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Access to a high resource environment protects against accelerated maturation following early life stress: A translational animal model of high, medium and low security settings

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Abstract

Early life exposure to a low security setting, charcterized by a scarcity of resources and limited food access, increases the risk for psychiatric illness and metabolic dysfunction. We utilized a translational rat model to mimic a low security environment and determined how this manipulation affected offspring behavior, metabolism, and puberty. Because food insecurity in humans is associated with reduced access to healthy food options the “low security” rat manipulation combined a Western diet with exposure to a limited bedding and nesting manipulation (WD-LB). In this setting, dams were provided with limited nesting materials during the pups’ early life (P2-P10). This manipulation was contrasted with standard rodent caging (SD) and environmental enrichment (EE), to model “medium security” and “high security” environments, respectively. To determine if transitioning from a low to high security environment improved outcomes, some juvenile WD-LB offspring were exposed to EE. Maternal care was impacted by these environments such that EE dams engaged in high quality care when on the nest, but spent less time on the nest than SD dams. Although WD-LB dams excessively chased their tails, they were very attentive to their pups, perhaps to compensate for limited resources. Offspring exposed to WD-LB only displayed subtle changes in behavior. However, WD-LB exposure resulted in significant metabolic dysfunction characterized by increased body weight, precoscious puberty and alterations in the hypothalamic kisspeptin system. These negative effects of WD-LB on puberty and weight regulation were mitigated by EE exposure. Collectively, these studies suggest that both compensatory maternal care and juvenile enrichment can reduce the impact of a low security

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environment. Moreover, they highlight how utilizing diverse models of resource (in)stability can reveal mechanisms that confer vulnerability and resilience to early life stress.

Keywords

early life stress; limited bedding; western diet; animal models; social economic status; environmental enrichment; precocious puberty; kisspeptin; maternal care

1. Introduction

Both basic and clinical research suggest that early-life stressors (e.g. abuse, neglect) can modify brain development and make an individual prone to mental illness and metabolic dysfunction in later life (Spencer, Korosi, Layé, Shukitt-Hale, & Barrientos, 2017; Walker, et al., 2017; Yam, et al., 2016; Korosi, et al., 2012; Pervanidou & Chrousos, 2012; Avishai-Eliner, Gilles, & Eghbal-Ahma, 2001). For example, exposure to early-life stress increases the risk for mental illnesses, such as mood and depressive disorders, anxiety disorders, and disruptive behavior disorders (Afari, et al., 2013; Green, et al., 2010; Heim, Newport, Mletzko, Miller, & Nemeroff, 2008; Chapman, et al., 2004; Anda, et al., 2002). Moreover, early-life adversity has a positive association with metabolic dysfunctions including metabolic syndrome and the occurrence of precocious puberty (Cowan & Richardson, 2018; Pervanidou & Chrousos, 2012; Björntorp, 2008). To fully understand the relationship between early-life stress and these later life outcomes, it is neccesary to develop ecologically relevant animal models in order to derive their mechanistic underpinnings. This step will be imperative for the design and testing of translational treatments and preventative methods.

The hypothalamic pituitary adrenal (HPA) axis is a fundamental feature of the stress response (Jacobson & Sapolsky, 1991). Normally each component of this axis develops simultaneously, connecting into a functional network. However severely stressful events during the perinatal period can cause them to develop out of sync, resulting in life-long modifications in how the organism responds to stressful experiences (Heim, et al., 2008; Levine, 1994). Even a short period of stressor exposure during the first week of life in rodents can cause behavioral deviations and a vulnerability to physiological disruptions that parallel the clinical symptoms associated with early-life adversity (Walker, et al., 2017; Champagne & Curley, 2007). Moreover, the hypothalamic-pituitary-gonadal (HPG) axis is also susceptible to early life programming, connecting the downstream effects of early adverse experiences on reproductive functioning and stress reponsivity to a larger interactive network (Kentner & Pittman, 2010).

Puberty is the process of hormones orchastrating physiological changes that turns an organism from its sexually immature to its sexually mature state. During early development, kisspeptin in particular plays a role in the secretion of sex steroids (e.g. lutenizing hormone and follicle stimulating hormone) and the onset of puberty (Kauffman, Clifton, & Steiner, 2007; Skorupskaite et al., 2014). Precocious puberty is when an organism experiences puberty significantly ealier than its species predicted time. In humans, the occurrence of precocious puberty is indicated by significant pubertal markers such as the development of secondary sex characteristics (e.g. pubic hair and breast development) before age 8 or

menarche before age 9 (Kaplowitz, et al., 2018; Baker & Kappy, 2011; Cesario & Hughes, 2007). Causes of precocious puberty include infection or trauma directed to the parts of the brain that control reproduction (Cowan & Richardson, 2018; Kaplowitz, et al., 2018), in addition to early life stress (Li et al., 2014; Kelly et al., 2017; Virdis et al., 1998). Precocious puberty is associated with short adult stature, emotional distress (i.e. depression), and other central nervous system abnormalaties (Kaplowitz, et al., 2018; Cesario & Hughes, 2007; Chalumeau, et al., 2002; Angold & Worthman, 1993). Both males and females can experience precocious puberty but it effects girls 10 times more frequently (Cesario & Hughes, 2007). Thus, precocious puberty could be considered a sex-dependent characteristic of humans who undergo early-life stress. Precocious puberty due to early life stress has also been reported in animal models of maternal separation and litter isolation (Cowan & Richardson, 2019; Kentner et al., 2018a; Grassi-Oliveira et al., 2016). Notably, sex-dependent precocial puberty is preventable by probiotic treatment (Cowan & Richardson, 2019) and sensory enrichment can also delay reproductive accerated maturation precipitated by early-life adversity (Kentner et al., 2018a).

Parental care has a critical influence on offspring development and negative experiences mediated through the parent-offspring relationship can significantly impair developmental outcomes. In the rodent laboratory, the dynamics of these relationships can be manipulated experimentally by changing the circumstances of the nest environment (Perry, et al., 2018; Walker, et al., 2017; McLaughlin, Verlezz, Gray, Hill, & Walker, 2016; Connors, et al., 2015; Kenny, et al., 2014; Champagne & Curley, 2007; Levine, 2001; Gilles, et al., 1996). One commonly used model of early-life adversity is the limited bedding model (LB) which involves reducing the availability of bedding materials that a dam uses to construct a nest for herself and her pups (Perry, et al., 2018; Walker, et al., 2017; Rice, Sandman, Lenjavi, & Baram, 2008; Gilles, et al., 1996). The lack of materials is a maternal stressor as it decreases the dam's ability to construct a satisfactory nest (Perry, et al., 2018; Walker, et al., 2017) which influences the way that she interacts with her offspring (Rice, et al., 2008; Champagne & Curley, 2007). Dams housed with limited bedding have lower nest construction quality scores, have poorer nursing habits, and have been reported to step-on and rough handle their pups (Perry, et al., 2018; Heun-Johnson & Levitt, 2016; Heun-Johnson & Levitt, 2016; Sullivan & Holman, 2010; Ivy, Brunson, Sandman, & Baram, 2008). These maternal behavioral patterns show that the LB paradigm can cause care fragmentation and maltreatment (Perry, et al., 2018; Walker, et al., 2017; Gilles, et al., 1996). There is evidence to support that this adversity causes long-term negative consequences on cognitive development, motor skills, and socialization of offspring (Gilles, et al., 1996).

As it stands, the LB protocol can be translated to the experience of a low resource or 'insecure' environment for humans. As established earlier, poor access to resources impacts how a dam interacts with her pups and this is also true of human parents. Socioeconomic status can affect parental care quality, for example high economic pressure has been positively correlated to the use of punitive or authoritarian parenting (Leinonen, Solantaus, & Punamaki, 2003). These parenting strategies are associated with conduct disorders and disruptive behavior disorders in children (Stormshak, Bierman, McMahon, & Lengua, 2010). Additionally, lower socioeconomic status is related to low birth weight, asthma, deficient language development, and fewer pre-academic skills (Burchinal, Peisner-

Feinberg, Bryant, & Clifford, 2000; Aber, Bennett, Conley, & Li, 1997;). The rodent LB protocol is most useful for modeling an impoverished environment because, in addition to the stress from lack of physical resources, it can cause behavioral modifications in the mother known to affect a child's development and adult outcomes.

On the other hand, environmental enrichment (EE) in the animal laboratory can translate to a high resource/high security situation and has been shown to rescue the effects of insecure "stressful" environments (Francis et al., 2002; Bredy et al., 2003; Bredy et al., 2004). Indeed, previous studies have shown that environmental complexity in early life can mitigate the effects of a number of early-life stressors including inflammation, poor maternal care, and neonatal brain injury (Kentner et. al, 2016; Schneider et al., 2006; Bredy et. al., 2004; Bredy et al., 2003; Pedrini Schuch et al., 2016; Durán-Carabali et al., 2018), highlighting its potential to mitigate the effects of LB, which to our knowledge has not been directly explored previously. Quantifying/mapping the therapeutic benefits of this housing condition has translational value in that enrichment protocols have been successfully utilized in clinical settings (Janssen et al., 2014; White et al., 2015; Woo et al., 2013; Woo et al., 2015), underscoring its acceptability and feasibility for use with patients.

Importantly, EE has also been shown to effect the quality of maternal care (Connors et al., 2015; Welberg et al., 2006; Sale et al., 2004; Durán-Carabali et al., 2018), but these effects have been underexplored. While there is some research focusing on defined periods of enrichment exposure (e.g. either pre- or postnatally; Cancedda et al., 2004; Rosenfeld and Weller, 2012), the number of studies evaluating dams in lifelong (or a combined pre- and postnatal) EE exposure are limited (Connors et al., 2015; Welberg et al., 2006; MacRae et al., 2015; Durán-Carabali et al., 2018). Moreover, some using this protocol have utilized co-parenting in the homecage, making the individual contribution of rodent dams difficult to assess. For this reason, we were interested in evaluating maternal care quality across the continuum of resource rich and poor animal laboratory conditions. Given the strength of maternal care quality in shaping offspring outcomes, it is important to understand differences in parent-offspring interactions as a function of environmental complexity. Environmental enrichment is an enhanced laboratory condition promoting the expression of species typical behaviors, when designing animal models for translational research it is imperative to consider environmental complexity and its impact on neurobiological indicies when trying to understand both the underlying etiology of disease and neurotypical development.

One imperative for improving animal models of early-life stressors is to simulate multiple adverse childhood experiences (ACEs). The 2016 National Survey of Children's Health reported that one in ten children experience three or more ACEs and that poverty is one of the most commonly experienced stressors (Sacks & Murphey, 2018). Experiencing multiple ACEs is associated with increased risk for both metabolic and psychiatric diseases in adulthood (Sacks & Murphey, 2018; Afari, et al., 2013; Pervanidou & Chrousos, 2012; Heim, et al., 2008; Chapman, et al., 2004; Anda, et al., 2002). While there are many early-life stressors that could be incorporated into the LB paradigm, diet is of special interest considering the association of both food insecurity and obesity with a variety of metabolic and psychological outcomes (Spencer, et al., 2017; Yam, et al., 2016). Food insecurity is the

condition of ones' diet being restricted in terms of variety, nutrition, and amount of food (Eicher-Miller & Zhao, 2018). Children who are food insecure are more likely to have poor outcomes including, but not limited to, generally poor health and greater number of hospitalizations, psychosocial and behavioral problems, worse developmental outcomes, and are more likely to suffer from childhood obesity (Gundersen & Kreider, 2009). When examining the relationship between food security and low-income women it was found that food insecurity and diet quality were inversely related; those who were identified as food insecure had lower levels of perceived neighborhood safety, had a higher body mass index, and had lower access to healthy food options (Sanjeevi, Freeland-Graves, & Hersh, 2018). A Western diet (WD) is characterized by high levels of refined sugars and saturated fats, and low fiber content which are all associated with obesity (Francis & Stevenson, 2012). In animal models, high-fat and sugar diets can cause impairments of the hippocampus and dysregulation of the HPA axis (Boitard, et al., 2015; Maniam, Antoniadis, & Morris, 2015). In humans there is a higher occurrence of psychological disturbances (e.g. mood, anxiety, somatoform and eating disorders) among adolescents who are obese in addition to reduced hippocampal volume, which is associated with depression (Kalyan-Masih, et al., 2016; Björntorp, 2008; Campbell, Marriott, Nahmias, & MacQueen, 2004; Britz, et al., 2000). On its own early-life stress causes increased vulnerability to metabolic syndromes which is only exacerbated by food insecurity (Yam, et al., 2016; Tilburg, et al., 2010). For example, early-life stress induced by the LB model paired with a WD in male rats was associated with higher body fat and a positive correlation between white adipose tissue and object recognition scores indicative of cognitive impairments (Yam, et al., 2016).

In the present study, we adapted rodent caging systems to simulate low, medium, and high security environments and evaluated their effects on maternal care and offspring development. The 'insecure' housing was modelled by a combination of WD-LB exposure, while standard rodent caging served as a 'medium security' environment. In contrast, a high resource environment was modelled by housing a subset of animals in EE. Between these settings we compared differences in maternal care and tested the hypothesis that early life stress caused by a low resource scenario would result in poor maternal care and associated metabolic dysfunction, including precoscious puberty in offspring. Finally, we tested whether weaning into EE could rescue some of the adverse outcomes associated with the early-life stress protocol.

2. Methods

2.1 Animals and housing

The experiment was approved by the MCPHS Institutional Animal Care and Use Committee and was carried out in compliance with the reccomendations from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Thirty-six female and twelve male virgin Sprague-Dawley rats were acquired from Charles River (Wilmington, MA) and habituated in same-sex pairs; vivarium kept at 20°C on a twelve-hour light/dark cycle (0700–1900 light) in larger sized cages (51 × 41 × 22 cm). These cages were a one level cage with access to corn bedding, one plastic tube, one chew bone, Nestlets® (Ancare) and *ad libitum* access to food and water. After a two-week habitation period, a subset of

female (n=14) and male (n=4) animals were allocated to a Western diet (WD; LabDiet® 5TJN). These females were assigned to later undergo LB housing (WD-LB; described below) in order to model a low resource or 'insecure' housing condition. A separate set of female (n = 22) and male (n = 6) rats were maintained on the standard diet (LabDiet® 5001). All animals were maintained on their respective diet for four weeks, prior to breeding, and remained on their assigned diet until weaning on postnatal day (P)22. One week prior to breeding ten females fed the standard diet were moved into EE (91.5 × 64 × 159 cm; Critter Nation, Muncie IN), maintaining their same-sex dyads. This EE cohort made up our 'high resource security' group. The EE housing units were multilevel cages with access to bedding, one tube, one chew bone, ample Nestlets® and toys. The location and type of toys used were changed two times weekly in order to stimulate novelty. The remaining standard chow fed females (n = 12) represented the medium or 'middle class' resource control group (standard housed; SD). Animals were weighed once weekly at 12pm. A timeline of the procedures can be found in Figure 1A. In line with initiatives to improve the reporting of experimental methods, we have complete the adapted reporting table from Kentner et al (2018b) and provided it as Supplementary Table 1.

2.2 Breeding and delivery

Breeding consisted of pairing one male with two females until pregnancy was verified by increased weight gain and the observation of visible teats. Pregnant females were kept in pairs until approximately gestational day (G)18, at which point they were housed individually in order to prevent the mixing of pups between litters. With respect to the WD-LB and SD groups, pregnant dams were placed into individual standard sized one level cages (27 × 48 × 20 cm). For the pregnant EE animals, a divider was built into the home cage so that litters could be separated – one litter housed in the top portion and one in the bottom until weaning. Each cage section had two levels (see Figure 1B). Toys were taken away from EE animals on G18 and returned on P14 in order to prevent the risk of pup injury during the early neonatal period. Day of birth was designated as P1; on P2 litters from all housing conditions were culled to n=12 (equal number of males and females where possible). Animals remained undisturbed until the morning of P10 at which point all cages were cleaned. In line with initiatives to reduce the number of animals in research (National Research Council, 2011), on P14, a small subset of male and female animals (n = 2) from the WD-LB litters were quickly administered a single dose of lipopolysaccharide (LPS; Escherichia coli; serotype 026:B6, L-3755; Sigma, St Louis, MO; 100mg/kg) so they could be included as part of a separate study (unpublished). It has previously been shown that this treatment does not disrupt maternal care (Spencer et al., 2006). One male and female animal from each litter was evaluated in the measurements described below. A list of offspring group designations can be found in Table 1.

2.3 Limited Bedding Model

On the late afternoon of P2 a stainless-steel grate was placed into the cage of WD fed animals (adapted from McLaughlin et al., 2016) creating the WD-LB housing group. In our case, the stainless steel grates sat on top of the bedding, restricting access of the dams to their bedding (see Figure 1C). Additionally, a single piece of paper towel was added in lieu of Nestlets® (Gilles, et al., 1996). Cages were left undisturbed until the morning of P10 at

which point the stainless-steel grates were removed and litters placed into fresh clean cages with the same level of access to nesting materials as the SD group.

2.4 Maternal care observations

The first week of life is a critical time for rodents (Champagne & Curley, 2007). Between P3-P6 passive home cage maternal behavior observations were conducted twice daily; once in the light phase (07:45–10:00 h) and once in the dark (19:45–22:00 h) to account for the nocturnality of rodents (Champagne & Curley, 2007). Each observational period consisted of five one-minute observations, with five minutes of no observation occurring between each one-minute bin. Recorded maternal behaviors included the frequency of pup retrievals, dam licking pup, passive/low crouch nursing, active/high crouch nursing, nest building/digging in bedding, dam self-grooms, dam sleeping, dam eating/drinking, dam chasing her tail, and total time on nest (seconds).

2.5 Assessment of puberty onset

Beginning on P25, female rats were evaluated daily for vaginal openings (VO) until all animals were scored as open. Animals were scored by their appearance as being either closed, or having a complete vaginal opening (Cowan & Richardson, 2018; Kentner et al., 2018a; Grassi-Oliveira et al., 2016).

2.6 Open field test

To evaluate the presence of anxiety-like behavior (Crawley, 2007), an open field test was conducted in dams on P22 and offspring on P50. Thirty minutes prior to testing animals were allowed to habituate to the testing room. Dams were placed into a black, square box (72 cm × 72 cm × 36 cm) and video recorded for ten minutes. Juvenile male and female offspring were similarly evaluated in a smaller arena (40 cm × 40 cm ×28 cm) and video recorded for five minutes. Between each recording session all of the equipment was thoroughly cleaned with Quatriside TB. Videos were then scored through an automated behavioral monitoring software program (Cleversys TopScan, Reston, VA) and evaluated for the percent of time (seconds) spent in the center of the arena [((total time in center)/(total time in center + total time in perimeter))*100] and total distance travelled (cm) in the arena.

2.7 Social preference test

Immediately following the open field test, which was used as a habituation period to the test arena, the social preference for a novel conspecific versus an inanimate object (Crawley, 2007) was evaluated. During this assessment, juvenile offspring were placed into the center of the arena and video recorded for five minutes. During this period animals had the choice to visit either a same sex/size/age novel conspecific or an object, each confined within a small wire cup on opposite ends of the arena. Placement of the novel conspecific and the object were counterbalanced between tests and experimental animals groups. Between each recording all of the equipment was thoroughly cleaned with Quatriside TB. Using the software program Stop Watch+ (Atlanta, Georgia), videos were then scored by two blinded observers with an established interrater reliability of >90%. The variables scored were the duration of time (seconds) spent with and frequency of visits to either the same sex/size/age

novel conspecific, or object. From these variables, a social preference score was calculated and expressed as a preference ratio arena [((total time with novel rat)/(total time with novel rat + total time with object))*100] where scores >0.5 indicated a preference for the novel rat compared to the object.

2.8 Tissue collection

Two hours after behavioral testing dams and their offspring underwent a 20-minute restraint stress procedure. During this period, animals were individually restrained in a soft open-ended plastic pastry bag (Daymark) and immediately euthanized with a mixture of Ketamine/Xylazine (40–80 mg/kg, i.p. 5–10 mg/kg, i.p.). Cardiac blood was collected in EDTA tubes and plasma collected. Brains were immediately collected and placed on a clean petri dish set on wet ice. Whole hypothalamus was quickly dissected from the freshly collected tissue and frozen on dry ice.All tissues were stored in −70°C for future processing.

2.9 ELISA

Plasma corticosterone and leptin levels were analyzed in duplicate by ELISA using the manufacturer's instructions. With respect to the plasma corticosterone ELISA we followed the small sample assay protocol provided with the testing kit (ADI-900–097, Enzo Life Sciences, Farmingdale, NY, USA). The minimum detectable concentration was 26.99 pg/ml, and the intra and inter-assay coefficients of variation were 6.6% and 7.8%, respectively. The minimum detectable leptin concentration (abcam ab100773, Abcam) was 30 pg/mL, intra and inter-assay coefficients of variation were <10% and <12%, respectively.

2.10 PCR

Total RNA was extracted from frozen hypothalamus with TRIzol reagent (Thermo Fisher). Isolated RNA was quantified utilizing a NanoDrop 2000 spectrophotometer (Thermo Fisher). cDNA synthesis and qPCR were performed using TaqMan assay probes to evaluate *Kiss1, Kiss1R, Gnrh1, Gnrhr* and *Gapdh* (Rn00710914_m1, Rn00576940_m1, Rn00562754_m1, Rn00578981_m1 and Rn01775763_g1, respectively) in a StepOne Plus station (Applied Biosystems) as described previously (Kentner et al., 2018a). Each sample was analyzed in duplicate and relative gene expression levels were evaluated using the ΔΔCt method with *Gapdh* as the housekeeping gene. Data are presented as mean expression relative to CON-nSaline animals, which have been normalized to 1 for each gene of interest.

2.11 Westerns

Microdissections of the medial prefrontal cortex (mPFC), centered on the infralimbic and prelimbic regions with the ventral anterior cingulate cortex included (A/P = 3.24mm and M/L = 0 mm from Bregma, D/V = −3.2 mm from the surface of the brain) were taken using a trephine from flash froze tissue. Tissue samples were homogenized in RIPA buffer (R0278, Sigma) and HaltTM protease and phosphatase inhibitor cocktail (ThermoFisher, 78440), spun at 40,000 × *g* for 30 min at 4°C, and the supernatant assayed with the BCA protein Assay (ThermoFisher, 23227). Samples (30ug) were loaded on 4–15% Mini-PROTEAN® TGX™ Precast Protein Gels (BioRad, 456–1084). Gels were run for 35 min, at 200V in 1× Tris/Glycine/SDS buffer (BioRad,1610771). Then gels were transferred to a PVDF

membrane (Immobilon-FL, IPFL00010) in 1× Tris/Glycine buffer (BioRad, 1610734) for 1.5 h at 55V. After transfer, membranes were stained with the REVERT Total Protein Stain Kit (BioRad, 926–11016) for total protein normalization according to manufacturer's instructions and immediately imaged with Odyssey FC Imaging System using Image Studio Life Version 4.0. Then membranes were blocked with Odyssey Blocking Buffer (927–50000) for 1h. Afterwards, membranes were probed with an anti-synaptophysin antibody, clone SY38 (1:1000, Millipore, MAB5258) diluted in 1:1 Odyssey Blocking Buffer:TBS overnight at room temperature. Membranes were washed three times for 5 minutes with TBST and them probed with anti-mouse IRDye 800CW (1:20,000, LiCor) 4 h at room temperature. After three additional 5 min TBST washes, membranes were placed in TBS before imaging with Odyssey FC Imaging System. After scanning, Odyssey Infrared Imaging software quantified the integrated intensity of each band and determined molecular weights based on Biorad Precision Plus Protein Standards (1610374). The ratio of synaptophysin to total protein for the lane was calculated (Kirshner & Gibbs, 2018). For the figures, each channel of the image was adjusted for brightness and contrast individually using the Odyssey Infrared Imaging software and adjustments were applied equally to the entire image.

2.12 Statistical analyses

Statistics were performed using the software package Statistical Software for the Social Sciences (SPSS) version 21.0 (IBM, Armonk, NY) or GraphPad Prism (version 7.0), in the case of the survival curves described below. Alpha levels were set to $p < 0.05$ for all omnibus tests. A one-or two-way repeated measures ANOVA was used to evaluate maternal (baseline, weeks 1–4, P22) and offspring body weights (P22, P29, P36, P43, P50) respectively. In each case, time was the repeated factor while either housing or housing and sex were the independent variables, as appropriate. The Greenhouse–Geisser correction procedure was applied due to violations to the assumption of sphericity for repeated measure designs. For maternal behavior, Chronbach's alpha was calculated and interrater reliability between two raters was found to be >80%. Due to the severity of skewness (Shapiro-Wilks) for maternal and offspring behaviors and plasma corticosterone levels, non-parametric Kruskal-Wallis tests (non-parametric equivalent to the one-way ANOVA) were employed. Pairwise comparisons were made using conservative adjusted alpha correction values. Day of VO and female offspring hypothalamic gene expression were evaluated using one way-ANOVAs. With respect to parametric data, LSD post hocs were applied except where there were fewer than three levels, in which case pairwise t-tests and Levene's (applied in the occurrence of unequal variances on the post hoc assessments) were utilized. The time to VO was also assessed, using the Log-rank (Mantel Cox) test, and plotted as a survival curve. For applicable offspring analyses, if there were no sex differences, then male and female data were collapsed together for analyses and presentation. Data were graphically expressed as mean ±SEM.

3. Results

3.1 Maternal body weights

A repeated measures ANOVA revealed a significant interaction between time and housing for maternal body weights ($F(3.956, 59.337) = 4.743$, $p = 0.002$; Figure 2). LSD post hocs did not show a difference across housing groups for maternal body weight at either baseline or P22, however significant group differences were observed across the course of the study with WD rats showing significantly greater weight gain compared to SD controls (Week 2: $p = 0.001$; Weeks 3 and 4: $p = 0.0001$) and EE animals (Week 3: $p = 0.05$; Week 4: $p = 0.023$), validating the western diet treatment. EE females also had significantly more weight gain than SD rats across several points of the study (Week 1: $p = 0.008$; Week 2: $p = 0.023$; Week 4: $p = 0.006$).

3.2 Maternal Care

With respect to P3–6 maternal behavior, although there were no treatment differences with respect to time on nest (seconds) during the light phase observation period ($X^2(2)=4.535$, p=0.104; Figure 3A), Kruskal-Wallis tests revealed a significant main effect of housing during the night ($X^2(2)=12.545$, p=0.002; Figure 3B) and for the combined total of the daylight and nighttime observations ($X^2(2)=11.319$, p=0.003; Figure 3C) on this measure. Follow-up tests demonstrated that SD (dark: p=0.021; light & dark: p=0.013) and WD-LB (dark: p=0.002; light & dark: p=0.007) dams both spent more time on nest than EE mothers.

During the light phase, there was a significant main effect of housing for licking/grooming ($X^2(2)=12.763$, p=0.002), active nursing ($X^2(2)=7.539$, p=0.023), passive nursing ($X^2(2)=7.954$, p=0.019), maternal self-grooming ($X^2(2)=14.779$, p=0.001), maternal eating and drinking ($X^2(2)=6.396$, p=0.041), and tail chasing ($X^2(2)=7.495$, p=0.024) behaviors (Figure 3D). Post hoc analysis revealed that WD-LB dams licked their pups more frequently than both SD (p=0.004) and EE (p=0.012) mothers. WD-LB dams also groomed themselves more than those reared in SD cages (p=0.0001). EE animals engaged in active nursing significantly more often than SD dams (p=0.019) and SD dams engaged in passive nursing significantly more frequently than EE dams (p=0.016). Finally, WD-LB animals demonstrated more tail chase behaviors than EE dams (p=0.024).

For observations during the dark phase, there were differences in the frequency of licking/grooming ($X^2(2)=10.478$, p=0.005), active nursing ($X^2(2)=6.491$, p=0.039), passive nursing ($X^2(2)=10.897$, p=0.004), and total nursing ($X^2(2)=13.772$, p=0.001) behaviors (Figure 3E). Pairwise comparisons revealed that WD-LB dams licked their pups significantly more than both EE (p=0.014) and SD (p=0.015) mothers. EE animals engaged in fewer bouts of passive nursing postures compared to SD (p=0.014) and WD-LB (p=0.007) animals. The same pattern was also true for the frequency of total nursing behaviors (WD-LB vs SD: p=0.026; WD-LB vs EE: p=0.001).

For the combined day and nighttime observation periods there were differences in licking/grooming pups ($X^2(2)=16.716$, p=0.0001), active nursing ($X^2(2)=10.235$, p=0.006), passive nursing ($X^2(2)=10.067$, p=0.007), total nursing ($X^2(2)=9.844$, p=0.007), maternal self-grooming ($X^2(2)=6.627$, p=0.036), and tail chase ($X^2(2)=6.337$, p=0.042) behaviors (Figure

3F). Post hocs demonstrated that WD-LB dams licked their pups significantly more often than both SD (p=0.001) and EE (p=0.002) mothers. For active nursing, SD dams engaged in significantly fewer bouts than both WD-LB (p=0.036) and EE (p=0.01) animals. SD dams engaged in significantly more passive nursing behaviors than the EE (p=0.006) dams. For total nursing behaviors, EE animals did not nurse as frequently as either the SD (p=0.019) or WD-LB (p=0.013) animals. With regards to maternal self-grooming, there were no significant differences across housing groups. Finally, WD-LB animals chased their tails significantly more than EE (p=0.042) dams.

3.3 Maternal open field test and stress-induced plasma corticosterone

No significant differences were observed across housing groups on measures of P22 maternal anxiety-like behavior and locomotor activity (open field test) or on maternal stress-induced (20 minute restraint stressor) plasma corticosterone (p>0.05, data not shown).

3.4 Offspring body weights

A two way repeated measures design (housing by sex) revealed a sex by time interaction ($F(2.199, 142.921) = 79.720$, $p = 0.0001$; n = 8–10; Figure 4AB). Post hocs showed that male offspring were heavier than females (p<0.0001) at P29, P36, P43, and P50. There was also a housing by time interaction ($F(6.596, 142.921) = 2.451$, $p = 0.023$; Figure 4AB). WD-LB animals were heavier than SD and EE offspring at weaning on P22 (SD: p =0.0001; EE: p=0.0001) and on P29 (SD: p =0.0001; EE: p=0.0009). WD-LB-EE animals had significantly higher body weights than SD (p=0.0001) and EE (p=0.0001), but were not different than WD-LB offspring on P22. On P29, EE offspring weighed more than SD rat (p=0.017). By P29, WD-LB-EE rats weighed significantly less than WD-LB offspring, suggesting that enrichment was able to quickly rescue the metabolic disruptions induced by a high fat diet. There were no differences in weight across the housing groups by P36, supporting the existence of a resilient weight regulation mechanism that may have turned on after the cessation of the western diet on P22.

3.5 Offspring behaviors

Non-parametric Kruskall-Wallis tests did not uncover sex or housing differences for either percent time spent in the center of the open field or for locotomor activity level (n = 8–10; p>0.05, data not shown). Non-parametric analysis revealed significant main effects of sex on the frequency of visits to the novel rat ($X^2(1) = 16.667$, $p = 0.0001$; Figure 4C) and object ($X^2(1) = 14.553$, $p = 0.0001$; Figure 4D) in the social preference test, in that females made a higher number of visits overall (p<0.05). There was also a main effect of housing for the total frequency of visits that female offspring made to the novel object ($X^2(3) = 17.970$, $p = 0.043$; Figure 4D) in that female SD rats visited the novel object more than WD-LB-EE rats. Kruskall-Wallis tests uncovered a main effect of housing on the social preference test ($X^2(3) = 8.282$, $p = 0.041$; Figure 4E), but follow-up tests did not confirm differences as a function of treatment (p>0.05).

3.6 Precoscious puberty,hypothalamic gene expression, and plasma leptin concentrations

One way ANOVA showed a significant effect of housing on pubertal maturation ($F(3, 34) = 3.235$, $p = 0.034$; n = 8–10, Figure 5A). LSD follow up tests revealed that full vaginal opening occurred significantly earlier in WD-LB female offspring compared to SD ($p = 0.025$), EE ($p = 0.040$), and WD-LB-EE ($p = 0.007$) rats. A more in depth evaluation of time to vaginal opening using the Log-rank (Mantel-Cox) test revealed a significant effect of housing ($X^2(3) = 11.83$, $p = 0.008$; Figure 5B). Comparisons of the survival curves revealed significant differences in day of vaginal opening (DVO) between SD (DVO: P38) vs. WD-LB (DVO: P36; $X^2(1) = 4.685$, $p = 0.0304$), WD-LB vs. WD-LB-EE (DVO: P38.5; $X^2(1) = 8.225$, $p = 0.0041$), as well as WD-LB vs. EE (DVO: P38); $X^2(1) = 4.628$, $p = 0.0315$). Animals from all treatment groups displayed full vaginal openings by P43 (Figure 5B).

The kisspeptin/gonadotropin releasing hormone (GnRh) system is an important pathway integral to puberty initiation (Kauffman, Clifton, & Steiner, 2007; Skorupskaite et al., 2014) so we evaluated the P50 expression of several related hypothalamic gene targets (n = 7–10). One way ANOVAs did not indicate differences as a function of housing for *Gnrh1* or for its receptor *Gnrhr* ($p>0.05$, Figure 5C). However, there were significant main effects of housing for *Kiss1* ($F(3,30) = 3.089$, $p = 0.043$; Figure 5C) and *Kiss1r* expression ($F(3, 30) = 7.708$, $p = 0.022$; Figure 5C). LSD follow-up tests showed that WD-LB female rats had significantly lower levels of *Kiss1* compared to SD rats ($p = 0.006$). Environmental enrichment did not completely rescue this effect (WD-LB vs WD-LB-EE: $p = 0.052$). However, WD-LB-EE animals were not significantly different from SD rats on this measure ($p>0.05$), suggesting some modest attenuation following enrichment. Hypothalamic *Kiss1r* was significantly elevated in WD-LB-EE animals compared to both SD ($p=0.005$) and WD-LB ($p = 0.016$) females. This suggests that the elevated levels of *Kiss1r* following EE may have compensated in part for the depleted *Kiss1* levels associated with WD-LB housing.

Given evidence that plasma leptin concentrations are correlated with precocious puberty we elected to measure this hormone in our female animals (Sominsky et al., 2016). A one-way ANOVA revealed a significant housing effect on plasma leptin concentrations ($F(3, 25) = 3.172$; $p = 0.042$; Figure 5D). LSD post hoc tests demonstrated that WD-LBM female rats had higher plasma leptin levels compared to WD-EE ($p=0.007$) and EE ($p = 0.033$) animals, respectively. There were no significant differences between SD and WD-LBM females ($p > 0.05$).

3.7 Offspring plasma corticosterone

No significant differences were observed across experimental groups on measures of stress-induced (20 minute restraint stressor) plasma corticosterone for P50 offspring (n = 7–10, $p>0.05$, data not shown).

3.8 Levels of synaptophysin in the mPFC

The mPFC continues to develop through puberty, and this development is characterized by alterations in synpases (Drzewiecki et al., 2016). To determine whether sex or housing influenced mPFC maturation we evaluated synaptophysin, a marker of synapses used to

assessed cortical synaptic development (Glantz et al., 2007). Values that exceeded 2 standard deviations above or below the group mean were considered outliers and dropped from the analysis. No significant differences were observed in synaptophysin levels in mPFC (n= 8–10, p> 0.05, Figure 6).

Discussion

The present experiments were designed to assess the impact of high, medium, and low security environments on maternal care quality and, in tandem, to evaluate the maturation and neurobehavioral outcomes of male and female offspring. The results show that resource insecurity and environmental complexity differentially affect maternal behavior, but not along a continuum ranging across defined categories from ‘low’ to ‘high’ quality, as one might expect. Instead, circadian timing greatly influenced the temporal pattern of behavioral expression, unveiling potential strengths and weaknesses in maternal care quality across the housing conditions. Offspring born to low resource WD-LB dams were resilient to substantative disruptions in behavior and mPFC plasticity. Only subtle perturbations were observed in the measures evaluated here, and were limited to differences in exploration of the novel object by female WD-LB rats in the social preference test. This did not translate into impairments of species typical preferences for social novelty. Instead, consequences of early life stress were most apparent by metabolic changes such as increased body weight and accelerated puberty, in addition to alterations in the levels of the kisspeptin gene and expression of its receptor. Importantly, placement into EE during the early juvenile period fast-tracked the regulation of body weight after cessation of the Western diet in WD-LB offspring. Moreover, the juvenile enrichment intervention prevented the precocial puberty associated with a history of early life stress. Given that enrichment is a translationally valid intervention for both animals and humans (Woo et al., 2013; Woo et al., 2015; Kentner et al., 2018c), this study offers further insight into additional unexplored uses for this tool. This is in addition to highlighting the utility of developing and incorporating clinically relevant animal models into our basic reserach.

While animal models can be used as a translational approach to evaluate the neurobehavioral effects of early-life stress, or even positive early life experiences, it is important to acknowledge their strengths and shortcomings in terms of test validity. For example, construct, face, and predictive validity satisfy the three-criteria required for an animal disease model: congruent risk factors, symptoms, and efficacy of treatment, respectively (Estes & McAllister, 2016; Belzung & Lemoine, 2011). Although early-life exposure to stressors do not always lead to a disease state, these adverse experiences do increase the risk for mental illnesses (e.g. depression, anxiety) in later life (Kumsta, et al., 2017; Burkholder, Koss, Hostinar, Johnson, & Gunnar, 2016; Bentall, Wickham, Shevlin, & Varese, 2012; Green, et al., 2010; Heim, et al., 2008; Chapman, et al., 2004). Employing animal models of early-life stress such as maternal separation or LB meet construct validity in that they mimic risk factors such as neglect or abuse. Moreover, they demonstrate face validity in that specific symptoms of mental illnesses (e.g. impaired emotional reactivity, disrupted HPA regulation, cognitive disturbances, anxiety-like behavior) can be observed in animals that have experienced these stressors during the perinatal period (Grassi-Oliveira, Honeycutt, Holland, Ganguly, & Brenhouse, 2016; Leussis, Freund, Brenhouse, Thompson, &

Andersen, 2012; Raineki, Cortés, Belnoue, & Sillivan, 2012; Chatterjee, et al., 2007; Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001; Fernandez-Teruel, Escorihuela, Driscoll, Tobena, & Battig, 1991).

Animal models of early-life stress can also be used to predict treatment efficacy. For example, in humans, skin-to-skin contact in the form of Kangaroo Care has been successful in treating the adverse effects of premature birth and improving parent-infant attachment when compared to traditional treatment (Charpak, et al., 2016; Baley, 2015; Tessier, et al., 2003). Similarly, when rat pups are isolated or artificially reared in the laboratory, maternal tactile contact can be simulated by stroking the animal with a paintbrush to emulate licking and grooming behaviors (Chatterjee, et al., 2007). Experimentally this has led to improvements in a number of neural proteins integral to development: higher densities of synaptophysin, neural cell adhesion molecule, growth associated protein 43, brain-derived neurotrophic factor and glucocorticoid receptor expression, when compared to control animals (Chatterjee, et al., 2007; Kentner et al., 2018c). Artificial rearing can have an intergenerational, detrimental behavioral effect. Artificially reared mothers will engage in the same maternal behaviors as control mothers but they will do so for less time, and additionally these dams will show an increase of non-maternal behaviors such as digging, biting at the cage floor, and chasing their own tail (Lomanowska & Melo, 2016; Barrett & Fleming, 2001; Burton, et al., 2007; Gonzalez, et al., 2001). Tactile stimulation during artificial rearing can improve the quality of maternal behaviors that artificially reared mothers provide to their own offspring, compared to artificially reared mothers that did not receive tactile stimulation (Gonzalez et al., 2001). For human infants, similar tactile contact in the form of Kangaroo Care has short-term improvements in birthweight and survival, and long-term improvements in academic success, and higher IQ (Charpak, et al., 2016; Baley, 2015; Tessier, et al., 2003). For human mothers there are improvements in breast milk production, reduced feelings of hopelessness, and better infant attachment (which also extends to fathers; Baley, 2015).

In a similar vein, children who were institutionalized and then moved to foster care showed improvements in cognitive, motor, and behavioral domains (as measured by the Bayley Scale of Infant Development) as well improvements in intellectual functioning in verbal and performance domains (as measured by the Weschler Preschool Primary Scale of Intelligence) when compared to their peers who remained institutionalized (Nelson, et al., 2007). When observing the differences between institutionalized vs never institutionalized children’s brains MRI revealed that those with a history of institutionalization had significantly reduced cortical gray matter volume (Sheridan, Fox, Zeanah, McLaughlin, & Nelson, 2012). Foster care is superior to institutionalization because of better caregiver contact, better access to resources, and a higher-quality environment all of which are beneficial to cognitive development (Sheridan, et al., 2012). Physical contact from their caregivers to their children is essential to the child’s development. Even in healthy infants, skin-to-skin contact with parents has been shown to stabilize body temperature and blood glucose concentrations, decreases crying, and supports cardiorespiratory stability (Feldman-Winter & Goldsmith, 2016). In animal models, EE has been demonstrated to reverse the negative effects of processive and systemic stressors (Champagne & Curley, 2016; Kentner, Khoury, Queiroz, & MacRae, 2016; Vivinetto, Suárez, & Rivarola, 2013; Schneider,

Turczak, & Przewlocki, 2006; Bredy, et al., 2004). Thus, improvements to one's environment can lead to better outcomes in both humans and rats. While it is important to model the effects of stress in animals, it is also important to model the effects of EE so that efficacy of potential interventions can be established. Indeed, in the present study, we moved juvenile LB animals into EE to measure whether the metabolic consequences of early life stress could be rescued. Overall, each animal model of early-life stress will have its own particular strengths and weaknesses, but the most translational ones will meet several of these tests of validity.

With respect to the current study,the maternal care quality of EE dams was similar to what our laboratory has previously reported (Connors et al., 2015), except here we evaluated individual litters in isolation, as opposed to as part of a co-parenting design. This allowed us to better control for the contributions of individual dams on their litters, which is important given reports that dams may 'take-turns' caring for pups in a colony nest setting (Sale et al., 2004). It should be noted that in these co-parenting conditions one dam could not sufficiently meet the needs of both litters on her own, and it is unlikely that pups would receive more maternal attention overall. However, one great strength of the colony nesting design is that group housing may more adequately mimic the living conditions of rats in the wild (see Schultz & Lore, 1993; Hayes, 2000), allowing for a semi-naturalistic assessment of offspring developmental outcomes. That said, evaluating individual litters in the present study revealed a clearer role of circadian rhythms on the temporal patterning of maternal care.

While EE mothers spent less time on their nest as a function of circadian timing, they were perhaps more efficient in their care when in direct contact with pups. Specifically, despite differences in total pup contact time, EE and SD dams did not differ in the quantity of pup licking behavior, which is in alignment with reports of others (Welberg et al., 2006; Connors et al., 2015). However, some investigators have observed higher rates of licking from EE dams (Sale et al., 2004). Additionally, enriched dams spent less time in passive nursing postures across the nycthemeron, compared to standard housed dams, and in turn demonstrated higher frequencies of active nursing, at least in the light phase. This effect is likely not surprising given the longer duration of time that SD dams remained with their litters. In terms of energy expenditure, it would be comparably more difficult to maintain long periods of active nursing postures when spending longer periods nursing on the nest (Sale et al., 2004).

Based on our previous work we had begun to equate SD dams with 'helicopter parents' given: a) the increased contact they maintain with their litters and b) observations that SD offspring may not be as 'adaptable' as EE animals when exposed to potentially threatening environments (Connors et al., 2015). This is comparable to the maladaptive coping and decision-making skills often assoiciated with children of helicopter parents (Leubbe et al., 2016). Similarly, WD-LB dams also spent longer durations in contact with their litters compared to EE mothers, however there were no indications of maladaptive offspring behaviors, at least among the behavioral tests administered here. Interestingly, WD-LB dams consistently engaged in higher frequencies of pup licking and grooming across both the light and dark periods. High levels of maternal licking and grooming are typically associated with

better HPA regulation by offspring in response to stressors, as mediated by upregulation of glucocorticoid receptor expression (Liu et al.,1997). This result raises the intriguing possibility that the the enhanced maternal licking behavior from WD-LB dams counteracted the effects that early life stress typically exerts on offspring behavior. Indeed, similarly to others (McLaughlin et al., 2016) we did not observe any instances of neglectful or abusive maternal behaviors from dams housed with LB, as previously described (Perry, et al., 2018; Heun-Johnson & Levitt, 2016; Sullivan & Holman, 2010; Ivy, Brunson, Sandman, & Baram, 2008). Overall, they appeared to be attentive mothers despite a reduced level of nesting resources. This may have been due in part to the WD as dams maintained on a high fat diet display higher quality maternal care (Purcell et al., 2011; Rincel et al., 2016).

Alongside the increased licking of pups, WD-LB dams also self-groomed significantly more often than EE and SD rats, at least in the light phase. Initially, we speculated that Western fed animals may lick more because of the taste of the diet on themselves and their pups. While this was not assessed directly, informal observations did not suggest that the fur of WD-LB animals was greasy; visually, these animals were no different in appearance from SD or EE dams/offspring. Morever, there were no differences in self-directed maternal grooming behaviors when both the light and dark cycles were considered together as an aggregate measure.

WD-LB mothers did, however, differ in the amount of non-maternal tail-chase behaviors compared to EE dams. Self-directed tail chase behaviors primarily emerge in rats that are individually housed (Hurst, 1997) and may represent a ‘surrogate social response’ in that the tail has spontaneous movements and so the rat responds as it would towards a social conspecific (Baenninger, 1967; Hurst et al., 1997). Higher rates of tail chase behaviors have also been reported in rat dams that underwent social isolation and received limited maternal care in early life (Gonzalez et al., 2001). Either way, tail chasing may suggest that the rat is seeking social interaction (Hurst et al., 1997) or may require additional sensory enrichment. Given the limited resources available to WD-LB dams, it is not surprising that these animals displayed higher levels of tail-chasing. However, we may have to address the question of whether the LB set-up is *too* impoverished as self-directed tail-chasing is not representative of species typical rat behavior. Additionally, laboratory SD housing as it stands is certainly not resource rich, even by comparison to LB conditions.

Despite a stressful postpartum period, WD-LB rat dams and their offspring demonstrated resiliency against the emergence of long-term behavioral disruptions. This finding is suggestive of compensatory mechanisms that may have made up for a lack of postnatal resources. In our hands, WD-LB dams did not display anxiety-like behaviors at weaning, nor did they or their offspring have exaggerated plasma corticosterone responses following a restraint stressor, though the later could be due to differences in sampling methodology (see Vahl et al., 2005), or a consequence of the high fat diet (see Rincel et al., 2016). While there have been ample investigations into the outcomes of offspring exposed to LB, studies evaluating the concsequences of LB on the dam have been limited. Of the work that has assessed dam behavioral performance after weaning, there has been no evidence of altered anxiety-like or fear-learning behaviors; aberrant behavioral expression appears to be limited to the early life stress period and specific to maternal care quality (Heun-Johnson & Levitt,

2016). In general, the wide variations in maternal behaviors we observed across the low, medium, and high resource settings underscores the need for the scientific community to more thoughtfully consider the utilization of translationally relevant housing conditions in the animal laboratory, particularly given the impact of parental nurturing on offspring outcomes.

Our WD-LB exposed offspring were resilient to behavioral peturbations, as has been similarly reported by others who exposed animals to a maternal high fat diet (Zieba et al., 2018). We only observed minor sex-specific disruptions in the frequency of novel object exploration in the social preference test. In contrast to the negligible behavioral effects reported here, animals exposed to limited resource conditions typically display robust deficits in attachment learning and impaired social behaviors, depressive and anxiety-like behaviors and disrupted maternal care (Moriceau et al., 2009; Raineki et al., 2010; Raineki et al., 2012; Dalle Molle et al., 2012; Brunson et al., 2005; Rincon-Cortes et al., 2016; Roth et al., 2009), although it is appears that whether or not abberant behavior is observed may be task specific (Ivy et al., 2010) or even dependent on age of testing or animal strain (Maniam et al., 2016; Brunson et al., 2005; Dalle Molle et al., 2012).

An important point to consider is that our LB animals were exposed to a WD preconceptionally and maintained on this diet across early development, until weaning, at which point it was replaced with regular chow. A post-weaning high fat diet has been demonstrated to reverse the anxiety-like effects associated with LB (Maniam et al., 2016), suggesting it may have value as an intervention tool (Maniam et al., 2016; Machado et al., 2013). Here, our data indicate that a Western style diet may also have *preventative* effects against the consequences of LB exposure. Indeed, maternal high-fat diet mitigated a variety of negative outcomes (e.g. anxiety, cognitive and social impairments, nociception and HPA dysregulation) associated with maternal separation including downregulation of *Bdnf* and *Crh* mRNA, spine loss and dendritic atrophy (Rincel et al., 2016; Rincel et al., 2018). This could account for the small impact that WD-LB housing had on offspring brain and behavior in the present work. Importantly, maternal high fat diet exposure is associated with increased maternal care (Purcell et al., 2011; Rincel et al., 2016) implicating an indirect pathway by which diet may influence offspring development. While our own WD-LB dams spent relatively less time in active nursing postures, compared to EE mothers, they engaged in higher rates of licking and grooming which conceivably may have counteracted the behavioral effects of early life stress through programming of the HPA-axis (Liu et al., 1997). This is important to note because although EE dams had lower total contact time with their nest, they made-up for this by participating in more active nursing postures. Together, this reminds us that despite the available resources no parent is perfect and for the most part the kids are (behaviorally) alright.

Although offspring of WD-LB animals appeared to be behaviorally resilient to the early life stress, metabolic consequences of this condition were more apparent. Specifically, these animals had higher body weights at weaning and female offspring displayed precocial puberty and downregulated *Kiss1* mRNA expression in the hypothalamus. Mutations and deletions of Kiss1 or its receptor are accompanied by impairments in reproductive functioning, including an interruption of pubertal development, associated with reductions in

GnRh (Kauffman, 2007). Moreover, prepubertal administration of kisspeptin initiates precoscious puberty, as indicated by LH release and advanced vaginal openings (Navarro et al., 2004). Therefore, we were surprised to measure diminished expression of *Kiss1* mRNA in our WD-LB animals. Indeed, we had anticipated that elevated levels of this gene would stimulate GnRh secretion, resulting in the accelerated pubertal onset associated with WD-LB. Since we evaluated whole hypothalamus, it should be considered that we overlooked the tissue specific nature regulating the expression of these genes. For example, sex sterioids inhibit or or stimulate *Kiss1* levels in a region specific manner (see Kauffman et al., 2007). We may have observed the predicted gene expression patterns (e.g. higher *Kiss1*) if we had targeted localized regions of the hypothalamus. It should also be noted that we collected tissue following puberty initation and we likely missed a critical window. Future work will need to include time-dependent sampling methods in order to capture a more accurate developmental picture of how early life stress affects the GnrH-Kisspeptin system and its influence on puberty. In addition to that, potential compensatory mechanisms such as differences in receptor sensitivity and regulatory-feedback systems will need to be evaluated. Finally, one must remember that the proportion of mRNA does not necessarily correlate to protein levels as this relationship is coordinated by several nuanced biological processes (Payne, 2015; Vogel & Marcotte, 2012).

In medicine, the pathophysiology of precocial puberty typically only considers trauma, genetic predisposition, illness or some physical cause for the accelerated maturation (Kaplowitz et al., 2018; Chalumeau et al., 2002; Strauss & Barbieri, 2009). However, early life stress is beginning to garner more attention as being a precipitator of early pubertal onset (Li et al., 2014; Kelly et al., 2017; Cowan & Richardson, 2018; Virdis et al., 1998). Recently, we observed precocious puberty in a rodent model of the neonatal intensive care unit (mimicking reduced parental contact, nosocomial infection, and medical manipulations). However, in this early life stress model we measured reduced *GnRh* receptor, as opposed to *Kiss1* (Kentner et al., 2018a), though this finding is vulnerable to the methodological limitations listed above. Alternatively, these data suggest that different early life stressors may result in similar physiological outcomes (in this case accelerated puberty) that are mediated via a diverse set of mechanistic pathways. This finding is similar to observations that males and females can have similar behavioral outputs mediated by different mechanisms (e.g. Sorge et al., 2015) and the future mapping of this will be interesting to follow. Even though the genes between our early life stress models are differentially affected, they are both suggestive of suppressed gonadotropin levels and possibly a peripheral GnRh-independent mechanism (Barker et al., 2011). The centrally measured changes in GnRh-Kisspeptin genes could consequently be due to disruptions in HPG feedback mechanisms but the underlying programing effects of early life challenges are mostly unclear (Kentner & Pittman, 2010).

We show that environmental enrichment, in the form of a more complex laboratory caging system, prevented several central and metabolic disrputions following early life stress. Indeed, enhanced environmental stimulation accelerated the restoration of body weight to levels of SD regular chow fed rats when access to the WD food ceased. This is a natural metabolic defence mechanism that occurs when regular chow is restored in rodents (Siersbaek et al., 2017; Fischer et al., 2018; Raun et al., 2007), and EE accelerated this

process. Moreover, the prepurbertal experience of enhanced laboratory stimulation was associated with elevated levels of hypothalamic *Kiss1r* in WD-LB-EE females, in addition to the normalization of species typical pubertal onset. Interventions in the form of sensory stimulation replacement and microbiome manipulation have also counteracted the occurance of precocial puberty as a consequency of early life adversity (Kentner et al., 2018a; Cowan & Richardson, 2018). In the low resource stress model used here, the pattern of changes in the kisspeptin system are suggestive of a compensatory mechanism to counteract the reduction in *Kiss1* observed in WD-LB exposed rats. While *Kiss1r* was also lower in SD compared to WD-LB-EE females, SD rats had elevated expression of *Kiss1*, indicating that hormone levels in these animals may have been sufficient to maintain homeostasis and typical pubertal development. In general, we hypothesize that the enrichment conferred protection in WD-LB-EE females, maintaining an adequate balance between the ligands and receptors of the kisspeptin system, preventing the pubertal timing disturbances associated with early life stress. What needs to be determined is whether this animal model of low resource availability interrupts factors such as sexual motivation and behavior, or even fertility, and if EE might prevent or even restore these systems. As mentioned, future work will also need to address potential changes in the trajectory of kisspeptin and GnRh associated genes expression across development and address the issue of tissue specificity.

In sum, these studies highlight the utility to develop better translational models of low, medium, and high insecurity environments. Our results suggest that the negative consequences of a high insecurity environment can be mitigated by parental care and environmental enrichment in the juvenile period. As these non-pharmacological interventions and their neurobiological effects are underexplored in the basic literature, employing these models can lead to novel insights into mechanisms that promote stress resilience.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

We compared translationally relevant rodent models of resource (in)stability to compare the effects of low, medium, and high security environments on maternal care and offspring outcomes

Maternal care was differentially impacted by each of these environments

The low security environment (Western diet/ limited bedding manipulation) resulted in metabolic dysfunction such as obesity, precocious puberty and disruptions in the hypothalamic kisspeptin system in offspring

These negative effects of early life stress on puberty and weight regulation were mitigated by environmental enrichment (high security)

These data highlight how utilizing diverse models of resource (in)stability can reveal mechanisms that confer vulnerability and resilience to early life stress.

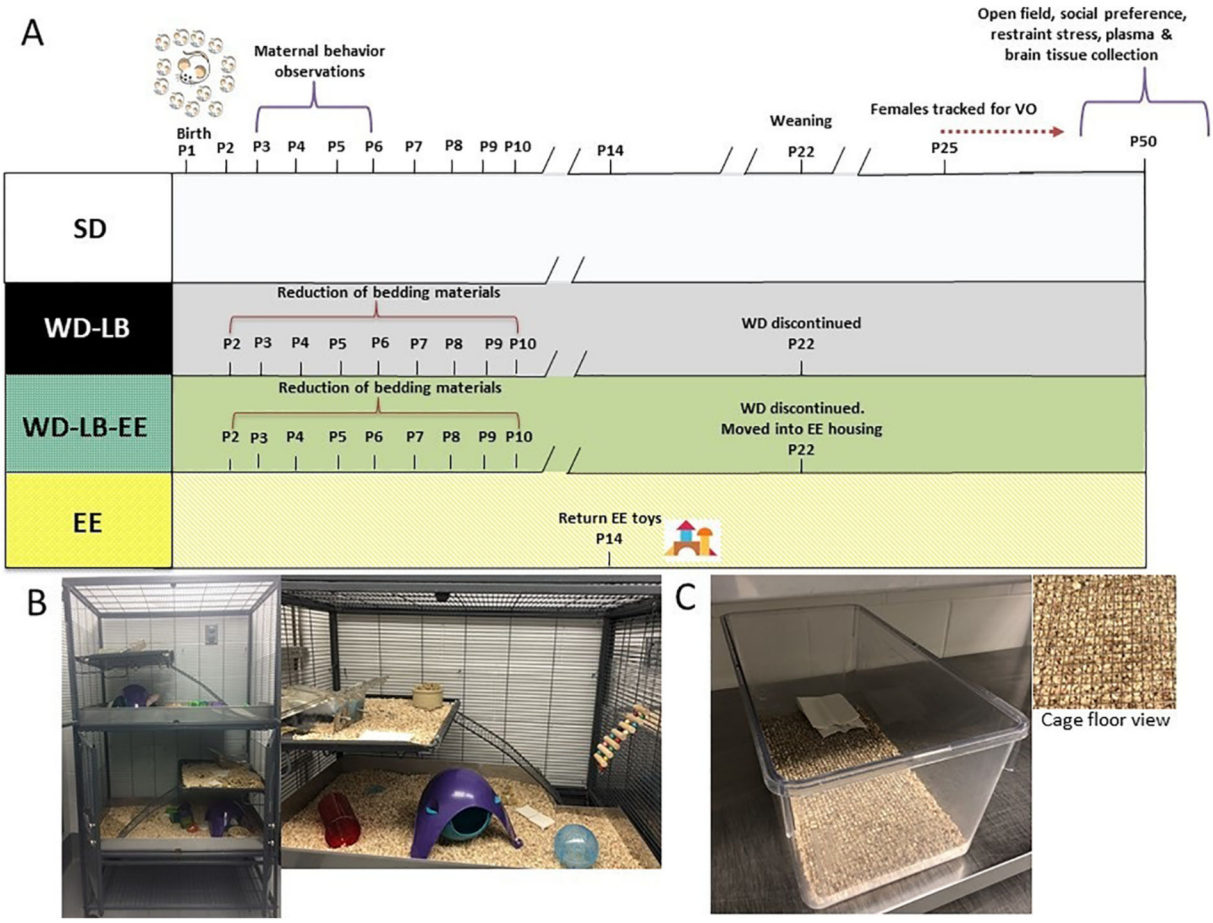

Figure 1.

A) Timeline of study and housing conditions. B) Multilevel environmental enrichment cage. C) Representative picture of the WD-LB cage and bedding floor (inset).

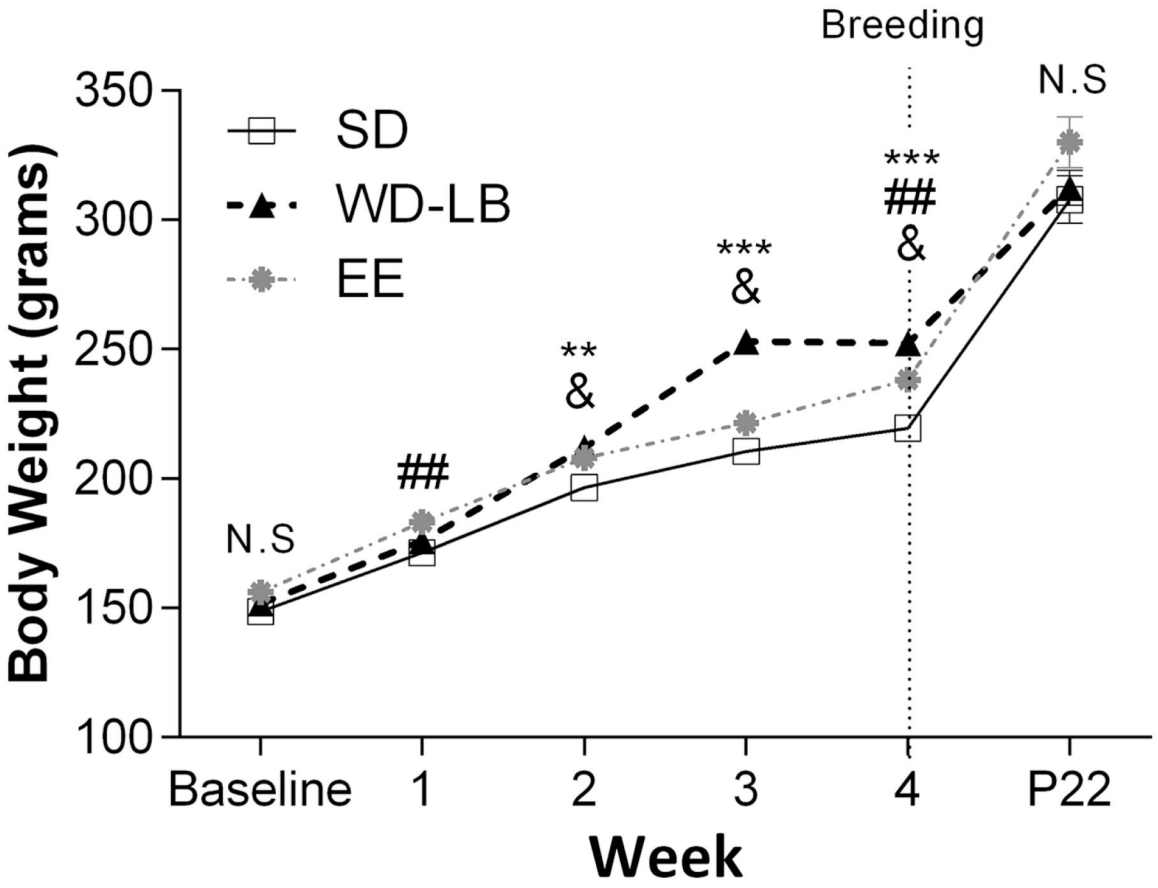

Figure 2.

Validation of Western diet on body weight gain (grams) across time. Baseline, weeks 1–4 (prior to breeding), and P22 (weaning). Data expressed as mean ±SEM; n = 8–10 per group. Various symbols are employed (e.g., $, &, *) to delineate differences between groups where *p < 0.05, **p <0.001, ***p<0.001, difference between SD and WD; #indicates differences between SD and EE; &indicates difference between WD and EE.

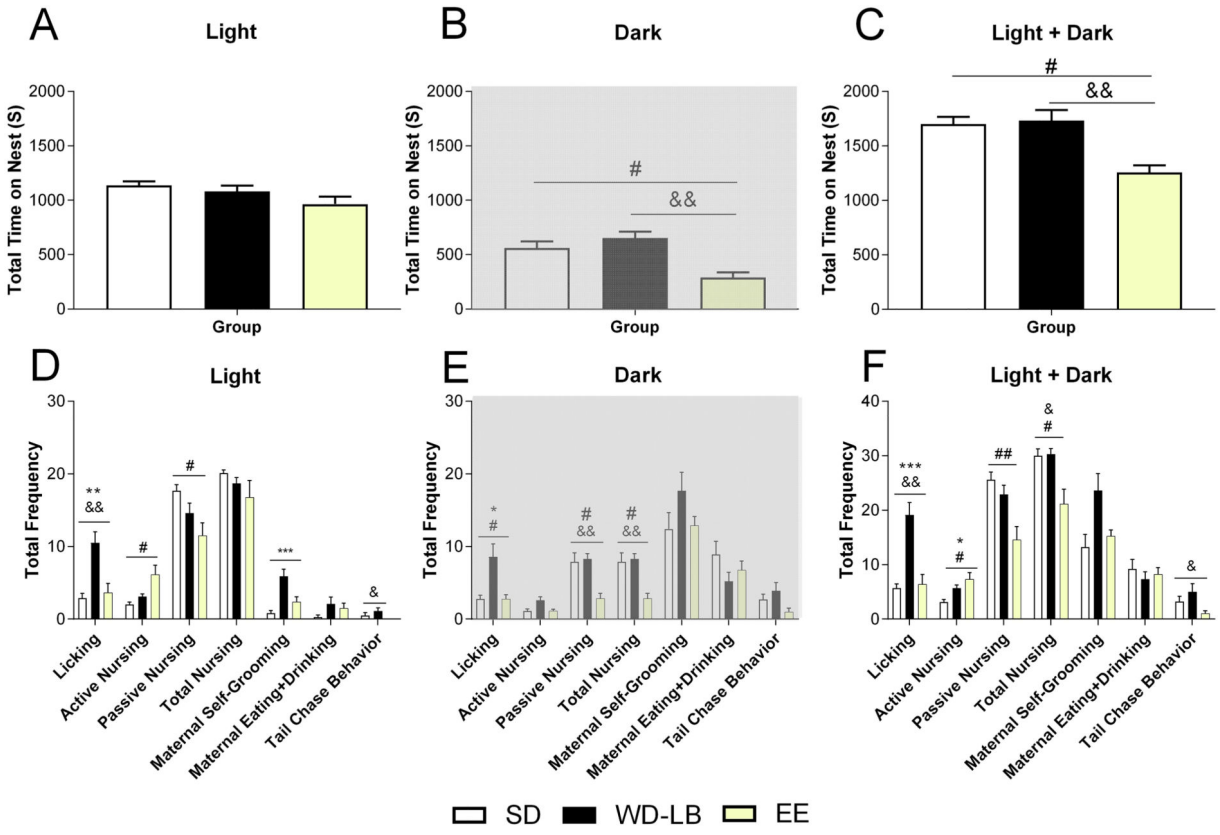

Figure 3.

Maternal behavior observations from standard (SD), Western diet-limited bedding (WD-LB), and environmentally enriched (EE) rat dams as a function of postnatal days in the light phase (left panels), dark phase (middle panels) and light and dark phases combined (right panels). Panels A-C display total time on nest (seconds). Panels D-F display the frequency of licking, active and passive nursing, total nursing, maternal self-grooming, maternal eating and drinking and tail chase behaviors. All maternal behavior data are expressed as mean ±SEM; n = 8–10 per group. Various symbols are employed (e.g., $, &, *) to delineate differences between groups where *p<0.05; **p<0.01; ***p<0.0001 indicates differences between SD and WD-LB, #indicates differences between SD and EE. &indicates differences between WD-LB and EE.

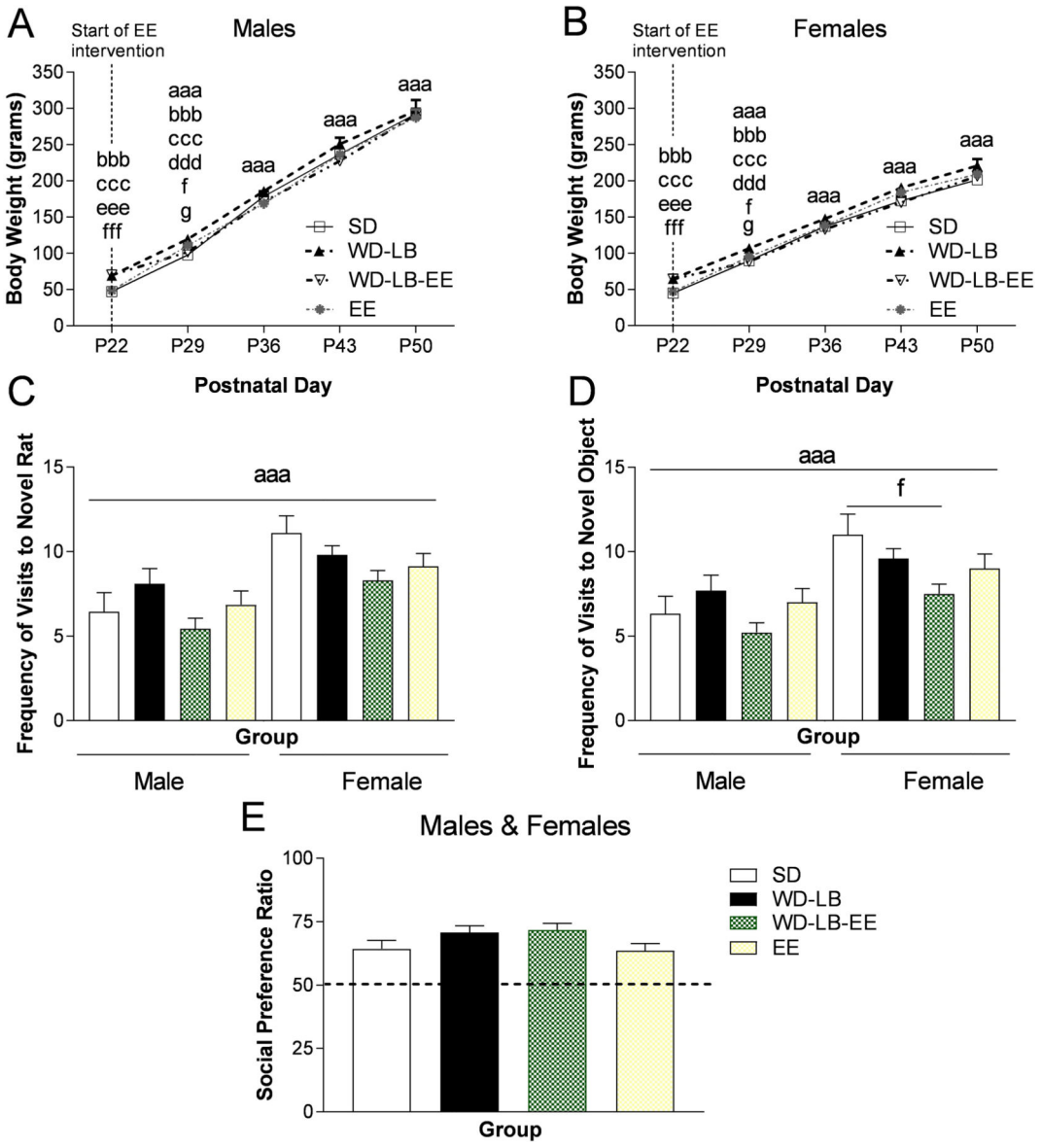

Figure 4.

Offspring body weights (grams) for male (A) and female (B) animals. C) Social preference ratios for male and female (collapsed) offspring. Values >0.05 indicate that the experimental rat spent more time visiting with the novel rat compared to the novel object. The total frequency of visits made to the D) novel rat and E) novel object in the social preference test. All data are expressed as mean ±SEM, n = 8–10. Various symbols are employed (e.g., a, b, c) to delineate differences between groups where $^{a}p<0.05$, $^{aa}p<0.01$, $^{aaa}p<0.001$ indicates differences between males and females; ^{b}indicates differences between SD and WD-LB, ^{c}indicates differences between WD-LB and EE, ^{d}indicates differences between WD-LB and WD-LB-EE, ^{e}indicates differences between WD-LB-EE and EE, ^{f}indicates differences between SD and WD-LB-EE, and ^{g}indicates differences between SD and EE.

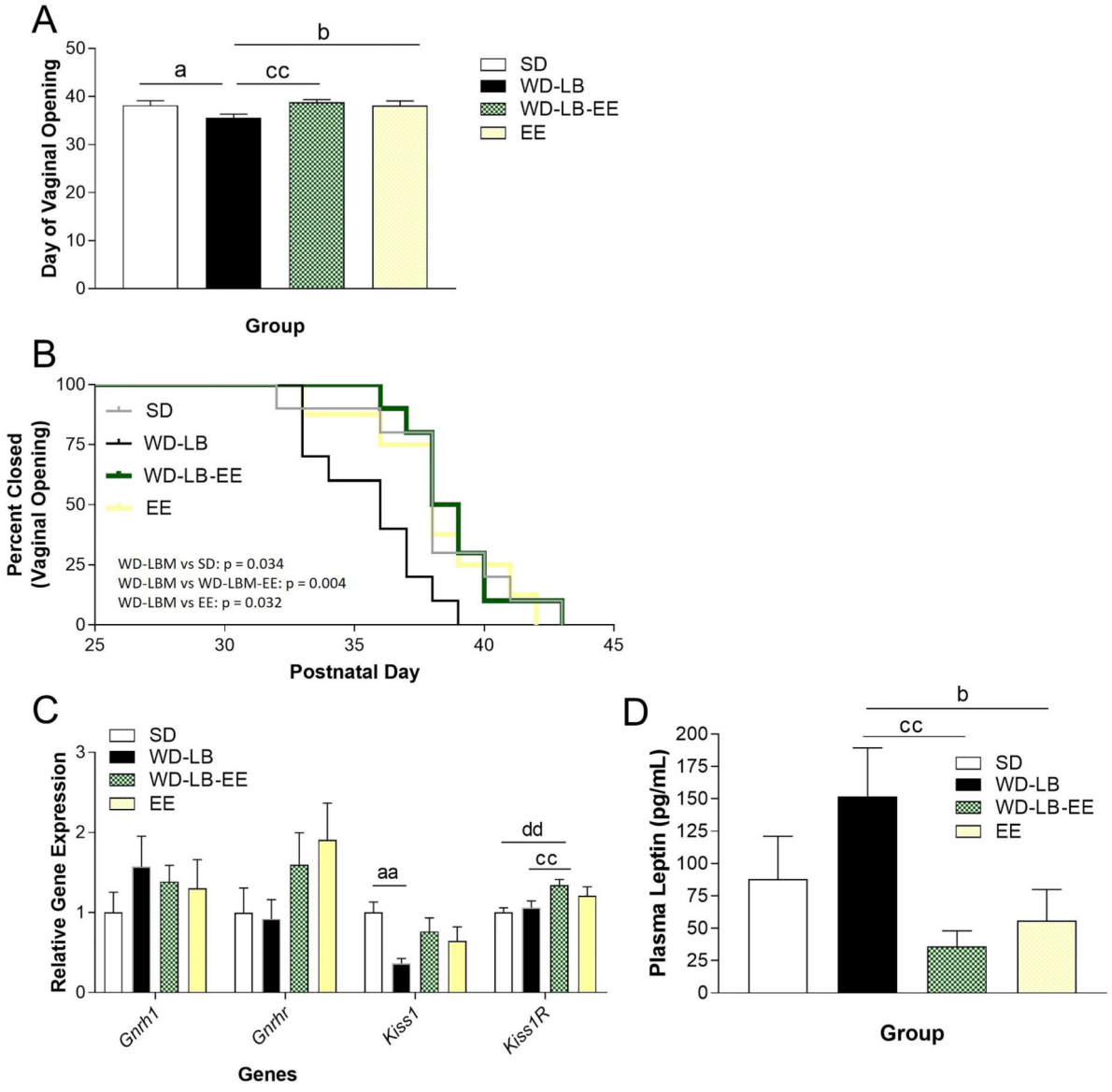

Figure 5.

A) Day of full vaginal opening for female offspring. B) Survival curves of full vaginal opening for each group expressed across time. C) Relative hypothalamic gene expression of *Gnrh1*, *Gnrhr1*, *Kiss1*, *Kiss1R* normalized to SD controls. D) Plasma leptin levels (pg/mL) on postnatal day 50. All data are expressed as mean ±SEM. Various symbols are employed (e.g., a, b, c) to delineate differences between groups where $^{a}p<0.05$, $^{aa}p<0.01$, $^{aaa}p<0.001$ indicates differences between WD-LB and SD; ^{b}indicates differences between WD-LB and EE, ^{c}indicates differences between WD-LB and WD-LB-EE; ^{d}indicates differences between SD and WD-LB-EE.

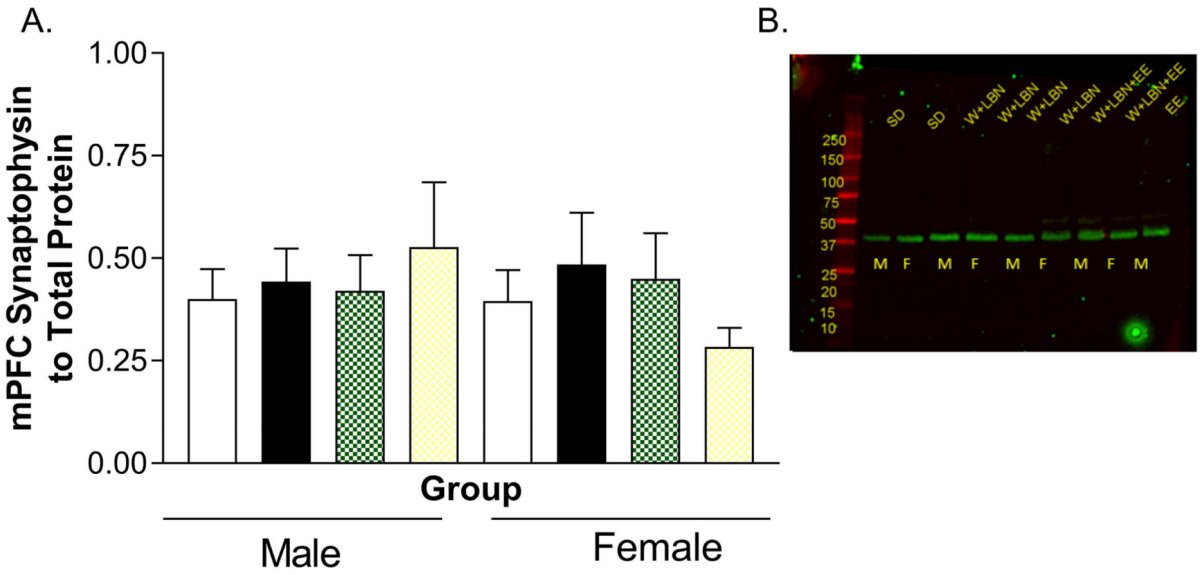

Figure 6.

Analysis for synaptophysin in the mPFC. A) Graph shows the mean ratio of the integrated intensity of each band of synaptophysin relative to the total protein signal for the lane of the same sample. There were no significant effects. B) Representative blot showing the levels of synaptophysin. These were normalized to the REVERT total protein stain depicted in Supplementary Figure 1. Please note that the second female EE animal is missing in the representative blot as the membrane only holds nine samples with the molecular weight marker.

Table 1:

Offspring Group Designations.

Group Name	Description
SD	Control male and female offspring fed a standard diet and housed in standard laboratory cages.
WD-LB	Male and female offspring fed a western diet and housed in standard laboratory cages. Animals were exposed to reduced bedding beginning on the late afternoon of P2 through until the morning of P10.
WD-LB- EE	Male and female offspring fed a western diet and housed in standard laboratory cages. Animals were exposed to reduced bedding beginning on the late afternoon of P2 through until the morning of P10.These animals were placed into environmental enrichment at weaning on P22.
EE	Male and female offspring fed standard diet housed in enriched multilevel cages; offspring were given novel enrichment toys twice weekly starting on P14.