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Maternal modulation of the experience of novelty on offspring hormonal stress reactivity

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MATERNAL MODULATION OF THE EFFECT OF NOVELTY ON OFFSPRING HORMONAL STRESS REACTIVITY

by

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MATERNAL MODULATION OF THE EXPERIENCE OF NOVELTY ON OFFSPRING HORMONAL STRESS REACTIVITY

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ABSTRACT

Background: Studies have shown that both maternal and non-maternal environmental influences affect the development of the hypothalamic-pituitary-adrenal (HPA) axis. It remains unclear how these influences jointly interact to program offspring HPA function. Here, we test the hypothesis that non-maternal environmental effects on offspring HPA function depend contextually on maternal self-stress regulation. **Methods:** To examine individual differences in maternal self-stress regulation, mothers' basal circulating and evoked corticosterone (CORT) response were measured shortly after weaning. The effects of the non-maternal environment were assessed by employing both the neonatal and the early adulthood novelty exposure procedures. The neonatal novelty exposure procedure occurred from post-natal day 1-20. Half the pups in a cohort of rat families were exposed to a novel, non-home environment daily for 3-min (Novel, *N=49* males), while their siblings remained in the home cage (Home, *N=45* males). From post-natal day 54-63, a subset of each neonatal group was exposed to early adulthood novelty exposure, in which the pups were exposed to a novel, non-home environment for 3-min

daily (Novel, *N=49*), while their siblings remained in their home cage (Home, *N=45*). Offspring self-stress regulation was measured in mid-adulthood (13 months of age).

Results: Compared to their Home siblings, neonatal Novel rats displayed a significantly higher initial evoked CORT response following a stressor. More importantly, maternal self-stress regulation interacted with this observed neonatal novelty exposure effect such that greater novelty-induced enhancement was found among offspring of mothers who were able to mount a larger evoked CORT response.

Conclusions: These findings support the maternal modulation hypothesis suggesting that the effects of early non-maternal environmental novelty on offspring HPA function is modulated by the context set by individual differences in maternal physiology. These results offer converging evidence with previously reported interaction effects found in spatial memory, behavioral inhibition, and early growth.

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INTRODUCTION

Many rodent studies have shown that early life experience can affect the development of the function of the hypothalamic–pituitary-adrenal (HPA) axis (Catalani, Marinelli, Scaccianoce, Nicolai, Muscolo, Porcu, Koranyi, et al., 1993; Levine, 1957; Liu et al., 1997; Meaney, Aitken, van Berkel, Bhatnagar, & Sapolsky, 1988). Two such early life environmental influences are those provided by the postnatal maternal and non-maternal environments. Aspects of maternal influence that have been shown to affect offspring's HPA function are the amount of care (Francis, Diorio, Liu, & Meaney, 1999; Liu et al., 1997), the consistency of care (Akers et al., 2008; Reeb-Sutherland & Tang, 2012) and maternal levels of circulating corticosterone (CORT), the end product of the HPA axis (Catalani, Marinelli, Scaccianoce, Nicolai, Muscolo, Porcu, Korányi, et al., 1993; Macrì et al., 2007). Specifically, these studies found that offspring develop a more adaptive stress response system (viz, lower basal CORT levels, rapid recovery to baseline after stress induction) as the result of an increased amount of maternal care (Liu et al., 1997; Meaney, 2001), increased consistency in maternal care (Akers et al., 2008; Tang, Dinces, Yang, Romeo, & McEwen, in preparation), and moderately increased levels of maternal circulating CORT received prior to weaning (Catalani, Marinelli, Scaccianoce, Nicolai, Muscolo, Porcu, Koranyi, et al., 1993; Macrì et al., 2007).

A non-maternal influence shown to affect development of offspring's HPA function is environmental novelty (Tang, 2001; Tang, Akers, Reeb, Romeo, & McEwen, 2006; Tang, Reeb, Romeo, & McEwen, 2003). Through the use of neonatal novelty exposure (Tang, 2001; Tang et al., 2006), an early life manipulation procedure that utilizes a split-litter design, one is able to isolate the novelty component from other

confounding factors, including experimenter handling, maternal handling and separation, and maternal stress, present in the more commonly used neonatal handling designs (Denenberg, 1964; Levine, 1957). Specifically, during this procedure half of the pups in a litter are exposed to a novel non-home environment for 3-min each day during the first 3 weeks of life while their siblings remain in the home cage. These brief and transient daily repeated exposures to novelty have been shown to affect the development of learning and memory (Reeb-Sutherland & Tang, 2011; Tang, 2001; Tang et al., 2003; Yang & Tang, 2011), hippocampal receptor sensitivity (Zou, Golarai, Connor, & Tang, 2001), and the stress response (Tang et al., 2006; Tang et al., 2003). Those neonates who were exposed to novelty early in life displayed lower basal CORT in adulthood (at 16-months) compared to their control littermates (Tang et al., 2003), and were able to mount an additional CORT response to a surprise stressor when already stressed (Tang et al., 2006).

Whereas early life experience has been shown to influence later outcome measures, it is unclear whether this is a critical period or whether similar interventions can have the same effects later in life. Therefore, it has been questioned whether early adulthood novelty exposure would have the same effect as neonatal novelty exposure. Recent studies examining the effects of early adulthood novelty exposure found that early adulthood novelty-exposed rats show a right turning bias at 15 months (Tang, Reeb-Sutherland, & Yang, 2011) and enhanced memory at 5 months (Yang & Tang, 2011) compared to their non-novelty-exposed home littermates. A potential underlying mechanism influencing this adulthood novelty-induced memory enhancement is subsequent alterations of the stress response system. It has been suggested that increased

density of the glucocorticoid receptors (GRs) in the hippocampus is related to a better adaptive stress response system and enhanced memory performance (de Kloet, Oitzl, & Joëls, 1999; Lupien & McEwen, 1997; Meaney et al., 1988). Neonatal novelty exposure has been shown to induce greater suppression of long-term potentiation (LTP) (Zou et al., 2001) which is likely caused by higher GR density which may underlie memory enhancements in neonatal novelty-exposed animals (Reeb-Sutherland & Tang, 2011; Tang, 2001; Tang et al., 2006; Tang, Reeb-Sutherland, Yang, Romeo, & McEwen, 2011). Given that similar enhancements of memory are induced by adulthood novelty exposure, it is probable that early adulthood novelty exposure also influences GR density as well as subsequent HPA output (i.e., CORT levels). To examine this possibility, the effects of early adulthood novelty exposure on HPA function were examined in the current study.

Whereas both the maternal and non-maternal environments influence the offspring's HPA function, it remains unclear how these influences may interact jointly to affect the offspring's HPA function. Interactions between maternal stress hormone levels and novelty exposure have been shown to affect offspring behavioral inhibition (Tang, Reeb-Sutherland, Romeo, & McEwen, 2012), early growth (Tang, Yang, Reeb-Sutherland, Romeo, & McEwen, 2012), and spatial memory (Tang, Reeb-Sutherland, et al., 2011). In these studies, maternal stress hormone levels modulated the effect of novelty exposure, such that offspring of mothers with better self-stress regulation (lower basal and high evoked CORT levels) displayed greater novelty-induced effects. These studies lend support for the maternal modulation hypothesis which states that the mother sets the context for how the offspring respond to their given environment (Smotherman, 1983). Specifically, the above studies demonstrated that mothers with lower basal

circulating CORT had novelty-exposed offspring with enhanced spatial memory (Tang, Reeb-Sutherland, et al., 2011), decreased behavioral inhibition (Tang, Reeb-Sutherland, et al., 2012), and greater body weight at weaning (Tang, Yang, et al., 2012) compared to their control littermates. These findings imply that maternal stress hormone levels may set the context for the novelty exposure effect. Differences in HPA function are known to influence behavioral inhibition (Fox, Henderson, Marshall, Nichols, & Ghera, 2005; Macrì, Zoratto, & Laviola, 2011), memory (de Kloet, Oitzl, & Joëls, 1993; Lupien & McEwen, 1997), and early growth (Dallman, 2010). Therefore, novelty-induced changes in HPA function may underlie these previous findings and may similarly interact with maternal context, specifically maternal self-stress regulation.

The goal of the current study is to examine whether novelty affects the development of offspring stress regulation. It was hypothesized that the effect of both neonatal and early adulthood novelty exposure will vary with individual differences in measures of maternal stress regulation. Previous studies reported significant relations between maternal basal and evoked CORT measures and offspring behavioral measures (Catalani et al., 2002; Catalani et al., 2000; Catalani, Marinelli, Scaccianoce, Nicolai, Muscolo, Porcu, Koranyi, et al., 1993; Macrì et al., 2009; Macrì et al., 2007). Maternal basal circulating CORT levels represent the levels of stress hormones when the animal is not threatened by a stressor and maternal rapid-evoked CORT levels represent how quickly an animal is able to respond to a stressor. These CORT measures can be used as a trait measure (Marquez, Nadal, & Armario, 2005; Tang, Reeb-Sutherland, et al., 2012) for the individual. These measures will be used to give a more complete profile of HPA function for both the mother and her offspring

METHODS

Neonatal Novelty Exposure.

Ninety-four male offspring from nineteen dams were used in this study. From post-natal day (PND) 1-20 (**FIG. 1A**), the neonatal novelty exposure procedure (Tang, 2001; Tang et al., 2006; Tang et al., 2003) was implemented. In order to carry out this procedure, the dam was first removed from the home cage and placed in a separate holding cage. Next, half of the litter was removed from the home cage and placed in a novel non-home cage for 3-min daily (Novel group), while the rest of the litter was kept in the home cage (Home group; **FIG. 1B**). To control for differences in experimenter handling, every time a Novel pup was picked up, a Home pup was also picked up and placed back into the home cage. This procedure is in marked difference from the popular neonatal handling method (Denenberg, 1964; Levine, 1957; Meaney et al., 1988) because it isolates the effects of novelty exposure from experimenter handling, maternal separation, and maternal stress by matching the amount of time both Novel and Home animals were handled by the experimenter and by separating the dam from both Novel as well as Home animals. Pups were weaned from their mothers on PND 21. For a more complete description of neonatal novelty exposure, see the supplemental methods.

Early Adulthood Novelty Exposure.

From PND 54-63 (**FIG. 1A**), the early adulthood novelty exposure procedure was implemented. In order to carry out this procedure, half of the neonatal Novel and half of the neonatal Home rats from each litter were removed from the housing room, transferred from their home cage to a novel open field environment for 3-min daily, and then returned to their home cage and housing room. The other half of the neonatal Novel and

Home siblings from each litter remained at home, resulting in four separate groups: those exposed to novelty only during infancy (NH), those exposed to novelty only during early adulthood (HN), those exposed to novelty during both infancy and early adulthood (NN), and those never exposed to novelty (HH; **FIG. 1C**). This later novelty exposure is not meant to exactly replicate the neonatal novelty exposure procedure, but is meant to give the animals a brief repeated stressor, like that which pups received with neonatal novelty exposure. Similar to neonatal novelty exposure, the amount of handling was controlled between the NH and NN groups. For a more detailed account of the procedure, see the supplemental methods.

Assessing Maternal Stress Regulation.

Maternal self-stress regulation measures were obtained on PND 28 and PND 29, seven and eight days after weaning (**FIG. 1A**). In order to fully assess maternal stress response, both basal (PND 28) and evoked CORT (PND 29) were analyzed from blood samples. Basal CORT ($CORT_B$) is defined as the CORT level obtained when the animal is in an undisturbed state, while evoked CORT ($CORT_E$) is collected shortly after a 1-min swim stressor (measured within 5-min after stressor). The $CORT_E$ measure is designed to capture the initial rising phase of the CORT response and is operationally defined as the percentage of CORT increase relative to $CORT_B$. Both measures are intended to reflect trait markers of the stress response, as they have been used as predictors for other behavioral and endocrine measures for not only the individual, but also for future generations (Marquez et al., 2005; Tang, H. Jiang, et al., 2011; Tang et al., 2003; Tang, Reeb-Sutherland, et al., 2012; Tang, Reeb-Sutherland, et al., 2011; Tang, Yang, et al., 2012). Seventeen of the 19 dams' basal and 16 of the 19 dams' evoked CORT samples

were characterized. For details on the collection procedure, see the supplemental methods.

Assessing Offspring Stress Regulation

The offspring's self-stress regulation was assessed using the same procedure as that used for maternal self-stress regulation. Blood samples were collected similarly on two consecutive days, at 13 months of age (**FIG. 1A**). CORT samples (both basal and evoked) from both Novel and Home rats were obtained from 15 of the 19 litters. For more information, see the supplemental methods.

Data Analysis.

Analysis of covariance (ANCOVA) was used to investigate how postnatal maternal self-stress regulation (maternal $CORT_B$ and $CORT_E$) interacts with neonatal and early adult novelty exposure to influence offspring's self-stress regulation (offspring $CORT_B$ and $CORT_E$). Litters were used as the unit of analysis with Neonatal Novel (NH), Adult Novel (HN), and the Combined Novel (NN) groups compared to the Home (HH) group. The two offspring CORT measures were the within factors and the two maternal CORT measures were the covariates; each covariate was separately investigated and ANCOVAs were run both before and after centering the variable. Centering the variable moves the mean of the covariate to the y-intercept, thus disambiguating the results. The data reported in the results section is from the centered maternal covariates. Using bag-plots (Rousseeuw, Ruts, & Tukey, 1999; Wolf, 2007), data were first checked for any violation of assumptions of normality and heterogeneity of variance. Outliers in the original data were detected, and various transformations were examined to determine the appropriate transformation for the final analysis. The transformations used were:

replacement with the maximum or minimum accepted value, replacement with the average, or square root transformation (where appropriate).

RESULTS

Neonatal Novelty Effect on Offspring CORT Measures

To quantify the within-family effect of neonatal novelty exposure on offspring self-stress regulation, for each litter, a novelty score (NE score) was computed for both basal and evoked CORT. This score is defined as the difference between the average CORT of all Novel pups and average CORT of all Home pups within that litter ($Litter\_AVG_{Novel}$ - $Litter\_AVG_{Home}$) (**FIG. BD**). Positive NE scores for $CORT_E$ and negative NE scores for $CORT_B$ reflect novelty-induced enhancement in the stress response in a given rat family (i.e., greater response and lower resting levels among Novel than Home siblings). Negative NE scores for $CORT_E$ and positive NE scores for $CORT_B$ reflect novelty-induced impairment (i.e., lesser stress response and higher resting levels among Novel than Home siblings). There is a wide range of neonatal novelty exposure effects across rat families were observed, with a majority showing a positive effect (**FIG. 2** left of dashed line for $CORT_E$ and right of the dashed line for $CORT_B$) and a minority showing a negative effect of a smaller magnitude (**FIG. 2,** right of dashed line for $CORT_E$ and left of the dashed line for $CORT_B$). There was a significant main effect of novelty exposure for $CORT_E$ (N=12, $F(1, 11)$=3.41, p=0.0475 one-tailed, f=1.486 (**FIG. 2BD**) — but no significant main effect was found for $CORT_B$ (N=13, $F(1,12)$=0.985, p>0.1 one-tailed, f=0.118) (**FIG. 2BD)**.

Interaction between Maternal Stress Response and Neonatal Novelty

In examining whether the mother's CORT levels (both basal and evoked) significantly affected the offspring's response to novelty exposure, it was found that when maternal $CORT_E$ was used as the covariate and offspring $CORT_E$ was used as the dependent measure, there was a significant interaction between maternal $CORT_E$ and the effect of neonatal novelty exposure on offspring $CORT_E$ (N=12, $F(1,11)$=5.078, p=0.048, f=1.933) (**FIG. 3D**). However, when offspring $CORT_B$ was used as the dependent measure, no significant interaction was found (N= 14, $F(1, 13)$=2.035, p>0.1, f=0.980) (**FIG. 3C**). When maternal $CORT_B$ was used as the covariate, no significant effect was found for either offspring $CORT_E$ (N=13, $F(1,12)$=3.356, p>0.05, f=1.475) or $CORT_B$ (N=14, $F(1,13)$=2.174, p>0.1, f=1.044) (**FIG. 3AB**). Therefore, the effect of neonatal novelty exposure on offspring $CORT_E$ varies according to maternal $CORT_E$, but not $CORT_B$.

Early Adulthood Novelty and Combined Novelty Effect on Offspring CORT Measures

No main effect of early adulthood (all ps> 0.15) or combined novelty (all ps>0.1) was found for offspring CORT levels. In addition, there was no interaction between maternal CORT levels and novelty for either of these novelty-exposed groups (all HN ps>0.50, all NN ps>0.7). Therefore, neither of these groups will be further discussed.

DISCUSSION

In a cohort of rat dams and their male offspring (dams: N=19, offspring: N=94), neonatal novelty was found to significantly increase adult offspring's rapid evoked CORT response, and this effect was positively correlated with the mothers' rapid evoked CORT response. Specifically, we found that those pups whose rapid evoked CORT response was increased by novelty, had mothers who had more adaptive self-stress

regulation (low $CORT_B$ and high $CORT_E$).

Maternal Modulation of Neonatal Novelty

Maternal and non-maternal environmental influences were investigated to determine whether they jointly affect the development of one's ability to regulate their own stress response. The novelty exposure effect was found to be differentially modulated by the maternal stress response. If the mother had a quick stress response (high rapid evoked CORT response), then her offspring were more likely to be positively influenced by early stimulation as adults. Conversely, if the mother had a slow stress response (low rapid evoked CORT response), then her offspring were more likely to be negatively influenced by early stimulation. The mother's ability to mount a quick response to stress modulates both the magnitude and the direction of the change induced by novelty exposure. Therefore, the mother plays a key role by helping to create the contextual background environment, which influences the individuals' response to future new situations (Boyce & Ellis, 2005; Essex, Klein, Cho, & Kalin, 2002; Stiller, Drugan, Hazi, & Kent, 2011).

Previously, it was shown that both the amount of maternal care (Francis et al., 1999; Liu et al., 1997; Weaver et al., 2004) and the consistency of maternal care (Akers et al., 2008) affect the offspring's stress response system. Here, similar to findings by both Catalani and colleagues (1993) and Macri and colleagues (2007), we see that in addition to maternal care, maternal physiology influences the offspring's physiology later in life. Together, these results suggest that the mother is a complex source of influence in the development of her offspring. To understand the full maternal influence on offspring

stress regulation, both maternal care and maternal physiology should be taken into consideration.

This maternal modulation finding not only provides converging evidence with previous offspring behavioral findings (Tang, Reeb-Sutherland, et al., 2012; Tang, Reeb-Sutherland, et al., 2011; Tang, Yang, et al., 2012), demonstrating that the mother's stress response sets the context for the early stimulation effect on offspring adult stress response, but also provides a possible mechanism for these previous findings. The stress response is influenced by the number of GRs present. Zou and colleagues' (2001) found that pups exposed to novelty have greater LTP suppression and therefore possibly higher GR density than Home pups. This higher GR concentration which is known to affect memory performance (de Kloet et al., 1999) may account for previously observed novelty-induced enhancement in spatial memory (Reeb-Sutherland & Tang, 2011; Tang, 2001; Tang et al., 2006; Tang, Reeb-Sutherland, et al., 2011). Since the maternal stress response (by changing the environmental context) affects how the offspring respond to new situations, the mother should be considered when examining the effects of early environmental stimulation.

Novelty Exposure-Induced Changes in Rapid Stress

The results from this study suggest that brief repeated exposure to novelty early in life enhances an individual's ability to regulate their own CORT response in mid-adulthood. We found a selective novelty-induced enhancement of offspring rapid evoked CORT, but not offspring basal CORT. This implies that the basal and evoked CORT are two distinct aspects of the HPA system. Therefore, it should be considered how this evoked CORT measure is unique from both basal and other evoked CORT measures. The

evoked measure used here is different from other evoked CORT measures in that: 1) it is proportionate to the basal measure for the animal, and 2) the blood sample is collected very soon after the stressor. One reason to use an evoked measure that is proportionate to the individual's basal CORT measure is that the neuronal response to heightened CORT levels is likely dependent upon the baseline levels of CORT. By making the measure proportionate, we are able to measure the relative rise in CORT. Also, because the measure is taken almost immediately after exposure to a stressor, the animal's CORT level is still rising and has not yet reached the peak CORT response. Therefore, in this case a high-evoked CORT response is desirable and indicative of being in control and having better adaptive stress regulation (Levine, 1960; Rose, Jenkins, Hurst, Herd, & Hall, 1982; Rose, Jenkins, Hurst, Kreger, et al., 1982; Rose, Jenkins, Hurst, Livingston, & Hall, 1982). To get a complete picture of the effect of a manipulation or intervention upon an individual's HPA-axis, both basal and evoked CORT should be analyzed.

Potential Relevance to Health Problems and Anxiety Disorders

The environmental influences that affect the HPA-axis function are important because of the health and behavioral implications. How an individual responds to a perceived threat is important to health (Harris & Seckl, 2011; Sapolsky, 2004), cognition (Lupien & McEwen, 1997), and behavior (Fox et al., 2005; Macrì et al., 2011). Being exposed to high circulating levels of CORT over long periods of time decreases the body's immune response, raising the chance of disease (Jemmott & Locke, 1984; Krantz, 1981; McKinnon, Weisse, Reynolds, Bowles, & Baum, 1989). Exposure to high levels of CORT also increases the chance that individuals will have a lower birth weight (Seckl, 1997), which increases the likelihood of the infant having both psychological and

physical illness (Barker et al., 1993; Famularo & Fenton, 1994; Jones, Rantakallio, Hartikainen, Isohanni, & Sipila, 1998; Räikkönen et al., 2008; Thompson, Syddall, Rodin, Osmond, & Barker, 2001; Wiles, Peters, Leon, & Lewis, 2005). High levels of baseline CORT can also impair memory (de Kloet et al., 1993; Lupien & McEwen, 1997) and increase behavioral inhibition, which is a defining feature of a temperament type related to the development of anxiety disorders (Biederman et al., 1993; Degnan, Almas, & Fox, 2010; Degnan & Fox, 2007; Pérez-Edgar & Fox, 2005; Schwartz, Snidman, & Kagan, 1999).

Since it is known that abnormal levels of CORT are related to a number of health and mental illnesses, it is important to identify what may affect CORT levels, especially leading to an enhancement of the HPA axis. Here, using an animal model, it was observed that brief and transient exposures to novelty repeated for a relatively short period of time have long-lasting, positive effects on the stress response for those offspring with mothers who are able to respond quickly to a stressor.

While some human studies have demonstrated that maternal stress hormone levels relate to the offspring's stress response (Davis, Glynn, Waffarn, & Sandman, 2011; Feldman et al., 2009; Papp, Pendry, & Adam, 2009), and that novel situations contribute to the stress response (Sumner, Bernard, & Dozier, 2010), to our knowledge, no studies have jointly looked at these contributing factors to see how they interact to affect the development of the stress response in children. Based upon human and non-human studies on the maternal influence of the offspring stress response and other behavioral characteristics, it seems promising that family-based and maternally-centered interventions may be successful in reducing anxiety-related disorders.

Limitations

Some limitations should be taken into account when discussing the findings from the current study. First, the current study only examined the effects of maternal self-stress regulation on novelty exposure-induced changes in male offspring. Therefore, the current findings are unable to be generalized to female rats. Given that early life manipulations differentially affect males and females (Frankola et al., 2010; Kosten, Lee, & Kim, 2006, 2007; Lehmann, Pryce, Bettschen, & Feldon, 1999), it is possible that maternal self-stress and novelty exposure would interact differently in the development of female HPA function.

Both genetics (Jiang, Wang, Luo, & Li, 2009) and prenatal maternal stress exposure (Weinstock, 2008) have been shown to influence offspring stress levels. Whereas the results from the current study allow us to make claims about the combined effect of the post-natal maternal stress response and neonatal novelty exposure on offspring stress response, we are unable to address the relative contributions of environmental and genetic interactions or the effect of prenatal maternal CORT exposure. The findings discussed here investigated post-natal maternal stress as another part of the maternal influence story, which is shown to also affect the offspring's stress response in adulthood. In future studies it would be beneficial to look at the relative contributions of both pre- and post- natal maternal stress levels.

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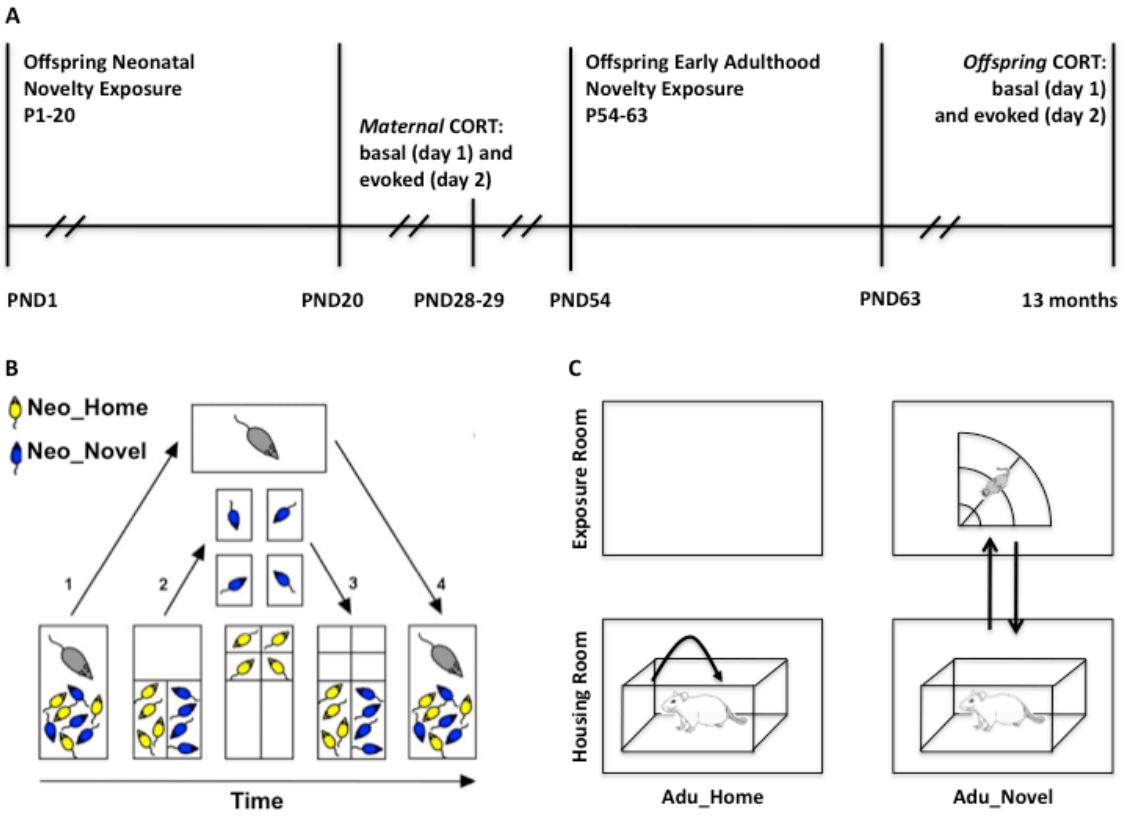
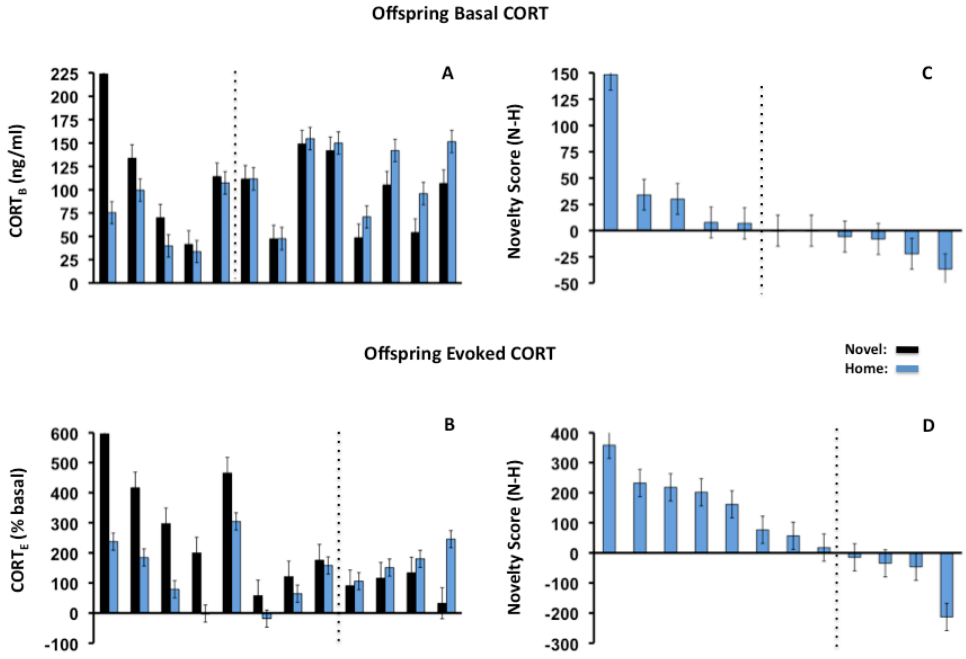
FIG 1., *Time*line and Protocol Diagrams



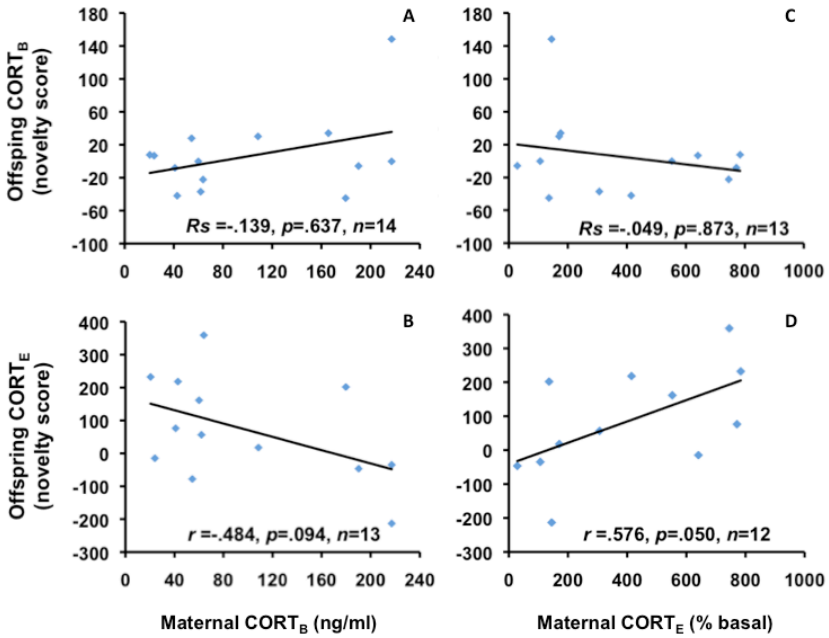
*Exp*erimental Methods. Experimental Timeline (**A**). Offspring neonatal novelty exposure: PND 1-20, Offspring early adulthood novelty exposure: PND 54-63, Maternal corticosterone (CORT) collection: PND 28-29, Offspring CORT collection: 13 months. Steps of Neonatal Novelty Exposure within litter design (**B**). These steps were followed for each litter: ***i.*** remove the dam from the home cage, ***ii.*** transfer novel pups to small individual cages, ***iii.*** return the novel pups to the home cage after 3 min of exposure to the non-home cages, ***iv.*** return the dam to the entire litter in the home cage. Steps of Early Adulthood Novelty Exposure (**C**). Right: Novel rats were transported to the exposure room and placed in the open field for 3-min; Left: Home rats were picked up twice and replaced back into their home cage in the housing room.

FIG 2., Family-to-Family Variations of the Novelty Effect



Family-to-family variations in novelty exposure effects on offspring stress response measures. (**A-B**) Litter averages for Novel (black bars) and Home (blue bars) animals, and (**C-D**) litter-based novelty effect scores (Litter_AVG$_{NOVEL}$ - Litter_AVG$_{HOME}$). Novelty exposure decreased basal corticosterone (CORT$_B$) in a majority of the litters (**A&C,** 7 litters to the right of the dashed line, n.s.) and increased evoked corticosterone (CORT$_E$) in a majority of the litters (**B&D,** 8 litters to the left of the dashed line, significant 1-tailed).

FIG 3., Maternal Influence of Family-to-Family Variations of the Novelty Effect



Associations between basal ($CORT_B$) and evoked ($CORT_E$) corticosterone measures of maternal self-stress regulation and family-to-family variations in novelty effect on offspring $CORT_B$ and $CORT_E$ measures of stress-regulation. (**A, C**) For measures of offspring $CORT_B$, there were no significant associations between maternal measures of self-stress regulation and novelty effect. (**B, D**) For measures of offspring $CORT_E$, there was a significant positive association between novelty-induced enhancement and maternal $CORT_E$ and a negative trend between novelty-induced enhancement and maternal $CORT_B$.

APPENDIX

Appendix A, Suppelmentary Methods

A. Supplementary Methods

Neonatal Novelty Exposure.

The neonatal novelty exposure procedure was created to eliminate possible confounds in Levine's (1960) classic neonatal handling procedure. Due to its between-litter design, the neonatal handling procedure creates a situation where pups in the handled group differ from the non-handled control pups by the amount of: 1) experimenter handling; 2) separation from the mother; 3) novelty associated with being removed from the familiar home environment and place in a new environment; and 4) maternal stress. Therefore, it is hard to identify which of these above aspects are responsible for the handling effect.

Recently, the novelty effect has been isolated by implementing neonatal novelty exposure, which employs a within-litter design where both the control and novelty-exposed siblings receive matching amounts of maternal separation/stress and experimenter handling and only differ by their exposure to a novel non-home environment. Neonatal novelty exposure has been shown to have a wide range of long-lasting effects during adulthood (Akers et al., 2008; Tang, 2001; Tang et al., 2006; Tang et al., 2003; Tang, Yang, et al., 2012; Yang & Tang, 2011; Zou et al., 2001).

To further investigate these novelty exposure-induced effects, we exposed individual rat pups to a novel cage for 3 minutes per day during the first three weeks of their life (Novel-NH) while their littermates remained in the home cage, but separated from their siblings (Home-HH). On PND 1, approximately one-half of each litter was pseudo-randomly assigned to Novel and the other half to Home conditions. The dam was first removed from the home cage. Then the Novel and Home pups were identified by

examining previously tattooed toe markings. Once identified, Novel rats were placed in a new cage lined with fresh sawdust, the normal bedding used in the home cages, for their 3-min exposure and subsequently returned to their home cage in which the Home rats remained. During this transfer, each Novel pup was yoked to a Home pup that received a similar amount of experimenter contact at approximately the same time as the Novel pup. The dam was returned to the litter after both the Novel and Home pups were joined in the home cage (for a diagram of the procedure see **Fig. 1B**). The amount of experimenter handling and the duration of maternal separation during this neonatal novelty exposure procedure were matched between the Novel and Home rats. This ensured that any differences between the stress regulation of the two groups was attributable to neither the separation from the dam nor the touching by the experimenter. The current study included 19 litters and 94 male offspring (Novel *N*=49, Home *N*=45).

Early Adulthood Novelty Exposure.

From PND 54–63, between 1200 and 1700h, half of the neonatal novelty-exposed and half of the home-staying rats in each litter were exposed to an additional novel experience in a sectored open field (radius: 75 cm) for 3-min daily. If a rat was in the early adulthood Novel group (HN or NN), it was first removed from the housing shelf to the transporting cart and then transported to a separate room for novelty exposure. As many as four rats were transported together and individually placed and remained in one of the four separate sectors of a circular open field for 3-min before being returned to the housing room (**FIG. 1C**). If a rat was assigned to the early adulthood Home group (NH, HH), it was also removed from the housing shelf to the transporting cart where it was twice picked up and replaced to the other end of their own home cage to match the

amount of handling to be experienced by the adult Novel rats during their exposure to the sector open field. The adult Home animals were returned directly to their housing shelf after this handling. Within each litter, the adult Home rats were first handled within the housing room and then the adult Novel rats were exposed to the sector open field. The orders of neonatal Home and neonatal Novel groups were counter-balanced within the adult Home and adult Novel groups.

Assessing Maternal Stress Regulation.

Blood samples were collected between 1300 and 1700h to measure both maternal basal CORT ($CORT_B$) and post-swim circulating CORT ($CORT_S$). Blood samples were collected via a tail-nicking procedure, which lasted between one to three minutes. All blood was collected in the same Blood Collection Room. All animals were kept in their home cage in a separate Holding Room and returned to the Housing Room only after all animals were tested, thus avoiding any potential effects of odor and ultra-sound signaling on the remaining rats. For basal samples, animals were transported from the Housing Room directly to the Blood Collection Room, while for the evoked CORT samples, animals were transported from the Swim Test Room to the Blood Collection Room 5-min after the onset of the 1-min swim (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: $[(CORT_S - CORT_B)/ CORT_B \times 100]$. The high $CORT_E$ measure used here is conceptually and operationally different from the "high CORT" measure used in Lupien et al (2000), where the CORT measure was obtained during children's regular class hours, which is neither resting nor

evoked by explicit and discrete event as was done in the current study. See Tang et al. (2012) for the full explanation of the differences between these evoked measures.

Each sample contained approximately 200 μL of blood and was centrifuged. The plasma was removed and stored at -20˚ C until the radioimmunoassay was performed. Plasma CORT concentration was measured in duplicate using the Coat-a-Count CORT Kit (Diagnostic Products, Los Angeles, CA). The lower limit of detectability for the mothers was 10.1 ng/ml, and the intra-assay coefficient of variation was 4.8%. The experimenter was blind to the sample identity. Out of the nineteen mothers who were sampled blood, basal CORT samples were obtained from seventeen and evoked CORT samples from sixteen.

Offspring Stress Response.

Before blood collection, the animals were tested to assess functional brain asymmetry and spatial memory at approximately 5 months of age (Tang et al., 2011; Yang & Tang, 2011). CORT collection at 13 months was chosen because it reflected our interest in assessing offspring stress regulation later in life and because of logistical constraints. From a total of 94 offspring alive at 13 months of age, 91 samples were collected under the basal condition ($CORT_B$) and 89 after the one-minute swim ($CORT_S$). The samples were processed and collected in the same manner as the maternal samples, with the lower limit of detectability at 9.5 ng/ml, and the intra-assay coefficient of variation at 5.4%.