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Neonatal resource scarcity alters maternal care and impacts offspring core temperature and growth in rats

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Abstract

Stressful experiences during childhood, including poverty and inconsistent parental care, can enhance vulnerability for worsened physical and mental health outcomes in adulthood. Using Sprague Dawley rats, the present study explored the impact of limited resource availability on maternal behavior and physiological and emotional behavior outcomes in the offspring. Early life adversity was induced by incorporating aspects of the Limited Bedding and Nesting (LBN) and Scarcity models, wherein limited resource availability has previously been shown to provoke unpredictable or adverse maternal care, respectively. In our hands, Neonatal Limited Bedding (NLB) stress during postnatal days (P)2–9 altered maternal care, augmenting pup-directed behaviors and reducing self-directed behaviors, and modestly increased the frequency of transitions between discrete behaviors across consecutive timed observations. NLB-exposed pups had lower core body temperatures immediately following the stressful manipulation and exhibited decreased body weight gain across development. However, NLB exposure did not impact adult offspring's social or emotional behavior outcomes in the three-chamber social interaction test, novelty-suppressed feeding test, splash test, or forced swim test. These findings add to literature demonstrating that early life adversity impacts maternal care in rodents and can disrupt certain metabolic and thermoregulatory outcomes in the offspring.

MeSH Keywords

Depression; Early Experience; Parental Care; Rat; Stress

INTRODUCTION

Research in rodents and humans alike has articulated the importance of early environment in shaping neurodevelopment, influencing adult health, and promoting vulnerability or resilience to future stress. Maternal sensory input is a crucial mechanism through which early environment influences neural development (Baram et al., 2012; Bolton et al., 2019). In experimental models, higher quality maternal care dampens offspring stress responses and

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Conflict of Interest Statement

The authors declare no competing financial interests.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

promotes resilience to behaviors relevant to depression, while minimal care is associated with cognitive and emotional deficits. However, the predictability of maternal signals – both in response to the infant as well as in response to changes in the environment – also plays a substantial role in determining offspring outcomes (Baram et al., 2012).

Adverse experiences during early life have the capacity to modify social, cognitive, and emotional responses, conferring augmented risk for developing a number of psychiatric disorders including depression, anxiety disorders, and substance use disorders (Anda et al., 2006; Chapman et al., 2004; Enoch, 2011; Felitti et al., 1998; Heim et al., 2010; Walker et al., 2017). Childhood poverty is a form of early-life adversity to which children and adolescents are particularly vulnerable. Growing up in a low socioeconomic status (SES) household has detrimental effects on mental health and well-being, augmenting both internalizing and externalizing behaviors (Bøe et al., 2014; Reiss, 2013). Furthermore, low SES is often coincident with other factors such as parental stress and maladaptive parenting that also directly or indirectly impact children's psychopathological outcomes (Belle & Doucet, 2003; Bøe et al., 2014; Pagliaccio & Barch, 2016).

More than a decade ago, the Baram Laboratory developed a rodent model of early-life adversity, the Limited Bedding and Nesting (LBN) model, to mimic the experience of a low-resource home environment (Ivy et al., 2008; Rice et al., 2008; Walker et al., 2017). As originally described, the LBN model entails transferring the dam and her neonatal litter to a scarce living environment consisting of wire mesh flooring and insufficient bedding and nesting materials (Ivy et al., 2008; Molet et al., 2014; Rice et al., 2008). The inability of the dam to construct a proper nest leads to greater fragmentation and reduced predictability of maternal care, which in turn invokes chronic stress in the developing pups (Bolton et al., 2019; Molet et al., 2014). A variation of the LBN model, known as the Scarcity Model, was later developed by Roth and Sullivan and subjects the dam and her litter to a less severe impoverished bedding environment. This model induces abusive behaviors by the dam (i.e., stepping on, dragging, and rough handling of pups) and is used to explore infant attachment learning and the impacts of caregiver maltreatment (Walker et al., 2017). The scarcity manipulation can be applied intermittently during 30-minute sessions from P1-P7 ("scarcity-adversity") (Asok et al., 2014; Blaze et al., 2013; Blaze & Roth, 2017; Doherty et al., 2017; Roth et al., 2009, 2014), as in the Roth laboratory, or continuously between P8-P12 (Moriceau et al., 2009; Rainekei et al., 2012, 2015; Yan et al., 2017), as in the Sullivan laboratory and others. Through these naturalistic approaches, laboratories can model aspects of adverse early-life experience and to study their respective impacts on offspring cognitive, physiological, and behavioral outcomes.

Although maternal care in the LBN model is fragmented as assessed by increased nest exits/entries or shorter bout lengths of individual caring behaviors, there are mixed findings in the literature with respect to the general quality of maternal care observed in this model (Eck et al., 2020). An early study reported that LBN dams spend overall less time licking and grooming pups and nursing relative to control dams (Ivy et al., 2008), but other reports suggest that these measures are either unaltered or increased in LBN dams (Eck et al., 2020; Hsiao et al., 2016; Knop et al., 2019; McLaughlin et al., 2016; Molet et al., 2016; Moussaoui et al., 2016). In the Scarcity model, dams show reduced time with offspring

and engage in more licking and grooming of pups (Moriceau et al., 2009; Raineki et al., 2010, 2012, 2015; Walker et al., 2017). Inconsistencies across studies could reflect any number of environmental or methodological discrepancies, including but not limited to pup age during the manipulation, differences among rodent strains, cage enrichment (or lack thereof) outside of the limited bedding manipulation, or use of 'spot checks' vs. continuous video monitoring of maternal care (Brenhouse & Bath, 2019; Eck et al., 2020). Procedural variability among studies implementing limited resource availability as a form of early-life adversity underscores the need to validate these procedures in each laboratory to ensure consistency with previously reported maternal and offspring behavioral outcomes (Brenhouse & Bath, 2019; Walker et al., 2017).

The present study implemented a rat model of early-life resource scarcity, hereafter referred to as Neonatal Limited Bedding (NLB), to examine maternal and offspring outcomes in Sprague Dawley rats. The NLB model, as applied in our hands, combines aspects of the LBN model and the Scarcity model. We first examined how NLB from offspring postnatal days (P)2–9 altered the quality and consistency of nurturing and non-care maternal behaviors during the first two postnatal weeks of life. We then measured offspring post-NLB core body temperature on P10 and growth across development as well as adult affective outcomes across a behavioral test battery. We hypothesized that NLB would result in worsened social and depressive-like outcomes for adult female offspring (relative to male counterparts) based on known sex differences in stress reactivity, vulnerability to early-life adversity, and diagnoses of anxiety and depressive disorders (Altemus et al., 2014; Bangasser et al., 2019; Bath, 2020; Hasin et al., 2018; Kessler, 2003; Kessler et al., 1994).

METHODS

Animals

Adult male and female virgin Sprague Dawley rats were purchased from Charles River (Wilmington, MA) and pair-housed by sex in a temperature-controlled facility (21–23°C, 50–55% humidity) with a 12/12-hour light-dark cycle (lights on at 7:00 a.m.). Animals were housed in flat-bottomed plastic cages on a ventilated housing rack (60 air changes per hour; Allentown Inc., Allentown, NJ, USA). Each cage was provided with ~3000 mL of highly absorbent bedding material (ALPHA-dri, Shepherd Specialty Papers, Watertown, TN) and Enviro-dri nesting material (Shepherd Specialty Papers). *Ad libitum* access to water and chow was provided throughout the study.

After two weeks of acclimation to the housing facility, including one week of habituation to experimenter handling, male and female rats ($n = 22$ per sex) were mated on P75 to produce offspring for studies examining the effect of early-life adversity on maternal care and adult offspring social and emotional behavior. Experiments were conducted in two cohorts containing 10 litters and 12 litters, respectively. Control and NLB group assignments were equally balanced across dams in each cohort. Offspring from Cohort #1 were sacrificed on P85, while offspring from Cohort #2 were sacrificed between P15–P21. Dams were placed on a breeder chow diet (Envigo 7904) from the onset of mating through weaning of the offspring. Evidence of successful mating was checked daily in the first two hours of the light cycle by vaginal lavage and visual evaluation for a sperm plug. Male breeders were

removed from the cage 4–8 days prior to birth of the offspring, and one plastic enrichment tube was supplemented to the home cage. Parturition was checked daily at 5:00 p.m., with the day of birth considered P0. Male and female offspring were weaned on P21 and housed in separate colony rooms with same-sex littermates that experienced the same early-life housing condition. Weaned offspring were handled twice weekly through P60 and remained group housed throughout the duration of behavior testing. All experiments were approved by the Institutional Animal Care and Use Committee at Virginia Polytechnic Institute and State University and conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Neonatal Limited Bedding (NLB) procedures

Dams in both cohorts were randomly assigned to either control (CON) or NLB conditions ($n = 11$ total litters per condition). Pups from all age-matched litters were gathered together on P2 and 10–12 pups (sex ratio approximately 1:1) were randomly assigned to each dam to minimize confounding effects due to genetics, litter size, or sex of the pups (Alleva et al., 1989; Moore & Chadwick-Dias, 1986; Moore & Morelli, 1979; Richmond & Sachs, 1984). Dams and litters assigned to the control housing condition on P2 were transferred to a fresh cage containing 3000 mL ALPHA-dri bedding material and one full-sized Brawny brand paper towel without additional enrichment. NLB dams and litters were transferred to a fresh cage with 400 mL ALPHA-dri bedding material and one-half paper towel. NLB cages were not outfitted with a raised wire mesh floor. Control and NLB cages were undisturbed from P2 through P9. Shortly after the onset of the light period of P10, dams and litters were transferred to a clean cage with ample bedding, Enviro-dri nesting material, and one enrichment tube, with weekly cage changes thereafter. One NLB litter from Cohort #1 was not cross-fostered due to lack of additional age-matched litters. One control dam and litter from Cohort #1 were excluded from all analyses due to low litter size, which can alter maternal behavior and, subsequently, offspring behavior.

The NLB manipulation was similar to protocols described by the Baram Laboratory in that (a) primiparous Sprague Dawley rats were used, (b) all pups, when possible, were cross fostered among dams on P2, and (c) pups were exposed to the limited bedding manipulation from P2 through P9 without any further experimenter intervention during the manipulation. Our protocol more closely modeled the Sullivan Laboratory's work in that (a) a wire mesh floor was not used and (b) rat dams were bred in our facility instead of being shipped during pregnancy (i.e., timed-pregnant).

Maternal behavior assessment

Maternal behavior of control and NLB dams was manually scored from P1 to P14 with a protocol adapted from our previous work (Glover et al., 2015) in dams from both cohorts ($n = 11$ litters per condition; one control litter excluded from analysis due to low litter size). Each cage was observed twice daily, with one 1-hour observation occurring during the light phase between 8:30 a.m. and 11:30 a.m. and another occurring during the dark phase between 8:30 p.m. and 11:30 p.m. Each one-hour observation period consisted of 20 5-second 'snapshot' observations of each cage taken approximately three minutes apart. Behaviors recorded during each observation included: (1) dam licking or grooming a pup;

(2) dam passively nursing pups; (3) dam arched-back nursing pups; (4) dam engaging in self-directed behaviors; and (5) non-care behaviors. During the light period on P2 and P10, maternal behavior was observed at least one hour after dams and litters were transferred to new cages to allow the dam time to acclimate to the new environment. Abusive behaviors toward the pups that are reported by the Roth and Sullivan Laboratories (Blaze et al., 2013; Blaze & Roth, 2017; Doherty et al., 2017; Raineke et al., 2012, 2015; Roth et al., 2009; Roth & Sullivan, 2005; Yan et al., 2017) were not observed during our protocol.

Passive nursing was defined as the mother lying on her side or back while nursing any number of pups. Arched-back nursing was classified as the dam having an arched posture, with legs extended, over nursing pups. Self-directed behaviors of the dam included eating, drinking, self-grooming, and resting away from the nest. Non-care behaviors by the dam included manipulating bedding, exploring the cage, and chasing her tail. After 14 days, each cage accumulated 560 total observations (20 observations per session \times 2 sessions per day \times 14 days).

Consistency in the patterns of maternal care was quantified based on methods described previously (Ivy et al., 2008; McLaughlin et al., 2016). Behavior between two independent observations was evaluated for whether it persisted or changed from the previous observation to the next. Categories of behavior considered for this measure included: (1) dam nursing pups (greater than 50% of pups); (2) dam away from nest and/or away from majority of pups (75% or more); (3) dam eating/drinking outside of nest; (4) dam self-grooming outside of nest; and (5) dam licking and grooming pups (both coincident with and independently of nursing). The total number of behavior changes was divided by the maximum possible number of changes ($N - 1$, where $N = 20$ observations) to calculate the proportion transitions between discrete behaviors. Higher scores reflect an increase in the number of transitions (or decreased consistency) between discrete behaviors from one observation to the next.

Offspring body weight and core temperature measurements

Pup body weights were recorded for all offspring in Cohort #1 on P2, P10, P14, P18, P21, P45, and P60 and in Cohort #2 on P2, P10, and P14. Three male and three female pups were randomly selected from each litter in Cohort #1 on P10 for core temperature measurements. Rectal temperature measurements were collected from pups during the transfer of each litter from control or NLB housing conditions to standard housing on the morning of P10. Temperatures were obtained using a Type J/K/T Thermocouple Thermometer (Cat. No. WD-20250-91; Kent Scientific, Torrington, CT, USA) equipped with a mouse rectal probe (Cat. No. RET-3; Kent Scientific).

Adult offspring social and emotional behavior assessment

Adult offspring from the control and NLB litters in Cohort #1 ($n = 3$ offspring per sex per litter; $n = 4$ control litters; $n = 5$ NLB litters) were randomly selected to undergo a behavioral test battery to examine social and emotional behaviors. The testing sequence included a social interaction test, novelty-suppressed feeding test (NSF), splash test, and forced swim test (FST). Each test was administered to females and males on alternating

days between the ages of P60-P74 (Figure 1). Social interaction, NSF, and splash test were performed in a dimly lit test room (approximately 30 lux), while FST was performed in a brightly lit test room (approximately 100 lux). For each of these tests, animals were transferred to a holding room on static cage racks to be housed overnight prior to behavioral testing on the following morning except for FST, for which animals were transferred to the holding room one hour prior to testing. All behavior testing occurred between 8:30 a.m. to 12:30 p.m. to avoid the diurnal rise in corticosterone that occurs in the afternoon in rodents. Ethovision XT 8.0 video tracking software (Noldus, Wageningen, The Netherlands) was used to record video for all tests. Testing arenas were disinfected between trials with 70% isopropyl alcohol.

Three-chamber social interaction test—Sociability and preference for social novelty were evaluated in an adaptation of the previously described three-chamber social interaction test (Kaidanovich-Beilin et al., 2010). On the first day of testing (*Habituation*), animals were habituated to a large, empty rectangular arena with opaque dividers separating the arena into three chambers (24×36×12-inch Plexiglas box with two partitions). Each divider had an open middle section allowing the test subject to freely access all chambers. The rat was placed along the edge of the middle chamber facing the center of the arena and allowed to explore freely for five minutes. On the second day (*Sociability*), a wire cup-like container (“interaction cage”; 6-inch diameter) was placed in each of the two side chambers. A novel, same-sex conspecific was placed inside the interaction cage on one side of the apparatus. The test rat was placed in the arena at the same starting point as previously and allowed to explore the chambers for 10 minutes. Time spent in each chamber and in the zones immediately adjacent to the interaction cages (Interaction Zone) was scored manually. On the third day (*Preference for social novelty*), the interaction cages were again placed in the side chambers. The previously novel (now ‘familiar’) conspecific was placed in the opposite interaction cage, while a new novel, same-sex conspecific was placed in the remaining interaction cage. The test rat started the trial in the middle chamber and its movements were recorded for ten minutes. Time spent in each chamber was manually scored. Measurements for each day were evaluated by an observer blinded to experimental conditions.

Novelty-suppressed feeding (NSF)—NSF was used to assess anxiety-like behavior in response to a novel environment with procedures adapted from previous work (Nam et al., 2014). Rats were food-deprived overnight prior to testing. A portion of pre-weighed chow was placed in the center of a clear Plexiglas chamber (45×45×60-cm Noldus PhenoTyper Box), and the subject began the 15-minute trial in the periphery of the chamber. Chow remaining at the conclusion of each trial was weighed. The latency of each rat to approach and investigate the chow in the novel test environment was scored by an observer blinded to experimental conditions.

Splash test—The splash test was used to identify depression-relevant phenotypes, namely, deficits in self-care and motivation, as previously reported in rodents (Isingrini et al., 2010; Sadeghi et al., 2016). Rats were singly transferred to a holding cage where their dorsal coats were sprayed five times with a 10% sucrose solution. Each rat was then placed in a Noldus PhenoTyper Box for 15 minutes. A blinded, trained observer scored the total grooming time

and latency to first grooming episode for each subject. Seven female subjects ($n = 3$ control; $n = 4$ NLB) were excluded from data analysis due to technical issues with video acquisition at the beginning of the trials.

Forced swim test (FST)—Porsolt's FST was performed as previously described across two sessions (Glover et al., 2019) using Plexiglas cylindrical containers (45 cm height \times 20 cm diameter). On both days, water depth was approximately 30 centimeters and the temperature was 25°C. Immobility in this test has been regarded as an indicator of passive coping or behavioral despair (Commons et al., 2017; Porsolt et al., 1977). On Day 1, rats were singly placed into the water-filled cylinder for 15 minutes. On Day 2 (24 hours later), the rats were returned to the water-filled cylinder for an additional five minutes. Water was changed after every session. Time spent immobile was scored by a trained observer blinded to experimental conditions.

Statistical analysis

Data are expressed as mean \pm SEM (standard error of the mean). Statistical analyses were performed in GraphPad Prism 8.3.0 (GraphPad Software, San Diego, CA, USA). Differences between groups were evaluated using unpaired two-tailed Student's t tests (offspring core temperature and body weight; maternal behavior, except tail chasing), two-way analysis of variance (ANOVA; adult offspring social and emotional behavior, with sex \times NLB as factors, each containing two levels), or three-way ANOVA (adult offspring social behavior, with sex \times NLB \times chamber as factors each containing two levels). Fisher's exact test was used to evaluate the proportion of dams that chased their tails during each portion of the diurnal cycle. Simple linear regression was used to determine the relationship between offspring core temperature on P10 and body weight (averaged by litter) in each sex. Outliers were screened using Grubbs' test and excluded from subsequent analysis. Significant interactions in ANOVA were followed up by *post hoc* Fisher's LSD tests between control and NLB conditions to ascertain effects of adverse early-life experience. All tests were two-tailed with statistical significance set at $p < 0.050$.

RESULTS

Maternal behavior in control vs. NLB dams

Aspects of maternal behavior intended to assess the quality and consistency of maternal care provided by the dams were recorded twice daily during the active and inactive periods.

Pup-directed behaviors: Observations of arched-back nursing, passive nursing, and licking and grooming of pups were quantified (Figure 2). Statistical comparisons of behavior were not performed between light cycles. NLB dams displayed significant increases in arched-back nursing during the light period on P4 ($t(18) = 2.209$, $p = 0.0404$; Figure 2C) and P8 ($t(18) = 5.113$, $p < 0.0001$; Figure 2G), as well as during the dark period on P2 ($t(19) = 3.209$, $p = 0.0046$; Figure 2A) and P4 ($t(19) = 2.682$, $p = 0.0148$; Figure 2C). NLB dams showed significant reductions in passive nursing during the light period on P8 ($t(19) = 3.523$, $p = 0.0023$; Figure 2G) and during the dark period on P12 ($t(18) = 2.379$, $p = 0.0287$; Figure 2K). NLB dams also showed a trend for significantly decreased passive nursing during the

dark period on P9 ($t(18) = 2.092, p = 0.0508$; Figure 2H) and P10 ($t(16) = 2.097, p = 0.0523$; Figure 2I). By contrast, NLB dams showed increased passive nursing during the dark period on P3 ($t(19) = 2.538, p = 0.0201$; Figure 2B) as well as a trend for an increase on P8 ($t(19) = 1.811, p = 0.0861$; Figure 2G). Licking and grooming of pups was only altered between control and NLB dams after the experimental housing manipulation. NLB dams engaged in decreased licking and grooming of pups during the light period on P10 ($t(18) = 2.162, p = 0.0443$; Figure 2I) and during the dark period on P11 ($t(19) = 2.325, p = 0.0313$; Figure 2J). The remainder of individual comparisons of arched-back nursing, passive nursing, and licking and grooming of pups within light cycle through P14 did not differ significantly between control and NLB dams (Student's t tests, $p > 0.112$).

When the sum of these behaviors were totaled for each observation period, NLB dams showed a significant increase in pup-directed behaviors during the light period on P5 ($t(18) = 2.535, p = 0.0207$; Figure 2D) and P9 ($t(18) = 2.396, p = 0.0276$; Figure 2H) relative to control dams. There was a trend for a significant increase in pup-directed behaviors by NLB dams during the light period on P8 ($t(18) = 1.924, p = 0.0704$; Figure 2G) and for a decrease on P12 ($t(19) = 1.947, p = 0.0664$; Figure 2K). During the dark period, NLB dams showed significant increases in pup-directed behaviors on P2 ($t(19) = 2.531, p = 0.0204$; Figure 2A) and P4 ($t(18) = 3.248, p = 0.004$; Figure 2C), as well as a trend for an increase on P3 ($t(19) = 1.974, p = 0.0631$; Figure 2B). Meanwhile, NLB dams showed overall decreased pup-directed behaviors during the dark period on P11 ($t(19) = 2.577, p = 0.0185$; Figure 2J) and P12 ($t(18) = 3.075, p = 0.0065$; Figure 2K). The remainder of comparisons through P14 were not statistically significant (Student's t tests, $p > 0.175$).

Self-directed behaviors: Observations of eating or drinking, self-grooming, and resting away from pups were evaluated in CON and NLB dams (Figure 3). Statistical comparisons of behavior were not performed between light cycles. Significant differences between control and NLB dams with respect to eating and drinking were observed during the dark period on P4, wherein NLB dams spent significantly less time engaged in this activity than control dams ($t(19) = 2.618, p = 0.0169$; Figure 3C). However, NLB dams showed a trend for an increase in eating/drinking during the light period on P5 ($t(18) = 1.852, p = 0.0805$; Figure 3D), P6 ($t(19) = 1.828, p = 0.0833$; Figure 3E), and P12 ($t(19) = 1.912, p = 0.0710$; Figure 3K), as well as during the dark period on P11 ($t(18) = 1.894, p = 0.0744$; Figure 3J). Self-grooming behavior was elevated in NLB dams relative to controls during the light period on P12 ($t(19) = 2.142, p = 0.0454$; Figure 3K), and trended for an increase in NLB dams on P4 ($t(19) = 2.086, p = 0.0507$; Figure 3C) and P5 ($t(18) = 1.863, p = 0.0789$; Figure 3D). Self-grooming decreased in NLB dams during the dark period on P2 ($t(18) = 2.175, p = 0.0432$; Figure 3A) and P3 ($t(19) = 2.656, p = 0.0156$; Figure 3B). NLB dams were observed to rest outside of the nest significantly less often than control dams during the light period on P4 ($t(18) = 2.393, p = 0.0278$; Figure 3C), P5 ($t(19) = 2.569, p = 0.0188$; Figure 3D), P6 ($t(17) = 2.565, p = 0.0201$; Figure 3E), and P7 ($t(17) = 2.781, p = 0.0128$; Figure 3F). Additionally, NLB dams rested less often outside the nest during the dark period on P4 ($t(19) = 2.609, p = 0.0173$; Figure 3C) and displayed a trend for a significant decrease in resting outside of the nest on P3 ($t(18) = 2.042, p = 0.0561$; Figure 3B) and P5 ($t(19) = 1.744, p = 0.0973$; Figure 3D). The remainder of individual comparisons of eating and

drinking, self-grooming, and resting outside of the nest did not differ significantly between control and NLB dams through P14 (Student's t tests, $p = 0.112$).

When the sum of these behaviors was calculated for each observation period, there were no differences in the frequency of all self-directed behaviors during the light period (Student's t tests, $p > 0.05$), although there was a trend for an increase in total self-directed behaviors by NLB dams on P12 ($t(19) = 1.929$; $p = 0.0688$; Figure 3K). During the dark period, NLB dams showed a significant decrease in summed self-directed behaviors on P2 ($t(19) = 2.296$; $p = 0.0332$; Figure 3A), P3 ($t(19) = 3.154$, $p = 0.0052$; Figure 3B) and P4 ($t(19) = 3.523$, $p = 0.0023$; Figure 3C), as well as a trend for a significant decrease in summed self-directed behaviors on P5 ($t(19) = 2.043$, $p = 0.0551$; Figure 3D). The remainder of comparisons through P14 were not statistically significant (Student's t tests, $p = 0.132$).

Nest building: Observations of nesting behavior were recorded in dams during the light (Figure 4A) and dark (Figure 4B) periods. Control and NLB dams both used the provided paper towels to construct a nest for their pups, although the nests built by NLB dams were of lower quality. During the light period, NLB dams spent significantly more time nest building on P3 ($t(18) = 3.207$; $p = 0.0049$) and P4 ($t(19) = 2.248$; $p = 0.0366$), and showed a trend for increased nesting on P5 ($t(19) = 1.741$; $p = 0.0978$), compared to control dams. However, NLB dams spent significantly less time nest building during the light period on P9 ($t(19) = 2.321$; $p = 0.0316$; Figure 4A). Nest building behavior was comparable between dams in both conditions during the dark period through P14 (Student's t tests, $p = 0.193$; Figure 4B).

Epochs outside of nest: The number of observations in which the dam was outside of the nest was recorded during the light (Figure 4C) and dark (Figure 4D) periods. While there were no significant differences between control and NLB dams with respect to epochs spent outside of the nest during the light period, we did observe a trend for a significant decrease in this measure in NLB dams on P5 ($t(18) = 2.025$; $p = 0.0579$) as well as a trend for a significant increase in this measure on P12, after the termination of the housing manipulation ($t(19) = 1.953$; $p = 0.0658$; Figure 4C). During the dark period, NLB dams spent significantly fewer epochs outside of the nest on P2 ($t(19) = 3.171$; $p = 0.0050$) and P4 ($t(19) = 2.925$; $p = 0.0087$), as well as a trend for fewer epochs outside the nest on P3 ($t(19) = 1.919$; $p = 0.0701$). The remainder of comparisons through P14 were not statistically different between groups (Student's t tests, $p = 0.101$).

Consistency in patterns of maternal care: Consistency in the pattern of maternal behaviors in control vs. NLB dams was quantified based on transitions between discrete behaviors across observations (Figure 4E–4F). During the light period, NLB dams displayed increased transitions between behaviors across timed observations on P3 ($t(19) = 2.100$; $p = 0.0494$) and P5 ($t(19) = 2.160$; $p = 0.0437$). Additionally, NLB dams transitioned between behaviors more often on P12, two days following the termination of the housing manipulation ($t(19) = 2.319$; $p = 0.0317$; Figure 4E). During the dark period, the quantity of transitions between maternal behaviors was significantly decreased in NLB dams on P13 ($t(19) = 2.377$; $p = 0.0281$), three days following the termination of the housing manipulation. Additionally, NLB dams showed a trend for decreased behavioral transitions on P2 ($t(19) = 1.930$; $p = 0.0686$) and a trend for increased behavioral transitions on P7

($t(19) = 1.815$; $p = 0.0853$; Figure 4F). The remainder of comparisons through P14 did not differ significantly between groups (Student's t tests, $p \geq 0.100$).

Tail chasing: The number of dams that chased their tail during at least one observation period during each diurnal cycle was compared between P2-P10 (Table 1). None of the dams displayed this behavior on P6, P8, P9, and P10, nor outside of the experimental manipulation. There were no differences between groups in the number of dams that chased their tails during the light period (Fisher's exact test, $p = 0.2143$). During the dark period, a significantly higher number of NLB dams chased their tails relative to CON dams on P3 ($p = 0.0351$). There was also a trend for a significant increase in the number of NLB dams observed chasing their tails on P4 relative to controls ($p = 0.0805$). There were no significant differences between groups on the remaining days ($p \geq 0.2099$).

Core temperature on P10 and offspring body weight across the lifespan

Core temperatures were assessed in a subset of pups upon returning to standard housing conditions on P10 (Figure 5A). Temperatures did not differ between male and female pups in any litter ($p > 0.05$), so an average temperature per litter was computed based on individual values for three male and three female pups. Pups exposed to limited bedding conditions between P2-P9 had significantly reduced core body temperatures on P10 relative to control-reared pups ($t(7) = 2.608$, $p = 0.0350$).

Offspring body weights were assessed at several time points throughout the lifespan (P10, P14, P21, P45, and P60; Figure 5B–5C). There were significant main effects of sex and NLB, in the absence of a significant interaction, on offspring pre-weaning body weights on P10 ($F_{\text{sex}}(1, 240) = 4.405$, $p = 0.0369$; $F_{\text{NLB}}(1, 240) = 33.48$, $p < 0.0001$; Interaction, $p = 0.5432$) and P14 ($F_{\text{sex}}(1, 240) = 5.204$, $p = 0.0234$; $F_{\text{NLB}}(1, 240) = 28.04$, $p < 0.0001$; Interaction, $p = 0.7017$). On the day of weaning (P21), there was a significant effect of NLB on body weight ($F_{\text{NLB}}(1, 98) = 15.95$, $p = 0.0001$) in the absence of a main effect of sex ($p = 0.4719$) or an interaction of these factors ($p = 0.9645$). In adolescent (P45) and early adult (P60) offspring, a main effect of NLB exposure persisted (P45: $F_{\text{NLB}}(1, 98) = 9.418$, $p = 0.0028$; P60: $F_{\text{NLB}}(1, 74) = 6.929$, $p = 0.0103$), as well as a main effect of sex (P45: $F_{\text{sex}}(1, 98) = 264.8$, $p < 0.0001$; P60: $F_{\text{sex}}(1, 74) = 667.6$, $p < 0.0001$), without significant interactions of these factors (P45: $p = 0.2563$; P60: $p = 0.1713$).

We performed correlational analysis to determine whether a relationship existed between degree of temperature difference and body weight (analyzed as averages by litter) on P10. Each sex was analyzed separately as sex differences in body weight were observed at this time point. No significant relationship emerged in males ($R^2 = 0.0420$, $p = 0.5968$) or females ($R^2 = 0.0297$, $p = 0.6577$).

Adult offspring social behavior: Sociability and preference for social novelty

During the three-chamber social interaction test, time spent in each of the three chambers was scored for each rat (Figure 6). Sociability was evaluated on the second day of testing (Figure 6A–6B). With respect to time spent in the social stimulus chamber vs. the object-containing chamber (Figure 6A), three-way ANOVA revealed a significant three-way

interaction of Sex \times NLB \times Chamber ($F_{(1,96)} = 4.769, p = 0.0314$), a two-way interaction of Sex \times Chamber ($F_{(1,96)} = 11.73, p = 0.0009$), and a main effect of chamber ($F_{(1,96)} = 541.6, p < 0.0001$). To better ascertain individual effects of sex and NLB, two-way ANOVA was performed with Sex \times NLB as factors. By two-way ANOVA, there was a significant main effect of sex on time spent in the social stimulus chamber ($F_{\text{sex}(1,48)} = 5.603, p = 0.0220$) and time spent in the object-containing chamber ($F_{\text{sex}(1,48)} = 6.289, p = 0.0156$). Females spent more time in the social stimulus chamber and less time in the object-containing chamber than males. However, there was not an effect of NLB (Social stimulus chamber: $p = 0.4913$; Object chamber: $p = 0.5760$), nor a significant interaction of Sex \times NLB (Social stimulus chamber: $p = 0.1495$; Object chamber: $p = 0.1050$), on time spent in either chamber (Figure 6A). Similarly, when evaluating time spent in the interaction zone of the social stimulus chamber (Figure 6B), there was a main effect of sex ($F_{\text{sex}(1,48)} = 4.944, p = 0.0309$), but not of NLB ($p = 0.7820$), nor an interaction ($p = 0.2169$). Additionally, there was significant main effect of sex ($F_{\text{sex}(1,47)} = 5.303, p = 0.0258$), as well as a trend for a significant interaction ($F_{\text{interaction}(1,47)} = 3.380, p = 0.0723$), on time spent in the interaction zone of the object-containing chamber, in the absence of a main effect of NLB ($p = 0.2482$; Figure 6B).

Preference for social novelty was quantified on the third day of testing (Figure 6C). There were no significant main effects of sex or NLB, nor an interaction of these factors, on time spent in the novel social stimulus chamber (Sex, $p = 0.1623$; NLB, $p = 0.1681$; Interaction, $p = 0.5884$). Likewise, there were no significant main effects of sex or NLB on time spent in the familiar social stimulus chamber (Sex, $p = 0.5369$; NLB, $p = 0.4726$). There was a trend for an interaction of these factors ($F_{\text{interaction}(1,48)} = 3.400, p = 0.0714$).

Adult offspring anxiety-like behavior

Anxiety-like behaviors were assessed in the NSF test by quantifying the latency of each animal to approach food in a novel environment and measuring total food consumed (Figure 7A–7B). There were no significant effects of sex or NLB, nor an interaction of these factors, on latency to approach food (Sex, $p = 0.2171$; NLB, $p = 0.3873$; Interaction, $p = 0.0720$). There was a main effect of sex on chow consumed ($F_{\text{sex}(1, 47)} = 30.67, p < 0.0001$), wherein males consumed more than females, but no significant main effect of NLB nor an interaction of these factors (NLB, $p = 0.5903$; Interaction, $p = 0.5709$) on this measure.

Adult offspring depressive-like behaviors

Measures of depression-relevant behavior – namely, lack of self-care and passive stress coping – were assessed in the splash test and FST, respectively. Self-care behavior in the splash test, quantified as time spent grooming, was unaltered by sex or NLB (Sex, $p = 0.3611$; NLB, $p = 0.2753$; Interaction, $p = 0.2555$; Figure 7C). There was a trend for a significant main effect of sex on latency to the first grooming episode in the splash test ($F_{\text{sex}(1, 43)} = 4.050, p = 0.0505$), but no main effect of NLB nor an interaction of these factors (NLB, $p = 0.1991$; Interaction, $p = 0.2864$; Figure 7D). In the FST, we observed a significant interaction between sex and NLB exposure ($F_{\text{interaction}(1, 50)} = 4.692, p = 0.0351$), as well as an effect of sex ($F_{\text{sex}(1, 50)} = 15.58, p = 0.0002$), on time spent immobile (Figure 7E). There was no main effect of NLB on time spent immobile in the FST ($p =$

0.6708). Post hoc analyses revealed significant differences in time spent immobile between control males and control females ($p = 0.0002$) as well as a trend for a significant difference between female CON vs. NLB rats ($p = 0.0726$).

DISCUSSION

The present study used a rodent model of limited resource availability during early life to investigate its impacts on maternal care as well as sex differences in offspring's physiological and behavioral outcomes. This model, Neonatal Limited Bedding (NLB), was an adaptation of two existing models in the literature: the Limited Bedding and Nesting (LBN) model and the Scarcity model. Each of these paradigms have previously been demonstrated to produce fragmented and unpredictable or adverse maternal care, respectively, and to alter offspring development and emotional behavior (Molet et al., 2014; Walker et al., 2017). In our hands, NLB altered the quality of maternal care in Sprague Dawley rats, particularly within the first several days after dams and litters were subjected to limited resource availability, and modestly impacted its overall pattern of consistency. Additionally, this adverse early-life experience led to early deficits in pup core temperature and stunted offspring body weight gain through early adulthood. However, a test battery surveying multiple aspects of social and emotional behavior in adult offspring illustrated that these measures were minimally impacted by NLB exposure. These findings highlight important methodological considerations for the application of models of early-life adversity and its effects on discrete offspring outcomes.

Clinical studies provide clear evidence that fragmented or unpredictable maternal signals influence children's brain development, cognitive ability, and emotional behavior (Davis et al., 2017; Glynn et al., 2018; Glynn & Baram, 2019). Importantly, these adverse offspring outcomes are observed even when adjusting for additional variables such as family SES. Low SES during childhood is itself a potent predictor of adult depression, even when controlling for family history of the disorder and low SES in adulthood. Additionally, women may be more susceptible to the deleterious impacts of low SES than men (Gilman et al., 2002, 2003; Pagliaccio & Barch, 2016; Spence et al., 2002). However, low SES is often coincident with other factors, including parental stress and abuse, that also directly or indirectly impact children's psychopathological outcomes (Belle & Doucet, 2003; Pagliaccio & Barch, 2016). A key feature of the LBN model is that the quality of maternal care provided by the dam is unaltered, but the pattern of care is abnormal. Disturbances in the pattern of maternal care by the dam in the LBN model are stress-induced behavioral changes arising from the inability of the dam to build a proper nest (Bolton et al., 2017; Ivy et al., 2008; Molet et al., 2016; Rice et al., 2008). An early report by the Baram Laboratory described dysfunctional maternal care in the model by evaluating transitions between discrete behaviors observed at set intervals of time (Ivy et al., 2008). Previous work in our laboratory has used a similar 'snapshot' observation approach to evaluate the quality and quantity of nurturing and non-care maternal behaviors in rats selectively bred for emotional behavior differences. We found that the 'snapshot' observation approach was effective in detecting these maternal behavior differences previously identified by continuous video (Clinton et al., 2007, 2010; Cohen et al., 2015).

By implementing the ‘snapshot’ observation approach in our current study, we found that NLB alters the quality of maternal care in Sprague Dawley rat dams without invoking behaviors reminiscent of abuse or maltreatment. These alterations in care were particularly notable during the dark cycle in the first several days of the housing manipulation. Pup-directed behaviors, in particular arched-back nursing, were increased in NLB dams. While NLB did not impact frequency of licking and grooming of pups during the manipulation period, this measure was reduced in NLB-exposed dams after standard housing conditions resumed. In addition, NLB lessened the overall frequency of self-directed behaviors, with NLB dams spending fewer observations engaged in consummatory behavior or self-grooming during the dark period and resting outside the nest less frequently during the light period. This general observation of possibly compensatory increases in nurturing behavior as a result of early-life adversity has also been recently described in Long-Evans rats (Eck et al., 2020). In our study, NLB dams’ early shifts in behavioral strategies coincided with the finding that NLB dams were observed outside of the nest less frequently than controls during these times. Although explicit behavioral comparisons between light cycles were not performed in this study, circadian variations in maternal care in the LBN model have been thoroughly documented elsewhere in mice (Gallo et al., 2019).

Even so, while ‘snapshot observations’ of maternal behavior are useful for identifying disparities in the quality of care, continuous video monitoring is more adept at assessing overall patterns of behavior (i.e., fragmentation or predictability of maternal care). In rodent studies, fragmentation of care is measured by quantifying the duration of individual bouts of behaviors such as licking and grooming or nursing episodes (Baram et al., 2012). In the LBN model, the duration of individual bouts of these behaviors is shorter in LBN dams, but the total duration of time that dams spend performing these behaviors is comparable to controls (Molet et al., 2016). Continuous video monitoring also allows for exact quantification of nest exits and entries, another measure of behavioral fragmentation, as well as determination of the exact sequence of behaviors performed by the dams. Information about the latter can be leveraged to assess whether individual behaviors by the dam occur in a predictable sequence over time (Baram et al., 2012; Molet et al., 2016). While past studies by our laboratory have not measured fragmentation or predictability of maternal care, we have attempted here to conceptualize the fragmented patterns of care provided by NLB dams by assessing transitions across timed behavioral observations (as in Ivy et al., 2008; McLaughlin et al., 2016). However, we acknowledge that ‘snapshot’ observations do not fully capture the nuanced, abnormal patterns of care described in the LBN model.

Evidence from clinical and rodent work supports the idea that early-life adversity contributes to metabolic and nutritional alterations (Felitti et al., 1998; Lapp, Mueller, et al., 2020; Murphy & Loria, 2017; Naninck et al., 2017; Walker et al., 2017; Yam et al., 2017, 2019). While changes in offspring body weight are not typically observed in the Scarcity model, we and many others using the LBN model have described reduced body weight in LBN-exposed pups immediately following the manipulation (Bath et al., 2016; Brunson et al., 2005; Eck et al., 2020; Goodwill et al., 2019; Lapp, Mueller, et al., 2020; McLaughlin et al., 2016; Moussaoui et al., 2016; Walker et al., 2017; Yam et al., 2017). Most laboratories assessing body weight at later times report that this effect does not persist, though at least three reports besides our own have described lasting impacts of this manipulation on

offspring growth (Bath et al., 2016; Eck et al., 2020; Goodwill et al., 2019). As in one recent study in Long-Evans rats, our data in Sprague Dawley rats illustrated that growth was stunted in NLB-exposed offspring despite increased nursing by NLB dams. These observations suggest the possibility that resource scarcity and associated stressors during the early postnatal period impacts the quality, rather than the quantity, of nutrition provided to the neonate offspring (Eck et al., 2020). Indeed, prior rodent studies have described reduced levels of certain micronutrients and blood glucose in LBN-exposed pups (Moussaoui et al., 2016; Naninck et al., 2017). Interestingly, maternal dietary supplementation restores pup micronutrient levels and rescues early life stress-induced cognitive deficits in the offspring (Naninck et al., 2017).

Differences in body weight between groups may also signal alterations in body fat. Brown adipose tissue (BAT) is essential for maintenance of core body temperature and thermogenesis (Cannon & Nedergaard, 2004). While prior work has reported that adversity-exposed rat pups do not experience cold stress in the LBN (Bolton et al., 2019) or Scarcity-Adversity models (Blaze et al., 2013), recent studies by others have identified alternative measures signaling perturbed physiological thermal regulation in LBN-exposed animals. LBN offspring show reduced white adipose tissue (WAT), increases in BAT and BAT thermogenesis-related gene expression, decreased plasma leptin, and changes in body fat distribution in adulthood (Lapp, Mueller, et al., 2020; Yam et al., 2017). Our core temperature observations suggest that the protocol described in this study did invoke cold stress in the animals, and in preliminary experiments, we observed that the impacts on core temperature were even more severe when offspring were raised in limited bedding conditions on wire mesh flooring (data not shown). The use of ventilated cage racks in our vivarium may have contributed to this outcome, as enhanced air circulation could create an overall cooler microclimate within the cage. Neonatal rodents are particularly vulnerable to these ambient conditions as they are not yet capable of many physiological thermoregulatory mechanisms observed in adult animals (Lapp, Mueller, et al., 2020). Sprague Dawley rats may also be more sensitive to these differences in cage microclimate in general, as this strain has a higher preferred ambient temperature (24.9 ± 0.4 °C) than others such as the Long-Evans rat (19.8 ± 0.3 °C) that are also used in LBN or scarcity manipulations (Gordon, 1987; Lapp, Mueller, et al., 2020).

Our correlational analysis of core temperature and body weight on P10 demonstrated no relationship between these variables in male or female offspring, suggesting that more rapid heat loss in individual animals may not account for core temperature differences in NLB-exposed pups. In place of more developed physiological mechanisms, neonatal rodents adopt behavioral strategies such as huddling to maintain temperature. Previous work has identified that LBN litter huddles are less cohesive in structure, with litters showing increased huddle area and perimeter and pups being visible more often than in control litters (Lapp, Mueller, et al., 2020). While we cannot comment on the huddle dynamics of litters in our study, it is possible that disparities in litter huddle temperature contributed to our core temperature findings. Decreased huddle coherence in combination with insufficient nesting material and higher ventilation in the ambient environment could create favorable conditions for accelerated heat loss.

The LBN and Scarcity models each provide an invaluable, naturalistic platform for understanding how abnormal maternal care in various forms contributes to deleterious cognitive and behavioral consequences (Molet et al., 2014; Walker et al., 2017). While the methodologies used in the present study were adapted from these two paradigms, it is important to acknowledge that they do not precisely replicate either model, undoubtedly contributing to incongruities in our reported behavior findings. Among these, the neonatal limited bedding protocol described in this study did not evoke changes in social behavior in adult offspring. Earlier work using the Scarcity model has described decreases in sociability using the two-chamber social interaction test in adolescent and adult Long-Evans rats (Raineke et al., 2012, 2015; Yan et al., 2017). Sex differences in social behavior outcomes due to early LBN exposure have also been reported (Guadagno et al., 2018; Lapp, Bartlett, et al., 2020). A possible explanation for our null findings could be the timing of the resource manipulation, which can produce distinct neurobehavioral outcomes (Walker et al., 2017). Most studies reporting differences in social behavior subjected animals to resource scarcity either later in development (i.e., P8-P12) or for a longer duration that also spanned that period (Lapp, Bartlett, et al., 2020; Raineke et al., 2012, 2015; Yan et al., 2017). In an LBN study in which the timing of the manipulation nearly matched our own, animals were singly housed prior to behavior testing to increase social interaction with a novel conspecific during testing (Guadagno et al., 2018), whereas animals were group-housed in the present study. Group-housing our animals during behavior may have also contributed to the decreased overall levels of social interaction on the third day of testing (preference for social novelty). Most protocols that measure preference for social novelty do so immediately after examining sociability. In our study, the novelty of a second non-familiar conspecific could have lost its impact after animals had the opportunity to interact with familiar cage mates between the second and third days of testing.

The present study also revealed an absence of anxiety- or depression-relevant phenotypes resulting from NLB during early life. This was consistent with a vast majority of studies using resource scarcity as a form of early-life adversity that also report no effect of exposure on anxiety-like behavior (Bolton et al., 2019; Bolton, Ruiz, et al., 2018; Doherty et al., 2017; Goodwill et al., 2019; Malter Cohen et al., 2013; Manzano Nieves et al., 2019; Rice et al., 2008). However, our findings conflict with published works that have documented elements of depressive-related behavior in rodents following the LBN or Scarcity manipulations. Although the present experiments did not assess anhedonic effects of NLB, numerous studies have reported this outcome using the sucrose preference test in offspring following both manipulations (Bolton, Molet, et al., 2018; Bolton, Ruiz, et al., 2018; Molet et al., 2016; Yan et al., 2017). Depressive-relevant phenotypes in the FST have also been described in rats in the Scarcity model (Raineke et al., 2012, 2015; Yan et al., 2017) and in mice (but not rats) in the LBN model (Bolton, Molet, et al., 2018; Goodwill et al., 2019). Classically, immobility in the FST has been regarded as an index of behavioral despair that is improved by anti-depressant treatment (Porsolt et al., 1977). However, more recent interpretations of immobility in the FST frame it as a measure of active or passive coping strategies toward an inescapable stressor, possibly as a means of energy conservation (Commons et al., 2017; West, 1990). It is important to acknowledge these limitations in the interpretation of depressive outcomes in the FST, as stress coping strategy is also altered in disorders

other than depression (e.g., autism spectrum disorders) (Commons et al., 2017). Likewise, while anhedonia is considered a core feature of clinical depression, it is also observed in other psychiatric conditions such as schizophrenia (Whitton et al., 2015). Far fewer studies have examined deficits in self-care or motivational behavior due to resource scarcity. In agreement with our own findings in rats, a recent report described no effect of LBN rearing in mice on grooming behavior in the splash test (Okhuarobo et al., 2020). However, as with the ‘core features’ assessed in the previously described tests, apathy or lack of motivation is not specific to depression and can be considered across cognitive, emotional, and behavioral contexts (Starkstein & Leentjens, 2008).

It may be surprising that none of the social or emotional phenotypes described above were altered by NLB experience when considering that models of early-life adversity produce well documented impacts on these outcomes. It is possible that affective outcomes are altered by the NLB manipulation described here but were simply not tested (i.e., anhedonia) or are affected at a time other than adulthood (i.e., adolescence). Performance in the splash test could also have been impacted by prior experience in the comparatively more stressful novelty-suppressed feeding test. However, the most salient possible explanation for these null findings could be that control dams and offspring also experienced stress during the postnatal period, thus neutralizing any behavioral impacts of resource scarcity relative to controls. While we are unable to comment on levels of stress-related hormones in the dams or offspring, our behavioral observations suggested another phenotypic indicator of this possibility. Tail-chasing behavior was observed in dams in both groups although it was more prevalent under NLB conditions. This behavior is a non-species typical phenomenon that has also been documented in other studies implementing resource scarcity as a form of early-life adversity (Lapp, Mueller, et al., 2020; Moriceau et al., 2009; Strzelewicz et al., 2019). Tail-chasing behavior can reflect that the dam was experiencing elevated stress or was lacking environmental or social stimulation (Hurst et al., 1997; Lapp, Bartlett, et al., 2020; Strzelewicz et al., 2019). The control conditions described for the LBN and Scarcity models (Molet et al., 2014; Walker et al., 2017) are less enriched compared to the standard housing conditions provided in our animal facility (i.e., Enviro-dri nesting material and a plastic enrichment tube). Adapting our protocol to better match the control condition with the standard provided in our facility, as has described recently elsewhere (Eck et al., 2020; Ordoñez Sanchez et al., 2021), may have alleviated maternal stress in controls and enhanced offspring differences in emotional behavioral outcomes between groups.

Taken together with the broader work interrogating the impacts of early-life adversity, this report adds to a growing literature demonstrating that methodological and environmental variability between laboratories influences outcomes on numerous measures relevant to maternal care and offspring development and behavior. These sources of variability can arise from factors intentional to the experimental design (i.e., rodent species and strain; timing and duration of housing manipulation; presence or absence of wire mesh flooring; timing and method of maternal care observations) as well as from factors outside of the experimenter’s direct control (i.e., sharing housing space with other investigators; caging type or housing conditions available in vivarium facilities). Within the parameters of this study, primiparous Sprague Dawley rat dams appeared to compensate for limited resource availability during the early postpartum period by providing augmented maternal care to

their offspring. While this stressful early life manipulation altered offspring physiological outcomes such as core body temperature and body weight throughout the lifespan, it did not impact the adult offspring social or emotional behavior outcomes assessed in the tests described. Our results underscore the importance of critically evaluating methodological and environmental factors that promote reproducibility across laboratories as well as thoughtfulness regarding the discrete outcomes expected and examined based on previous reports. Such considerations will facilitate meaningful comparisons between studies and ensure translatability of preclinical work to the human condition.

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LITERATURE CITED

- Alleva E, Caprioli A, & Laviola G (1989). Litter gender composition affects maternal behavior of the primiparous mouse dam (*Mus musculus*). *Journal of Comparative Psychology*, 103(1), 83–87. 10.1037/0735-7036.103.1.83 [PubMed: 2924532]
- Altemus M, Sarvaiya N, & Epperson CN (2014). Sex differences in anxiety and depression clinical perspectives. *Frontiers in Neuroendocrinology*, 35(3), 320–330. 10.1016/j.yfrne.2014.05.004 [PubMed: 24887405]
- Anda RF, Felitti VJ, Bremner JD, Walker JD, Whitfield C, Perry BD, Dube SR, & Giles WH (2006). The enduring effects of abuse and related adverse experiences in childhood: A convergence of evidence from neurobiology and epidemiology. *European Archives of Psychiatry and Clinical Neuroscience*, 256(3), 174–186. 10.1007/s00406-005-0624-4 [PubMed: 16311898]
- Asok A, Bernard K, Rosen JB, Dozier M, & Roth TL (2014). Infant-caregiver experiences alter telomere length in the brain. *PLoS ONE*, 9(7), e101437. 10.1371/journal.pone.0101437 [PubMed: 24983884]
- Bangasser DA, Eck SR, & Ordoñez Sanchez E (2019). Sex differences in stress reactivity in arousal and attention systems. *Neuropsychopharmacology*, 44(1), 129–139. 10.1038/s41386-018-0137-2 [PubMed: 30022063]
- Baram TZ, Davis EP, Obenaus A, Sandman CA, Small SL, Solodkin A, & Stern HS (2012). Fragmentation and unpredictability of early-life experience in mental disorders. In *American Journal of Psychiatry* (Vol. 169, Issue 9, pp. 907–915). American Psychiatric Association. 10.1176/appi.ajp.2012.11091347
- Bath KG (2020). Synthesizing Views to Understand Sex Differences in Response to Early Life Adversity. In *Trends in Neurosciences* (Vol. 43, Issue 5, pp. 300–310). Elsevier Ltd. 10.1016/j.tins.2020.02.004 [PubMed: 32353334]
- Bath KG, Manzano-Nieves G, & Goodwill HL (2016). Early life stress accelerates behavioral and neural maturation of the hippocampus in male mice. *Hormones and Behavior*, 82, 64–71. 10.1016/J.YHBEH.2016.04.010 [PubMed: 27155103]
- Belle D, & Doucet J (2003). Poverty, inequality, and discrimination as sources of depression among U.S. women. *Psychology of Women Quarterly*, 27(2), 101–113. 10.1111/1471-6402.00090
- Blaze J, & Roth TL (2017). Caregiver maltreatment causes altered neuronal DNA methylation in female rodents. *Development and Psychopathology*, 29(02), 477–489. 10.1017/S0954579417000128 [PubMed: 28401839]
- Blaze J, Scheuing L, & Roth TL (2013). Differential methylation of genes in the medial prefrontal cortex of developing and adult rats following exposure to maltreatment or nurturing care during infancy. *Developmental Neuroscience*, 35(4), 306–316. 10.1159/000350716 [PubMed: 23751776]

- Bøe T, Sivertsen B, Heiervang E, Goodman R, Lundervold AJ, & Hysing M (2014). Socioeconomic status and child mental health: The role of parental emotional well-being and parenting practices. *Journal of Abnormal Child Psychology*, 42(5), 705–715. 10.1007/s10802-013-9818-9 [PubMed: 24150864]
- Bolton JL, Molet J, Ivy AS, & Baram TZ (2017). New insights into early-life stress and behavioral outcomes. *Current Opinion in Behavioral Sciences*, 14, 133–139. 10.1016/j.cobeha.2016.12.012 [PubMed: 28413813]
- Bolton JL, Molet J, Regev L, Chen Y, Rismanchi N, Haddad E, Yang DZ, Obenaus A, & Baram TZ (2018). Anhedonia following early-life adversity involves aberrant interaction of reward and anxiety circuits and is reversed by partial silencing of amygdala corticotropin-releasing hormone gene. *Biological Psychiatry*, 83(2), 137–147. 10.1016/j.biopsych.2017.08.023 [PubMed: 29033027]
- Bolton JL, Ruiz CM, Rismanchi N, Sanchez GA, Castillo E, Huang J, Cross C, Baram TZ, & Mahler SV (2018). Early-life adversity facilitates acquisition of cocaine self-administration and induces persistent anhedonia. *Neurobiology of Stress*, 8, 57–67. 10.1016/j.ynstr.2018.01.002 [PubMed: 29888304]
- Bolton JL, Short AK, Simeone KA, Daglian J, & Baram TZ (2019). Programming of stress-sensitive neurons and circuits by early-life experiences. *Frontiers in Behavioral Neuroscience*, 13, 30. 10.3389/fnbeh.2019.00030 [PubMed: 30833892]
- Brenhouse HC, & Bath KG (2019). Bundling the haystack to find the needle: Challenges and opportunities in modeling risk and resilience following early life stress. *Frontiers in Neuroendocrinology*, 54, 100768. 10.1016/j.yfrne.2019.100768 [PubMed: 31175880]
- Brunson KL, Kramar E, Lin B, Chen Y, Colgin LL, Yanagihara TK, Lynch G, & Baram TZ (2005). Mechanisms of late-onset cognitive decline after early-life stress. *Journal of Neuroscience*, 25(41), 9328–9338. 10.1523/JNEUROSCI.2281-05.2005 [PubMed: 16221841]
- Cannon B, & Nedergaard J (2004). Brown adipose tissue: Function and physiological significance. *Physiological Reviews*, 84(1), 277–359. 10.1152/physrev.00015.2003 [PubMed: 14715917]
- Chapman DP, Whitfield CL, Felitti VJ, Dube SR, Edwards VJ, & Anda RF (2004). Adverse childhood experiences and the risk of depressive disorders in adulthood. *Journal of Affective Disorders*, 82(2), 217–225. 10.1016/j.jad.2003.12.013 [PubMed: 15488250]
- Clinton SM, Bedrosian TA, Abraham AD, Watson SJ, & Akil H (2010). Neural and environmental factors impacting maternal behavior differences in high- versus low-novelty-seeking rats. *Hormones and Behavior*, 57(4–5), 463–473. 10.1016/j.yhbeh.2010.02.004 [PubMed: 20156440]
- Clinton SM, Vázquez DM, Kabbaj M, Kabbaj MH, Watson SJ, & Akil H (2007). Individual differences in novelty-seeking and emotional reactivity correlate with variation in maternal behavior. *Hormones and Behavior*, 51(5), 655–664. 10.1016/j.yhbeh.2007.03.009 [PubMed: 17462647]
- Cohen JL, Glover ME, Pugh PC, Fant AD, Simmons RK, Akil H, Kerman IA, & Clinton SM (2015). Maternal style selectively shapes amygdalar development and social behavior in rats genetically prone to high anxiety. *Developmental Neuroscience*, 37(3), 203–214. 10.1159/000374108 [PubMed: 25791846]
- Commons KG, Cholanians AB, Babb JA, & Ehlinger DG (2017). The rodent forced swim test measures stress-coping strategy, not depression-like behavior. *ACS Chemical Neuroscience*, 8(5), 955–960. 10.1021/acscchemneuro.7b00042 [PubMed: 28287253]
- Davis EP, Stout SA, Molet J, Vegetabile B, Glynn LM, Sandman CA, Heins K, Stern HS, & Baram TZ (2017). Exposure to unpredictable maternal sensory signals influences cognitive development across species. *Proceedings of the National Academy of Sciences of the United States of America*, 114(39), 10390–10395. 10.1073/pnas.1703444114 [PubMed: 28893979]
- Doherty TS, Blaze J, Keller SM, & Roth TL (2017). Phenotypic outcomes in adolescence and adulthood in the scarcity-adversity model of low nesting resources outside the home cage. *Developmental Psychobiology*, 59(6), 703–714. 10.1002/dev.21547 [PubMed: 28767135]
- Eck SR, Ardekani CS, Salvatore M, Luz S, Kim ED, Rogers CM, Hall A, Lee DE, Famularo ST, Bhatnagar S, & Bangasser DA (2020). The effects of early life adversity on growth, maturation, and steroid hormones in male and female rats. *European Journal of Neuroscience*, 52(1), 2664–2680. 10.1111/ejn.14609

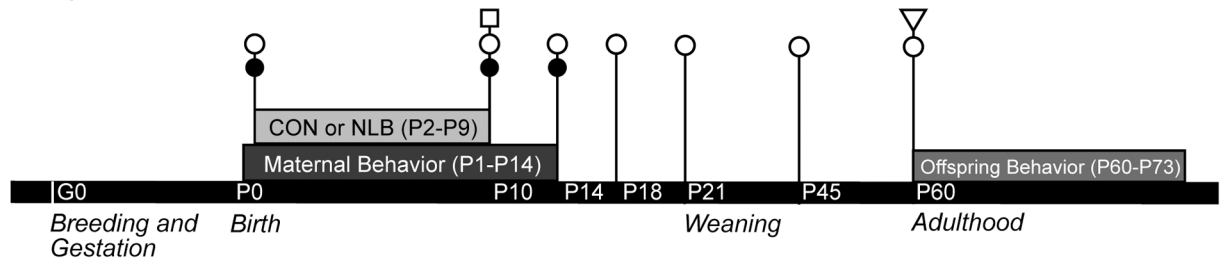
- Enoch MA (2011). The role of early life stress as a predictor for alcohol and drug dependence. *Psychopharmacology*, 214(1), 17–31. 10.1007/s00213-010-1916-6 [PubMed: 20596857]
- Felitti VJ, Anda RF, Nordenberg D, Williamson DF, Spitz AM, Edwards V, Koss MP, & Marks JS (1998). Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults: The adverse childhood experiences (ACE) study. *American Journal of Preventive Medicine*, 14(4), 245–258. 10.1016/S0749-3797(98)00017-8 [PubMed: 9635069]
- Gallo M, Shleifer DG, Godoy LD, Ofray D, Olaniyan A, Campbell T, & Bath KG (2019). Limited bedding and nesting induces maternal behavior resembling both hypervigilance and abuse. *Frontiers in Behavioral Neuroscience*, 13, 167. 10.3389/fnbeh.2019.00167 [PubMed: 31402857]
- Gilman SE, Kawachi I, Fitzmaurice GM, & Buka SL (2002). Socioeconomic status in childhood and the lifetime risk of major depression. *International Journal of Epidemiology*, 31(2), 359–367. 10.1093/ije/31.2.359 [PubMed: 11980797]
- Gilman SE, Kawachi I, Fitzmaurice GM, & Buka SL (2003). Socio-economic status, family disruption and residential stability in childhood: Relation to onset, recurrence and remission of major depression. *Psychological Medicine*, 33(8), 1341–1355. 10.1017/S0033291703008377 [PubMed: 14672243]
- Glover ME, McCoy CR, Shupe EA, Unroe KA, Jackson NL, & Clinton SM (2019). Perinatal exposure to the SSRI paroxetine alters the methylome landscape of the developing dentate gyrus. *European Journal of Neuroscience*, 50(2). 10.1111/ejn.14315
- Glover ME, Pugh PC, Jackson NL, Cohen JL, Fant AD, Akil H, & Clinton SM (2015). Early-life exposure to the SSRI paroxetine exacerbates depression-like behavior in anxiety/depression-prone rats. 10.1016/j.neuroscience.2014.10.044
- Glynn LM, & Baram TZ (2019). The influence of unpredictable, fragmented parental signals on the developing brain. *Frontiers in Neuroendocrinology*, 53, 100736. 10.1016/J.YFRNE.2019.01.002 [PubMed: 30711600]
- Glynn LM, Howland MA, Sandman CA, Davis EP, Phelan M, Baram TZ, & Stern HS (2018). Prenatal maternal mood patterns predict child temperament and adolescent mental health. *Journal of Affective Disorders*, 228, 83–90.
- Goodwill HL, Manzano-Nieves G, Gallo M, Lee H-I, Oyerinde E, Serre T, & Bath KG (2019). Early life stress leads to sex differences in development of depressive-like outcomes in a mouse model. *Neuropsychopharmacology*, 44(4), 711–720. 10.1038/s41386-018-0195-5 [PubMed: 30188513]
- Gordon CJ (1987). Relationship between preferred ambient temperature and autonomic thermoregulatory function in rat. *American Journal of Physiology*, 252(6), R1130–R1137. 10.1152/ajpregu.1987.252.6.r1130
- Guadagno A, Wong TP, & Walker CD (2018). Morphological and functional changes in the preweaning basolateral amygdala induced by early chronic stress associate with anxiety and fear behavior in adult male, but not female rats. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 81, 25–37. 10.1016/j.pnpbp.2017.09.025 [PubMed: 28963066]
- Hasin DS, Sarvet AL, Meyers JL, Saha TD, Ruan WJ, Stohl M, & Grant BF (2018). Epidemiology of adult DSM-5 major depressive disorder and its specifiers in the United States. *JAMA Psychiatry*, 75(4), 336–346. 10.1001/jamapsychiatry.2017.4602 [PubMed: 29450462]
- Heim CM, Shugart M, Craighead WE, & Nemeroff CB (2010). Neurobiological and psychiatric consequences of child abuse and neglect. *Developmental Psychobiology*, 52(7), 671–690. 10.1002/dev.20494 [PubMed: 20882586]
- Hsiao Y-M, Tsai T-C, Lin Y-T, Chen C-C, Huang C-C, & Hsu K-S (2016). Early life stress dampens stress responsiveness in adolescence: Evaluation of neuroendocrine reactivity and coping behavior. *Psychoneuroendocrinology*, 67, 86–99. 10.1016/J.PSYNEUEN.2016.02.004 [PubMed: 26881834]
- Hurst JL, Barnard CJ, Nevison CM, & West CD (1997). Housing and welfare in laboratory rats: welfare implications of isolation and social contact among caged males. *Animal Welfare*, 6(4), 329–347.
- Isingrini E, Camus V, Le Guisquet A-M, Pingaud M, Verine Devers S, & Belzung C (2010). Association between repeated Unpredictable Chronic Mild Stress (UCMS) procedures with a high fat diet: A model of fluoxetine resistance in mice. *PLoS One*, 5(4), e10404-undefined. 10.1371/journal.pone.0010404 [PubMed: 20436931]

- Ivy AS, Brunson KL, Sandman CA, & Baram TZ (2008). Dysfunctional nurturing behavior in rat dams with limited access to nesting material: A clinically relevant model for early-life stress. *Neuroscience*, 154(3), 1132–1142. 10.1016/j.neuroscience.2008.04.019 [PubMed: 18501521]
- Kaidanovich-Beilin O, Lipina T, Vukobradovic I, Roder J, & Woodgett JR (2010). Assessment of social interaction behaviors. In *Journal of Visualized Experiments* (Issue 48, p. 2473). *Journal of Visualized Experiments*. 10.3791/2473
- Kessler RC (2003). Epidemiology of women and depression. *Journal of Affective Disorders*, 74(1), 5–13. 10.1016/S0165-0327(02)00426-3 [PubMed: 12646294]
- Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S, Wittchen HU, & Kendler KS (1994). Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States: Results from the National Comorbidity Survey. *Archives of General Psychiatry*, 51(1), 8–19. 10.1001/archpsyc.1994.03950010008002 [PubMed: 8279933]
- Knop J, van IJendoorn MH, Bakermans-Kranenburg MJ, Joëls M, & van der Veen R (2019). The effects of different rearing conditions on sexual maturation and maternal care in heterozygous mineralocorticoid receptor knockout mice. *Hormones and Behavior*, 112, 54–64. 10.1016/j.yhbeh.2019.04.001 [PubMed: 30953639]
- Lapp HE, Bartlett AA, Zup SL, Hunter RG, & Moore CL (2020). Early experience alters developmental trajectory of central oxytocin systems involved in hypothalamic-pituitary-adrenal axis regulation in Long-Evans rats. *Hormones and Behavior*, 126, 104822. 10.1016/J.YHBEH.2020.104822 [PubMed: 32730760]
- Lapp HE, Mueller I, & Moore CL (2020). Limited bedding and nesting material changes indices of cellular metabolism and behavioral thermal regulation in Long-Evans rats during the first two weeks of life. *Physiology and Behavior*, 222. 10.1016/j.physbeh.2020.112957
- Malter Cohen M, Jing D, Yang RR, Tottenham N, Lee FS, & Casey BJ (2013). Early-life stress has persistent effects on amygdala function and development in mice and humans. *Proceedings of the National Academy of Sciences of the United States of America*, 110(45), 18274–18278. 10.1073/pnas.1310163110 [PubMed: 24145410]
- Manzano Nieves G, Schilit Nitenson A, Lee HI, Gallo M, Aguilar Z, Johnsen A, Bravo M, & Bath KG (2019). Early life stress delays sexual maturation in female mice. *Frontiers in Molecular Neuroscience*, 12. 10.3389/fnmol.2019.00027
- McLaughlin RJ, Verleza S, Gray JM, Hill MN, & Walker CD (2016). Inhibition of anandamide hydrolysis dampens the neuroendocrine response to stress in neonatal rats subjected to suboptimal rearing conditions. *Stress*, 19(1), 114–124. 10.3109/10253890.2015.1117448 [PubMed: 26552023]
- Molet J, Heins K, Zhuo X, Mei YT, Regev L, Baram TZ, & Stern HS (2016). Fragmentation and high entropy of neonatal experience predict adolescent emotional outcome. *Translational Psychiatry*, 6. 10.1038/tp.2015.200
- Molet J, Maras PM, Avishai-Eliner S, & Baram TZ (2014). Naturalistic rodent models of chronic early-life stress. *Developmental Psychobiology*, 56(8), 1675–1688. 10.1002/dev.21230 [PubMed: 24910169]
- Moore CL, & Chadwick-Dias A-M (1986). Behavioral responses of infant rats to maternal licking: Variations with age and sex. *Developmental Psychobiology*, 19(5), 427–438. 10.1002/dev.420190504 [PubMed: 3758488]
- Moore CL, & Morelli GA (1979). Mother rats interact differently with male and female offspring. *Journal of Comparative and Physiological Psychology*, 93(4), 677–684. 10.1037/h0077599 [PubMed: 479402]
- Moriceau S, Shionoya K, Jakubs K, & Sullivan RM (2009). Early-life stress disrupts attachment learning: The role of amygdala corticosterone, locus ceruleus corticotropin releasing hormone, and olfactory bulb norepinephrine. *Journal of Neuroscience*, 29(50), 15745–15755. 10.1523/JNEUROSCI.4106-09.2009 [PubMed: 20016090]
- Moussaoui N, Larauche M, Biraud M, Molet J, Million M, Mayer E, & Taché Y (2016). Limited nesting stress alters maternal behavior and in vivo intestinal permeability in male Wistar pup rats. *PLOS ONE*, 11(5), e0155037. 10.1371/journal.pone.0155037 [PubMed: 27149676]

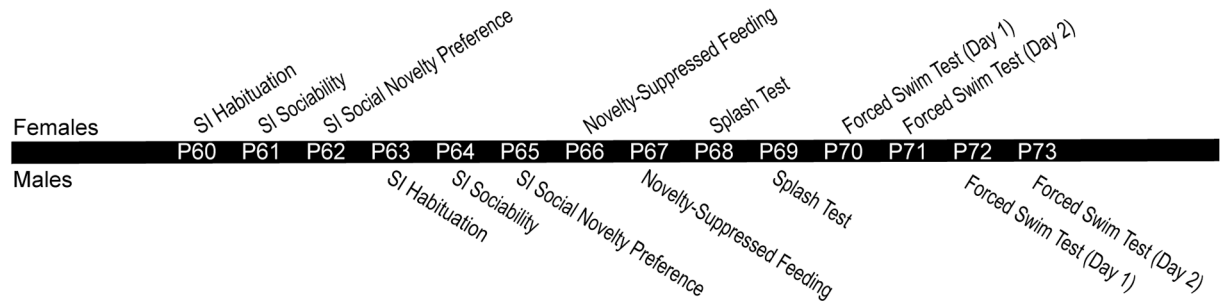
- Murphy MO, & Loria AS (2017). Sex-specific effects of stress on metabolic and cardiovascular disease: Are women at higher risk? *American Journal of Physiology*, 313(1), R1–R9. 10.1152/ajpregu.00185.2016 [PubMed: 28468942]
- Nam H, Clinton SM, Jackson NL, & Kerman IA (2014). Learned helplessness and social avoidance in the Wistar-Kyoto rat. *Frontiers in Behavioral Neuroscience*, 8(109). 10.3389/fnbeh.2014.00109
- Naninck EFG, Oosterink JE, Yam KY, De Vries LP, Schierbeek H, Van Goudoever JB, Verkaik-Schakel RN, Plantinga JA, Plosch T, Lucassen PJ, & Korosi A (2017). Early micronutrient supplementation protects against early stress-induced cognitive impairments. *FASEB Journal*, 31(2), 505–518. 10.1096/fj.201600834R [PubMed: 27770020]
- Okhuarobo A, Bolton JL, Igbe I, Zorrilla EP, Baram TZ, & Contet C (2020). A novel mouse model for vulnerability to alcohol dependence induced by early-life adversity. *Neurobiology of Stress*, 13, 100269. 10.1016/j.ynstr.2020.100269 [PubMed: 33344722]
- Ordoñez Sanchez E, Bavley CC, Deutschmann AU, Carpenter R, Peterson DR, Karbalaie R, Flowers J, Rogers CM, Langrehr MG, Ardekani CS, Famularo ST, Bongiovanni AR, Knouse MC, Floresco SB, Briand LA, Wimmer ME, & Bangasser DA (2021). Early life adversity promotes resilience to opioid addiction-related phenotypes in male rats and sex-specific transcriptional changes. *Proceedings of the National Academy of Sciences*, 118(8), e2020173118. 10.1073/pnas.2020173118
- Pagliaccio D, & Barch DM (2016). Early life adversity and risk for depression: Alterations in cortisol and brain structure and function as mediating mechanisms. In Frodl T (Ed.), *Systems Neuroscience in Depression* (pp. 29–77). Academic Press. 10.1016/B978-0-12-802456-0.00002-9
- Porsolt RD, Le Pichon M, & Jalfre M (1977). Depression: A new animal model sensitive to antidepressant treatments. *Nature*, 266(5604), 730–732. 10.1038/266730a0 [PubMed: 559941]
- Raineki C, Cortés MR, Belnoue L, & Sullivan RM (2012). Effects of early-life abuse differ across development: infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala. *Journal of Neuroscience*, 32(22), 7758–7765. 10.1523/JNEUROSCI.5843-11.2012 [PubMed: 22649253]
- Raineki C, Moriceau S, & Sullivan RM (2010). Developing a neurobehavioral animal model of infant attachment to an abusive caregiver. *Biological Psychiatry*, 67(12), 1137–1145. 10.1016/j.biopsych.2009.12.019 [PubMed: 20163787]
- Raineki C, Sarro E, Rincón-Cortés M, Perry R, Boggs J, Holman CJ, Wilson DA, & Sullivan RM (2015). Paradoxical neurobehavioral rescue by memories of early-life abuse: The safety signal value of odors learned during abusive attachment. *Neuropsychopharmacology*, 40(4), 906–914. 10.1038/npp.2014.266 [PubMed: 25284320]
- Reiss F (2013). Socioeconomic inequalities and mental health problems in children and adolescents: A systematic review. *Social Science and Medicine*, 90, 24–31. 10.1016/j.socscimed.2013.04.026 [PubMed: 23746605]
- Rice CJ, Sandman CA, Lenjavi MR, & Baram TZ (2008). A Novel Mouse Model for Acute and Long-Lasting Consequences of Early Life Stress. *Endocrinology*, 149(10), 4892–4900. 10.1210/en.2008-0633 [PubMed: 18566122]
- Richmond G, & Sachs BD (1984). Maternal discrimination of pup sex in rats. *Developmental Psychobiology*, 17(1), 87–89. 10.1002/dev.420170108 [PubMed: 6698313]
- Roth TL, Lubin FD, Funk AJ, & Sweatt JD (2009). Lasting epigenetic influence of early-life adversity on the BDNF gene. *Biological Psychiatry*, 65(9), 760–769. 10.1016/J.BIOPSYCH.2008.11.028 [PubMed: 19150054]
- Roth TL, Matt S, Chen K, & Blaze J (2014). Bdnf DNA methylation modifications in the hippocampus and amygdala of male and female rats exposed to different caregiving environments outside the homecage. *Developmental Psychobiology*, 56(8), 1755–1763. 10.1002/dev.21218 [PubMed: 24752649]
- Roth TL, & Sullivan RM (2005). Memory of early maltreatment: Neonatal behavioral and neural correlates of maternal maltreatment within the context of classical conditioning. *Biological Psychiatry*, 57(8), 823–831. 10.1016/j.biopsych.2005.01.032 [PubMed: 15820702]
- Sadeghi M, Peeri M, & Hosseini MJ (2016). Adolescent voluntary exercise attenuated hippocampal innate immunity responses and depressive-like behaviors following maternal separation stress

- in male rats. *Physiology and Behavior*, 163, 177–183. 10.1016/j.physbeh.2016.05.017 [PubMed: 27184238]
- Spence SH, Najman JM, Bor W, O'Callaghan MJ, & Williams GM (2002). Maternal anxiety and depression, poverty and marital relationships factors during early childhood as predictors of anxiety and depressive symptoms in adolescence. *Journal of Child Psychology and Psychiatry*, 43(4), 457–469. 10.1111/1469-7610.00037 [PubMed: 12030592]
- Starkstein SE, & Leentjens AFG (2008). The nosological position of apathy in clinical practice. *Journal of Neurology, Neurosurgery and Psychiatry*, 79(10), 1088–1092. 10.1136/jnnp.2007.136895
- Strzelewicz AR, Ordoñez Sanchez E, Rondón-Ortiz AN, Raneri A, Famularo ST, Bangasser DA, & Kentner AC (2019). 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. *Hormones and Behavior*, 111, 46–59. 10.1016/j.yhbeh.2019.01.003 [PubMed: 30708031]
- Walker C-D, Bath KG, Joels M, Korosi A, Larauche M, Lucassen PJ, Morris MJ, Raineke C, Roth TL, Sullivan RM, Taché Y, & Baram TZ (2017). Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress*, 20(5), 421–448. 10.1080/10253890.2017.1343296 [PubMed: 28617197]
- West AP (1990). Neurobehavioral studies of forced swimming: The role of learning and memory in the forced swim test. *Progress in Neuropsychopharmacology and Biological Psychiatry*, 14(6), 863–877. 10.1016/0278-5846(90)90073-P
- Whitton AE, Treadway MT, & Pizzagalli DA (2015). Reward processing dysfunction in major depression, bipolar disorder and schizophrenia. *Current Opinion in Psychiatry*, 28(1), 7–12. 10.1097/YCO.000000000000122 [PubMed: 25415499]
- Yam KY, Naninck EFG, Abbink MR, la Fleur SE, Schipper L, van den Beukel JC, Grefhorst A, Oosting A, Van Der Beek EM, Lucassen PJ, & Korosi A (2017). Exposure to chronic early-life stress lastingly alters the adipose tissue, the leptin system and changes the vulnerability to western-style diet later in life in mice. *Psychoneuroendocrinology*, 77, 186–195. 10.1016/j.psyneuen.2016.12.012 [PubMed: 28088658]
- Yam KY, Schipper L, Reemst K, Ruigrok SR, Abbink MR, Hoeijmakers L, Naninck EFG, Zarekiani P, Oosting A, Van Der Beek EM, Lucassen PJ, & Korosi A (2019). Increasing availability of ω -3 fatty acid in the early-life diet prevents the early-life stress-induced cognitive impairments without affecting metabolic alterations. *FASEB Journal*, 33(4), 5729–5740. 10.1096/fj.201802297R [PubMed: 30673509]
- Yan C-G, Rincón-Cortés M, Raineke C, Sarro E, Colcombe S, Guilfoyle DN, Yang Z, Gerum S, Biswal BB, Milham MP, Sullivan RM, & Castellanos FX (2017). Aberrant development of intrinsic brain activity in a rat model of caregiver maltreatment of offspring. *Translational Psychiatry*, 7(1), e1005–e1005. 10.1038/tp.2016.276 [PubMed: 28094810]

A. Experimental Timeline



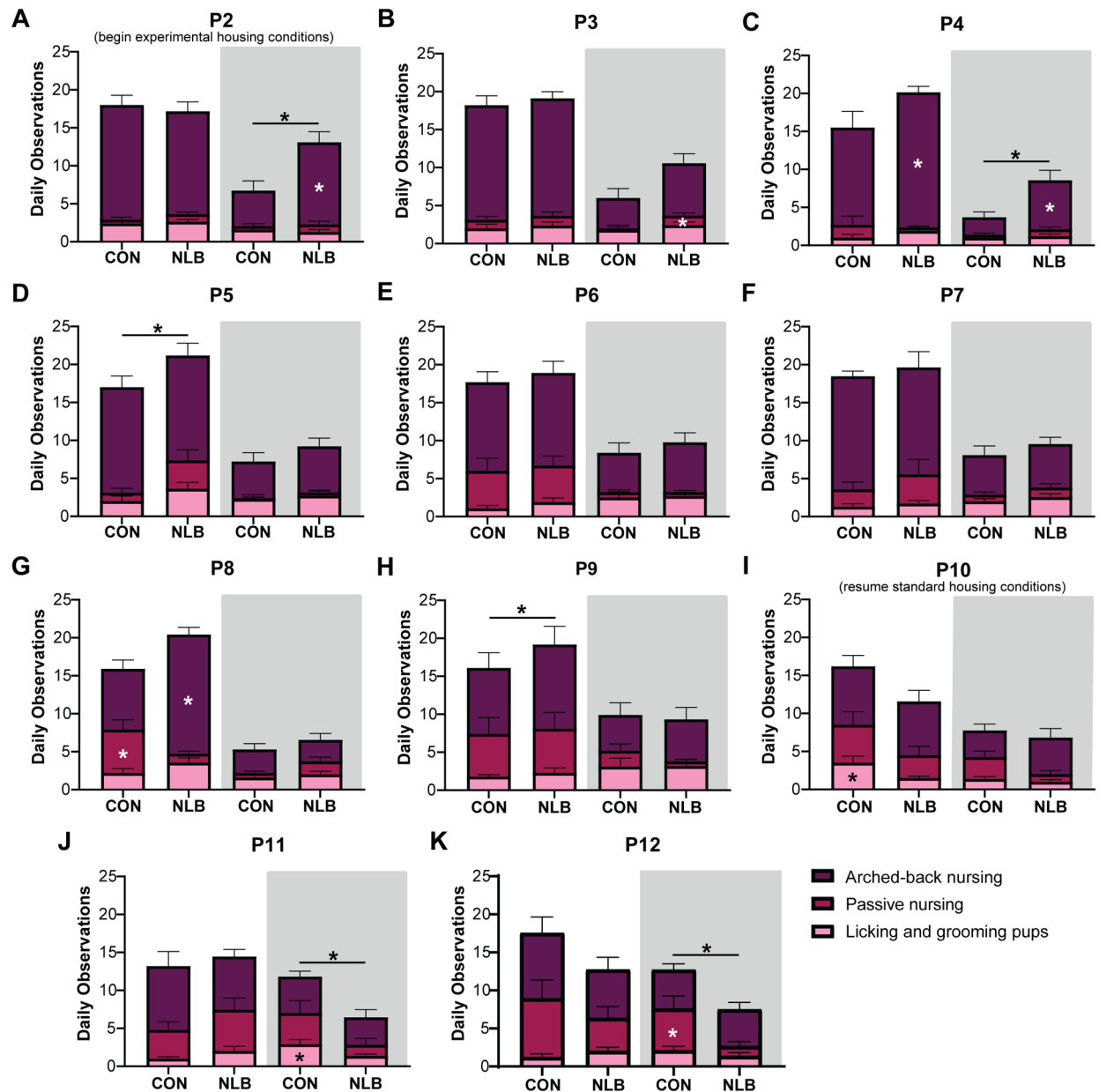
B. Offspring Emotional Behavior Test Battery



Cohort #1	Cohort #2	Offspring Measurements
○	●	Body Weight
□	N/A	Core Body Temperature (P10)
▽	N/A	Emotional Behavior (P60-P74)

Figure 1.

Timeline of experimental procedures (A) and behavioral tests used to assess offspring emotional behavior (B). Two cohorts of Sprague Dawley rats were used for these experiments. Dams and their litters in Cohort #1 (N = 10 litters) and Cohort #2 (N = 12 litters) were randomly assigned to either control (CON) or neonatal limited bedding (NLB) conditions from postnatal day (P)2 through P9. Maternal behavior was observed in dams from both cohorts from P1-P14. Body weight, core body temperature (P10), and emotional behavior outcomes were assessed in offspring from Cohort #1 (open symbols; see Key for specific measurements). Body weight measurements were collected from offspring in Cohort #2 (dark symbol; see Key for specific measurement). Cohort #1 offspring were sacrificed on P85, while offspring from Cohort #2 were sacrificed between P15-P21.

**Figure 2.**

Daily observations of pup-directed maternal behaviors by control (CON, $n = 9-10$) and neonatal limited bedding (NLB, $n = 10-11$) dams during the light period (left; white background) and dark period (right; shaded background) from P2-P12. Data are not shown for P13-P14 due to lack of statistically significant differences between groups. Number of daily observations for individual behaviors by the dam, including arched-back nursing, passive nursing, and licking and grooming of pups, are expressed as mean \pm SEM. Frequencies of individual behaviors were compared between CON and NLB dams within a light cycle, but not between light cycles (i.e., light vs. dark). Significant differences between CON and NLB dams with respect to individual behaviors are shown as asterisks (*) within the colored bar corresponding to a particular behavior, whereas differences in summed

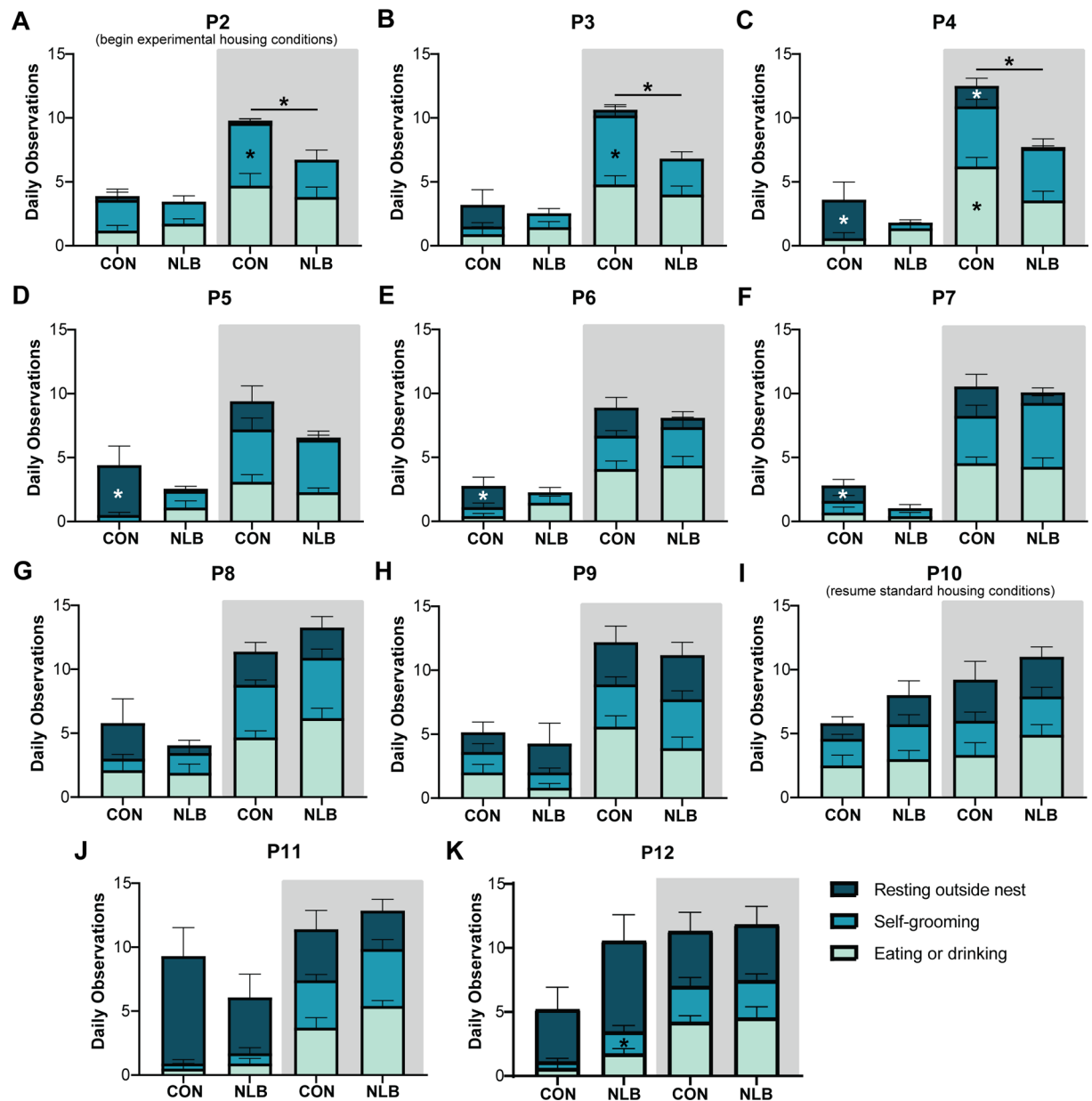
pup-directed behaviors are shown as asterisks above the bars. Significance was set at $p < 0.05$: *, $p < 0.05$.

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**Figure 3.**

Daily observations of self-directed maternal behaviors by control (CON, $n = 9-10$) and neonatal limited bedding (NLB, $n = 10-11$) dams during the light period (left, white background) and dark period (right, shaded background) from P2-P12. Data are not shown for P13-P14 due to lack of statistically significant differences between groups. Number of daily observations for individual behaviors by the dam, including eating and drinking, self-grooming, and resting outside of the nest, are expressed as mean \pm SEM. Frequencies of individual behaviors were compared between CON and NLB dams within a light cycle, but not between light cycles (i.e., light vs. dark). Significant differences between CON and NLB dams with respect to individual behaviors are shown as asterisks (*) within the colored bar corresponding to a particular behavior, whereas differences in summed self-directed

behaviors are shown as asterisks above the bars. Significance was set at $p < 0.05$: *, $p < 0.05$.

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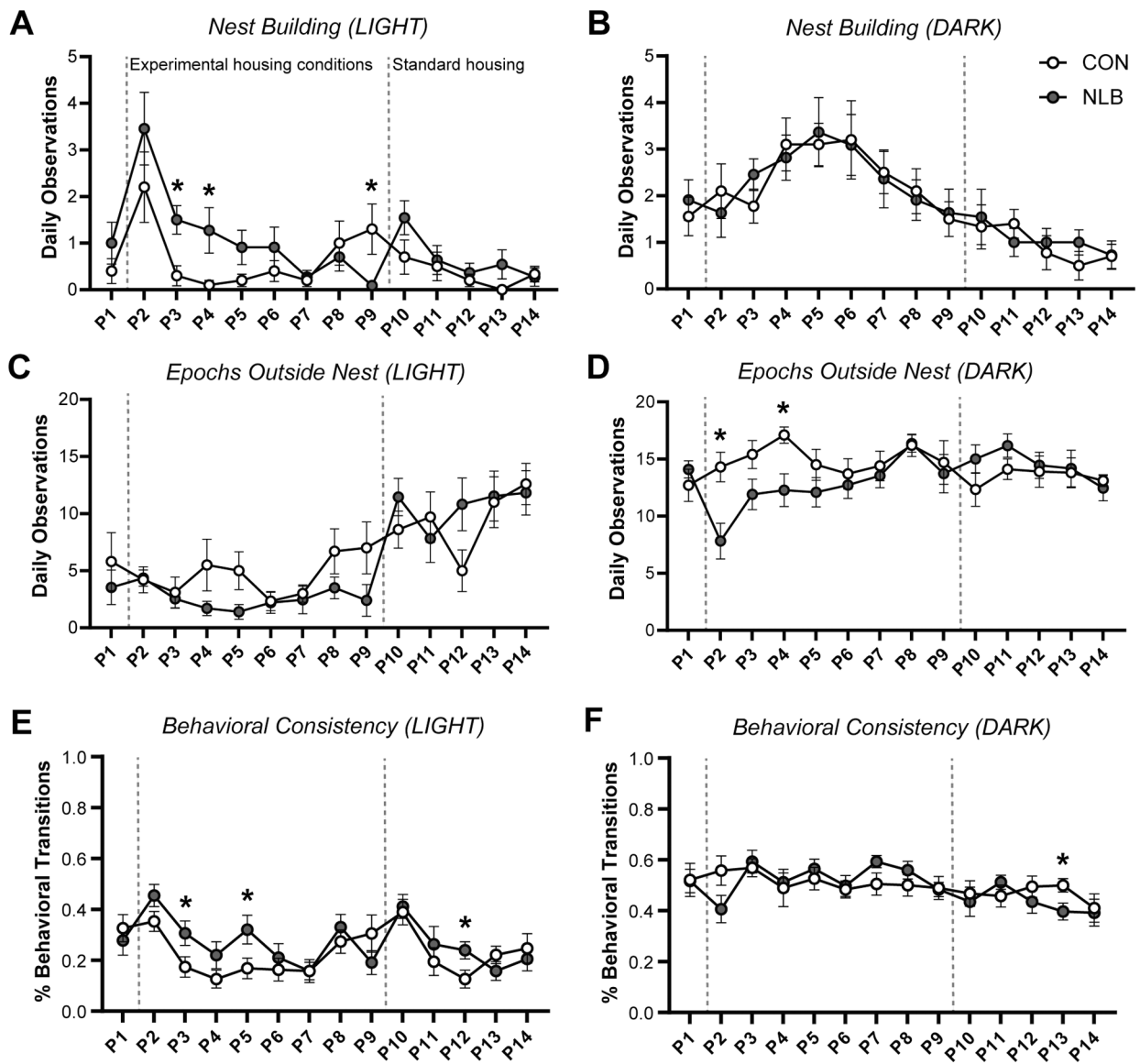
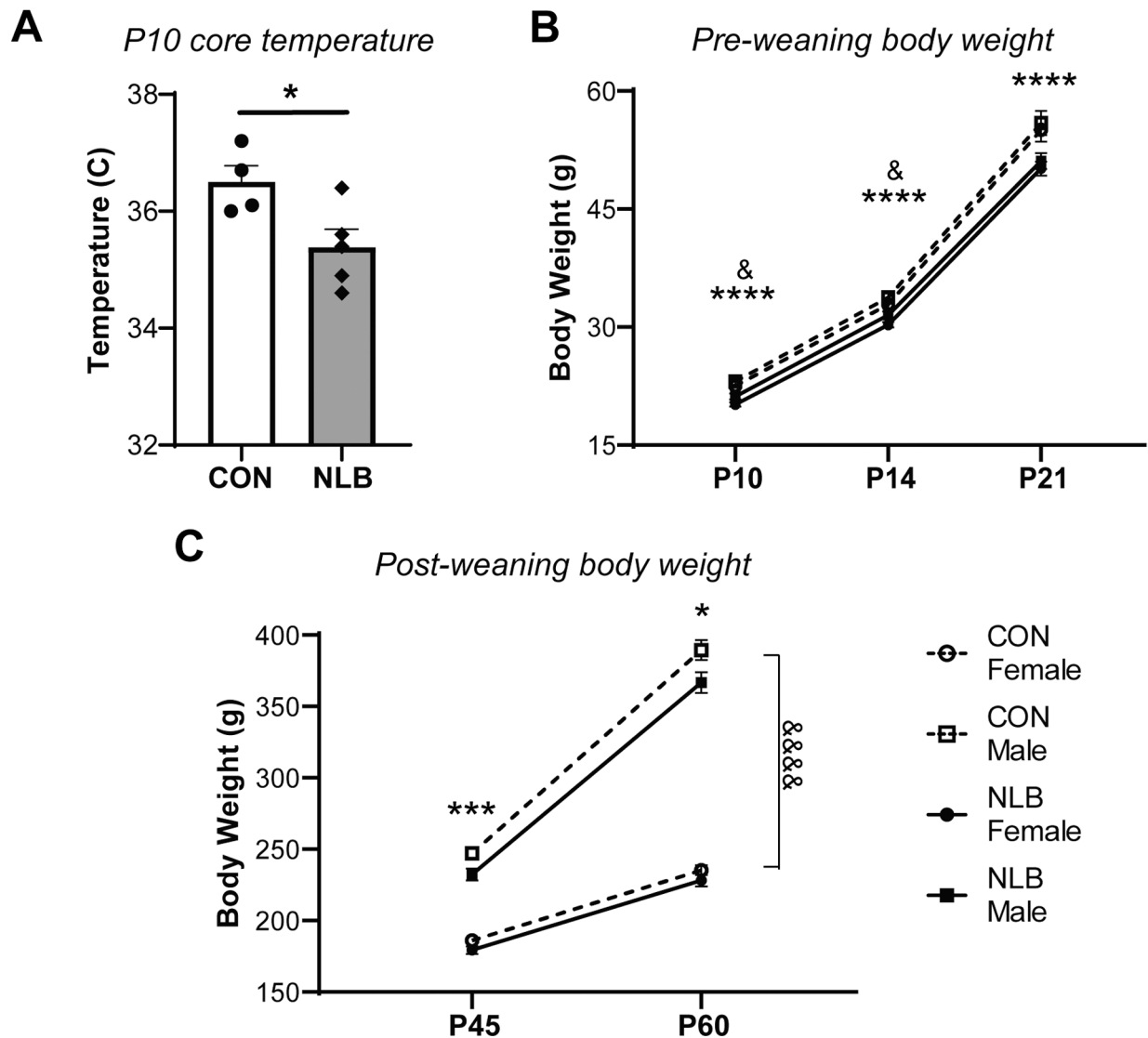


Figure 4.

Daily observations of nesting behavior (A-B), epochs spent outside the nest (C-D), and consistency in the patterns of overall maternal behaviors (E-F) by control (CON, $n = 9-10$) and neonatal limited bedding (NLB, $n = 10-11$) dams during the light period (left) and dark period (right) from P1-P14. (A) During the light period, NLB dams were observed engaging in nest building activities significantly more often on P3 and P4, and significantly less often on P9, than control dams. (B) During the dark period, NLB and CON dams spent a comparable number of observations engaging in nest building between P1-P14. (C) During the light period, NLB and CON dams spent comparable amounts of time outside of the nest between P1-P14. (D) During the dark period, NLB dams were observed outside of the nest less often on P2 and P4 relative to control dams. (E) During the light period, NLB dams showed an increase in the number of transitions between discrete behaviors on P3, P5, and P12, indicative of less consistency in the overall pattern of care. (F) During the dark period,

NLB and CON displayed a similar pattern of transitions between discrete behaviors except on P13, three days after the termination of the housing manipulation. Significance was set at $p < 0.05$: *, $p < 0.05$.

**Figure 5.**

Core temperature and body weights of offspring exposed to control (CON) or neonatal limited bedding (NLB) conditions from P2-P9. (A) Core body temperature on P10, averaged by litter, was significantly reduced in NLB-reared offspring ($n = 5$ litters) relative to control-reared offspring ($n = 4$ litters). (B) NLB-reared offspring of both sexes showed significant decreases in body weight at P10, P14, and P21 compared to controls. Additionally, male offspring weighed more than female offspring. (P10 and P14: CON females, $n = 60$; CON males, $n = 57$; NLB females, $n = 65$; NLB males, $n = 62$. P21: CON females, $n = 24$; CON males, $n = 23$; NLB females, $n = 29$; NLB males, $n = 26$.) (C) Male, but not female, NLB-reared offspring displayed significantly reduced body weight at P45 (adolescence) and P60 (early adulthood) relative to controls. (P45: CON females, $n = 24$; CON males, $n = 23$; NLB females, $n = 29$; NLB males, $n = 26$. P60: CON females, $n = 18$; CON males, $n = 17$; NLB females, $n = 23$; NLB males, $n = 20$.) Main effects of sex were denoted as “&.” Significance was set at $p < 0.05$; *, $p < 0.05$; ****, $p < 0.0001$.

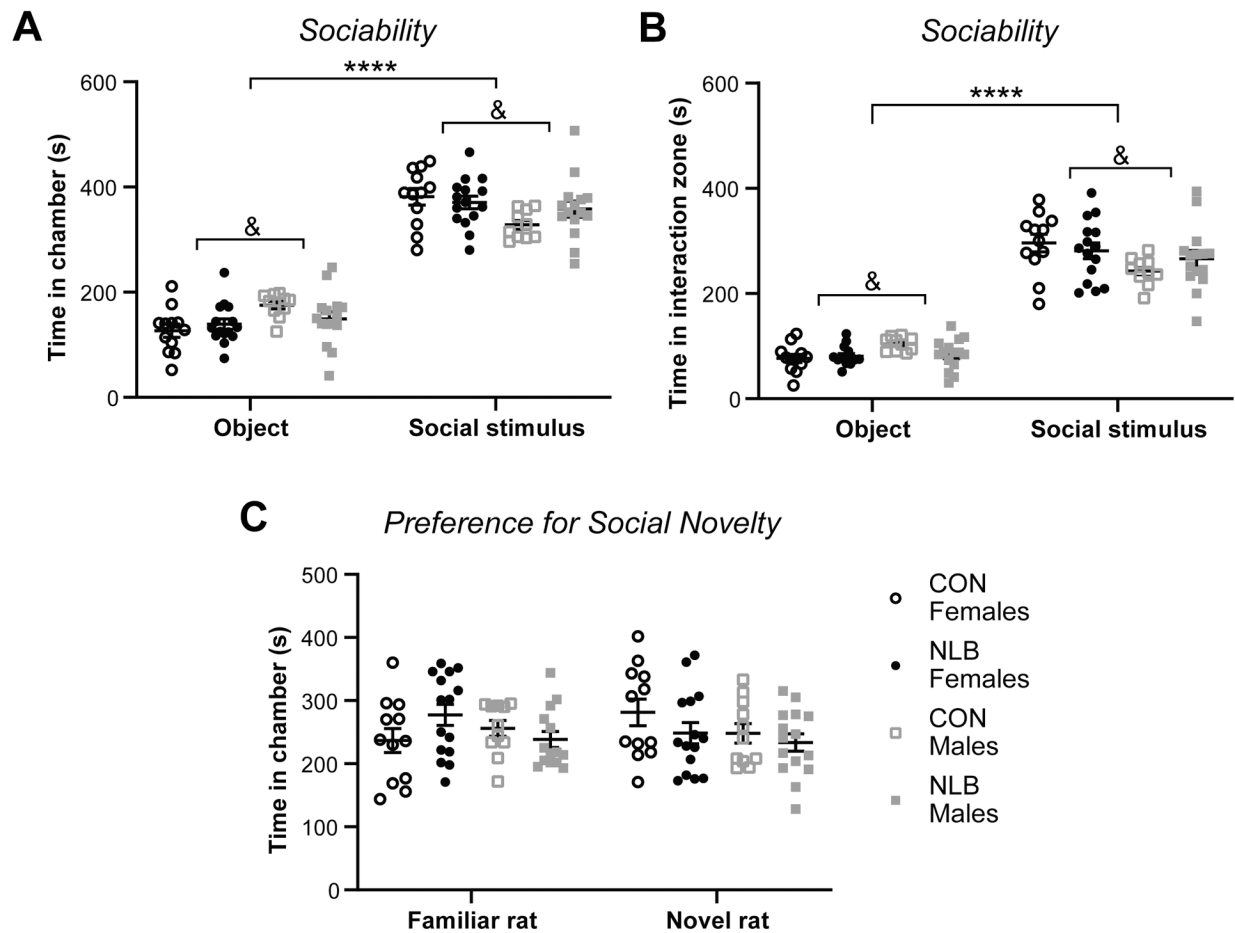
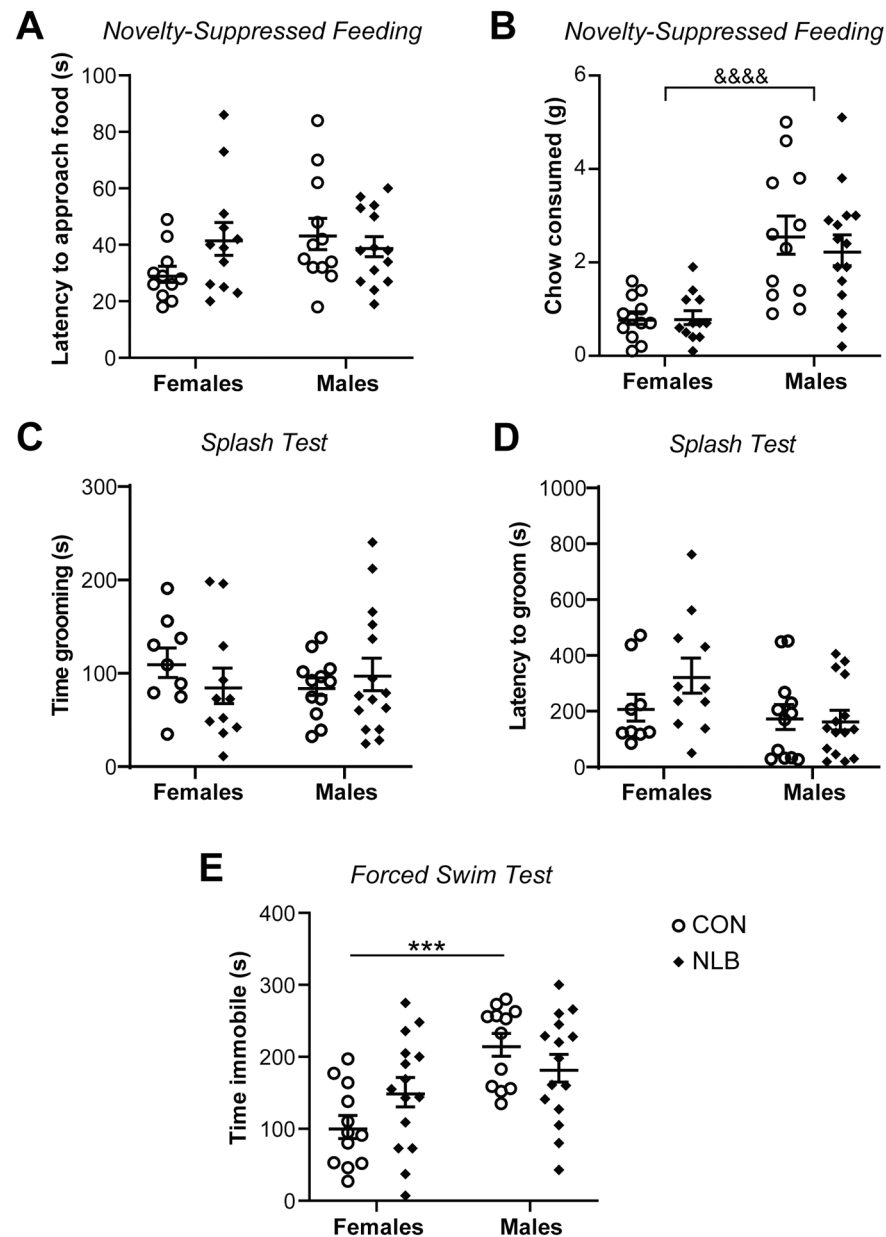


Figure 6.

Comparison of adult social behavior in offspring reared under control (CON) or neonatal limited bedding (NLB) conditions. (A-B) Sociability on Day 2 was assessed by comparing time spent by CON (female, $n = 12$; male, $n = 10$) vs. NLB ($n = 15$ per sex) offspring in the chambers containing the novel social stimulus or an object. All adult offspring spent more time in the chamber containing the social stimulus, as well as its corresponding interaction zone, than in the chamber containing an object. Females, regardless of exposure to early-life adversity, spent more time in the social stimulus chamber and less time in the object-containing chamber, as well as in the respective interaction zones of these chambers. (C) Neither CON (female, $n = 12$; male, $n = 11$) nor NLB-exposed (female, $n = 15$; male, $n = 14$ – 15) offspring displayed a preference for social novelty, based on time spent in the chamber containing either a novel or familiar social stimulus. Main effects of sex are denoted as “&.” Significance was set at $p < 0.05$: *, $p < 0.05$; ****, $p < 0.0001$.

**Figure 7.**

Comparison of adult anxiety- and depression-relevant behaviors in offspring reared under control (CON) or neonatal limited bedding (NLB) conditions. (A) Anxiety-like behavior, measured as latency to approach food in a novel environment in the Novelty-Suppressed Feeding (NSF) test, did not differ between CON (female, $n = 11$; male, $n = 12$) and NLB (female, $n = 14$; male, $n = 12$) offspring of either sex. (B) Male offspring (CON, $n = 12$; NLB, $n = 15$) consumed more chow in the NSF test than female offspring ($n = 12$ per group) regardless of exposure to early-life adversity. (C-D) Self-care behavior in the splash test, measured as total time spent grooming (C) and latency to first grooming episode (D), was unaltered in either sex between CON (female, $n = 9$; male, $n = 12$) and NLB (female, $n = 11$; male, $n = 15$) offspring. (E) An interaction for the effects of sex and NLB was

observed for passive stress coping, measured as time immobile, in the Forced Swim Test (FST). Female CON ($n = 12$) offspring spent significantly less time immobile than male CON ($n = 15$) offspring. None of the remaining comparisons with female ($n = 12$) or male ($n = 15$) NLB-exposed offspring revealed statistically significant differences. Main effects of sex are denoted as “&.” Significance was set at $p < 0.05$: ***, $p < 0.001$; ****, $p < 0.0001$.

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Table 1.

Number of control (CON) and neonatal limited bedding (NLB)-exposed dams observed chasing their tails during at least one ‘snapshot’ observation of maternal behavior during the light and dark periods from offspring postnatal days (P)2-P10.

Postnatal Day	Light Cycle	No. of CON dams chasing their tail	No. of NLB dams chasing their tail	P-value (Fisher's exact test)
P2	<i>Light</i>	0 of 10	2 of 11	0.4762
	<i>Dark</i>	1 of 10	3 of 11	0.5865
P3	<i>Light</i>	0 of 10	3 of 11	0.2143
	<i>Dark</i>	0 of 10	5 of 11	0.0351
P4	<i>Light</i>	1 of 10	1 of 11	>0.9999
	<i>Dark</i>	2 of 10	7 of 11	0.0805
P5	<i>Light</i>	2 of 10	0 of 11	0.2143
	<i>Dark</i>	3 of 10	4 of 11	>0.9999
P6	<i>Light</i>	0 of 10	0 of 11	>0.9999
	<i>Dark</i>	2 of 10	1 of 11	0.5865
P7	<i>Light</i>	0 of 10	2 of 11	0.4762
	<i>Dark</i>	0 of 10	2 of 11	0.4762
P8	<i>Light</i>	0 of 10	0 of 11	>0.9999
	<i>Dark</i>	1 of 10	1 of 10	>0.9999
P9	<i>Light</i>	0 of 10	0 of 11	>0.9999
	<i>Dark</i>	0 of 10	0 of 11	>0.9999
P10	<i>Light</i>	0 of 10	0 of 11	>0.9999
	<i>Dark</i>	0 of 9	0 of 11	>0.9999