Supporting Information

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SI Methods

Animals. Sixty-six male pups were born from 14 Long-Evans hooded dams (Harlan) that arrived at the University of New Mexico Psychology Department vivarium 10 d before giving birth. After birth, litters were culled to eight pups, keeping as many male pups as possible. A 12-h light/dark cycle was used with lights on at 0700. Rats were fed with a regular diet (Harlan-Tekland Global Diets), food and water was ad libitum. All experimental procedures were in accordance with the Institutional Animal Care and Use Committee at the University of New Mexico.

Using the neonatal novelty exposure (NNE) procedure based on a split-litter design (1), we exposed approximately half of the siblings from each litter to a relatively novel nonhome environment and kept the remaining half in only the home cage during postnatal day (PND) 1–21. Offspring body weight was measured at weaning (PND 22, W1), early (PND 100, W2), and late adulthood (13 mo and 14 mo of age: W3 and W4) (see experimental timeline in Fig. S1*A*).

Neonatal Novelty Exposure. NNE is a neonatal intervention or stimulation paradigm that is derived from the postnatal handling paradigm but fundamentally different from the handling paradigm. As have been shown in classic (2, 3) as well as recent (4, 5) discussions of relevant literature, the handling treatment was typically implemented as a between-litter design and pups of the handled litters differ from the nonhandled litters by: (*i*) additional experimenter handling; (*ii*) additional experience of novelty associated with a brief exposure to a nonhome cage; (*iii*) additional separation from the dams; and (*iv*) additional maternal stress which may simultaneously activate her own hypothalamic-pituitary-adrenal (HPA) response and modify her care-giving behavior. Because of the fact that handling treatment is not just experimenter handling but confounded by at least three additional factors, these previous studies do not allow determination of which of the four factors, or which of the many combination of four factors, are the cause of the observed developmental differences between the "handled" and "nonhandled" offspring, therefore seriously limiting our ability to interpret findings associated with such a treatment.

To dissociate these factors, the NNE procedure was introduced to isolate the effect of the novelty exposure component by using a split-litter design, as opposed to the between-litter design (1, 4–7). This alternative early-stimulation paradigm involves exposing half of each litter to a relatively nonhome environment (Novel rats), while the remaining half remains in the home cage (Home rats). In addition, the duration of maternal separation and the amount of handling of both groups were carefully matched between the Novel and Home siblings. This procedure has been previously shown to produce not only a wide range of first-order enhancement effects on cognitive, social, emotional, and neuroendocrine functions throughout the animals' lifespan (1, 4–10), but also second-order effects: maternal circulating stress corticosterone (CORT) level provides a distinct context to allow neonatal novelty exposure to have an effect of differential enhancement on spatial memory performance (7) and fear reduction (11) across offspring from different families; maternal care reliability provides similar family-specific effects on offspring's social competitiveness and plasticity in stress response (12), as well as spatial working memory (13). Specifically, on PND 0, approximately half of each litter was randomly assigned to Novel and the other half to Home conditions (split-litter design). Group membership was marked by tattooing the hindpaws. On PND 1–21, the NNE procedure (Fig. S1*B*) was carried out in the housing room (ambient temperature 22 °C; humidity 25%) (for additional details see refs. 1, 5, 7).

Body Weight Measurement. Body weight was measured both at weaning (W1) and late adulthood (W2–4) (Fig. S1*A*). To maximize the sensitivity of detecting weight difference between Novel and Home offspring, rats within the same litters were measured one immediately after another and the order of measurement between the Novel and Home rats was counterbalanced within each litter. This process was intended to minimize variations because of food consumption, defecation, and urination during the weighing process. At PND 22 (W1) and PND 100 (W2), body weight was directly measured; at 13 mo of age (W3), body weight were measured for 2 consecutive days and an average weight was obtained as the final dependent measure and daily food consumption was recorded to partial out its contribution at the time of analysis; at 14 mo of age (W4), with the use of another variant of measuring procedure, the contribution of food consumption, water intake, defecation, and urination was minimized by 15 h of food and water restriction (from 1800 to 0900 hours) before weighing. The 14-mo measure was obtained for the purpose of confirming findings made for the 13-mo measure. All weight measurements were made between 0830 and 1400 hours.

Maternal Physiological Measure. Shortly after weaning, maternal basal CORT ($CORT_B$) and postswim circulating CORT ($CORT_S$) levels were measured from blood samples collected on PND 26 and PND 27 between 1300 and 1700 hours. For $CORT_B$ samples, dams were transported from the Housing Room directly to the Blood Collection Room, and for $CORT_S$ samples, dams were transported from the Swim Test Room to the Blood Collection Room 5 min after the onset of the 1-min swim test (water temperature of ~21 °C). The maternal evoked-CORT response ($CORT_E$) was quantified as the difference between $CORT_S$ and $CORT_B$ normalized by the baseline measure $CORT_B$ [$(CORT_S - CORT_B)/CORT_B \times 100$]. Each sample, containing ~200 μL of blood, was centrifuged, and the plasma was extracted and then stored at −20 °C until radioimmunoassay (RIA) was performed. Plasma CORT concentration was measured using the Coat-a-Count CORT Kit (Diagnostic Products). The lower limit of detection was 10.41 ng/mL, and the intra-assay coefficient of variation was 13%. The descriptive statistic for $CORT_B$ is 39.22 ± 9.7 ng/mL and for $CORT_E$ is 242.33 ± 22.3 ng/mL. These measures are intended to serve as "trait" as opposed to "state" measures, because they reflect the dam's ability to regulate her own level of circulating stress hormone. Note that a high $CORT_E$ measure used here is conceptually and operationally different from the "high CORT" measure used in Lupien et al. (14), where the CORT measure was obtained during children's regular class hours, which is neither resting nor evoked by an explicit and discrete event as was done in the current study. Rather, that study follows work on dominant vs. subordinate baboons (15, 16), as well as successful vs. unhappy air-traffic controllers, showing that a robust CORT response from a low basal level is an index of a sense of control and good stress regulation (17–19). It should also be noted that, in the present results, it is the maternal $CORT_E$ measure that relates to the offspring body weight outcomes; the $CORT_B$ measure did not have any predictive value, although in other studies it is maternal $CORT_B$ measure that relates to spatial memory (7) and long-term habituation of acoustic startle reflex-related (11) outcomes. Fur-

ther studies involving more offspring outcome measures may provide additional information regarding selectivity of these two aspects of maternal stress regulation—the ability to maintain a low $CORT_B$ and the ability to mount a rapid initial CORT response ($CORT_E$) to stressor onset—in influencing offspring development.

Maternal Care Measure. The frequency of maternal licking and grooming behavior was measured from the first 5 min of video recording made on PND 1–10 immediately after the return of the dam to the home cage following novelty exposure. Two maternal care measures were obtained: average amount and variability of licking and grooming over 10 d. The maternal-care variability measure was computed as the standard deviations of the residuals after removing the increasing trend over the 10-d repeated novelty exposure (12, 13). An interrater reliability of 0.89 was obtained from two coders using data from 1 d. For ease of interpretation, we used the reversed rank order of this variability measure to represent maternal care reliability in data plotting. For additional details, see refs. 12 and 13.

Spatial Working Memory Measure. At the onset of juvenility, we used the Morris water task (WM) to investigate spatial learning and memory on 2 d: day1 as the training day and day 2 as the testing day. In the working memory version of the Morris water task (20), the platform location varies from day to day such that the rats must acquire a new platform location on each day. Spatial working memory performance was measured by one-trial learning, defined as the decrease in swim latency between trial 1 and trial 2 on the testing day. For additional details, see refs. 4–6.

Statistical Analysis. Novelty-related growth was measured for each litter by computing a novelty effect (NE) score defined as the difference between the Novel rats' litter average and the Home rats' litter average normalized by the average body weight of the entire litter. Data were checked for violation of assumptions of normality and heterogeneity of variance and appropriate transformations were performed before statistical testing. Repeated-measure ANOVA was used to analyze the Novelty-related growth. Univariate ANCOVA was used to examine the relation between maternal measures and average body weight of her entire litter. To determine whether individual differences in these maternal measures contribute to little-to-litter variations in novelty-related growth, we used repeated-measure ANCOVA with novelty as a within factor and each of the maternal measures as a covariate. The main results from the ANCOVA represent interaction effects between postnatal maternal stress regulation measures (a) and novelty exposure (b). In presenting these interaction effects graphically, we remind the reader that an interaction effect on the dependent variable (dv) between factors a and b can be viewed in two ways: (i) as a's influence on b's effect on the dv (denoted $a > b$); or (ii) b's influence on a's effect on the dv (denoted $b > a$). Along with report of F and P values, effect sizes are also reported and indicated as large, medium or small accordingly (effect size f: small: $0.25 > f >= 0.1$; medium: $0.4 > f >= 0.25$; large: $f >= 0.4$) (21).

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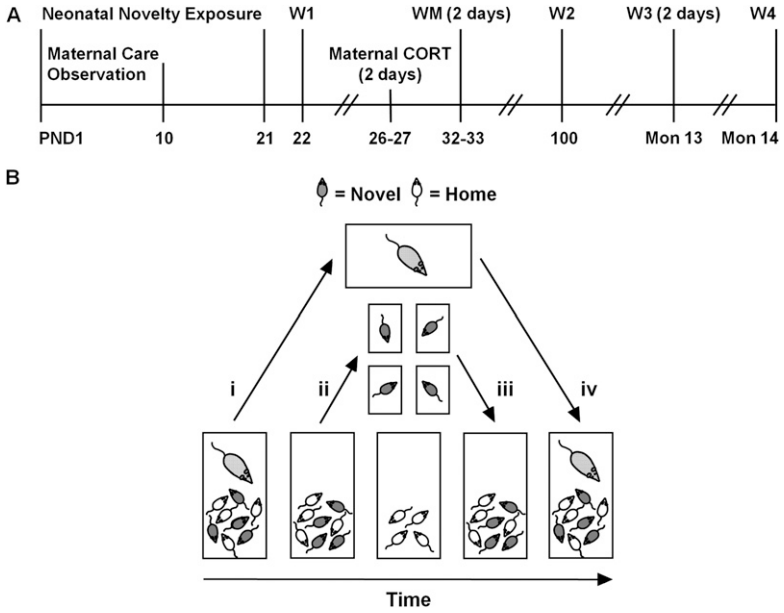

Fig. S1. (*A*) Timeline of the longitudinal study. (*B*) Sequential steps in neonatal novelty exposure using a split-litter design. For each litter: (i) Removal of dam from home cage. (ii) Transfer of Novel pups (solid color) to individual nonhome cages (small rectangles). (iii) Return of Novel pups to home cage after 3-min exposure to nonhome cages. (iv) Return of the dam to entire litter in home cage.