Reducing Behavioral Inhibition to Novelty via Systematic Neonatal Novelty Exposure: The Influence of Maternal Hypothalamic-Pituitary-Adrenal Regulation

Akaysha C. Tang, Bethany C. Reeb-Sutherland, Russell D. Romeo, and Bruce S. McEwen

Background: Behavioral inhibition (BI) to novelty is thought to be a stable temperament type that appears early in life and is a major risk factor for anxiety disorders. In the rat, habituation of such inhibition can be facilitated via neonatal novelty exposure (NNE), thus reducing BI to novelty. Here, we tested the hypothesis that this early intervention effect is modulated by the context of maternal self-stress regulation.

Methods: The NNE was carried out during postnatal days 1–21, in which one half of each litter was exposed to a relatively novel nonhome environment for 3-min daily while the remaining one half stayed in the home cage. After weaning, BI to novelty was assessed in an open field with a measure of disinhibition defined as a greater increase in exploration across two brief trials. Maternal context was characterized by trait measures of hypothalamic-pituitary-adrenal (HPA) axis reactivity, including basal and stress-evoked corticosterone (CORT) responses.

Results: Family-to-family variations in the NNE effect were associated with variations in maternal HPA function—a low-basal CORT and high-evoked CORT response profile constituting the context for a novelty-induced facilitation of disinhibition (i.e., a greater increase in exploratory activity over repeated trials) and an opposite HPA profile constituting the context for a novelty-induced reduction of disinhibition.

Conclusions: This result is consistent with the hypothesis that maternal self-stress regulation modulates the effect of early life intervention on BI to novelty and suggests that effective interventions should include strategies to help mothers improve their self-stress regulation.

Key Words: Behavioral inhibition, early experience, maternal modulation, neonatal handling, novelty, open field

When encountering novelty, humans as well as nonhuman animals typically show behavioral withdrawal. As the novel situation becomes familiar over time, ongoing behaviors are expected to resume. Although this initial behavioral inhibition might be viewed as adaptive due to potential threats associated with a novel and uncertain environment, prolonged or heightened behavioral inhibition to the once-novel entity might become maladaptive. In humans, this heightened tendency to withdraw from novelty in a physical or social environment (1,2) is the defining feature of a specific temperamental type known to be associated with anxiety disorders (3–7). Yet due to obvious ethical constraints, animal models are needed to tease apart causal factors, ultimately leading to a better understanding of the brain mechanisms and underlying neurochemical and molecular factors linked to the manifestation of anxiety in humans. One common theme emerging from parallel human studies and animal models is an association between the hypothalamic-pituitary-adrenal (HPA) function of an individual and an inhibited behavioral phenotype (8–14), suggesting that individual differences in stress responsiveness are part of the underlying mechanisms related to both normal and abnormal novelty responses. Yet, relatively little is known with regard to what specific early life interventions can promote normal novelty responses by reducing the otherwise abnormal behavioral inhibition in novel situations.

One promising lead comes from the work of Denenberg *et al.* (15–17) nearly a half century ago. With the neonatal handling procedure as an early life stimulation paradigm, they showed that rats that received early "handling treatment" displayed higher activity levels compared with those that received the "nonhandling treatment" in the open field, an environment considered to be novel relative to the familiarity of the home cage. Such greater activity level could be construed as an indicator for less behavioral inhibition, thus suggesting that behavioral inhibition to novelty might be reduced via early life intervention. The use of this encouraging finding was initially limited, because the "handling treatment" is a combination of several confounded factors as discussed in classic (18,19) as well as recent (20–22) reviews of the literature. Due to the nature of the between-litter design of the handling procedure, pups from the handled litters differ from pups from the nonhandled litter by receiving additional: 1) experimenter handling; 2) separation from the mother; 3) experience of novelty associated with brief exposures to a nonhome cage and activates the HPA axis (23,24); and 4) maternal stress, which might acutely activate her HPA axis, subsequently activating the HPA axes of her pups via her milk (25,26). This compounded treatment makes it difficult to determine which one or combination of the aforementioned produces the desirable effect of reduced behavioral inhibition in the open field.

More recently, the effect of the novelty component has been isolated via the use of a within-litter design with the novelty-exposed and their matched control siblings sharing the same mother and having matched maternal separation and experimenter handling ([20–22,27,28] and Supplement 1). Systematic brief neonatal exposures to novelty afforded by a nonhome environment induced greater disinhibition (i.e., reduction in inhibition

From the Department of Psychology (ACT, BCR-S); Department of Neurosciences (ACT), University of New Mexico, Albuquerque, New Mexico; Department of Human Development (BCR-S), University of Maryland, College Park, Maryland; Department of Psychology and Neuroscience and Behavior Program (RDR), Barnard College; and the Laboratory of Neuroendocrinology (BSM), Rockefeller University, New York, New York.

Address correspondence to Akaysha C. Tang, Ph.D., Department of Psychology, Logan Hall, University of New Mexico, Albuquerque, NM 87131; E-mail: akaysha@unm.edu.

Received Jan 4, 2012; revised Feb 28, 2012; accepted Mar 18, 2012.

0006-3223/$36.00
http://dx.doi.org/10.1016/j.biopsych.2012.03.021

defined by an in increase in activity) over repeated exposures to a novel environment in offspring compared with their home-staying siblings from the same mother (20,21,29). This novelty effect on behavior was paralleled by a novelty-related reduction in basal corticosterone (CORT) concentration (27) and increase in CORT response to unexpected changes in the environment (22). Nonhuman primate studies also found that repeated time away from the familiarity of the home environment led to greater habituation of fearful responses to repeated novelty tests accompanied by a low plasma cortisol concentration (30). Finally, human developmental studies have recently offered initial evidence that children who are exposed to daycare, which inevitably involves increased exposure to novelty and surprises, are more likely to transition from being behaviorally inhibited to noninhibited (31). These cross-species findings raise the possibility that small doses of stress induced by systematic early exposures to novelty might offer a form of inoculation to future novelty-related stress, thereby curtailing the prevalence of prolonged behavioral inhibition to novel situations. This notion that early life experience of stress can have a long-lasting positive impact is currently under-explored (30) and forms a sharp contrast with the overwhelmingly large body of literature documenting detrimental effects of early life adversity (32).

One challenge in translating findings in animal models to solutions for developmental psychopathology in humans is the rich context of the human maternal environment that is clearly multidimensional, including, at the very least, the ability of the mother to regulate her own stress response system in addition to her behavior toward her infants. This complexity has received some attention but is clearly under-investigated in current animal models of human psychopathology. For example, although rodent noncorrelational studies have demonstrated that individual differences in maternal circulating stress hormone concentration have a causal influence on the HPA function of offspring that is dissociable from the quantity of maternal care (25,26,33), correlations between maternal care quantity and offspring developmental differences continue to be interpreted as evidence for maternal care being the causal factor (34–37). As an alternative to this maternal care-centric view, an integrative view assumes that both the maternal physiological and behavioral environment and nonmaternal environment converge on the HPA axis of the offspring to jointly program its development (38). According to this integrative view, the mother is thought to modulate the impact of nonmaternal aspects of the neonatal environment by setting up a unique physiological context to enable the same early intervention to produce distinct effects from one rat family to another.

Here, after the report of interaction effects between maternal HPA function and early stimulation effect via novelty exposure on physical and cognitive development (28,38), we further test this maternal modulation hypothesis with offspring emotional development as an endpoint. Specifically, we conducted a follow-up analysis of a previously reported population-level finding with regard to novelty exposure-induced reduction in behavioral inhibition to novelty (21) to test a similar interaction effect on measures of change in open field activity. It should be pointed out that we previously considered the role of maternal care but found evidence for neither preferential maternal care toward the novelty-exposed offspring compared with their home-staying siblings (21) nor a significant modulation effect by maternal care quantity (39). The present study specifically emphasizes the importance of viewing neonatal experience of novelty and maternal circulating CORT as two interacting factors and focuses on uncovering the conditionality of an early intervention effect upon the self-stress regulation of the mother.

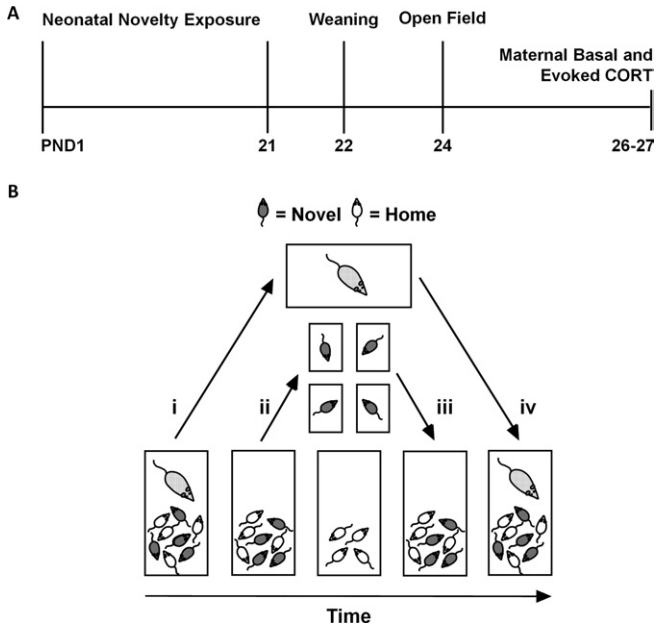

Figure 1. (A) Timeline of the study. **(B)** Sequential steps in neonatal novelty exposure with a split-litter design. For each litter: i) removal of dam from home cage; ii) transfer of Novel pups (solid color) to individual nonhome cages (small rectangles); iii) return of Novel pups to home cage after 3-min exposure to nonhome cages; and iv) return of the dam to the entire litter in home cage. CORT, corticosterone; PND, postnatal day.

Methods and Materials

Neonatal Novelty Exposure

Long Evans male offspring ($n = 106$) from 22 dams (Harlan, Indianapolis, Indiana) participated in the present study. During postnatal days 1–21 (Figure 1A), the neonatal novelty exposure (NNE) procedure was carried out in the animal housing room and involved taking a subset of pups away from the familiarity of their home cage for 3 min daily (Novel) while their matched control siblings (Home) remained in the home cage (Figure 1B). With this split-litter or within-litter design, the novelty exposure effect is isolated from the effects of experimenter handling, maternal separation of the pups, and maternal stress ([20–22,27,28] and Supplement 1).

Assessing Maternal Individual Differences in HPA Function

Following the early research of Levine (40) as well as research on baboons (41,42) and air traffic controllers (43–45) showing that a robust CORT response from a low basal level is an index of a sense of control and good stress regulation, we focused on two aspects of maternal HPA function, the maintenance of the basal CORT level and the ability to mount a timely response to a stressor. The basal CORT measure ($CORT_B$) is operationally defined here as that obtained in an undisturbed state. The stress-evoked CORT response measures ($CORT_E$) are aimed at capturing the rising phase of the CORT response (38) and are defined as the percentage of CORT increase relative to $CORT_B$ at 5, 15, and 30 min after the dam was exposed to a 1-min swim stress ($CORT_{E5}$, $CORT_{E15}$, $CORT_{E30}$). Both CORT measures are intended to serve as trait as opposed to mere state measures in the following sense: 1) they reflect the ability of the dam to regulate her own level of circulating stress hormone; and 2) they have been used as markers or predictors for other behavioral and endocrine measures within the same individual

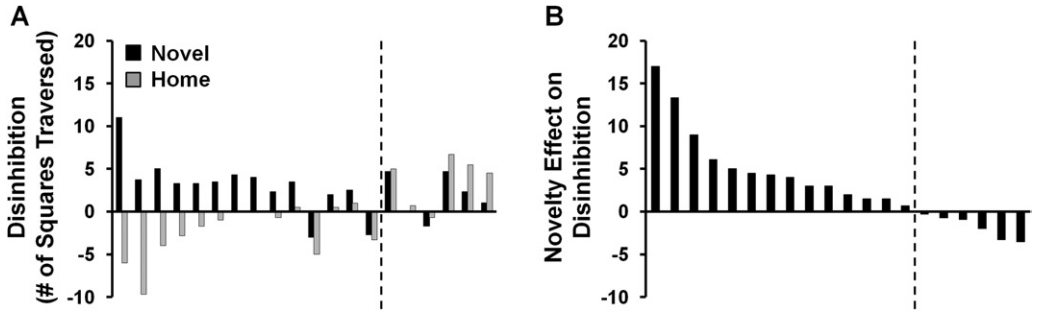

Figure 2. Family-to-family variations in novelty exposure effects in open field disinhibition: novelty exposure increased disinhibition in a majority (14 litters, left of dashed line) and decreased disinhibition in a minority of litters (6 litters, right of dashed line). (**A**) Litter averages for Novel (black bars) and Home (gray bars) animals, and **(B)** litter-based novelty effect scores ($Litter\_AVG_{NOVEL} - Litter\_AVG_{HOME}$).

(27,28,38,46,47) or from one generation to another (11,12). For more details, see Supplement 1.

Open Field Disinhibition

We assessed the behavioral inhibition of offspring to novelty (postnatal day 24; Figure 1A) with a second order measure of activity level (i.e. an increase in the number of squared areas traversed over two brief trials of open field exposure [20-sec duration and approximately 5 min inter-trial interval] shortly after weaning) (21). The sensitivity of this type of change-based measure in general has long been pointed out in reviews based on a large number of open-field studies (48,49), and the sensitivity of this specific disinhibition measure to the NNE manipulation has been confirmed in several previous studies (20,21,29). Conceptually, this operational definition aims to capture an essential feature of human behavioral inhibition—a lack of habituation to novel stimuli. An individual with a greater disinhibition to novelty (i.e., a greater increase in activity over repeated exposures) is an individual who quickly habituates to novelty becoming uninhibited, and an individual with small or no disinhibition is an individual who remains inhibited even when the environment becomes increasingly familiar and safety has been confirmed.

Statistical Analysis

To examine whether maternal HPA function modulates previously reported novelty exposure effects on disinhibition in the open field (21), repeated measures analysis of covariance (ANCOVA) was performed with litter as the unit of analysis, novelty exposure treatment (Novelty) as the within factor, and the four maternal CORT measures ($CORT_B$, and $CORT_{E(5,\ 15,\ 30)}$) as separate covariates. Litter average scores for Novel ($Litter\_AVG_{Novel}$) and Home ($Litter\_AVG_{Home}$) rats of each individual litter were computed (Figure 2A, B). For more details, see Supplement 1.

Results

To quantify the within-family effect of neonatal novelty exposure on open-field disinhibition, for each litter, we computed a novelty effect score (NE score), defined as the difference between the average disinhibition of all Novel pups and average disinhibition of all Home pups within that litter ($Litter\_AVG_{Novel} - Litter\_AVG_{Home}$) (Figure 2B). Positive NE scores reflect novelty-induced enhancement in disinhibition in a given rat family (i.e., greater disinhibition among Novel than Home siblings), and negative NE scores reflect novelty-induced impairment (i.e., lesser disinhibition among Novel than Home siblings). It is apparent that a wide range of NNE effects across rat families were observed, with a majority showing a positive effect (Figure 2AB, left of dashed line) and a minority showing a negative effect of a smaller magnitude (Figure 2AB, right of dashed line). There is a significant main effect of novelty exposure [$t(20) = 2.517$, $p = .02$]—results were previously published in Reeb-Sutherland and Tang (21)—indicating enhancement in disinhibition at the population level, meaning a greater reduction of fear to a novel environment among Novel than Home rats.

In testing the hypothesis that family-to-family variations in this NNE effect might in part originate from maternal individual differences in HPA function, we found that the ANCOVA with novelty exposure as a within factor and maternal basal CORT measure as a covariate revealed a significant Novelty × $CORT_B$ interaction effect [$F(1,17) = 4.449$, $p = .049$, $f = .512$] (Figure 3A). Specifically, for mothers with lower $CORT_B$, NNE produced greater facilitation in

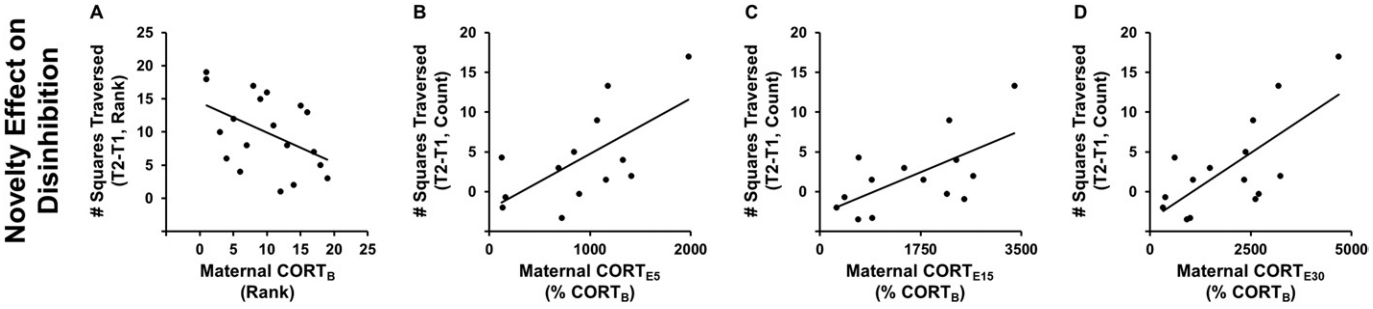

Figure 3. Association between basal and evoked corticosterone (CORT) measures of maternal self-stress regulation and family-to-family variations in novelty effect on open field disinhibition. **(A)** Offspring of mothers with lower postnatal basal CORT ($CORT_B$) measure showed greater novelty-induced enhancement in disinhibition, whereas those with higher basal CORT showed impairment. **(B–D)** Opposite patterns of prediction by maternal evoked CORT ($CORT_E$) measures after a 1-min swim stressor: **(B)** $CORT_{E5}$; **(C)** $CORT_{E15}$; **(D)** $CORT_{E30}$.

disinhibition to the open field environment among their offspring; for mothers with higher $CORT_B$, less facilitation and even a small reduction in open field disinhibition was produced.

Dams with low $CORT_B$ tended to be able to mount a large normalized response to the 1-min swim stressor ($CORT_B$ with $CORT_{E5}$: $r = -.798$, $p = .001$, $n = 13$; $CORT_B$ with $CORT_{E15}$: $r = -.808$, $p < .001$, $n = 14$; $CORT_B$ with $CORT_{E30}$: $r = -.786$, $p = .001$, $n = 15$). These negative correlations between $CORT_B$ and $CORT_E$ are consistent with the directions of results from the following ANCOVAs with separate $CORT_E$ as the covariate. Significant Novelty × $CORT_E$ interaction effects were found for all three maternal post-swim CORT measures [$CORT_{E5}$: $F(1,11) = 7.785$, $p = .018$, $f = .841$, Figure 3B; $CORT_{E15}$: $F(1,12) = 7.907$, $p = .016$, $f = .812$, Figure 3C; $CORT_{E30}$: $F(1,13) = 13.245$, $p = .003$, $f = 1.009$, Figure 3D)[1], indicating a positive relation between maternal $CORT_E$ and NNE facilitation of open field disinhibition, which is in contrast to the negative relation between maternal $CORT_B$ and NNE facilitation of disinhibition (Figure 3A).

These complementary (opposite) trends between patterns of interaction involving basal (Figure 3A) and evoked (Figures 3B–D) CORT measures offer converging evidence from two distinct yet inter-related aspects of HPA function in support of the hypothesis that maternal individual differences in HPA function is a possible source of maternal modulation that sets the critical context to determine both the direction and magnitude of the NNE effect on behavioral inhibition to novelty. Similar directions of trend in correlations between NNE effects and multiple evoked CORT measures (Figures 3B–D) are expected, as they reflect similar aspects of HPA function, and the observed similarity thus offers support for the temporal reliability of this finding.

Discussion

Similar to that of human (9,50) and nonhuman primate (51,52) infants, male infant rats also display initial inhibition of ongoing activity upon encountering a relatively novel situation (e.g., an unfamiliar open field). This behavioral inhibition to novelty is reduced as the animal becomes familiarized with the initially novel situation upon a second exposure. This disinhibition over repeated trials of exposure can be enhanced by NNE (21,29), demonstrating a constructive impact of early life experience that might be viewed as stressful (53). Here, by taking into consideration maternal individual differences in HPA function (28,38,47), we report that family-to-family variations in this novelty-induced enhancement in disinhibition are associated with aspects of maternal self-stress regulation, with the greatest enhancement of disinhibition found among offspring of mothers with a low-basal CORT and high-evoked CORT response profile.

These findings do not imply that the specific form of behavioral inhibition to the specific novel context in this specific experiment maps exactly onto the varying forms of expressions of behavioral inhibition in children across a wide range of novel situations, because novelty is a moving target—the situation that creates the quality of novelty is inevitably variable as the individual accumulates experience (9). Nor do the findings imply that the potential impact of maternal HPA function captures all factors contributing to human behavioral inhibition. What the findings do suggest is the possibility that individual differences in habituation to novelty are a part of the underlying mechanism contributing to individual differences in the inhibited versus noninhibited temperament types and that this mechanism is subject to modification. A note of caution is that, although the current study focused on the maternal contextual effects, it does not imply that the dam-pup interaction is unidirectional, because the physiology and behavior of the dams are clearly under the influence of the behavior of the pups (54).

Maternal Modulation of Early Experience Effects

Many decades ago it was proposed that maternal care mediates the effects of early stimulation, specifically the effects of neonatal handling (55–59). However, recent findings suggest that maternal care alone is neither sufficient nor necessary for the production of stimulation-induced effects (22,33,60–62). Other environmental variables that affect the offspring or the HPA function of the mother can also powerfully influence offspring development, including stress activation of HPA axis (63), maternal presence (64,65), maternal circulating stress hormone (25,28,38,47), and maternal care reliability (38,39,66). Most critical is that even if using a cross-fostering experimental design (59), maternal care is intrinsically confounded by both the stress of the dam and offspring (18–20) and such confounding factors cannot be ruled out within the handling paradigm. Here, by controlling for these confounding factors (22,67), we found evidence consistent with the claim that the mother modulates instead of mediates the effect of early stimulation, with better maternal self-stress regulation providing the context for greater NNE-induced facilitation of disinhibition to novelty.

Such maternal modulation of NNE effects has also been found across multiple functional domains, supporting the notion that the mother can function as not only a binary switch, setting the direction of an early stimulation effect on her offspring (65), but also a bidirectional dial to set continuous and finer control of the magnitude of these effects (28,38,47). Maternal modulation of the early experience effect on her offspring via her ability to maintain a low basal CORT and to mount a large CORT response is consistent with findings showing that the regulatory capacity of the maternal stress response system has important consequences for offspring behavioral and brain development (25,68,69) and consistent with the theoretical considerations that the relation between two variables can change depending on the context set by other critical contextual variables (70–72).

Reframing Maternal Influence: From Reductionism to Integration

Since the early work on the detrimental effects of mother-infant separation (73–76), there has been an enduring interest in identifying what specific caregiving properties are critical for infant development (77). This effort begins with considering the mother as a source of food (73,74), security (78,79), tactile stimulation (59,80), and hormonal inputs (25,65). Each line of research explicitly or inexplicitly claims that one aspect of maternal caregiving is a sufficient cause for offspring differences. This tends to create an impression that there exists a one-size-fits-all manipulation that would produce, unconditionally, a desirable developmental consequence. Yet, within each line of study, the presumed cause produces variable effects across different individuals, and sometimes these individual effects occur in opposite directions, resulting in the cancellation of the effect across a population (38). Such conditionality of the NNE effect upon maternal context suggests that a reductionist approach is insufficient to capture the reality of development and an integrative approach, in which the effect of one variable is evaluated within the natural contexts of other potentially relevant variables, is needed.

Recent rodent studies also showed that the maternal context is not singular but multidimensional, because the effects of early stimulation on offspring development are sensitive to the context

[1]No significant interaction between novelty exposure and maternal care measures were found (39).

of both maternal self-stress regulation (28,38,47) and maternal care reliability (38,39,66) and that the two sources of maternal influences are distinct and dissociable (38,39). Maternal self-stress regulation might exert its effect on the HPA axis of the offspring, by a direct hormonal signaling via the placenta prenatally (81) and via maternal milk postnatally (25). A low basal maternal CORT offers the nursing pup both a low level of baseline CORT from which to mount its own stress response to other environmental challenges and toward which the stress response can recover. A high evoked maternal CORT response to various environmental stressors might produce transient repeated CORT surges that activate the HPA axis of the offspring in ways similar to that induced by direct environmental stimulation, as has been shown in rats via repeated neonatal novelty exposure procedure (22), in nonhuman primates via intermittent stress inoculation (63), and in humans via daycare (82). In parallel and at the level of behavior, post-stress maternal care reliability (39,63,66) indirectly exerts an effect on the HPA axis of the offspring by defining the level of environmental uncertainty against which other nonmaternal events are interpreted and acted upon by the developing HPA axis. Within this integrative model, both sources of maternal influences and nonmaternal stimulations that activate the HPA axis can converge to jointly shape the development of the offspring's HPA function.

Modifiability of Novelty Response by Early Life Experience

A longstanding notion with regard to the behavioral tendency of an individual toward novelty and uncertainty is its resistance to change over time (14). This temperamental characteristic is considered moderately stable over development (83), and up to 70% of variation in this characteristic has been reported accountable by genetic differences (50). Kagan *et al.* (2,9) have documented substantial stability in behavioral inhibition to novel stimuli over the course of development, with inhibited toddlers rarely becoming noninhibited adolescents. If a 4-month-old infant displays increased motor arousal and negative affect when presented with novel visual and auditory stimuli, there is an increased likelihood that, during toddlerhood, this same infant will show increased avoidance and withdrawal behaviors when presented with an unfamiliar adult or strange complex objects, such as a robot that moves and produces noises in an unpredictable manner (84,85). Most of these inhibited toddlers continue to show increased reticence and shyness during novel social situations during childhood (9,31).

In contrast, direct evidence for developmental plasticity in this characteristic has been relatively scarce. Caregiving context might contribute to the switching of a handful of individuals from being behaviorally inhibited to noninhibited (5,6), and maternal stress history can affect offspring behavioral inhibition to novelty (86–88). Interestingly, among children who changed from being inhibited to noninhibited between 1–2 years and 4 years of age, more received nonparental care with nonsibling children at daycare than those who remained inhibited (31). Incidentally, daycare is known to elevate the cortisol of children (82), and such a cortisol increase seemed to be transient—rising in the afternoon hours, and returning to baseline levels in the evening similar to those observed at home on nonday care days (89).

Although such cortisol increase has been mostly interpreted as a negative stress indicator, it is possible that such daycare-induced transient cortisol surge can, over time, have a positive impact—by repeatedly activating the developing HPA axis in ways similar to the systematic stimulation induced by the neonatal novelty exposure procedure used in the present rat study and by the intermittent stress procedure used in the nonhuman primate study by Parker *et al.* (63), ultimately leading to more effective stress regulation later in life. The present findings add to this emerging story of plasticity by offering a clear demonstration that, in the rat, not only the response of an individual to novelty is definitively modifiable via systematic and brief repeated neonatal exposures to novelty associated with a nonhome environment but also the degree and direction of this plasticity is dependent upon characteristics of maternal self-stress regulation. Here, the infants might be viewed as using the environmental signals transmitted via maternal stress hormone to determine the direction and magnitude of its behavioral plasticity (90).

Potential Relevance to the Prevention of Anxiety Disorder

Given that behavioral inhibition is a major risk factor in the development of anxiety during childhood and adolescence (3–7) and that approximately 28% of adults in the United States alone will experience at least one anxiety disorder during their lifetime (91) thus leading to significant economic burden, costing approximately $44 billion annually (92), it has become necessary to not only understand the conditions that produce anxiety disorders but also design early life interventions and identify early life environmental conditions that might ultimately lead to prevention. The present findings might contribute to prevention and understanding by demonstrating, in an animal model, a positive early experience effect—when systematic brief neonatal exposures to novelty are provided within the context of a mother who is good at regulating her own stress response system, behavioral inhibition to novelty can be reduced among her offspring.

In humans, to our knowledge, only separate evidence is available for the influence of maternal stress (typically assessed via maternal report, without direct assessment of maternal HPA response [68]) and early environmental (daycare [31]) effects on behavioral inhibition. The present findings at least serve to generate parallel hypotheses that are clearly testable in the human population. Parallel evidence from both nonhuman animal models and human participants would suggest that a family-specific and maternal stress-regulation–centered approach to the prevention of offspring anxiety disorders is promising. Developmental scientists from diverse fields have come to the conclusion that behavior arises from a multitude of underlying contributing elements and no one element alone has causal primacy or forms the basis for behavior (70). Across distinct functional domains, the effects of early stimulation via novelty exposure are dependent upon maternal context (28,38,39,47,66), demonstrating the necessity and feasibility of incorporating this principle in the design of intervention studies and particularly in translating these research findings into practice.

The authors report no biomedical financial interests or potential conflicts of interest. We thank K.G. Akers and M. Nakazawa for their assistance.

Supplementary material cited in this article is available online.

1. Fox NA, Henderson HA, Marshall PJ, Nichols KE, Ghera MM (2005): Behavioral inhibition: Linking biology and behavior within a developmental framework. *Ann Rev Psychol* 56:235–262.
2. Kagan J, Snidman N, Kahn V, Towsley S (2007): The preservation of two infant temperaments into adolescence. *Monogr Soc Res Child Dev* 72:1–75.
3. Biederman J, Rosenbaum JF, Bolduc-Murphy EA, Faraone SV, Chaloff J, Hirshfeld DR, *et al.* (1993): A 3-year follow-up of children with and without behavioral inhibition. *J Am Acad Child Adolesc Psychiatry* 32:814–821.
4. Schwartz CE, Snidman N, Kagan J (1999): Adolescent social anxiety as an outcome of inhibited temperament in childhood. *J Am Acad Child Adolesc Psychiatry* 38:1008–1015.

5. Degnan KA, Fox NA (2007): Behavioral inhibition and anxiety disorders: Multiple levels of resilience process. *Dev Psychopathol* 19:729–746.
6. Degnan KA, Almas AN, Fox NA (2010): Temperament and the environment in the etiology of childhood anxiety. *J Child Psychol Psychiatry* 51:497–517.
7. Perez-Edgar K, Fox NA (2005): Temperament and anxiety disorders. *Child Adolesc Psychiatric Clin N Am* 14:681–706.
8. Kagan J, Reznick JS, Snidman N (1987): The physiology and psychology of behavioral inhibition in children. *Child Dev* 58:1459–1473.
9. Kagan J, Reznick JS, Snidman N (1988): Biological bases of childhood shyness. *Science* 240:167–171.
10. Gunnar M (1994): Psychoendocrine studies of temperament and stress in early childhood: Expanding current models. In: Bates JE, editor. *Temperament: Individual Differences at the Interface of Biology and Behavior*. Washington, DC: American Psychological Association, 175–198.
11. Kalin NH, Shelton SE, Rickman M, Davidson RJ (1998): Individual differences in freezing and cortisol in infant and mother rhesus monkeys. *Behav Neurosci* 112:1–4.
12. Kalin NH, Larson C, Shelton SE, Davidson RJ (1998): Asymmetric frontal brain activity, cortisol, and behavior associated with fearful temperament in rhesus monkeys. *Behav Neurosci* 112:286–292.
13. Cavigelli SA, McClintock MK (2003): Fear of novelty in infant rats predicts adult corticosterone dynamics and an early death. *Proc Natl Acad Sci U S A* 100:16131–16136.
14. Cavigelli SA, Stine MM, Kovacsics C, Jefferson A, Diep MN, Barrett CE (2007): Behavioral inhibition and glucocorticoid dynamics in a rodent model. *Physiol Behav* 92:897–905.
15. Denenberg VH (1964): Critical periods, stimulus input, and emotional reactivity: A theory of infantile stimulation. *Psychol Rev* 71:335–351.
16. Denenberg VH (1969): Open field behavior in the rat: What does it mean? *Ann N Y Acad Sci* 159:852–859.
17. Denenberg V, Garbanati J, Sherman G, Yutzey D, Kaplan R (1978): Infantile stimulation induces brain lateralization in rats. *Science* 201: 1150–1152.
18. Russell P (1971): Infantile stimulation in rodents: A consideration of possible mechanisms. *Psychol Bull* 75:192–202.
19. Daly M (1973): Early stimulation of rodents: A critical review of present interpretations. *Br J Psychol* 64:435–460.
20. Tang AC (2001): Neonatal exposure to novel environment enhances hippocampal-dependent memory function during infancy and adulthood. *Learn Mem* 8:257–264.
21. Reeb-Sutherland BC, Tang AC (2011): Dissociation between neonatal novelty-induced preferential maternal care and enhancement in cognitive, social, and emotional functions. *Behav Brain Res* 224:318–325.
22. Tang AC, Akers KG, Reeb BC, Romeo RD, McEwen BS (2006): Programming social, cognitive, and neuroendocrine development by early exposure to novelty. *Proc Natl Acad Sci U S A* 103:15716–15721.
23. Smotherman WP (1983): Mother-infant interaction and the modulation of pituitary-adrenal activity in rat pups after early stimulation. *Dev Psychobiol* 16:169–176.
24. Denenberg VH, Brumaghim JT, Haltmeyer GC, Zarrow MX (1967): Increased adrenocortical activity in the neonatal rat following handling. *Endocrinology* 81:1047–1052.
25. Catalani A, Alemà GS, Cinque C, Zuena AR, Casolini P (2011): Maternal corticosterone effects on hypothalamus-pituitary-adrenal axis regulation and behavior of the offspring in rodents. *Neurosci Biobehav Rev* 35:1502–1517.
26. Macrì S, Zoratto F, Laviola G (2011): Early-stress regulates resilience, vulnerability and experimental validity in laboratory rodents through mother-offspring hormonal transfer. *Neurosci Biobehav Rev* 35:1534–1543.
27. Tang AC, Reeb BC, Romeo RD, McEwen BS (2003): Modification of social memory, hypothalamic-pituitary-adrenal axis, and brain asymmetry by neonatal novelty exposure. *J Neurosci* 23:8254–8260.
28. Tang AC, Reeb-Sutherland BC, Yang Z, Romeo RD, McEwen BS (2011): Neonatal novelty-induced persistent enhancement in offspring spatial memory and the modulatory role of maternal self-stress regulation. *J Neurosci* 31:5348–5352.
29. Tang AC, Nakazawa M, Reeb BC (2003): Neonatal novelty exposure affects sex difference in open field disinhibition. *Neuroreport* 14: 1553–1556.
30. Lyons DM, Parker KJ, Schatzberg AF (2010): Animal models of early life stress: Implications for understanding resilience. *Dev Psychobiol* 52:402–410.
31. Fox NA, Henderson HA, Rubin KH, Calkins SD, Schmidt LA (2001): Continuity and discontinuity of behavioral inhibition and exuberance: Psychophysiological and behavioral influences across the first four years of life. *Child Dev* 72:1–21.
32. McCall RB, Van Ijzendoorn MH, Juffer F, Groark CJ, Groza VK (2011): Children without permanent parents: Research, practice, and policy. *Monographs of the Society for Research in Child Development* Boston: Wiley.
33. Macrì S, Würbel H (2006): Developmental plasticity of HPA and fear responses in rats: A critical review of the maternal mediation hypothesis. *Horm Behav* 50:667–680.
34. Cameron NM, Shahrokh D, Del Corpo A, Dhir SK, Szyf M, Champagne FA, *et al.* (2008): Epigenetic programming of phenotypic variations in reproductive strategies in the rat through maternal care. *J Neuroendocrinol* 20:795–801.
35. Meaney MJ (2010): Epigenetics and the biological definition of gene x environment interactions. *Child Dev* 81:41–79.
36. Zhang TY, Meaney MJ (2010): Epigenetics and the environmental regulation of the genome and its function. *Annu Reve Psychol* 61:439–466.
37. Bagot RC, Meaney MJ (2010): Epigenetics and the biological basis of gene x environment interactions. *J Am Acad Child Adolesc Psychiatry* 49:752–771.
38. Tang AC, Yang Z, Reeb-Sutherland BC, Romeo RD, McEwen BS (2012): Maternal modulation of novelty effects on physical development. *Proc Natl Acad Sci U S A* 109:2120–2125.
39. Reeb-Sutherland BC, Tang AC (2012): Functional specificity in the modulation of novelty exposure effects by reliability of maternal care. *Behav Br Res* 226:345–350.
40. Levine S (1962): Plasma-free corticosteroid response to electric shock in rats stimulated in infancy. *Science* 135:795–796.
41. Sapolsky R (1990): Stress in the wild. *Sci Am* 262:116–123.
42. Sapolsky RM (1991): Endocrinology al fresco: Psychoneuroendocrinology of wild baboons. *Recent Prog Horm Res* 48:437–467.
43. Rose RM, Jenkins CD, Hurst M, Livingston L, Hall RP (1982): Endocrine activity in air traffic controllers at work. I. Characterization of cortisol and growth hormone levels during the day. *Psychoneuroendocrinology* 7:101–111.
44. Rose RM, Jenkins CD, Hurst M, Herd JA, Hall RP (1982): Endocrine activity in air traffic controllers at work. II. Biological, psychological and work correlates. *Psychoneuroendocrinology* 7:113–123.
45. Rose RM, Jenkins CD, Hurst M, Kreger BE, Barrett J, Hall RP (1982): Endocrine activity in air traffic controllers at work. III. Relationship to physical and psychiatric morbidity. *Psychoneuroendocrinology* 7:125–134.
46. Marquez C, Nadal R, Armario A (2005): Responsiveness of the hypothalamic-pituitary-adrenal axis to different novel environments is a consistent individual trait in adult male outbred rats. *Psychoneuroendocrinology* 30:179–187.
47. Tang AC, Jiang H, Yang Z, Zhang Y, Romeo RD, McEwen BS (2011): Converging influence of neonatal novelty experience and maternal self-stress regulation on the plasticity of offspring acoustic startle response latency. *Behav Brain Res* 221:253–260.
48. Walsh RN, Cummins RA (1976): The open-field test: A critical review. *Psychol Bull* 83:482–502.
49. Whimbey AE, Denenberg VH (1967): Two independent behavioral dimensions in open-field performance. *J Comp Physiol Psychol* 63: 500–504.
50. Bokhorst CL, Bakermans-Kranenburg MJ, Fearon RMP, van Ijzendoorn MH, Fonagy P, Schuengel C (2003): The importance of shared environment in mother-infant attachment security: A behavioral genetic study. *Child Dev* 74:1769–1782.
51. Kalin NH, Shelton SE (1989): Defensive behaviors in infant rhesus monkeys: Environmental cues and neurochemical regulation. *Science* 243: 1718–1721.
52. Kalin NH, Shelton SE, Takahashi LK (1991): Defensive behaviors in infant rhesus monkeys: Ontogeny and context-dependent selective expression. *Child Dev* 62:1175–1183.
53. Lyons DM, Macrì S (2011): Resilience and adaptive aspects of stress in neurobehavioral development. *Neurosci Biobehav Rev* 35:1451.

54. Stern JM (1998): Offspring-induced nurturance: Animal-human parallels. *Dev Psychobiol* 31:19–37.
55. Barnett SA, Burn J (1967): Early stimulation and maternal behaviour. *Nature* 213:150–152.
56. Lee MHS, Williams DI (1974): Changes in licking behavior of rat mother following handling of young. *Anim Behav* 22:679–681.
57. Levine S (1975): The potential influence of infantile stimulation on emotional disorders. In: Levi L, editor. *Society, Stress and Disease*. London: Oxford University Press, 411–415.
58. Smotherman WP, Bell RW (1980): Maternal mediation of early experience. In: Bell RW, Smotherman WP, editors. *Maternal Influences and Early Behavior*. New York: Spectrum, 201–210.
59. Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, *et al.* (1997): Maternal care, hippocampal glucocorticoid receptor gene expression and hypothalamic-pituitary-adrenal responses to stress. *Science* 277:1659–1662.
60. Denenberg VH (1999): Commentary: Is maternal stimulation the mediator of the handling effect in infancy? *Dev Psychobiol* 34:1–3.
61. Pryce CR, Feldon J (2003): Long-term neurobehavioral impact of the postnatal environment in rats: Manipulations, effects, and mediating mechanisms. *Neurosci Biobehav Rev* 27:57–71.
62. Ragan CM, Loken E, Stifter CA, Cavigelli SA (2012): Within-litter variance in early pup-mother interactions and adult offspring responses to novelty. *Dev Psychobiol* 54:199–206.
63. Parker KJ, Buckmaster CL, Sundlass K, Schatzberg AF, Lyons DM (2006): Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. *Proc Natl Acad Sci U S A* 103: 3000–3005.
64. Stanton ME, Wallstrom J, Levine S (1987): Maternal contact inhibits pituitary-adrenal stress responses in preweanling rats. *Dev Psychobiol* 20:131–145.
65. Moriceau S, Sullivan RM (2006): Maternal presence serves as a switch between learning fear and attraction in infancy. *Nat Neurosci* 9:1004–1006.
66. Akers KG, Yang Z, DelVecchio DP, Reeb BC, Romeo RD, McEwen BS, *et al.* (2008): Social competitiveness and plasticity of neuroendocrine function in old age: Influence of neonatal novelty exposure and maternal care reliability. *PLoS ONE* 7:e2840.
67. Benetti F, Andrade de Araujo P, Sanvitto GL, Lucion AB (2007): Effects of neonatal novelty exposure on sexual behavior, fear, and stress-response in adult rats. *Dev Psychobiol* 49:258–264.
68. Essex MJ, Klein MH, Cho E, Kalin NH (2002): Maternal stress beginning in infancy may sensitize children to later stress exposure: Effects on cortisol and behavior. *Biol Psychiatry* 52:776–784.
69. Lupien SJ, Parent S, Evans AC, Tremblay RE, Zelazo PD, Corbo V, *et al.* (2011): Larger amygdala but no change in hippocampal volume in 10-year-old children exposed to maternal depressive symptomatology since birth. *Proc Natl Acad Sci U S A* 108:14324–14329.
70. Thelen E, Smith LB (1994): *A Dynamic Systems Approach to the Development of Cognition and Action*. Cambridge, MA: MIT Press.
71. Boyce WT, Ellis BJ (2005): Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity. *Dev Neuropsychol* 17:271–301.
72. Mischel W (2009): From personality and assessment (1968) to personality science. *J Res Pers* 43:282–290.
73. Harlow HF, Zimmerman R (1959): Affectional responses in the infant monkey. *Science* 130:421–432.
74. Harlow HF, Dodsworth RO, Harlow MK (1965): Total social isolation in monkeys. *Proc Natl Acad Sci U S A* 54:90–97.
75. Sanchez MM, Ladd CO, Plotsky PM (2001): Early adverse experience as a developmental risk factor for later psychopathology: Evidence from rodent and primate models. *Dev Psychopathol* 13:419–449.
76. Stevens HE, Leckman JF, Coplan JD, Suomi SJ (2009): Risk and resilience: Early manipulation of macaque social experience and persistent behavioral and neurophysiological outcomes. *J Am Acad Child Adolesc Psychiatry* 48:114–127.
77. Bowlby J (1991): Ethological light on psychoanalytical problems. In: Bateson P, editor. *The Development and Integration of Behaviour: Essays in Honour of Robert Hinde*. London: Cambridge University Press, 301–313.
78. Bowlby J (1969): *Attachment and Loss*. New York: Basic Books.
79. Ainsworth MDS, Blehar MC, Waters E, Wall S (1978): *Patterns of Attachment: A Psychological Study of the Strange Situation*. Hillsdale, NJ: Lawrence Erlbaum.
80. Champagne FC, Francis DD, Mar A, Meaney MJ (2003): Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiol Behav* 79:359–371.
81. Glover V, O'Connor TG, O'Donnell K (2010): Prenatal stress and the programming of the HPA axis. *Neurosci Biobehav Rev* 35:17–22.
82. Gunnar MR, Kryzer E, Van Ryzin MJ, Phillips DA (2010): The rise in cortisol in family day care: Associations with aspects of care quality, child behavior, and child sex. *Child Dev* 81:851–869.
83. Stevenson-Hinde J (2005): On the interplay between attachment, temperament, and maternal style. In: Grossman KE, Grossman K, Waters E, editors. *The Power and Dynamics of Longitudinal Attachment Research*. New York: Guilford Press, 198–222.
84. Kagan J, Snidman N (1991): Infant predictors of inhibited and uninhibited profiles. *Psych Sci* 2:40–44.
85. Calkins SD, Fox NA, Marshall TR (1996): Behavioral and physiological antecedants of inhibited and uninhibited behavior. *Child Dev* 67: 523–540.
86. Moehler E, Kagan J, Parzer P, Brunner R, Reck C, Wiebel A, *et al.* (2007): Childhood behavioral inhibition and maternal symptoms of depression. *Psychopathology* 40:446–452.
87. Lupien SJ, King S, Meaney MJ, McEwen BS (2000): Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. *Biol Psychiatry* 48:976–980.
88. Volbrecht MM, Goldsmith HH (2010): Early tempermental and family predictors of shyness and anxiety. *Dev Psychol* 46:1192–1205.
89. Sumner MM, Bernard K, Dozier M (2010): Young children's full-day patterns of cortisol production on child care days. *Arch Pediatr Adolesc Med* 164:567–571.
90. Hinde RA (1986): Some implications of evolutionary theory and comparative data for the study of human prosocial and aggressive behavior. In: Olweus D, Block J, Radke-Yarrow M, editors. *Development of Anti-Social and Prosocial Behaviour*. Orlando, FL: Academic Press, 13–32.
91. Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE (2005): Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 62:593–602.
92. Greenberg PE, Sisitsky T, Kessler RC, Finkelstein SN, Berndt ER, Davidson JR, *et al.* (1999): The economic burden of anxiety disorders in the 1990s. *J Clin Psychiatry* 60:427–435.