NR) at day 14 after parturition against age-matched control female mice (n = 3) shows that the postpartum liver increases NAD synthesis and NAD turnover to mobilize NAD metabolites. Although postpartum alone is sufficient to drive liver NAD^+ down and blood NAD metabolites up, NR supplementation leads to recovery of liver NAD^+ and superinduction of blood NAD metabolites. Gray plungers denote nonlactating females, white plungers denote lactating dams, and black plungers denote lactating dams supplemented with NR.

(A) Liver NAD^+ is depressed in postpartum but is increased by NR supplementation with respect to liver NAD^+ in age-matched females.

(B–D) Liver NAAD (B), NAM (C), and MeNAM (D) are increased by oral NR supplementation.

(E) Expression of enzymes involved in NAD synthesis and turnover is increased at day 14 after parturition (qPCR with GAPDH control). For the most part, the condition of lactation upregulates gene expression. In the case of NRK1, lactation on NR upregulates gene expression.

(F) Protein expression levels of key enzymes involved in NAD synthesis and turnover is increased at day 14 after parturition (western blot with tubulin control).

(G–J) Blood NAD metabolites are increased in postpartum lactating dams and are superinduced by oral NR supplementation: (G) NAD^+, (H) NAAD, (I) NAM, and (J) MeNAM.

*p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. Data are represented mean ± SEM.

hepatic $NADP^+$ and NADPH (Trammell et al., 2016b), we aimed to determine whether postpartum might alter the liver NAD system. To address this question, we generated a cohort of new mouse dams that were fed either NC or NR after parturition. Though they were kept with pups in order to allow the normal postpartum and lactation program to proceed, they were sacrificed at day 14, which is at peak lactation. As shown in Figure 2A, at day 14 after parturition, in NC-fed mothers, hepatic NAD^+ is significantly depressed with respect to the NAD^+ level in age-matched females. However, supplementation of new mothers with NR allowed them to increase their liver NAD^+ and increase levels of hepatic NAAD, NAM, and *N*-methylnicotinamide (MeNAM) (Figures 2B–2D). These NAD metabolomic changes were accompanied by the simultaneous increase in mRNA and protein expression of key enzymes responsible for forming and catabolizing NAD. As shown in Figure 2E, at the RNA level, postpartum females increase expression of NAMPT and NMNAT1, which form NAD, while they simultaneously increase expression of SIRT1 and CD73, which turn over NAD metabolites. For the most part, the fold-change increase in expression is a function of the female lactating. In the cases of NRK1, NMNAT1, and SIRT1, the addition of NR to the lactating dam's chow superinduces liver NAD biosynthetic and catabolic programs. At the protein level, NMNAT1 is increased, while NAD-consuming enzymes, especially CD38 and PARP1, are highly upregulated in postpartum liver (Figure 2F). Consistent with a higher rate of synthesis and turnover of NAD in the liver of lactating postpartum females, circulating NAD metabolites were higher in the blood of lactating dams than nonlactating females (Figures 2G–2J). These increases reached statistical significance for blood NAAD and MeNAM without NR supplementation.

Supplementation of maternal chow with NR boosted blood NAD^+ and NAM by 2-fold and increased levels of blood NAAD and MeNAM by 10-fold. Thus, during lactation, the liver sacrifices levels of NAD^+ to increase circulation of NAD metabolites to other tissues. By virtue of maternal supplementation with NR, the NAD metabolome can be maintained at levels at or

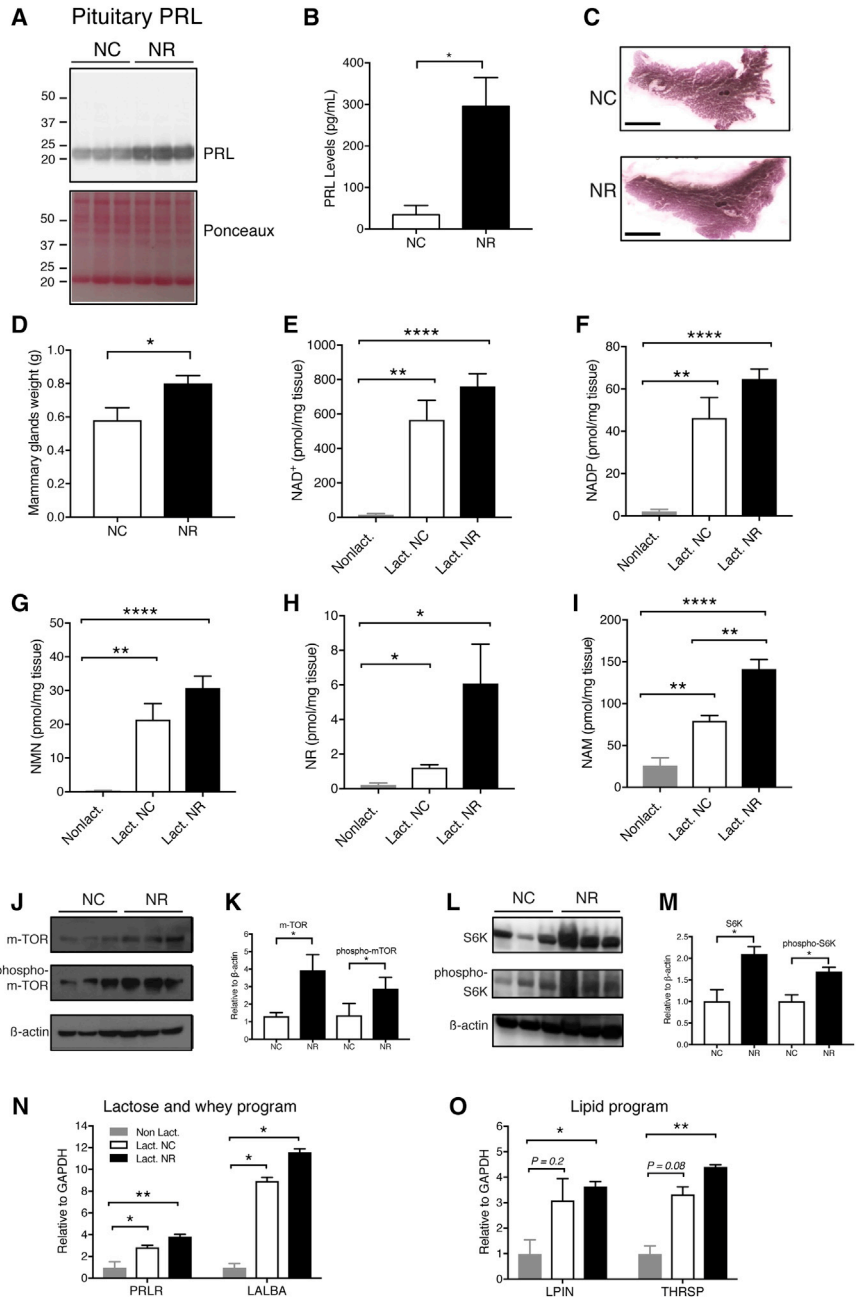

Figure 3. NR Superinduces the Pituitary and Mammary Lactation Programs

(A) On postpartum day 14, pituitary prolactin (PRL) levels are enhanced by NR (n = 5 in each group).

(B) Plasma PRL is highly increased by NR (n = 4 in each group).

(C) Representative images of mammary glands 4 and 5 of dams at postpartum day 22 show that NR increases mammary development. Scale bars, 1 cm.

(D) Average weight of mammary glands 1 to 5 is greater on postpartum day 14 when mothers are on NR (n = 5 in each group).

(E–I) Postpartum greatly increases levels of mammary NAD metabolites with respect to mammary from age-matched females: (E) NAD^+, (F) $NADP^+$, (G) NMN, (H) NR, and (I) NAM. The data further show that NR supplementation superinduces the mammary NAD metabolome (n = 3 nonlactating, n = 5 on NC, n = 5 on NR).

(J–O) Mammary biosynthetic programs are increased by maternal NR as evidenced by western blotting (J) and quantification (K) of mTOR and phospho-mTOR (phosphoSer2448), western blotting (L) and quantification (M) of S6 kinase, and phospho-S6 kinase (phosphoThr389), and qPCR for genes involved in lactose and whey (N) and lipid (O) biosynthesis.

Gray plungers denote control female mice, white plungers denote lactating female mice, and black plungers denote lactating female mice fed NR. *p < 0.05, **p < 0.01, and ****p < 0.0001. Data are represented as mean ± SEM.

greater than levels of a nonlactating female and the circulating NAD metabolome is superinduced.

NR Superinduces the Pituitary and Mammary Lactation Programs

PRL is the principal hormone responsible for driving lactation and plays an important role in driving maternal behaviors (Frantz, 1978). It was previously shown that MeNAM induces expression of PRL in a pituitary-derived cell line (Kimura et al., 1983). To connect our observation of increased lactation with NR supplementation, we asked whether NR supplementation increases PRL expression. As shown in Figure 3A, PRL protein expression is 2-fold induced by NR in the pituitary of mouse mothers at day 14 after parturition. Moreover, as shown in Figure 3B, PRL circulation is increased by 6-fold. Plasma estrogen, oxytocin, and progesterone were also tested and showed a tendency for elevated estrogen in NR-supplemented mothers (Figures S2A–S2C). As shown in Figures 3C and 3D, NR supplementation promotes mammary tissue development. In addition, the mammary NAD program is strikingly upregulated by lactation. As shown in Figures 3E and 3F, compared with age-matched mammary tissue from a nonlactating female, lactation induces accumulation of NAD^+ and $NADP^+$ by 30- and 20-fold, respectively. NAD precursors such as NMN, NR, and NAM were also highly induced (Figures 3G–3I). In each case, lactation without supplementation induces the NAD metabolite, while NR supplementation superinduces the NAD metabolite during lactation. Consistent with enhanced lactation of NR-supplemented dams and their higher level of PRL, mammary tissue from NR-supplemented mothers is superinduced for all biosynthetic programs. Consistent with the increased protein biosynthetic program required to increase lactation, as shown in Figures 3J–3M, levels of mTOR, phosphor-mTOR, S6 kinase, and phospho-S6 were increased by NR

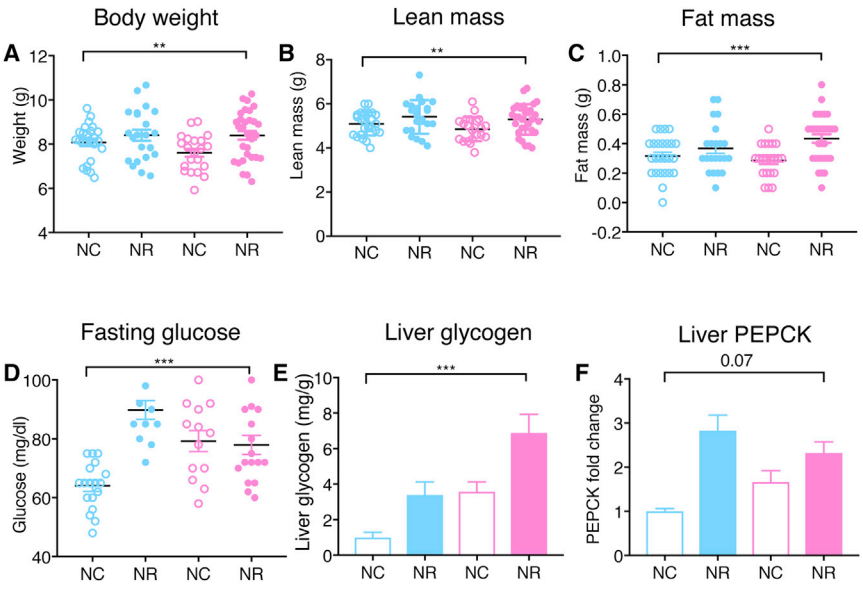

Figure 4. Maternal NR Produces Larger Weanlings that Resist Fasting-Induced Hypoglycemia

Weanling offspring of NC-fed (open circles and plungers) and NR-supplemented (filled circles and plungers) are represented by sex (light blue, male; pink, female). Indicated statistics are the two-way ANOVA main effect of maternal supplementation. p values for effects of maternal supplementation on each sex are provided in Table S1.

(A) Offspring of NR-supplemented mothers are larger at weaning (n = 25 males from NC mothers, 22 males from NR mothers, 21 females from NC mothers, and 31 females from NR mothers). Note that with 46 pups from NC mothers and 53 pups from NR mothers, the size advantage cannot be explained by fewer pups of NR-fed mothers.

(B) Offspring of NR-supplemented mothers have greater fat mass at weaning (n = 25 males from NC mothers, 22 males from NR mothers, 21 females from NC mothers, and 31 females from NR mothers).

(C) Offspring of NR-supplemented mothers have greater lean mass at weaning (n = 25 males from NC mothers, 22 males from NR mothers, 21 females from NC mothers, and 31 females from NR mothers).

(D) Fasting blood glucose of mouse pups at day 22 is protected if mothers were NR supplemented (n = 18 male pups of NC mothers, n = 12 male pups of NR-fed mothers, n = 13 female pups of NC mothers, n = 17 female pups of NR-fed mothers). A Holm-Sidak post hoc multiple-comparisons test is included.

(E) Liver glycogen content is greater if mothers were NR supplemented (n = 6 males from NC mothers, 6 males from NR mothers, 5 females from NC mothers and 6 females from NR mothers).

(F) Liver PEPCK mRNA expression assessed by qPCR against GAPDH tends to be greater if mother was NR supplemented (n = 12 males from NC mothers, 16 males from NR mothers, 7 females from NC mothers, and 8 females from NR mothers).

Data were analyzed using two-way ANOVA. **p < 0.01, ***p < 0.001, and ****p < 0.0001. Data are represented as individuals with mean or mean ± SEM.

supplementation. As shown in Figures 3N and 3O, expression of genes involved in lactose, whey protein, and lipid synthesis are also superinduced by NR supplementation. These data provide a connection between endogenous liver and mammary NAD programming and lactation and a means to further increase lactation by supplementation with NR.

Maternal NR Enhances Juvenile Growth and Glycemic Control

As schematized in Figure 1A, at 21 days postpartum, offspring were weaned to group housing with littermates and all offspring were put on an NC diet. As shown in Figures 4A–4C and Table S1, weanlings of NR-supplemented mothers are bigger than those of nonsupplemented mothers with more lean mass and fat mass. As shown in Table S1 and Figure S3A, weanling brain weight controlled for body weight also tended to be greater in the offspring of NR-supplemented mothers. In rats, weanlings of NR-supplemented mothers tended to be larger and have more lean mass (Figures S3B and S3C). Table S1 provides p values for sex-based main differences and for main differences on the basis of the maternal diet during the nursing and co-housing period. In addition, Table S1 identifies the assays for which there was an interaction between offspring sex and maternal diet.

We subjected 21-day-old mice to a 16 h fast to measure their susceptibility to hypoglycemia. As shown in Figure 4D and as seen in the preliminary study in which mothers were fed NR during pregnancy (Figure S1B), male pups reared by mothers on NC had an average fasting glucose level of 64 mg/dL, whereas males from cages with NR-supplemented mothers had a fasting glucose level of 90 mg/dL. Female pups were resistant to fasting hypoglycemia. As shown in Table S1, there were highly significant main effects of sex and maternal supplementation on resistance to hypoglycemia. To probe the mechanisms by which maternal NR protects weanlings from hypoglycemia, we raised another cohort of pregnant females that were split into NR versus NC on the day of parturition. All pups were weaned into NC cages on day 21. Mice (22 days of age) were sacrificed after a 16 h fast, and livers were isolated for analysis of gene expression and glycogen storage. As shown in Figures 4E and 4F, pups raised by NR-supplemented mothers store >2-fold more glycogen in their livers and tend to have higher RNA expression of phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme for hepatic gluconeogenesis. As shown in Table S1, by two-way ANOVA, the higher weanling body mass, lean mass, fat mass, glycemic control, and glycogen storage measurements all had maternal supplementation as a statistically significant main effect independent of sex. Glycogen storage was the only assay for which there was a statistically significant effect of sex independent of maternal supplementation. Thus, maternal NR not only increases lactation but also allows mothers to produce larger and more physically resilient weanlings.

Maternal NR Enhances Juvenile Learning and Synaptic Pruning

We initially noticed that 2-week-old pups of NR-supplemented mothers were more active in their cages and upon handling. To

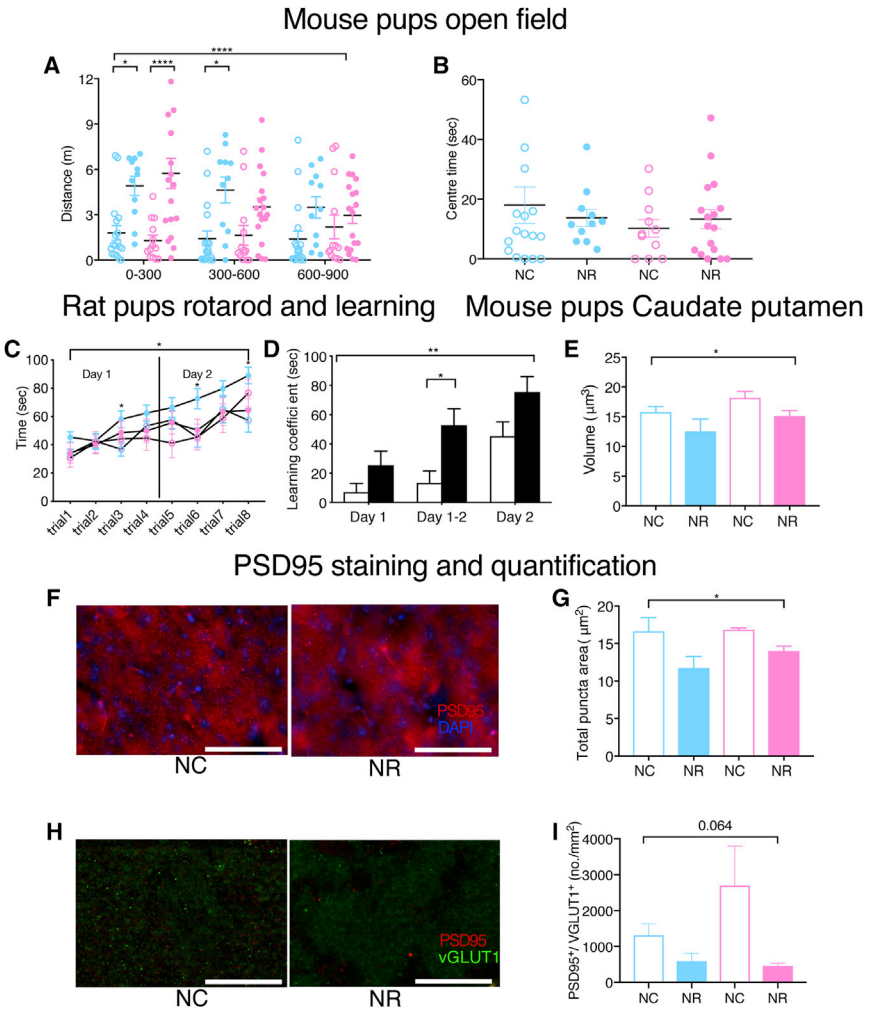

Figure 5. Maternal NR Confers Neurobehavioral and Neurodevelopmental Advantages to Juvenile Offspring

Juvenile offspring of NC-fed (open circles and plungers) and NR-supplemented (filled circles and plungers) are represented by sex (light blue, male; pink, female). Indicated overall statistics are the two-way ANOVA main effect of maternal supplementation. p values for effects of maternal supplementation on each sex are provided in Table S1.

(A) Open-field activity of 15-day-old mouse pups was measured in three 5 min epochs. Offspring of NR-fed mothers are more active (n = 18 males from NC mothers, 11 males from NR mothers, 13 females from NC mothers, and 18 females from NR mothers). Holm-Sidak multiple-comparisons analysis shows that this effect remains significant for males in the second epoch.

(B) Open-field center time in the first 5 min epoch. Offspring of NR-fed mothers have normal behavior with respect to the center of the open field (n = 17 males from NC mothers, 11 males from NR mothers, 11 females from NC mothers, and 17 females from NR mothers).

(C) Rotarod performance of 21-day-old rat pups. Offspring of NR-fed mothers have significantly better overall performance (n = 24 males from NC mothers, 20 males from NR mothers, 11 females from NC mothers, and 13 females from NR mothers).

(D) Additional seconds of performance on day 1 (trial 4 – trial 1), days 1–2 (trial 8 – trial 4), and day 2 (trial 8 – trial 5) show that offspring of NR-fed mothers are significantly advantaged in overall and day 1–2 rotarod learning (n = 32 offspring of NC mothers and n = 18 offspring of NR mothers).

(E) CP volume is more compact in the brains of 22-day-old offspring of NR-supplemented mothers (n = 4 in each group).

(F) Representative PSD95 CP immunostaining of female mice at 22 days. Scale bars, 50 µM.

(G) PSD95 puncta area quantification shows evidence of advanced pruning in the CP of offspring of NR-supplemented mothers (n = 3 in each group).

(H) Representative colocalization of PSD95 with vGLUT1 in 22-day-old female CPs. Scale bars, 20 µM.

(I) Maternal supplementation shows a strong trend toward lowering CP synapse number in 22-day-old offspring of NR-supplemented mothers (n = 2 in each group, i.e., 4 offspring of NC-fed and 4 offspring of NR-supplemented mothers).

*p < 0.05, **p < 0.01, and ****p < 0.0001. Data are represented as individuals with mean or mean ± SEM.

quantify this observation, we performed an open field test with 15-day-old mice (Carola et al., 2002). As shown in Figure 5A, male and female offspring of NR-supplemented mothers were consistently more mobile, covering substantially more distance, particularly in the initial two 5 min epochs of observation. Because mice may increase activity because of changes in motor ability or anxiety-like behavior (Carola et al., 2002), we evaluated initial center time as a measure of anxiety and found no differences (Figure 5B). In addition, when juvenile rats were assessed with open-field testing on postnatal day 19, they showed no evidence of altered anxiety-like behavior as a function of maternal diet.

Juvenile rats were assessed for motor learning on the rotarod (Carter et al., 2001) on postnatal days 21 and 22. Overall performance across trials and performance in trials 3, 6, and 8 was better in offspring of NR-fed mothers (Figure 5C). In addition, although all animals learned, the offspring of NR-supplemented mothers showed greater learning across all phases of rotarod training compared with the offspring of NC-fed mothers (Figure 5D).

To determine whether maternal diet might be responsible for advanced neuroanatomical development of motor learning circuitry, we sacrificed some of the weanlings after performance testing and analyzed the caudate-putamen (CP) in 22-day-old mice. As shown in Figure 5E, CP volume was smaller in the offspring of NR-supplemented mothers. Assessing postsynaptic PSD95 immunostaining, we discovered that in offspring of NR-supplemented mothers, the area of postsynaptic density puncta was reduced (Figures 5F and 5G). In addition, colocalization of presynaptic vGLUT1 with postsynaptic PSD95 revealed a >2-fold reduction in puncta number in the offspring of NR-supplemented mothers versus offspring of NC-fed mothers that nearly

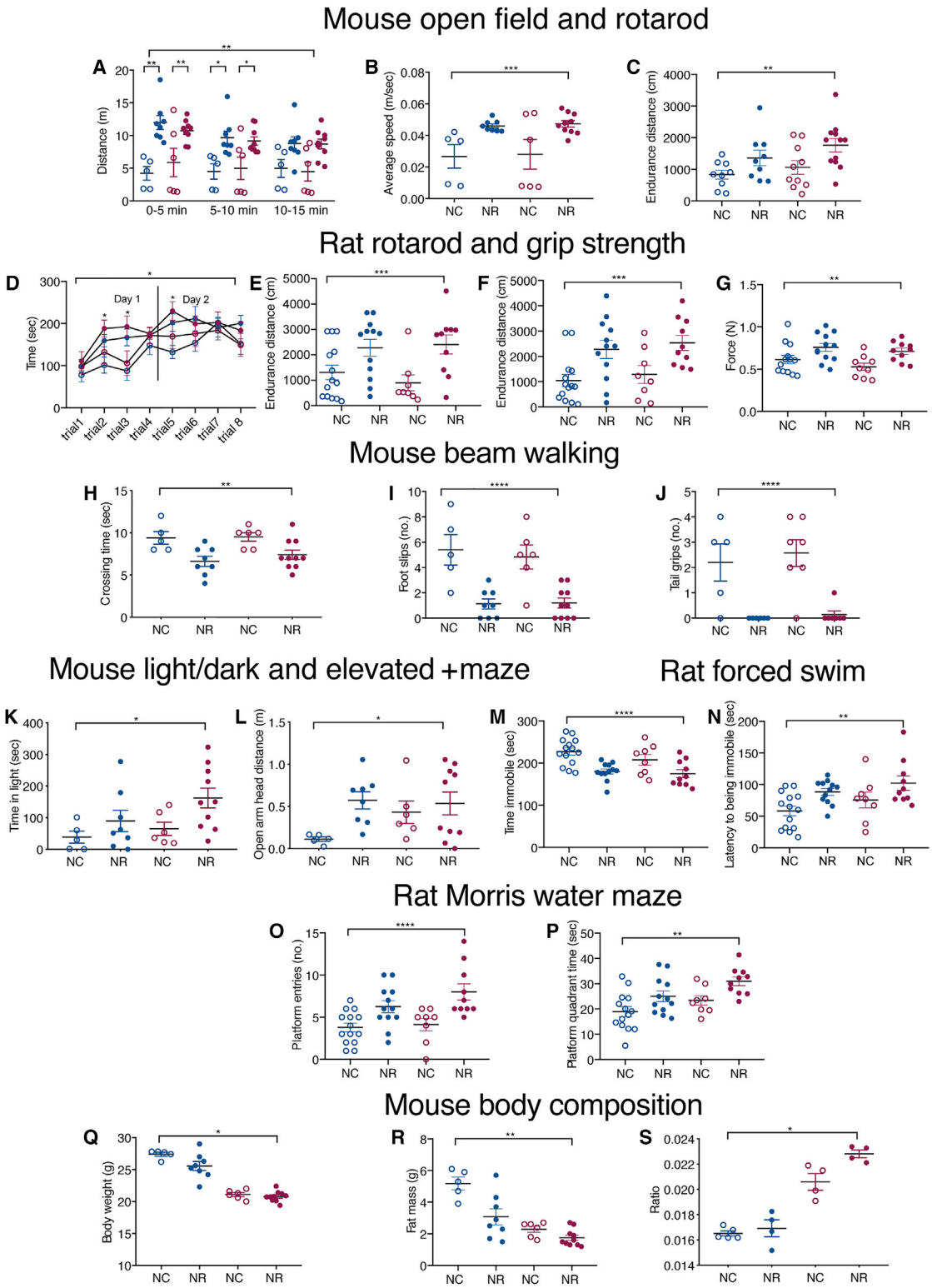

Figure 6. Maternal NR Produces Lasting Benefits in Adult Offspring Physical Performance, Memory, Resiliency, Anti-anxiety and Body Composition

Adult mouse and rat offspring that had been weaned into cages with NC were put through a battery of behavioral assays and ultimately sacrificed. The data show lasting and wide-ranging advantages of being raised by an NR-supplemented mother. Adult offspring of NC-fed (open circles) and NR-supplemented (filled

(legend continued on next page)

reached statistical significance (Figures 5H and 5I). This advanced pruning is temporally significant because peak levels of synaptic structures in mouse striatum occur in the first postnatal week (Pellegrini-Giampietro et al., 1991). Thus, maternal NR supplementation enhances juvenile behaviors including locomotor exploration, motor learning, and motor performance, and advances juvenile synaptic pruning in the CP, which is apparent at the time of weaning.

Maternal NR Produces Lasting Benefits in Physical Performance of Adult Offspring

We considered the possibility that the changes we observed in juvenile liver function, body mass, brain, and behaviors might translate into more capable and/or resilient adults. After the 21 day maternal postpartum dietary intervention, all mice and rats were maintained on NC diets for an additional 69–89 days.

A battery of adult behavioral assays was used, including open-field testing and rotarod endurance, beam walking, elevated plus maze, and light/dark box testing for mice. We used rotarod trials, a grip strength test, a forced swim, and the Morris water maze for rats. Our rats were largely immobile in open-field testing and declined to perform a beam walk. Rats completed an elevated plus maze but did not show an effect of maternal NR. For all other assays, performance data are plotted in Figure 6 as a function of maternal diet and sex, and two-way ANOVA significance data are provided in Table S1. In brief, maternal NR provided significant advantages for both sexes in all the adult behavioral and body composition assays of Figure 6. Sex was an additional main effect only in the case of Morris water maze platform entries (females had better spatial memory) and body composition (males are bigger with more lean mass, while females have bigger brains normalized to body weight).

As shown in Figure 6A, the open-field distance explored of adult (70-day-old) mouse offspring of NR-supplemented mothers was twice as great across all epochs observed. Similarly, as shown in Figure 6B, adult offspring of NR-supplemented mothers moved twice as quickly as offspring of NC-fed mothers ($p < 0.001$). To test whether adult offspring of NR-supplemented mothers would also show increased motor endurance compared with the offspring of NC-fed mothers, we measured the rotarod endurance of 85-day-old mice. As shown in Figure 6C, the 21 day intervention to postpartum mothers predisposes their adult offspring to superior rotarod endurance independent of sex.

circles) mothers are represented by sex (blue, male; red, female). Indicated statistics are the two-way ANOVA main effect of maternal supplementation. p values for effects of maternal supplementation on each sex are provided in Table S1.

(A) Open-field activity of 70-day-old mouse adults was measured in three 5 min epochs. Adult offspring of NR-fed mothers are more active (n = 5 males from NC mothers, 8 males from NR mothers, 6 females from NC mothers, and 9 females from NR mothers).

(B) Average speed of adult offspring of NR-fed mothers is significantly greater in the open-field assay (n = 5 males from NC mothers, 8 males from NR mothers, 6 females from NC mothers, and 10 females from NR mothers).

(C) At 85 days of age, adult offspring of NR-fed mothers have advantages in rotarod endurance (n = 9 males from NC mothers, 9 males from NR mothers, 10 females from NC mothers, and 12 females from NR mothers).

(D) At 77 and 78 days of age, adult offspring of NR-fed mothers have superior overall rotarod performance and outperformed on their second, third, and fifth trials (n = 14 males from NC mothers, 12 males from NR mothers, 8 females from NC mothers, and 10 females from NR mothers).

(E and F) After four training trials on day 1 (E) and four training trials on day 2 (F), adult offspring of NR-fed mothers have superior rotarod endurance compared with the endurance of adult offspring of NC-fed mothers (n = 14 males from NC mothers, 12 males from NR mothers, 8 females from NC mothers, and 10 females from NR mothers).

(G) At 76 days of age, adult rat offspring of NR-fed mothers have greater forelimb grip strength than offspring of NC-fed mothers (n = 14 males from NC mothers, 12 males from NR mothers, 8 females from NC mothers, and 10 females from NR mothers).

(H–J) At 86 days of age, adult mice were subjected to a beam walking assay. After one training run, in which offspring of NR-supplemented mothers outperformed offspring of NC-fed mothers (Figure S4A), male (blue) and female (red) offspring of NR-fed mothers (filled) outperformed the offspring of NC-fed mothers (open) in faster crossing times (H), fewer foot slips (I), and fewer tail grips (J) (n = 5 male offspring of NC-fed mothers, n = 8 male offspring of NR-fed mothers, n = 6 female offspring of NC-fed mothers, and n = 10 female offspring of NR-fed mothers). See Video S1 for representative beam crosses.

(K) At 70 days of age, the adult offspring of NR-fed mothers spend significantly more time in light in a light/dark box, consistent with an advantage in anti-anxiety (n = 5 male offspring of NC-fed mothers, n = 8 male offspring of NR-fed mothers, n = 6 female offspring of NC-fed mothers, and n = 10 female offspring of NR-fed mothers).

(L) At 85 days of age, adult mice were tested in an elevated plus maze. As judged by increased head distance traveled on the open arm, the adult offspring of NR-supplemented mothers are less anxious than control adults (n = 5 male offspring of NC-fed mothers, n = 8 male offspring of NR-fed mothers, n = 6 female offspring of NC-fed mothers, and n = 10 female offspring of NR-fed mothers).

(M and N) At 82 days of age, adult rats were subjected to a forced swim assay. As judged by lower times immobile (M) and greater latency to first immobility (N), the adult offspring of NR-supplemented mothers are more resilient to this challenge (n = 14 male offspring of NC-fed mothers, n = 12 male offspring of NR-fed mothers, n = 8 female offspring of NC-fed mothers, and n = 10 female offspring of NR-fed mothers).

(O and P) At 81 days of age, adult rats were subjected to 12 training trials in a Morris water maze to learn the placement of a platform. Offspring of NR-supplemented mothers had more rapid times to the platform across these trials (Figure S4B). In the 13th, in which the platform was removed, the adult offspring of NR-supplemented mothers have a strong advantage in platform entries (O) and platform quadrant time (P), indicating superior spatial memory (n = 14 male offspring of NC-fed mothers, n = 12 male offspring of NR-fed mothers, n = 8 female offspring of NC-fed mothers, and n = 10 female offspring of NR-fed mothers).

(Q–S) At 90 days of age, mice were analyze for body composition and sacrificed. Adult offspring of NR-supplemented mothers have lower body weight (Q) and fat mass (R) and have a larger brain (S) as a proportion of body weight than adult offspring of NC-fed mothers (n = 5 male offspring of NC-fed mothers, n = 8 male offspring of NR-supplemented mothers, n = 6 female offspring of NC-fed mothers, and n = 10 female offspring of NR-fed mothers).

(S) Adult mouse brain weight normalized to body weight (n = 5 male offspring of NC-fed mothers, n = 4 male offspring of NR-fed mothers, n = 4 female offspring of NC-fed, and n = 4 female offspring of NR-fed mothers).

Filled symbols are individuals whose mothers were fed NR. Data were analyzed using two-way ANOVA with the Holm-Sidak multiple-comparisons test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$. Data are represented as individuals with mean except in (D), which is mean ± SEM.

The rotarod advantages of adult offspring of NR-supplemented mice were directly recapitulated in rats. As shown in Figure 6D, in 77-day-old rats (nearly 2 months after the end of the maternal nutritional intervention), the offspring of NR-supplemented mothers showed enhanced motor performance on the rotarod. Additionally, we found that learning occurred faster with respect to offspring of nonsupplemented mothers (significantly increased latency to fall in the second, third, and fifth trials). In addition to the four daily learning trials, each rat was probed on the first and second day for maximal rotarod endurance. As shown in Figures 6E and 6F, endurance on the rotarod was strikingly increased for the offspring of NR-supplemented mothers on both days independent of sex ($p < 0.001$).

Rotarod navigation is a complex task involving strength and balance. We used our 76-day-old rats to test grip strength (Meyer et al., 1979) and our 86-day-old mice to test balance in a beam walking test (Carter et al., 2001). As shown in Figure 6G, having been nursed by an NR-supplemented mother conferred greater grip strength to adults ($p < 0.01$). Male and female offspring of NR-supplemented mothers were subjected to a training run on a 6 mm wide × 1 m beam (Figure S4A) and were videotaped on a second trial. As shown in Figures 6H–6J and Video S1, offspring that had been nursed by NR-supplemented mothers had profoundly superior beam walking skills. Whereas control adults crossed the beam in 9.5 s with an average of 5.4 foot slips and 2.2 tail grips, the adult offspring of supplemented mothers crossed the beam in 6.6 s with an average of 1.1 foot slip and almost no observed tail grips. These long-term advantages of maternal NR-supplementation were highly statistically significant for the physical performance of adult offspring independent of sex ($p < 0.0001$ for fewer foot slips and tail grips).

Maternal NR Produces Adult Offspring that Exhibit Decreased Anxiety-like and Depressive Behaviors

As nursing pups, the offspring of NR-supplemented mice are more active in exploring an open field. To determine whether offspring of NR-supplemented mothers develop into adults with greater capacity for exploration and resiliency than control adults, we subjected 70-day-old mice to a light/dark box test (Bourin and Hascoët, 2003), 85-day-old mice to an elevated plus maze (Carola et al., 2002), and 82-day-old rats to a forced swim (Slattery and Cryan, 2012).

As shown in Figures 6K and 6L, adult murine offspring of NR-supplemented mothers exhibited substantially less anxiety-like behavior than control adults (Table S1) with statistically significantly greater willingness to explore light and put their heads out on the open arm of a plus sign-shaped maze.

In the forced swim test of adult rats (Figures 6M and 6N), the mothers' NR supplementation during the nursing period dramatically reduced behavioral immobility in adult offspring assayed more than 2 months after weaning. Whereas control adults average 220 s of immobility after an average latency period of 64 s, the offspring of NR-supplemented mothers average only 177 s of immobility after a 92 s latency. These differences, typically interpreted as a lack of depressive behavior (Slattery and Cryan, 2012), were significant at $p < 0.0001$ and $p < 0.01$, respectively, across all offspring (Table S1).

Maternal NR Results in Adult Offspring with Superior Spatial Learning

We used 81-day-old rats in a Morris water maze to assess spatial learning and memory. As shown in Figure S4C, offspring of control and NR-supplemented mothers readily learn to swim more quickly to the platform, lowering their times from 33.4 s in the first trial to 8.7 s in the 12th trial with the offspring of NR-fed mothers showing overall superior performance. When the platform was removed for the 13th/probe trial, offspring of NR-supplemented mothers made more platform entries (Figure 6O; $p < 0.0001$ for both sexes) and spent more time in the platform quadrant (Figure 6P; $p < 0.01$ for both sexes), indicating that mothers' postgestational micronutrition confers greater spatial memory to adult offspring (Table S1).

Maternal NR Results in Adult Offspring with Lower Body Fat and Higher Brain Mass

At weaning, mice nursed by NR-supplemented mothers are bigger than those of NC-fed mothers, carrying greater body fat and lean mass. Whereas increased weight at weaning might be protective, rodents with greater access to milk prior to weaning have been shown to be at greater risk for inflammatory conditions and metabolic dysfunction as adults (Habbout et al., 2013). At 90 days of age, mice were analyzed for body composition by MRI and then sacrificed. As shown in Figures 6Q and 6R, offspring of NR-supplemented mothers are smaller with lower body fat and, as shown in Figure S4C, the same lean mass as offspring of NC-fed mothers. Finally, the weight of adult brains as a proportion of body weight is greater in the adult offspring of NR-supplemented mothers (Figure 6S).

NR-Supplemented Mothers Exhibit Enhanced Nursing Behavior

We aimed to identify the mechanisms responsible for the lasting effects of maternal NR on physical and neurobehavioral functions of rodent adults. Maternal care is known to provide lasting neurodevelopmental benefits (Caldji et al., 1998; Liu et al., 2000). To quantify maternal care, we trained a blinded investigator to recognize arched-back nursing, licking/grooming, and other behaviors in a set of 1 h recordings of home cage activity taken on days 11 and 15 post-parturition. Recordings were taken from 9–10 a.m. and 6–7 p.m. on both days to sample maternal behavior in the early resting and early waking periods, respectively (Jensen Peña and Champagne, 2013). Our morning and evening data were not different and were therefore summed to produce 2 h observations on each of 2 days for $n = 6$ rat mothers in each group. As shown in Figure 7A, rat mothers eating NR-supplemented food spend 35% more time in arched-back nursing ($p = 0.025$) and tend to spend increased time licking and grooming offspring (Figure S5A), thereby conferring not only increased nutrition but the potential for care-based programming for neurodevelopmental advantages in anti-anxiety (Caldji et al., 1998) and spatial learning (Liu et al., 2000). We noticed that whereas all six mothers exhibited an elevated time of arched-back nursing (Figure 7A), three mothers exhibited high licking/grooming activity, while three did not (Figure S5A). Because high licking/grooming have been linked to improved neurodevelopment (Champagne et al., 2008; Champagne and

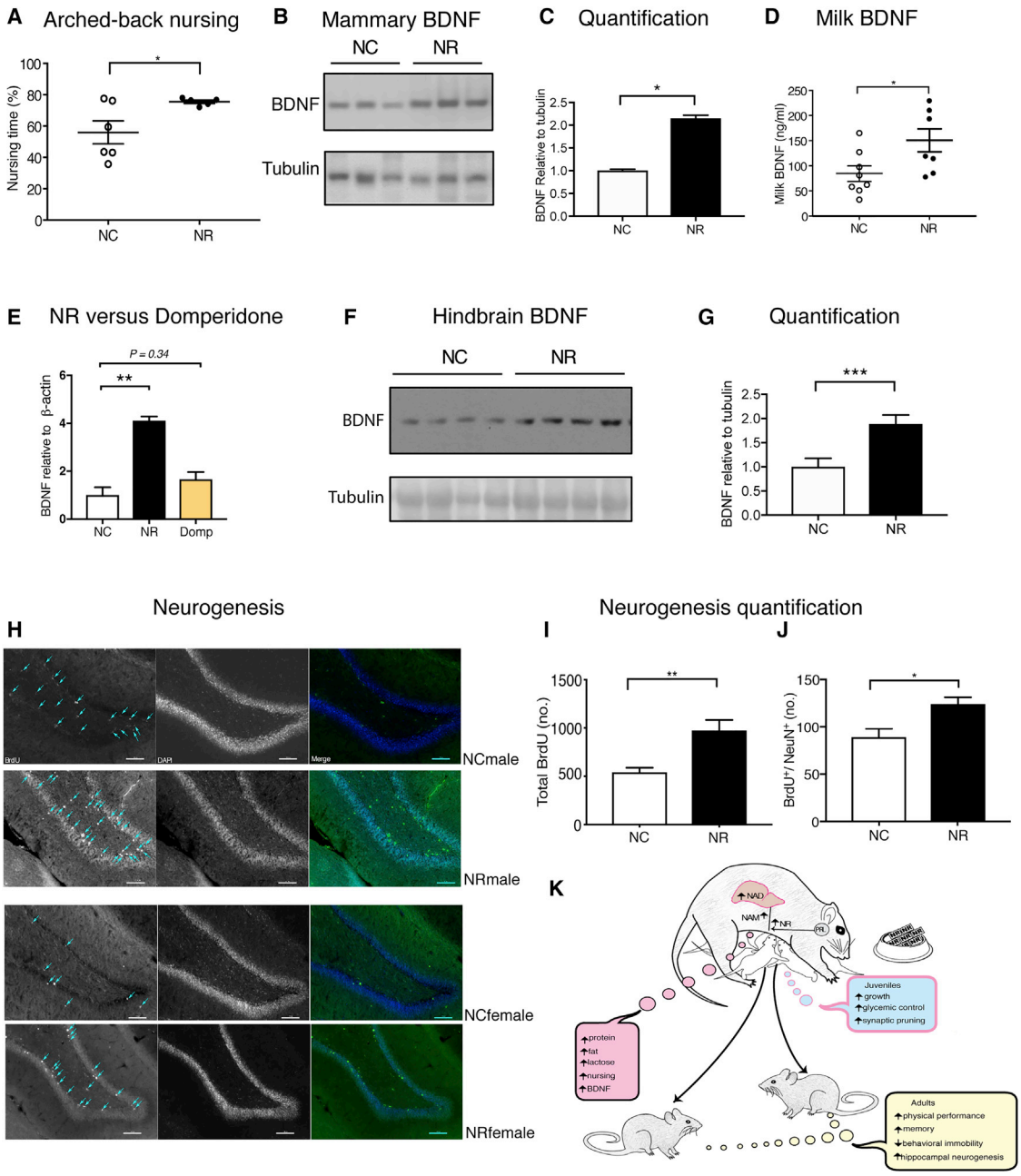

Figure 7. NR Increases Maternal Nursing Behavior and Mammary BDNF Expression into Milk and Produces Adult Offspring with Greater Hippocampal Neurogenesis

(A) At day 15 post-parturition, rat mothers supplemented with NR spend more time in arched-back nursing (n = 6 mothers fed NC and n = 5 mothers supplemented with NR).

(B and C) Western analysis (B) and quantification (C) of mammary of mouse mothers at day 14 post-parturition shows that NR-supplementation increases expression of BDNF (n = 3 in each group).

(D) ELISA analysis of BDNF concentration in day 14 mouse milk shows that NR-supplementation increases BDNF deposition into milk (n = 8 in each group).

(E) In a blinded study, 12-week-old female rats were mated and, upon parturition, assigned to be fed NC, NR, or NC + 0.1 g domperidone/kg of NC. At a dose of domperidone that produced the same increase in lactation as NR, the mammary expression (14 days after parturition) of BDNF by qPCR is 4-fold greater in NR-supplemented mothers than in NC-fed mothers. In contrast, domperidone does not elevate expression of BDNF (n = 6 fed NC, n = 5 fed NR, and n = 4 fed domperidone).

(F and G) At 14 days of age, male mouse pups were sacrificed, and the extracted hindbrain was probed for BDNF by western (F). BDNF protein, quantified after normalization to tubulin (G), normalized to tubulin, is more abundant in the offspring of NR-supplemented mothers (n = 4).

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Meaney, 2006), we looked for correlations between maternal licking/grooming with enhanced adult behaviors. As shown in Figures S5B–S5I, high licking/grooming mothers did not produce adult offspring with learning, physical or neurobehavioral advantages with respect to the offspring of low licking/grooming mothers, suggesting the existence of bioactive factor(s) in the milk of NR-supplemented mothers responsible for transgeneration developmental programming.

NR-Supplemented Mothers but Not Domperidone-Treated Mothers Increase Expression of BDNF into Milk

The greater size at weaning of offspring of NR-supplemented mothers, the increased lactation of NR-supplemented mothers, and the increased weight loss of NR-supplemented mothers clearly indicate that offspring of NR-supplemented mothers obtain more macronutrients than control offspring. Although increased milk quantity is easy to link to enhanced protection against hypoglycemia in weanlings, greater access to milk has been linked to long-lasting cardiovascular and physical performance deficits (Habbout et al., 2013) rather than the physical performance advantages and improved adult body composition observed in this study. We therefore hypothesized that the advanced neuroanatomical and neurobehavioral development is driven by specific bioactive factors. BDNF, which plays critical roles in neurodevelopment and protection from anxiety and depression (Egan et al., 2003; Huang and Reichardt, 2001; Martinowich et al., 2007), has been reported to be present in human milk and proposed to be among the bioactive factors that confer the developmental benefits of lactation (Li et al., 2011). As shown in Figures 7B–7D, in comparison with tissue and milk from NC-fed mothers, mammary tissue from NR-supplemented mothers at 14 days after parturition expresses a higher level of BDNF protein and day 14 milk from NR-supplemented mothers has a higher level of BDNF.

To test whether enhanced BDNF was simply a bystander effect of increased lactation, we performed a blinded experiment with 12 new rat mothers that were randomized to NC, NR-supplemented chow, or chow supplemented with domperidone at 0.1 g/kg of NC. Domperidone is a dopamine D2 receptor antagonist, which is used in some parts of the world as a prescription drug to increase lactation (Paul et al., 2015). As shown in Figures S5J and 7E, at a dose of domperidone that functioned as an equal galactagogue to NR, maternal NR elevates mammary expression of proBDNF, whereas domperidone does not.

Nursing Offspring of NR-Supplemented Mothers Have Higher BDNF

BDNF in the hindbrain has been shown to play a role in promoting appetite suppression, locomotor activity, and resistance to weight gain (Noble et al., 2011), making it an attractive potential mediator of the beneficial effects of maternal NR supplementation, particularly because offspring are larger at weaning, more active, and grow into leaner adults. We sacrificed male 14-day-old mice and probed for BDNF in the hindbrain. Consistent with their higher exposure to milk-derived BDNF, as shown in Figures 7F and 7G, the offspring of NR-supplemented mothers have consistently higher levels of BDNF in the hindbrain.

Adult Neurogenesis Is Enhanced in Rats Nursed by NR-Supplemented Mothers

The higher concentration of BDNF in milk and the brains of nursing offspring could be a casual mechanism for advanced synaptic pruning and a priming mechanism for enhanced neurobehavioral functions of adult offspring of NR-supplemented mothers. Because of the strong associations between hippocampal neurogenesis, learning, and behavioral resilience (Deng et al., 2010; Masi and Brovedani, 2011), we tested whether 90-day-old rats that differed only in their mother's diet from their birth to weaning might differ in their rate of incorporation of bromodeoxyuridine (BrdU) into cells that become new neurons of the dentate gyrus. As shown in Figures 7H–7J, adult hippocampal neurogenesis quantified 3 weeks after 6 days of BrdU injection was enhanced in male and female adult offspring of NR-supplemented mothers with respect to the offspring of NC-fed mothers. Thus, NR-supplemented mothers confer long-lasting neurodevelopmental benefits to their offspring.

DISCUSSION

The common theme that unites models in which NR has provided efficacy is that the NAD metabolome, particularly levels of NAD^+ and/or NADPH, is dysregulated by particular types of metabolic stress. Here we investigated postpartum, which is widely understood to be an episode of major metabolic stress, for its effect on the NAD metabolome and its potential modulation by NR.

In postpartum, maternal metabolism is programmed for self-sacrifice with homeorhetic transfer of macronutrients from the mother to her offspring. We discovered that the postpartum liver sacrifices levels of NAD^+ (Figure 2A) to circulate higher levels of

(H–J) Adult rats (95 days old) were injected with BrdU for 6 consecutive days and were sacrificed 10 days after the last injection. Representative images (H) of adult rat dentate gyrus and quantification data show that NR supplementation of mothers predisposes adults to greater hippocampal proliferation (I) and neurogenesis (J) (n = 5 male and 4 female offspring of NC-fed and n = 5 male and 4 female offspring of NR-fed mothers). Scale bars, 100 μM.

(K) In summary, provision of NR to postgestational female rodents increases hepatic NAD synthesis and systemic circulation of the NAD metabolome and stimulates PRL production by the pituitary. The lactation program greatly induces mammary NAD synthesis and mammary macronutrient biosynthetic programs, but mammary NAD and macronutrient synthesis are further induced by oral NR. NR-fed mothers increase arched-back nursing behavior and mammary production of BDNF, which is transmitted to milk. Juvenile offspring are larger at weaning, are more mobile, learn faster, and have advanced pruning in the CP. Adult offspring of NR-supplemented mothers have enhanced physical prowess, motor learning, and spatial memory and test as less anxious and more resistant to behavioral immobility than control adults. Finally, the adult offspring of NR-supplemented mothers are advantaged in adult hippocampal neurogenesis.

Open symbols and plungers represent mothers fed NC (except in F–J, in which they refer to their offspring). Black-filled symbols represent mothers fed NR (except F–J, in which they refer to offspring of NR-fed mothers). Filled gold plunger denotes mothers given domperidone. Data were analyzed using unpaired Student's t test and two-way ANOVA with the Holm-Sidak multiple-comparisons test. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. Data are represented as individuals with mean or mean ± SEM.

NAD metabolites systemically (Figures 2I and 2J). One of the primary circulating NAD metabolites is formed by *N*-methylation of NAM (Trammell et al., 2016a). MeNAM has been previously shown to activate transcription of PRL in pituitary cells (Kimura et al., 1983). We found that MeNAM is increased during postpartum and is superinduced by maternal NR supplementation.

As shown in Figures 3E and 3F, we discovered that NAD^+ and $NADP^+$ are more than 20-fold increased in the lactating mammary without NR supplementation. With export of NAD metabolites from the liver, there are increased NAD metabolites in circulation, resulting in a huge induction of the mammary NAD program. The postpartum drop in liver NAD can be more than corrected by NR supplementation, which in turn superinduces blood circulation of NAD metabolites and mammary increases in the NAD metabolome. The >20-fold induction of mammary NAD^+ and $NADP^+$ constitutes a remarkable development-mediated expansion of the NAD metabolome, which appears to be linked to the expanded biosynthetic capacity of the lactating mammary.

As schematized in Figure 7K, enhanced PRL expression is postulated to be a key driver of all of the reported phenotypes. By circulation more PRL, mammary biosynthetic programs are increased, and there would be an obligately greater homeorhetic transfer of calories to offspring (Bauman and Currie, 1980; Frantz, 1978). In addition, with greater milk production, maternal behavior would tend to increase. However, by culling litters in mice and rats, one can produce offspring that are overfed; such rodents have defective hypothalamic programming as well as impaired glucose tolerance and renal and cardiovascular function (Habbout et al., 2013). Thus, simply increasing milk quantity without an increase in milk quality cannot account for the physical performance differences observed here.

Because NR is found in milk and is stable in milk (Bieganowski and Brenner, 2004; Trammell et al., 2016c), it was straightforward to test whether maternal NR results in greater transmission of NR or other NAD metabolites to offspring. Though NAM and NR are transmitted at higher levels by NR-supplemented mothers, the mother's oral NR is not directly transmitted to her milk. Given the complexity of milk, we postulated that milk from NR-supplemented mothers might be enhanced in bioactive components. Earlier, lactoferrin was found to confer a higher degree of BDNF expression in neonatal hippocampus (Chen et al., 2015), and time the mother invests in arched-back nursing was linked to higher neonatal expression of BDNF (Liu et al., 2000). BDNF is one of many compact, disulfide-linked growth factors thought to be orally available to target tissues through the neonatal digestive system (Playford et al., 2000). Our data establish that NR-supplemented mothers express more BDNF than control mothers and mothers whose milk production was increased by administration of domperidone. We further show that offspring sacrificed at the peak of milk production have higher levels of BDNF in their hindbrains. The powerful effects of BDNF on development, learning, and protection against psychological imbalances are well known (Egan et al., 2003; Huang and Reichardt, 2001; Huang et al., 1999; Martinowich et al., 2007). Because NR but not domperidone increased mammary BDNF expression, this bioactive factor is specifically linked to increased NAD metabolism during postpartum.

As schematized in Figure 7G, the nature of the physical and behavioral advantages we observed are striking when one considers their breadth. In the wild, neonates and juveniles are vulnerable to starvation; maternal NR provides advantages in size and protection from hypoglycemia to weaned pups. We further documented lasting advantages in learning, strength, balance, resistance to conditions that make rodents anxious, and resistance to behavioral immobility, which has been interpreted as despair or depression. Whereas mice raised by NR-supplemented mothers had greater lean and fat mass at weaning, as 90-day-old adults, the same animals had less fat while preserving their lean mass.

Though pregnancy-associated niacin deficiency is reportedly common (Baker et al., 2002) and B3 supplementation during pregnancy could potentially be used to prevent birth defects (Shi et al., 2017), it is striking that an increase in NAD metabolism strictly during the postpartum period was sufficient for increased lactation, increased production of BDNF, and long-lasting neurodevelopmental benefits. Thus, this work creates the potential for NR to emerge as a safe and effective galactagogue that may also promote human childhood development.

Future studies will determine whether gestational NR protects offspring against the maternal effects of obesity, probe the means by which PRL expression is induced by NR, and determine the set of transmissible factors, and the developmental roles of these factors, that are coordinately induced in the NR-supplemented mammary. These factors and their modulation by dietary NR have the potential to explain some of the benefits of breastfeeding. In addition, the work suggests avenues for clinical investigation that would probe whether NR will increase lactation and/or weight loss in postpartum women, the degree to which NR may increase transmission of BDNF or other bioactive components into human milk, and whether maternal NR supplementation may improve human childhood and adult development.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - NAD metabolomics
  - Physiological characterization
  - Neuroanatomical characterization
  - Behavioral characterization
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures, two tables, and one video and can be found with this article online at https://doi.org/10.1016/j.celrep.2019.01.007.

ACKNOWLEDGMENTS

We thank Alicia Ortiz and Edenia da Cunha Menezes for technical assistance and Amy Sindler and Carlos Chan for helpful discussions. This work was supported by grants from the Roy J. Carver Trust and ChromaDex to C.B.

AUTHOR CONTRIBUTIONS

M.E.M., H.E.S., and C.B. designed the study. A.C., P.H.E., S.B.G., M.S.S., S.V., J.M., J.K., and M.M.M. performed experiments. A.C. took the lead on the neurodevelopmental and behavioral effects of maternal NR on offspring. P.H.E. took the lead on the effect of NR on maternal physiology. A.C., P.H.E., and H.E.S. analyzed data. C.B. wrote the manuscript. H.E.S. supervised neuroscience and statistics. P.H.E., A.C., M.S.S., S.B.G., M.E.M., H.E.S., and C.B. reviewed and edited the manuscript.

DECLARATION OF INTERESTS

C.B. is the inventor of intellectual property on the nutritional and therapeutic uses of NR. He serves as chief scientific advisor of ChromaDex, which licensed, developed, and commercialized NR technologies, and holds stock in ChromaDex. C.B., P.H.E., A.C., M.E.M., and H.E.S. have applied for a patent for the use of NR to improve the health of new mothers and their offspring.

Received: August 30, 2018
Revised: December 9, 2018
Accepted: January 2, 2019
Published: January 22, 2019

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-CD38	Cell Signaling Technology	Cat#14637S
Anti-PARP1	Cell Signaling Technology	Cat# 9542S
Anti-SIRT1	Cell Signaling Technology	Cat# 9475
Anti-Nampt	Bethyl	Cat# A300-372A
Anti-NMNAT1	Bethyl	Cat# A304-317A
Anti-phospho-mTOR (Ser 2448)	Cell Signaling Technology	Cat# 5536S
Anti-mTOR	Cell Signaling Technology	Cat# 2983S
Anti-S6K	Cell Signaling Technology	Cat# 2708T
Anti-phospho-S6K (Thr 389)	Cell Signaling Technology	Cat# 9234T
Anti-NeuN (D4G40)	Cell Signaling Technology	Cat# 24307S
Anti-β-actin	Sigma	Cat# 2066
Anti-GAPDH	Cell Signaling Technology	Cat# 5174S
Anti-β-Tubulin	Cell Signaling Technology	Cat# 15115S
Anti-BDNF	Abcam	Cat# ab108319
Anti-mouse IgG, HRP conjugated	Cell Signaling Technology	Cat# 7076
Anti-rabbit IgG, HRP conjugated	Cell Signaling Technology	Cat# 7074
Anti-rabbit IgG, HRP conjugated	Sigma	Cat# 12-348
Anti-BrdU Clone B44 (RUO (GMP))	BD biosciences	Cat# 347580
Anti-Mouse IgG1, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-21121
Anti-Rabbit IgG (H+L), Alexa Fluor 594	Thermo Fisher Scientific	Cat# A-21207
Anti-PSD95 (7E3) Mouse mAb	Cell Signaling technology	Cat# 36233S
Anti-vGlut1 Guinea Pig polyclonal Ab	Millipore	Cat# AB5905
Anti-Rabbit IgG (H+L), Alexa Fluor 488	Thermo Fisher Scientific	Cat# A27034
Anti-Mouse IgG (H+L), Alexa Fluor 594	Thermo Fisher Scientific	Cat# R37115
Chemicals, Peptides, and Recombinant Proteins		
Teklad global soy protein-free extruded 2920X	Envigo	https://www.envigo.com/
Nicotinamide riboside Cl	Chromadex	NR Cl bulk
Domperidone	Arkpharmic	AK608060
iScript Reverse Transcription Supermix	Bio-Rad Laboratories	Cat# 1708840
iQ SYBR Green Supermix	Bio-Rad Laboratories	Cat#1708880
qScript cDNA SuperMix	Quantabio	Cat# 95048-100
PerfeCTa SYBR Green SuperMix	Quantabio	Cat# 95054-100
TRIzol	Thermo Fisher Scientific	Cat# 15596026
E.Z.N.A Total RNA Kit I	Omega Biotek	Cat# R6834-02
NAM	Sigma	Cat# B3376
cOmplete, EDTA-free Protease Inhibitor Cocktail	Roche	Cat# 1187580001
Immobilon PVDF membrane	EMD Milipore	Cat# IPVH00010
Sodium Orthovanadate	Sigma	Cat# S6508
Clarity Western ECL Blotting Substrates	Bio-Rad	Cat# 1705060
NuPAGE 4-12% Bis-Tris protein gels	Thermo Fisher Scientific	Cat# NP0336BOX
Carmine	Sigma	Cat#1022
Aluminum potassium sulfate dodecahydrate	Sigma	Cat# 237086

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
PRL ELISA KIT	Thermo Fisher Scientific	Cat# EMPRL
17β-Estradiol ELISA KIT	Enzo	Cat# ADI-900-008
Oxytocin ELISA KIT	Enzo	Cat# ADI-900-153A
Progesterone ELISA KIT	Thermo Fisher Scientific	Cat# EIAP4C21
BDNF ELISA KIT	AVIVA	Cat #OKEH00022
Total carbohydrate assay	Biovision	Cat # K645
Glycogen assay kit	Cayman	Cat # 700480
Pierce BCA assay kit	Thermo Fisher Scientific	Cat# 23225
Experimental Models: Organisms/Strains		
C57BL/6NJ	The Jackson Laboratory	Strain: 005304
C57BL/6J	The Jackson Laboratory	Strain: 000664
Fisher 344	Charles River	Strain: 403
Oligonucleotides (see Table S2)		
Software and Algorithms		
Adobe Creative Suite	Adobe	https://www.adobe.com/
GraphPad Prism v7	GraphPad Software	https://www.graphpad.com/
ImageJ	NIH	https://imagej.nih.gov/
Steroinvestigator	Microbrightfield Bioscience	https://www.mbfbioscience.com
Anymaze	Stoelting	http://www.anymaze.co.uk/index.htm

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Charles Brenner (charles-brenner@uiowa.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All animal experiments were done according to procedures approved by the University of Iowa Institutional Animal Care and Use Committee. Rodents were housed in a temperature-controlled facility with 12 hour day/light cycles and *ad libitim* access to food and water. Parental mice (C57BL6/NJ) were bred at 18-weeks old in the experiments of Figure S1. Parental mice were bred at 12-weeks old in all other experiments. The mouse strain was C57BL6/NJ except in Figure 2, Figure 3A, 3D-O and Figure 7F-G, which employed C57BL6/J mice. Parental rats (Fisher 344) were bred at 9-weeks old. Mothers were analyzed at parturition and on indicated days post-parturition. Offspring were analyzed at indicated ages.

METHOD DETAILS

NAD metabolomics

Quantitative targeted NAD metabolomics was as previously described (Trammell and Brenner, 2013; Trammell et al., 2016a; Trammell et al., 2016c).

Physiological characterization

Mammary tissue was dissected, mounted and stained as described (Plante et al., 2011). Body composition of mice and rats was determined using Bruker Minispec LF90 and LF-90II.

Total RNA was isolated from tissues using TRIzol and further purified using column purification (Omega Biotek EZNA Total RNA Kit I). cDNA was synthesized using 1-2 μg of total RNA using iScript Reverse Transcription Supermix or qScript (Quantabio). Quantitative PCR was done using the BioRad CFX96 Touch system with SYBR Green mix (BioRad). SDS-PAGE and western blot analysis was performed by separating proteins in 4%–20% gels (Invitrogen), were transferred to PVDF membranes, incubation with respective antibodies, and detected with Clarity Western ECL Blotting Substrate (BioRad).

Milk was collected in lactating mice (n = 8) in each of two groups and rats (n = 6) in each of two groups on days 7, 14 and 21 post-parturition. In the domperidone study, comparison study, there were n = 6 rats on NC, n = 5 rats on NR and n = 4 rats on NC plus

domperidone. Before collection, dams and pups were separated for a period of 4 hours. Dams were anesthetized using isoflurane and injected with oxytocin at 2 IU/kg (mice) or 4 IU/kg (rats). Milk was collected by manual expression for 15 min and stored at −80°C after measuring volumes. Double-labeled NR, which incorporates a deuterated ribose and ^{13}C NAM moeity (Ratajczak et al., 2016), was gavaged at a dose of 185 mg/kg to lactating mice to determine whether oral NR is transmitted intact into milk. Milk was collected at 0, 2, 4, 6, 12 and 24 hours after gavage from individual mice.

Total carbohydrate in mouse and rat milk was determined by colorimetric assay (K645 BioVision). Total protein was determined using the bicinchoninic assay and milk fat was determined as described (Forcato et al., 2005). For assays of macronutrients, there were n = 4 mice in each group and n = 6 rats. Mouse milk BDNF was determined by ELISA (OKEH00022, Aviva Systems Biology) with n = 8 in each group.

Mouse blood glucose was measured after 16-hour fasts with blood from tail tips. Glycogen from snap-frozen liver tissue was determined with kit 700480 from Cayman.

Neuroanatomical characterization

To characterize CP volume and PSD95 staining, mouse offspring of NC-fed (n = 8) and NR-fed (n = 8) mothers at postnatal day 22 and 90 were euthanized by decapitation. Brains were removed, post-fixed in 4% paraformaldehyde for 24 hr, and stored in 20% sucrose/PBS. Brains were frozen in OCT, cryosectioned (50 μm), and stored in vials with PBS plus 2% sodium azide at 4°C until processing for immunohistochemistry. Free-floating brain sections were treated with blocking solution of 0.2% triton, 0.1% tween and 10% horse serum after which they were stained with PSD-95 (1:200) and vGlut1 primary antibodies (1:1000) overnight and secondary rabbit 488 and mouse 594 antibodies (1:500). Sections were mounted on slides and coverslipped with DAPI for nuclear staining. PSD95+ puntal area and PSD95+/vGlut1+ colocalized puncta number were evaluated with two 63x z stack maximum projection Apotome images (3 planes within 1 micron section thickness) within the CP from two sections per brain taken with equivalent light, exposure, and gain across samples using StereoInvestigator software (Microbrightfield, Colchester, VT) coupled to a Zeiss AxioImager 2 Mot Plus (Carl Zeiss, Oberkochen, Germany) with a digital camera and motorized stage controller. Equivalently thresholded images were evaluated for total area of PSD95 puncta and double labeled (PSD95/vGlut1) synapse number using ImageJ with the Puncta Analyzer v2.0 plugin.

90 day-old Fisher 344 offspring of NC-fed and NR-fed mothers were injected intraperitoneally six times with BrdU (100 mg/kg once every 24 hr and were euthanized 10 days after the last injection. There were n = 9 per group including 5 males and 4 females. After an anesthetic overdose of 100 mg/ml ketamine and 10 mg/ml xylazene, all animals were transcardially perfused with 4% paraformaldehyde. Brains were processed, cryosectioned, stored and blocked as above. Sections were stained with NeuN (1:500) primary and secondary rabbit 594 (1:500) antibodies, again fixed in 4% paraformaldehyde, and then treated with 1.5 M HCl at 37°C for 1.5 hours for antigen unmasking. After washing in PBS, sections were treated with a BrdU primary antibody (1:100) overnight and then a secondary 488 antibody (1:500) for one hour, mounted on slides, and coverslipped with DAPI for nuclear staining.

An average of 12 pairs of sections from each brain (n = 9) in each group were used to count the number of BrdU-positive nuclei in the dentate gyrus region of the hippocampus. A subset of these (n = 4 per group) were then also assessed for BrdU co-labeling with NeuN to determine neuronal identity. All cells contained in the pre-defined dentate gyrus region were counted using Stereoinvestigator software.

Behavioral characterization

Maternal behavior was recorded in home cages in rats (n = 6) in each group on days 11 and day 15 after parturition. The behavior of each dam was video recorded for two 60-minute observation periods on each day. Within each 60-min observation period, the behavior of each mother was scored every 5 min for arched back nursing, licking, grooming, eating, self-grooming and nest-making by a blinded investigator.

Open field activity was assessed using Anymaze software with an overhead camera. The set-up allowed the simultaneous tracking of two rats or four mice, using separate square observation arenas (100 cm length x 100 cm width x 30 cm height) for rats and (50 cm length x 30 cm width x 30 cm height) for mice. At the beginning of the trial, the subject was placed in the center of the arena. Spontaneous activity was measured over a 30-minute period for rats or a 15-minute period for mice.

Grip strength was measured by tension force using a Columbus instruments 1027DR.

The sum of six consecutive trials was taken as an index of forelimb grip strength.

Rotarod accelerating and endurance was tested with Panlab Harvard apparatus model number LE8305 at acceleration rate 30 s to 10 minutes with 1 s increments. All rats were tested for 8 trials with 4 trials on day 1 and 4 trials on day 2. Mice were trained for rotarod endurance on two successive days and tested for endurance on day 3.

The elevated plus maze is made of medium-density fiberboard with a matte black acrylic surface and consists of four arms (two open without walls and two enclosed by 16 cm high wall in mice). Both open and closed arms were 25 cm long and 5 cm wide in mice. Behavior was videotaped using an overhead camera and tracked using Anymaze software for five min.

The Morris water maze consist of a pool 150 cm in diameter and 50 cm in height divided into 4 quadrants. The pool was filled with water at a temperature of 25 ± 1°C, and a square platform (diameter = 10 cm) was positioned 1.5 cm below the water surface in the platform quadrant. During training trials, each rat had to escape the water by climbing onto the square platform, which was not visible to the rat. The time to locate the platform was recorded. If the rat failed to locate the platform within 60 s, the trial was terminated and

the unsuccessful rat was guided onto the platform. All the rats were allowed to remain on the platform for 20 s. After a final 12th training trial on same day, the rats were subjected to a probe trial (60 s) in the maze without the presence of a platform. Anymaze tracking system with overhead camera was used to videotape the behavior.

The forced swim test consisted of individually placing the rats into a cylindrical tank (transparent acrylic, 60 cm height × 20 cm diameter) containing clean water at 25°C (25 cm deep). They were allowed to swim in the tank for 6 minutes and the behavior was videotaped and analyzed later by an investigator who was blinded to groups.

Aversion to well-lit spaces was tested using a box made of two compartments one-third dark and two-third light with an exterior size 46 × 27 × 30 cm. Behavior was videotaped using an overhead camera and time spent and distance covered in light or dark was analyzed using Anymaze software.

The beam walking apparatus consisted of a 1-m beam of 6 mm width resting 50 cm above the table top on two poles. A black box is placed at the end of the beam as the finish point. Time taken to cross the beam and number of paw slips were later analyzed using videos by the investigator who was blinded to the groups.

QUANTIFICATION AND STATISTICAL ANALYSIS

In the experimental design of Figure S1A, mothers were randomly assigned to four different diets. In the experimental design of Figure 1A, after the first two mothers had litters and were alternately assigned to NC or NR, subsequent mothers were assigned to a diet in order to equalize the initial post-gestational body weight. Data are presented as individuals with means or as means ± SEM and statistical significance was performed using Student's t test, repeated-measures analysis of variance, and two-way ANOVA was used to identify significance of main effects. The effect of maternal NR within sex groups was determined by the Holm Sidak approach. All analyses were performed in GraphPad Prism using the false discovery rate-based ROUT method to identify outliers. P values of $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$ are indicated as *, **, ***, and ****, respectively. Number of individuals analyzed and statistical details are provided in figure legends.

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Supplemental Information

Maternal Nicotinamide Riboside Enhances Postpartum Weight Loss, Juvenile Offspring Development, and Neurogenesis of Adult Offspring

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Table S1. Statistical Summary. Related to Figures 4-6 and S4.

Assay	sex main effect ANOVA p-value	mom's NR main effect ANOVA p-value	Interaction
Body weight of mouse pups greater Fig. 4A	0.2363	0.0073	0.7375
Lean mass of mouse pups greater Fig. 4B	0.1835	0.0049	0.5602
Fat mass of mouse pups greater Fig. 4C	0.5337	0.0010	0.1029
Maintenance of fasting glucose by mouse pups Fig. 4D	0.5769	0.0001	<0.0001
Liver glycogen protection of mouse pups Fig. 4E	0.0005	0.0009	0.5450
Increased liver PEPCK of mouse pups Fig. 4F	0.9544	0.0698	0.4358
Increased mobility of mouse pups in open field Fig. 5A	0.2695	< 0.0001	0.6016
No difference in time spent in center of open field by mouse pups Fig. 5B	0.3793	0.4363	0.4308
Enhanced rotarod performance by rat pups Fig. 5C	0.0176	0.0475	0.9101
Reduced caudate-putamen volume of mouse pups Fig. 5E	0.0663	0.0254	0.9449
Compacted PSD95 staining in caudate-putamen of mouse pups Fig. 5G	0.3462	0.0149	0.4365
Fewer PSD95/VGLUT1colocalized puncta in caudate-putamen of mouse pups Fig. 5I	0.3423	0.0644	0.2648
Increased mobility of adult mouse in open field Fig. 6A	0.7818	0.0035	0.0021
Increased average speed of adult mouse in open field Fig. 6B	0.7961	0.0007	0.9975
Enhanced endurance rotarod performance of adult mouse Fig. 6C	0.1449	0.0063	0.6867
Enhanced accelerating rotarod performance of adult rat Fig. 6D	0.0590	0.0190	0.3247
Enhanced endurance rotarod performance by adult rat on day 1 Fig. 6E	0.6772	0.0007	0.4243
Enhanced endurance rotarod performance by adult rat on day 2 Fig. 6F	0.4337	0.0004	0.6867
Increased grip strength performance by adult rat Fig. 6G	0.1653	0.0013	0.6743
Increased distance traveled in second trial of beam walking by adult mouse Fig. 6H	0.4935	0.0007	0.5966
Reduced foot slips in second trial of beam walking by adult mouse Fig. 6I	0.7214	< 0.0001	0.6419
Reduced tail grips in second trial of beam walking by adult mouse Fig. 6J	0.5526	< 0.0001	0.7911
Increased time spent in light of light/dark by adult mouse Fig. 6K	0.1347	0.0281	0.4770
Increased distance covered in open arm of elevated plus maze by adult mouse Fig. 6	0.2802	0.0375	0.1763
Reduced time immobile in forced swim by adult rat Fig. 6M	0.1825	< 0.0001	0.4477
Increased latency to immobile in forced swim by adult rat Fig. 6N	0.0946	0.0033	0.5667
Increased platform entries in Morris water maze by adult rat Fig. 6O	0.1647	0.0001	0.5233
Increased platform quadrant time in Morris water maze by adult rat Fig. 6P	0.0155	0.0019	0.7311
Body weight of adult mouse reduced Fig. 6Q	<0.0001	0.0332	0.1206
Fat mass of adult mouse reduced Fig. 6R	<0.0001	0.0010	0.0353
Normalized brain/body weight in adult mouse greater Fig. 6S	<0.0001	0.0167	0.0793
Increased distance traveled in first trial of beam walking by adult mouse Fig. S4A	0.0584	< 0.0001	0.3836
Lean mass of adult mouse Fig. S4B	<0.0001	0.8628	0.8081
Superior Morris water maze learning by adult rat Fig. S4C	0.3070	0.0364	0.7054

Table S2. Oligonucleotides Used for Quantitative PCR. Related to STAR Methods.

PEPCK forward: ACACACACACATGCTCACAC	(Zou et al., 2015)
PEPCK reverse: ATCACCGCATAGTCTCTGAAA	(Zou et al., 2015)
GAPDH forward: GAGGCCGGTGCTGAGTATGTCGTG	(Ronai et al., 2005)
GAPDH reverse: TCGGCAGAAGGGGCGGAGAT	(Ronai et al., 2005)
NRK1 forward: CCCAACTGCAGCGTCATATC	(Ratajczak et al., 2016)
NRK1 reverse: CCTTGAGCACTTTCCAAGGC	(Ratajczak et al., 2016)
NRK2 forward: GCCGTATGAGGAATGCAAGC	(Ratajczak et al., 2016)
NRK2 reverse: TGACCATCAAACAGGCCAGG	(Ratajczak et al., 2016)
NAMPT forward: AGTGGCCACAAATTCCAGAGA	(Ratajczak et al., 2016)
NAMPT reverse: CCGCCACAGTATCTGTTCCTT	(Ratajczak et al., 2016)
NMNAT1 forward: TGGCTCTTTTAACCCCATCAC	(Ratajczak et al., 2016)
NMNAT1 reverse: TCTTCTTGTACGCATCACCGA	(Ratajczak et al., 2016)
NNMT forward: GATTGCACGCCTCAACTTCT	https://mouseprimerdepot.nci.nih.gov
NNMT reverse: GAACCAGGAGCCTTTGACTG	https://mouseprimerdepot.nci.nih.gov
CD38 forward: TTGCAAGGGTTCTTGGAAAC	https://mouseprimerdepot.nci.nih.gov
CD38 reverse: CGCTGCCTCATCTACACTCA	https://mouseprimerdepot.nci.nih.gov
CD73 forward: AACGTTTCTGAGGAGGGGAT	https://mouseprimerdepot.nci.nih.gov
CD73 reverse: CTTCATGAACATCCTGGGCT	https://mouseprimerdepot.nci.nih.gov
PARP1 forward: CACCTTCCAGAAGCAGGAGA	https://mouseprimerdepot.nci.nih.gov
PARP1 reverse: GCAGCGAGAGTATTCCCAAG	https://mouseprimerdepot.nci.nih.gov
SIRT1 forward: GACACAGAGACGGCTGGAAC	https://mouseprimerdepot.nci.nih.gov
SIRT1 reverse: CAGACCCTCAAGCCATGTTT	https://mouseprimerdepot.nci.nih.gov
PRLR forward: TTCAGGGTTCATGTGCAAAA	https://mouseprimerdepot.nci.nih.gov
PRLR reverse: GCAAGAAGTGCTCAATCCCT	https://mouseprimerdepot.nci.nih.gov
LALBA forward: ACGCCACTGTTCAAGCTTCT	https://mouseprimerdepot.nci.nih.gov
LALBA reverse: ATGACATAGCGTGTGCCAAG	https://mouseprimerdepot.nci.nih.gov

LPIN forward: TTCACCGTCACAAACACCTG	https://mouseprimerdepot.nci.nih.gov
LPIN reverse: TTTTTGCATACAAAGGCAGC	https://mouseprimerdepot.nci.nih.gov
THRSP forward: TCGGGGTCTTCATCAGTCTT	https://mouseprimerdepot.nci.nih.gov
THRSP reverse: GCGGAAATACCAGGAAATGA	https://mouseprimerdepot.nci.nih.gov
Rat BDNF forward: CAGGGGCATAGACAAAAG	(Kondo et al., 2013)
Rat BDNF reverse: CTTCCCCTTTTAATGGTC	(Kondo et al., 2013)
Rat beta-actin forward: CCTGTATGCCTCTGGTCGTA	(Kondo et al., 2013)
Rat beta-actin reverse: CCATCTCTTGCTCGAAGTCT	(Kondo et al., 2013)

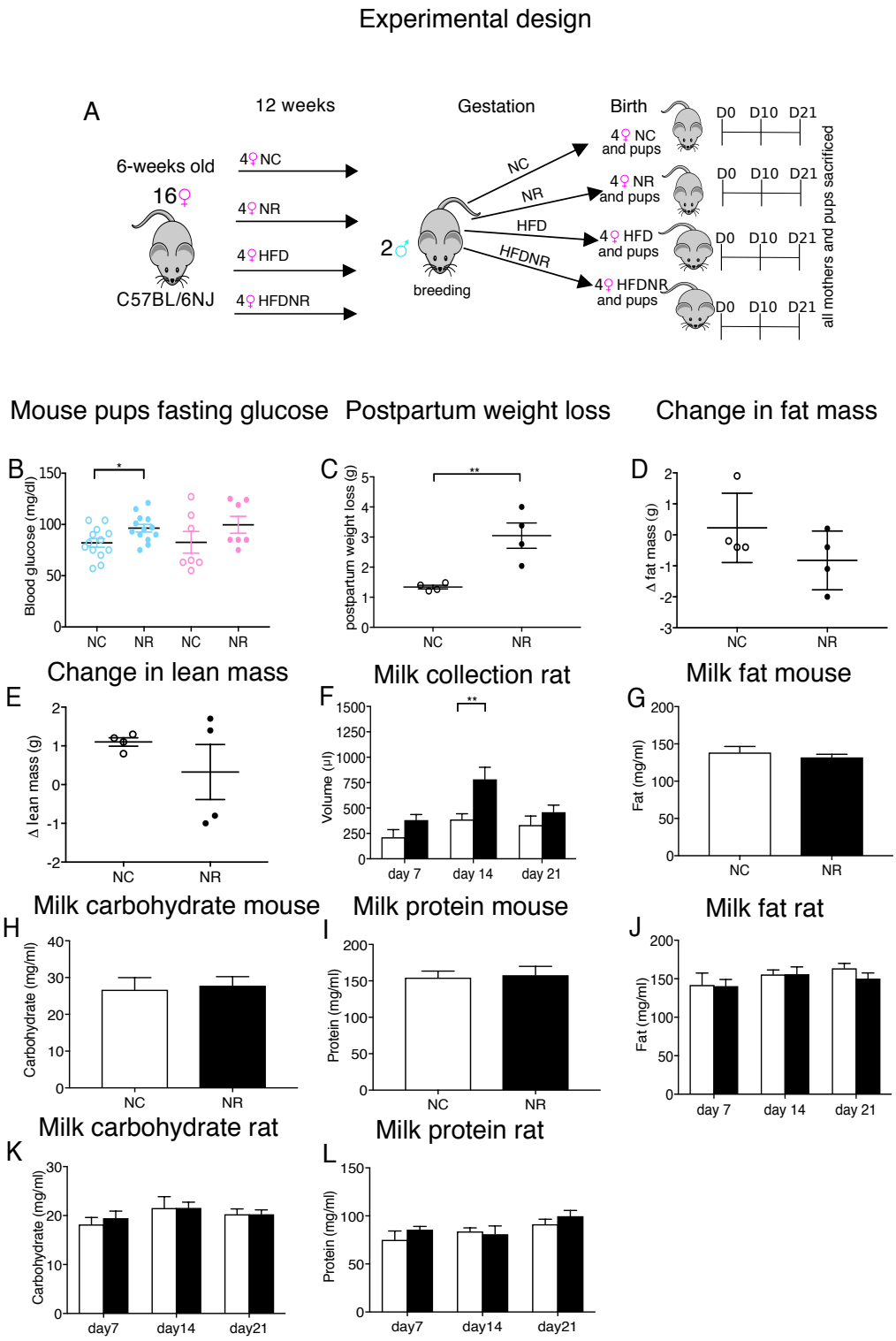

Figure S1

Maternal NR Promotes Weight Loss and Lactation. Related to Figure 1.

Figure S1. Maternal NR Promotes Weight Loss and Lactation. Related to Figure 1.

(A) Experimental design in which NR and HFD were introduced to female mice before breeding and during gestation.
(B) Fasting glucose in day-22 mouse pups of NC and NR mothers from the S1 experimental design (n = 13 male pups of NC-fed mothers, n = 13 male pups of NR-fed mothers, n = 7 female pups of NC-fed mothers and n = 7 female pups of NR-fed mothers).
(C) Postpartum weight loss of NC and NR mothers from the S1 experimental design (n = 4 in each group).
(D-E) Change in fat mass and lean mass of NC and NR-fed mothers from the Figure 1 experimental design (n = 4 in each group).
(F) Rat lactation data from the Figure 1 experimental design (n =6 in each group).
(G-L) Mouse and rat milk macronutrient content from the Figure 1 experimental design (n = 4 mouse mothers or n=6 rat mothers in each group).
Data were analyzed by unpaired Student's t-test or a 2-way ANOVA with Holm-Sidak multiple comparisons test. P values of < 0.05 and < 0.01 and < 0.001 are indicated as * and **, respectively. Data are represented as individuals with mean or mean ± SEM.

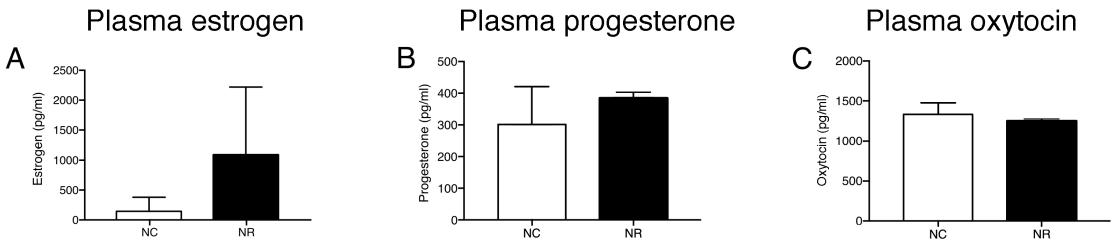

Figure S2. Effect of Maternal NR on Circulating Hormones. Related to Figure 3.

(A-C) Levels of circulating estrogen, progesterone and oxytocin in female mice 22 days after parturition that had been fed NC or NR (n = 4 in each group). Data are represented as mean ± SEM.

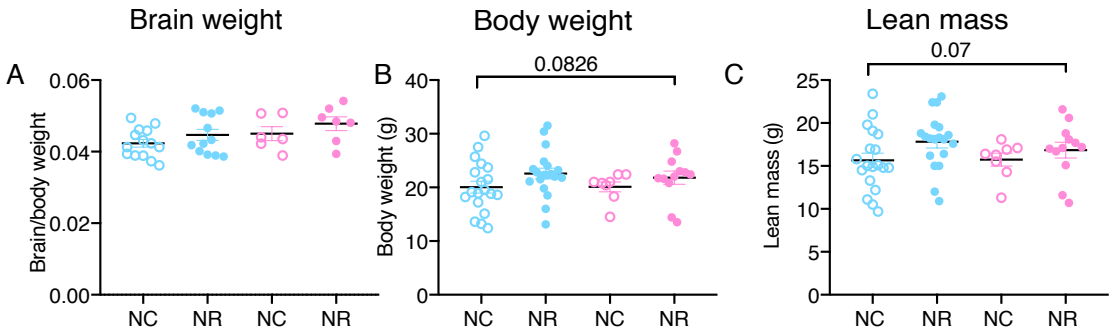

Figure S3. Effect of Maternal NR on Weanling Brain and Body Weight. Related to Figure 4.

(A) Brain weight from Figure 4A-C (n = 25 males from NC mothers, 22 males from NR mothers, 21 females from NC mothers and 31 females from NR mothers).
(B-C) Body weight and lean mass of weaned rat pups at day 23 (n = 19 males from NC mothers, 19 males from NR mothers, 8 females from NC-fed mothers and 12 females from NR-fed mothers).
Data were analyzed by 2-way ANOVA and are represented as individuals with mean.

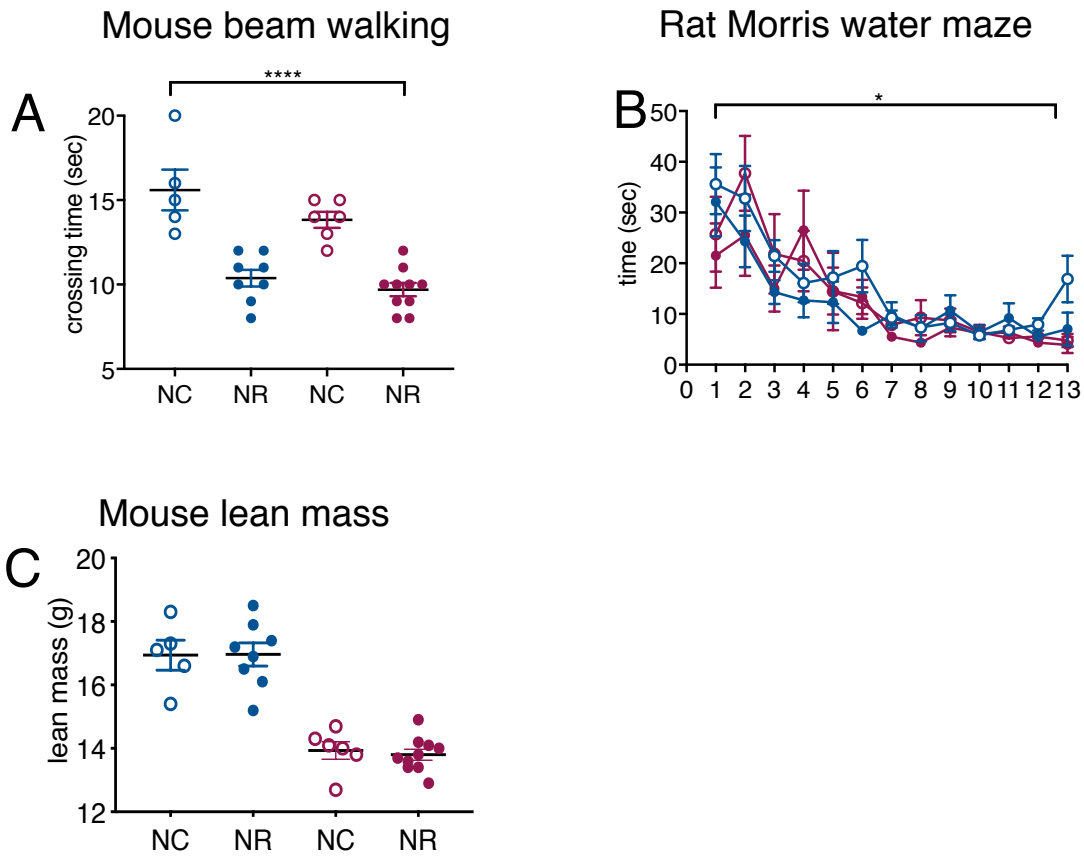

Figure S4. Maternal NR Produces Lasting Benefits in Adult Offspring Physical Performance and Body Composition. Related to Figure 6.

(A) Data from the first mouse beam walking training run that preceded assays shown in Figure 6H-J and Supplemental Movie S1 (n = 5 male offspring of NC-fed mothers, n = 8 male offspring of NR-fed mothers, n = 6 female offspring of NC-fed mothers, and n =10 female offspring of NR-fed mothers).
(B) Adult rat Morris water maze training runs corresponding to Figure 6O-P (n = 14 male offspring of NC-fed mothers, n = 12 male offspring of NR-fed mothers, n = 8 female offspring of NC-fed mothers, and n = 10 female offspring of NR-fed mothers).
(C) Adult mouse lean mass corresponding to body composition data in Figure 6Q-R (n = 14 male offspring of NC-fed mothers, n = 12 male offspring of NR-fed mothers, n = 8 female offspring of NC-fed mothers, and n = 10 female offspring of NR-fed mothers).
Data were analyzed two-way ANOVA. P-values of < 0.05 and <0.0001 are represented by * and ****, respectively. Data are represented as individuals with mean or mean ± SEM.

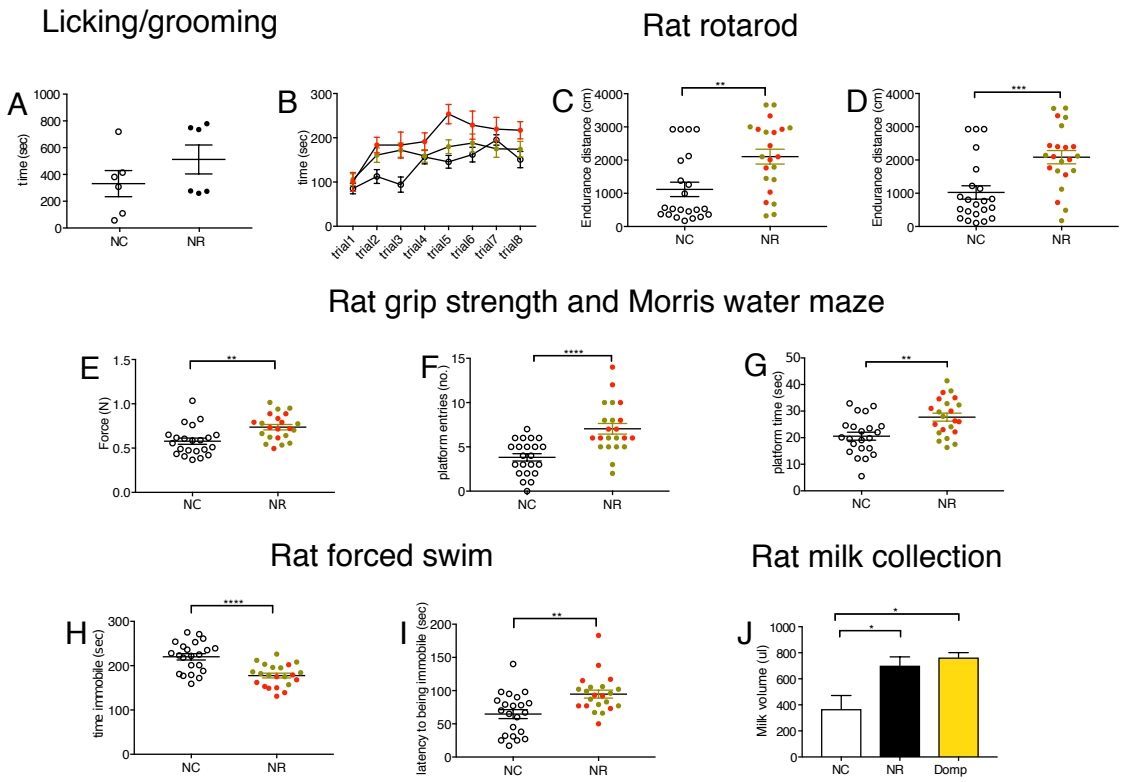

Figure S5. Increased Maternal Licking/Grooming Does not Account for Superior Adult Performance While Increased Mammary BDNF Expression is a Specific Effect of Maternal NR. Related to Figures 6 and 7.

(A) At day 15 post-parturition, rat mothers supplemented with NR tend to spend more time licking/grooming pups (n = 6 mothers fed NC and n = 6 mothers supplemented with NR). Though all NR mothers exhibited increased time of arched-back nursing (Figure 7A), note that three NR mothers had high licking/grooming behavior while 3 had licking/grooming times similar to that of the mean NC mothers.
(B-I) Here the adult offspring of NR-supplemented mothers were coded as green filled circles (offspring of high licking/grooming mothers) and red filled circles (offspring of low licking/grooming mothers). Being an offspring of a high licking/grooming mother does not predispose to higher adult performance than being offspring of a low licking/grooming mother. Sex-specific data are provided in Figure 6D-G, Figure 6M-P.
(J) Day 14 milk production by rats fed NR, NC or domperidone corresponding to Figure 7E.
Data were analyzed by unpaired t-test. There were no differences attributable to licking/grooming behaviors of NR-fed mothers. P-values of < 0.05, < 0.01, < 0.001 and <0.0001 are represented by *, **, *** and ****, respectively. Data are represented as individuals with mean or mean ± SEM.