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# Early Adversity and Serotonin Transporter Genotype Interact With Hippocampal Glucocorticoid Receptor mRNA Expression, Corticosterone, and Behavior in Adult Male Rats

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Despite its importance for development, relatively little is known about how allelic variation interacts with both pre- and postnatal stress. We examined the interaction between serotonin transporter (5-HTT) genotype, prenatal and postnatal stress on glucocorticoid receptor (GR) mRNA expression, corticosteroid stress responses, and behavior in adult male rats. Prenatal stress involved a daily restraint of pregnant dams from gestational Day 10–21. Postnatal stress involved raising pups after parturition either by their mothers (MR) or in the artificial rearing (AR) paradigm, with or without additional "licking-like" stroking stimulation. 5-HTT genotype, hippocampal GR mRNA level, corticosteroid stress response, and behaviors including startle response, prepulse inhibition (PPI), and locomotor activity were measured in adult male rat offspring. We found significant genotype by prenatal stress interactions for hippocampal GR mRNA levels and for the corticosterone stress responses in adulthood. In contrast, behavioral endpoints tended to be more clearly affected by an interaction between genotype and postnatal environment. These findings suggest that allelic variation in the 5-HTT gene interacts with the prenatal environment to affect the hypothalamic-pituitary-adrenal (HPA) axis physiology and the postnatal environment to affect behavior. These results are the first to indicate a role for genetic variation in the 5-HTT gene in physiology and behavior in the rat.

*Keywords:* prenatal stress, acoustic startle, glucocorticoid receptor, HPA axis, rat maternal separation

Mothers influence the physiology and behavior of their offspring (Champagne, 2008; Fleming et al., 2002; Weinstock, 2005; Zhang, Parent, Weaver, & Meaney, 2004). These effects are mediated prenatally through, among other signals, maternal hormones and nutrients and postnatally through the quality of the mother's interactions with her young and her milk (Champagne, Francis, Mar, & Meaney, 2003; Fleming et al., 2002; Francis & Meaney, 1999; Hofer, 1994; Kapoor, Dunn, Kostaki, Andrews, & Matthews, 2006; Levine, Haltmeyer, Karas, & Denenberg, 1967; Melo et al., 2009; O'Donnell, O'Connor, & Glover, 2009; Seckl & Meaney, 2004; Weinstock, 2008). The genotype of the offspring can also modulate the effects of both the prenatal and the postnatal environments on the offspring (e.g., Bennett et al., 2002; Gerra et al., 2010; Kraemer, Moore, Newman, Barr, & Schneider, 2008). In the present study, we focused on the combined effects of prenatal stress, postnatal separation from mother, and serotonin transporter (5-HTT) genotype on the behavior and physiology of the developing offspring. This model also examined whether any individual differences in "resilience/vulnerability" to prenatal or postnatal adversity was mediated by the 5-HTT polymorphism. This study is

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All the procedures described in this report conformed to the guidelines set by the Canadian Council on Animal Care and in accordance with Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research. The procedures were approved by the UTM Animal Care Committee. This work is part of the Program in Maternal Adversity, Vulnerability and Neurodevelopment (MAVAN), funded by Canadian Institutes for Health Research (CIHR) and by a CIHR Strategic Training Grant.

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the first to examine the importance of genetic variation in the 5-HTT gene for the development of rat phenotypes.

Prenatal stress in animals produces a wide range of behavioral and physiological changes in both the mother and her offspring (Beydoun & Saftlas, 2008; Knackstedt, Hamelmann, & Arck, 2005; Moore & Power, 1986; Thompson, 1957; Weinstock, 2008). Behavioral changes in the offspring include altered social behavior, conditioned avoidance, prepulse inhibition (PPI), and increased aggressive behavior, emotionality, propensity to administer drugs, and locomotor activity (Chapillon, Patin, Roy, Vincent, & Caston, 2002; Deminiere et al., 1992; Koenig et al., 2005; Lee, Brady, Shapiro, Dorsa, & Koenig, 2007; Lehmann, Stohr, & Feldon, 2000; Mintz, Yovel, Gigi, & Myslobodsky, 1998; Thomas, Hu, Lee, Bhatnagar, & Becker, 2009; Wakshlak & Weinstock, 1990). Prenatal stress also produces a wide range of effects on both endocrine and central nervous systems (CNS) that likely mediate the effects of stress on behavior. These include altered prenatal gonadal activity (Ward, 1972), decreased neurogenesis (Lemaire, Koehl, Le Moal, & Abrous, 2000), and hypothalamic-pituitary-adrenal axis (HPA) responses to stress (Fride, Dan, Feldon, Halevy, & Weinstock, 1986; Glover, O'Connor, & O'Donnell, 2009; Louvart, Maccari, Vaiva, & Darnaudery, 2009; Maccari et al., 1995; McCormick, Smythe, Sharma, & Meaney, 1995; Vallee et al., 1999, 1997).

Postnatal maternal care also has pronounced effects of on a wide range of phenotypic outcomes. Short periods of separation from the mother (i.e., 5–20 min) or a daily "handling" of pups appear to dampen behavioral and endocrine responses to stress (Lehmann et al., 2000; Levine, 1967; Levine et al., 1967; Liu, Caldji, Sharma, Plotsky, & Meaney, 2000; Macri, Mason, & Wurbel, 2004; Meaney et al., 1985; Vallee et al., 1997), whereas longer periods ($>$1 hr) of daily separations produce endocrine states that inhibit physical growth (Schanberg, Evonluk, & Kuhn, 1984; Stone, Bonnet, & Hofer, 1976; Zimmerberg & Shartrand, 1992) and enhance behavioral and endocrine responses to stress in adulthood (Biagini, Pich, Carani, Marrama, & Agnati, 1998; Ellenbroek, van den Kroonenberg, & Cools, 1998; Francis, Diorio, Plotsky, & Meaney, 2002; Kalinichev, Easterling, Plotsky, & Holtzman, 2002; Ladd, Owens, & Nemeroff, 1996; Plotsky et al., 2005).

Complete separation of young from the mother and siblings using an artificial rearing (AR) paradigm throughout the preweaning period produces still greater behavioral and physiological effects. In comparison to mother-reared (MR) pups, rats reared without their mothers, siblings, and nest in the AR (pup-in-a-cup) paradigm show increased activity in the open field, reduced maternal care toward their own offspring, reduced attention, and impaired sensorimotor gating (Gonzalez, Lovic, Ward, Wainwright, & Fleming., 2001; Lovic & Fleming, 2004; Novakov & Fleming, 2005). However, when additional somatosensory stroking stimulation is provided to AR pups, their behavioral profiles in adulthood are not significantly different from mother-reared rats (Gonzalez, Lovic, Ward, Wainwright, & Fleming, 2001; Lovic & Fleming, 2004; Novakov & Fleming, 2005). Moreover, in a recent study using the AR paradigm, we found clear interactions of both prenatal stress and AR condition on behavior, especially for locomotor activity (Burton, Lovic, & Fleming, 2006). Among AR groups, additional stroking increased locomotor activity in prenatal stress rats, but reduced locomotor activity in controls (Burton, Lovic, & Fleming, 2006). These findings combined with studies examining normal variations in maternal licking behavior on offspring neural development and behavior demonstrate that postnatal somatosensory stimulation plays an important role in the development of rat behavior (e.g., Brake, Zhang, Diorio, Meaney, & Gratton, 2004; Caldji, Diorio, & Meaney, 2003; Caldji et al., 1998; Cameron, Fish, & Meaney, 2008; Champagne, 2008; Champagne et al., 2003; Francis & Meaney, 1999; Zhang & Meaney, 2010).

Studies with nonhuman primates show profound effects of both prenatal (Roughton, Schneider, Bromley & Coe, 1998) and postnatal (Suomi, 1991, 1997) environments on behavior. Interestingly, the effects of the postnatal environment on behavior can vary as a function of genotype. One gene that has been focused on in the literature is the 5-HTT gene. In humans and the rhesus monkey a naturally occurring repeat length polymorphism is found in the promoter region of the 5-HTT gene and this polymorphism has been suggested to exert its effects through differential transcriptional regulation of the 5-HTT gene related to allele dependent differences in 5-HTT promoter activity (Heils et al., 1996).

Studies in rhesus monkey infants that carry the short allele of the 5-HTT gene and are exposed to peer rather than maternal rearing show delayed infant orientation development, decreased looking at and attention toward orienting objects (Champoux et al., 2002), increased anxiety and depression (Spinelli et al., 2007), altered stress-induced HPA responses (Barr, Newman, Lindell et al., 2004; Barr, Newman, Shannon et al., 2004; Erickson et al., 2005; Shannon, Champoux, & Suomi, 1998) and altered serotonergic function (Bennett et al., 2002; Erickson et al., 2005) compared to monkeys homozygous for the long allele of 5-HTT. A comparable gene by environment interaction involving the SLC6A4 gene that codes for the 5-HTT is apparent in the effects of childhood maltreatment on the risk for depression in humans (Caspi et al., 2003; Stein, Schork, & Gelernter, 2008).

Analysis of the 5-HTT promoter polymorphism in mice (Carneiro et al., 2009) and rats (present study) showed that this repeat length polymorphism is absent in rodents. However, there are polymorphisms at other regions of the SLC6A4 gene. To investigate if any polymorphisms found in the rat 5-HTT gene have similar influences on modulating the effect of maternal environment on the behavior of offspring, we genotyped the only known single nucleotide polymorphism (SNP) in the rat, rs8154473, found in the coding region of the rat 5-HTT homologue, SLC6A4. This C3598T SNP is a synonymous polymorphism located in the third exon of the rat 5-HTT gene.

The primary focus of this study was to investigate interactions between 5-HTT genotype, prenatal stress, and postnatal adversity and their interaction on hippocampal glucocorticoid receptor (GR) expression, the HPA stress response, and behaviors, all known to be influenced by early adversity and/or by the 5-HT system, including startle responses, PPI (Borrell, Vela, Arevalo-Martin, Molina-Holgado, & Guaza, 2002; Farid, Martinez, Geyer, & Swerdlow, 2000; Lovic & Fleming, 2004; Swerdlow et al., 2006), and locomotor activity in rats (Burton et al., 2006).

## Method

### Subjects

Fifty-eight Sprague-Dawley rats were used in this study. The rats were born and raised at the University of Toronto at Mississauga from a stock originally obtained from Charles River Farms

(St. Constan, Quebec, Canada). The colony was maintained on a 12-hr light–dark cycle with lights at 0800 in a room maintained at approximately 22 °C, and 50–60% humidity. Beginning on postnatal day (PND) 21, rats were housed two per cage (clear, 20 × 43 × 22 cm), with food (Purina Rat Chow) and water available ad libitum.

## Apparatus

**Locomotor activity boxes.** Locomotor activity was measured in one of eight activity boxes (clear Plexiglas cages, 22 × 44 × 30 cm; custom made by the Centre for Addiction and Mental Health, Toronto) with an array of 16 infrared photocells extended lengthwise along the cages. The photocells were spaced 2.5 cm apart and 2 cm above the floor of the cage.

**Acoustic startle response and prepulse inhibition chambers.** Rats were tested in one of four acoustic startle cubicles (55.9 cm × 38.1 cm × 35.6 cm internal dimensions; MED Associates, St. Albans, VT, U.S.A.), lit with a red light and fan ventilated. A grid floor rat holder (16.5 cm × 7.6 cm × 8.9 cm) was mounted on top of a startle platform which detected and transduced the movement of the rat. All acoustic stimuli were delivered through two Radio Shack Supertweeters located along the back wall which were controlled by a PC computer equipped with Windows-based Startle Reflex Software (3.35, MED associates).

## Procedures

**Timed mating.** Nineteen virgin females were housed two per cage and vaginal smears were taken over several days at approximately 1400. Once in proestrus, females were placed with a sexually experienced male for 24 hr. Presence of a spermatozoa in a vaginal smear after mating was considered gestational day (GD) 0.

**Prenatal stress.** Dams were left undisturbed until GD 10 and then randomly assigned to prenatal stress (PS) or nonstressed (NS) groups. From GD 10 to 21, PS dams were weighed and then placed in a Plexiglas restrainer (8 cm diameter × 20 cm length) for 4 hr per day starting at a variety of different times between 900 and 1400. Restrainers were designed to restrain, but not prohibit movement of the pregnant female or to constrict her abdomen. The length of the chamber was flexible (15 to 18 cm) to accommodate the range of sizes. After each session, the restrainers were cleaned thoroughly with 30% alcohol. This type of stressor has been used previously (Fujioka et al., 1999; Hashimoto et al., 2001; Lehmann et al., 2000; Lemaire et al., 2000; Vallee et al., 1997). Rats were monitored to ensure appropriate weight gain and any signs of poor health. NS dams were left undisturbed.

## General Procedures

Dams gave birth undisturbed. On the day of parturition (PND 0), litters were culled to 14 pups (seven males and seven females). On PND 4, three male and three female pups were removed from the nest. Of these pups, two males and two females were implanted with a cheek cannula and raised artificially (see Surgery and Treatments and Groups below) while the third male and female were sham operated, labeled (marker on their ear), and returned to the mother (MR-SHAM). One pup of each sex was sham operated. The remaining pups were not manipulated and one pup of each sex constituted control subjects—mother-reared control (MR-CON). PS refers to Prenatal Stress; NS refers to No (Prenatal) Stress; MR-SHAM and MR-CON refer to the two Mother-Reared control groups; AR-MIN and AR-MAX refer to Artificially Reared groups receiving either minimal strokings per day or maximal strokings per day. Groups and sample sizes that were created were: PS AR-MIN = 9, PS AR-MAX = 6, PS MR-SHAM = 9, PS MR-CON = 9, NS AR-MIN = 4, NS AR-MAX = 7, NS MR-SHAM = 7, NS MR-CON = 7. These rats were derived from 19 litters with only one rat from each litter per group. Hence all subjects within a group came from different litters.

**Surgery and artificial rearing.** Prior to surgery, pups were weighed and a topical anesthetic (EMLA) was applied to their right cheek. A leader wire (stainless steel 0.25 mm in diameter), sheathed in lubricated (mineral oil) silastic tubing and PE 10 tubing, was used to pierce the cheek. Once the flared end of the tubing contacted the inside of the cheek, the leader wire and silastic tubing were removed and polysporin was applied topically to the site of penetration. Another leader wire was then used to insert a flat and a t-washer that were secured in place with superglue. MR-SHAM pups had their cheeks pierced but the P10 tubing was removed. Polysporin was applied to the site of penetration and black marker was applied to their ears prior to placing them back with the litter for later identification. After surgery, AR pups were placed individually in plastic cups (11 cm diameter × 15 cm deep) containing corn cob bedding (Bed O'Cobs) and were placed inside another weighted cup. The cups floated in temperature controlled (36–40 °C) water directly below time-controlled infusion pumps (Harvard Apparatus Syringe, PHD 2000) to which they were connected with the cheek cannula tubing. The pumps delivered milk (Messer diet) for 10 min every hour, 24 hr a day. The amount infused was calculated based on mean pup body weight. Beginning on PND 4, pups received a volume of milk equal to 33% of the mean body weight, and this amount increased by 1% daily. Each morning, the pups were disconnected from the pumps, their weight recorded, and all tubing was flushed with double distilled water. New syringes with fresh formula were prepared and the new infusion rates programmed based on the new weights.

**Treatment and groups.** AR rats were randomly assigned to either AR-MIN or AR-MAX groups. Each day, AR-MIN pups were stimulated twice for 30 s each (morning and night) with a wet camelhair paintbrush in the anogenital region to stimulate urination and defecation. AR-MAX pups also received the same anogenital stimulation twice daily, as well as 2 min of dorsal stimulation with a dry camelhair paintbrush 8 times a day. Stimulations for both groups occurred daily from PND 4–16. On PND 17–18, pups were removed from the pumps and given milk formula, rat chow, and a mixture of the two. On PND 21, all rats were weighed and paired with a social partner of the same sex from another litter that was not tested. Rats were left undisturbed until PND 60 when they were tested on the sensorimotor gating, attentional task-PPI, and locomotor activity in a novel environment. Procedures for PPI are based on Lovic and Fleming (2004). All behavioral testing occurred during the light cycle.

**Acoustic startle and prepulse inhibition testing.** Acoustic startle and PPI were measured during a single testing session after PND 60. Prior to testing, rats were brought into an adjacent suite and allowed to acclimatize for 30 min. Rats were weighed and

placed into a metal grid floor rat holder that attached to the startle platform. The test lasted 25.5 min, beginning with a 7 min acclimatization period with a 70 dB background noise that was presented throughout the test. In block 1, there were 4 pulse/startle trials (120 dB, 10 kHz, 30 ms). In block 2, there were 60 trials presented in random fashion but consistent between rats. These trials consisted of 8 startle trials (120 dB, 10 kHz, 30 ms long), 16 prepulse alone trials, (4 for each intensity; 72, 74, 80, 84 dB, 10 kHz, 20 ms long), 4 no-stimulus (null) trials, and 32 prepulse and startle trials (8 of each prepulse intensity; 120 dB startle pulse, 100 ms between prepulse and pulse). To calculate the percentage of prepulse inhibition, the following formula was used: [1-(prepulse and startle response/startle response)] × 100. Averaged responses during the 100 ms following the termination of sound stimulus were the dependant measures. The metal grid rat holder was cleaned thoroughly between each test with 70% alcohol.

**Locomotor activity.** Locomotor activity was measured in three daily 1 hr sessions in a dimly lit room. The number of beam breaks in 5 min intervals was measured. Cages were cleaned thoroughly with 70% alcohol between each rat.

**Blood collection and corticosterone (CORT) radioimmunoassay (RIA).** Between 1400 and 1600 (midway into the light phase), rats were hand-restrained and blood was collected from a nick in the tail into nonheparinized capillary tubes (baseline; 4 tubes; approximately 200 μl per rat) and immediately placed on ice. Rats were then placed into Plexiglas restrainers (described above in Prenatal Stress section) and 20 min later, a second sample of blood was collected as stated above while rats were in the restrainer. Rats were then returned to their homecage, without their conspecific, and left undisturbed for 70 min in the same room where the stress and blood collection occurred. Ninety min after rats were again hand-restrained and a third sample of blood was collected as stated above.

Blood was allowed to sit on ice for at least 30 min before samples were centrifuged (Eppendorf 5804R) at 4 °C, 4000 rpm for 20 min. Then serum was extracted and stored in 0.5 ml tubes at −80 °C. Finally, bloods were analyzed for CORT levels using RIA. CORT was determined by a solid phase (I-125) RIA (Coat-a-Count, Diagnostic Products Corporation; interassay variability = 8.5%; intraassay variability = 6.83%). The antiserum is highly specific for rat corticosterone, with very low cross-reactivity to other compounds that might be present in rat serum samples. For the antibody: The Coat-a-Count Rat corticosterone procedure is a solid-phase radioimmunoassay in which $^{I}125$ labeled rat corticosterone competes for a fixed time with corticosterone in the sample for antibody sites. The sensitivity of the assay is 5.7 ng/ml, intrassay has 3 CV, low value is 12.2%, mid value 4.3% and high values 4.0%, the interassay variation; low value 14.9%, mid values 5.8% and high values 4.8%.

**Tissue collection.** Rats were decapitated postbehavioral analysis on ~PND 110 (±10). Brain and heart tissues were collected and immediately frozen in dry ice. Tissues were then stored at −80°C until further processing.

## Glucocorticoid Receptor mRNA: In Situ Hybridization

For all in situ hybridization studies, animals were decapitated under resting-state conditions directly from the home cage. After rapid decapitation, brains were removed and quickly frozen in isopentane maintained on dry ice. Brains were blocked, and 15 mm cryostat sections were mounted onto poly-D-lysine-coated slides, desiccated under vacuum, and stored at −80 °C. Preparation and description of GR riboprobes as well as the in situ hybridization procedure have been described elsewhere (Liu et al., 1997). The GR cRNA was transcribed from a 674-bp Pst I- Eco RI fragment of the rat GR cDNA (steroid binding domain), linearized with Ava I, and transcribed with T7 RNA polymerase. The hybridization signal within the parvocellular subregion of the paraventricular region of the hypothalamus as well as hippocampus (i.e., the dentate gyrus and CA1 and CA3 regions of Ammon's horn) was quantified for GR mRNA levels by means of densitometry of autoradiograms with an image analysis system (MCID, Imaging Research, Inc., St. Catherines, Ontario). The data are presented as arbitrary optical density (OD; absorbance) units after correction for background. The anatomical level of analysis was verified with the Paxinos and Watson rat brain atlas (Paxinos & Watson, 1996) and Nissl-staining of alternate sections.

**DNA extraction and PCR.** Genomic DNA was extracted from 25 mg heart tissues of 58 rats using Sigma's GenElute Mammalian Genomic DNA Miniprep kit (St. Louis, MO, U.S.A.) according to the manufacturer's instruction. DNA amounts were determined using Nanodrop ND-1000 spectrohotometer (Wilmington, DE, U.S.A.). A specific primer pair, forward primer 5′-TCTGCCCGATTTTCAAAG/reverse primer 5′-GTGAGAGACTCCAAGCTGAAA, was designed to amplify a 548 bp region in the rat SLC6A4 gene that codes for the 5-HTT. This region included the third exon of SLC6A4 gene, which contained single nucleotide polymorphism described in NCBI as rs8154473. Polymerase chain reaction (PCR) was performed in 50 μl reaction mixtures containing 0.5 U Biolase DNA polymerase, 1× Biolase buffer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$ and 0.2 μmol/L of each primer (Bioline, London, U.K.) to amplify the region of interest under the following conditions: 5 min at 95 °C; 35 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s; and a final extension of 7 min at 72 °C. The final PCR products were then purified using Qiaquick PCR Purification Kit (Qiagen, Maryland, U.S.A.).

**DNA sequencing and genotyping.** Purified PCR products containing 5–10 ng DNA were sequenced on ABI 3100 genetic analyzer using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). The following conditions were employed for cycle sequencing with the Big Dye Terminator kit: 25 cycles of 95 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min. Princeton's CENTRI.SEP 96 well purification plates (Princeton Separations, NJ, U.S.A.) were used to remove excess dye from sequencing products. Purified sequence products were analyzed and nucleotide sequences were determined on ABI 3100 genetic analyzer using the ABI Sequencing Analysis Software v1.0 (Applied Biosystems). All samples were sequenced in both directions using the corresponding primer. The genotype of each individual was then determined postsequencing analysis.

The allelic distribution of the single nucleotide polymorphism in the coding region of the rat 5-HTT gene, identified in NCBI as rs8154473, was analyzed in our population of 58 Sprague-Dawley rats. This synonymous C3598T polymorphism is found in the third exon. We found 33 rats homozygous for the C allele whereas the 21 were heterozygous (CT). One animal could not be genotyped. The frequency of TT was very low with only 3 of 58 rats exhib-

iting the genotype and, consequently, these rats were omitted from further analysis in correlation studies. We tested for a correlation between the CC and CT genotypic frequencies and a number of physiological and behavioral measures obtained using these rats.

### Statistical Analysis

We previously reported on prenatal × postnatal effects on behavior in males and females (see Burton et al., 2006). In the present report we only focused on male offspring. No significant differences were found between the MR-SHAM and MR-CON groups ($p > .05$) for any physiological measure or behavioral test. Therefore, these groups were combined and will be referred to as MR in our analyses. Locomotor activity levels and startle data were examined using a 2 (stress: prenatal stress, nonstressed) × 3 (rearing: AR-MIN, AR-MAX, MR) × 2 (5-HTT genotype: CT, CC) analysis of variance (ANOVA). For analysis of PPI and the acoustic startle response, body weight was employed as a covariate because body weight can affect the recorded magnitude of the startle response. Because fewer brains were analyzed for the in situ hybridization procedure than was the case for other measures, for analyses of GR mRNA we combined the AR-MAX ($n = 2$) and AR-MIN groups into a single AR group and compared these with the MR groups. GR mRNA was analyzed using a 2 (stress) or 2 (rearing: AR vs. MR) × 2 (genotype) × 4 (brain site; within) ANOVA. Finally, the values analyzed in these ANOVAs included both the OD units for the more anterior sections within the hippocampus and the OD units derived as a mean score of both anterior and more posterior sections. Since the outcomes were essentially the same, we reported the results of analyses for the anterior sections (since due to technical difficulties some sections were lost from the posterior sections, hence reducing degrees of freedom using the combined values). CORT levels were analyzed in a 2 (stress) × 3 (rearing) × 3 (CORT across 3 time-points) × 2 (genotype) repeated measures ANOVA. Tukey's post hoc analysis was utilized to examine group differences. The accepted level of statistical significance was $p < .05$. Since our primary interest is in the effects of genotype and of genotype by pre and/or postnatal experience, main effects are only reported on variables where no interactions involving genotype are found.

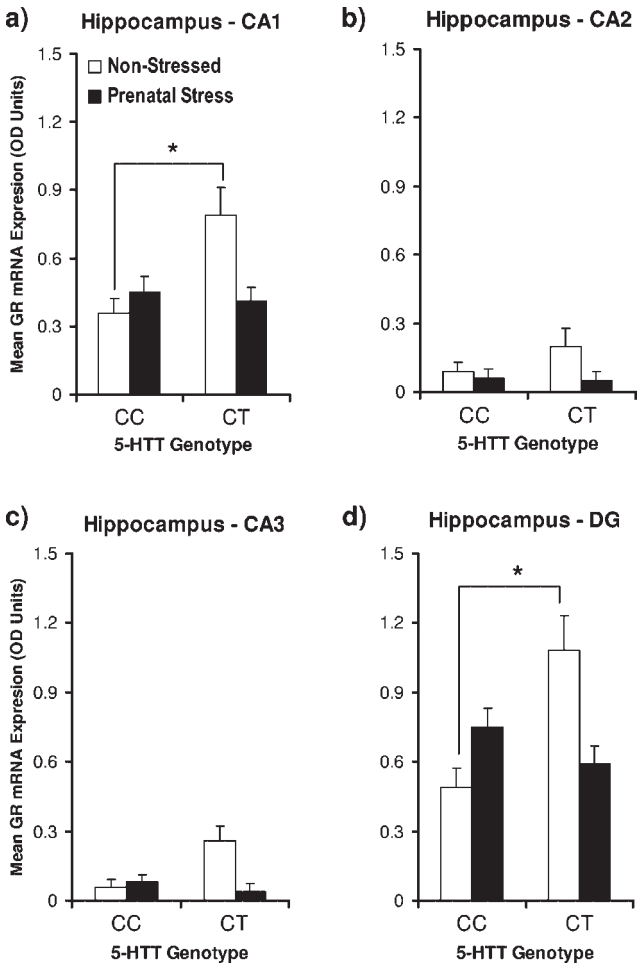


*Figure 1.* The figure depicts mean GR mRNA (+sem) levels within different regions of the hippocampus (a-e). There were no main effects or significant 3-way interactions. However, there was significant genotype × prenatal condition (NS vs. PS) interaction. * Post hoc analyses indicated that CC rats were different from CT rats, within the NS condition but not within the PS condition, for GR mRNA levels in the DG region ($p < .05$); marginal differences were also found in the CA1 region.

## Results

### GR mRNA Levels

**Gene by prenatal stress interactions.** A series of ANOVAs were performed on GR mRNA levels within the brain sites CA1, CA2, CA3, and DG (dentate gyrus) of the hippocampus and these sites were included as a within factor in the analyses. As shown in Figure 1, for analyses including prenatal stress, there was a significant site × gene × prenatal stress interaction, $F(1, 25) = 7.6$, $p < .01$ and a significant gene × prenatal stress interaction, $F(1, 25) = 6.5$, $p < .01$. Subsequent analyses on each brain site separately indicated that for regions CA1, CA3, and DG, there were significant interactions between prenatal stress and genotype in the dentate gyrus [$F(1, 25) = 11.53$, $p < .01$]. Post hoc tests of individual means for the DG indicated that the groups that differed were CC versus CT rats, within the NS condition but not within the PS condition ($p < .05$). Within the NS condition, CT animals showed higher density of GRs than did the CC animals. There were no significant gene effects or gene × prenatal stress interactions for the other brain sites. For a photomicrographic representation of brain sections of a representative animal in each of the groups, prenatal nonstress (NS)/CT and prenatal stress (PS)/CT, see Figure 2.

### CORT Stress Response

**Gene by prenatal stress interactions.** Similar to the GR mRNA expression results, there was a significant prenatal stress × 5-HTT genotype interaction [$F(1, 43) = 4.82$, $p < .033$]. As shown in Figure 3a and 3b, for NS rats, 5-HTT genotype significantly influenced rats' CORT levels; among NS rats, those carrying the CT genotype showed greater CORT levels 20 and 90 min poststressor compared to those carrying the CC genotype. In contrast, CORT levels were consistently lower in PS rats regard-

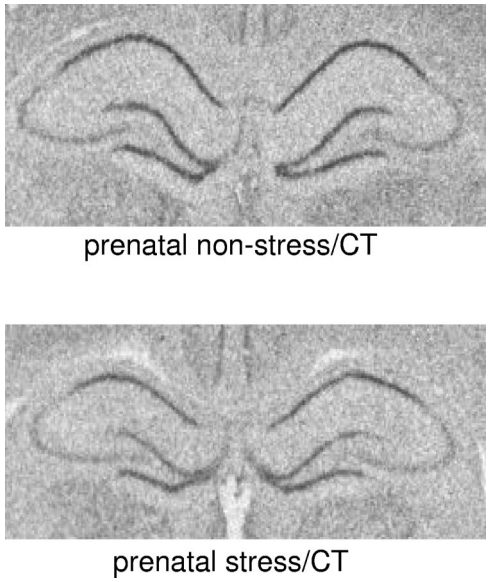


*Figure 2.* Photomicrograph of brain sections showing hippocampal and dentate gyrus regions that were quantified for glucocorticoid mRNA within the hippocampus.Two brain sections are from representative animals within groups prenatal nonstress/CT and prenatal stress/CT. Darkened regions are regions of high receptor density.

less of their genotype. Post hoc tests indicated that the groups that differed were the 2 NS groups ($p < .05$; CT vs. CC); differences were not found between CC and CT PS animals. When different groups were compared with one another only on baseline CORT levels, there were no overall significant differences.

**Main effects.** As shown in Figure 3a and 3b, there were also main effects of time on CORT levels [$F(2, 86) = 102.43$, $p < .0001$]; all groups showed increased CORT (from baseline) with the stressor [$F(1, 43) = 11.11$, $p < .01$]. As shown in Figure 3c there was a main effect of postnatal adversity [$F(2, 43) = 5.70$, $p < .006$]; AR-MIN rats maintained higher CORT levels than AR-MAX or MR rats. These two groups did not differ from one another.

## Acoustic Startle Response and Prepulse Inhibition

**Gene by postnatal experience interactions.** As can be seen in Figure 4 there was a significant interaction between genotype and postnatal adversity on the initial startle response, prior to the PPI condition [$F(2, 43) = 3.30$, $p < .05$]. Post hoc tests indicated that there were no group differences in the groups carrying the CC genotype; however, there were group differences among rats carrying the CT genotype ($p < .05$). CT AR-MIN rats showed significantly higher startle responses than all other CT or CC groups with the exception of the CT AR-MAX group. There were no main effects or interactions in analyses of PPI.

**Main effects.** No significant main effect of prenatal stress was found for acoustic startle responses.

## Locomotor Activity

**Gene by postnatal experience interactions.** As shown in Figure 5, there was a significant genotype by postnatal experience interaction for activity [$F(2, 43) = 4.1$, $p < .05$]. Post hoc analyses showed that MR rats carrying the CT genotype differed from all other AR groups with the exception of the AR-MAX group carrying the CT genotype. MR rats carrying the CC genotype differed also from Groups CC AR-MAX and CT AR-MIN ($p < .05$).

## Discussion

The results of the present study demonstrate that polymorphisms for the 5-HTT gene interact with prenatal and postnatal environmental influences to determine individual differences in multiple levels of phenotype. Generally, the prenatal environment inter-

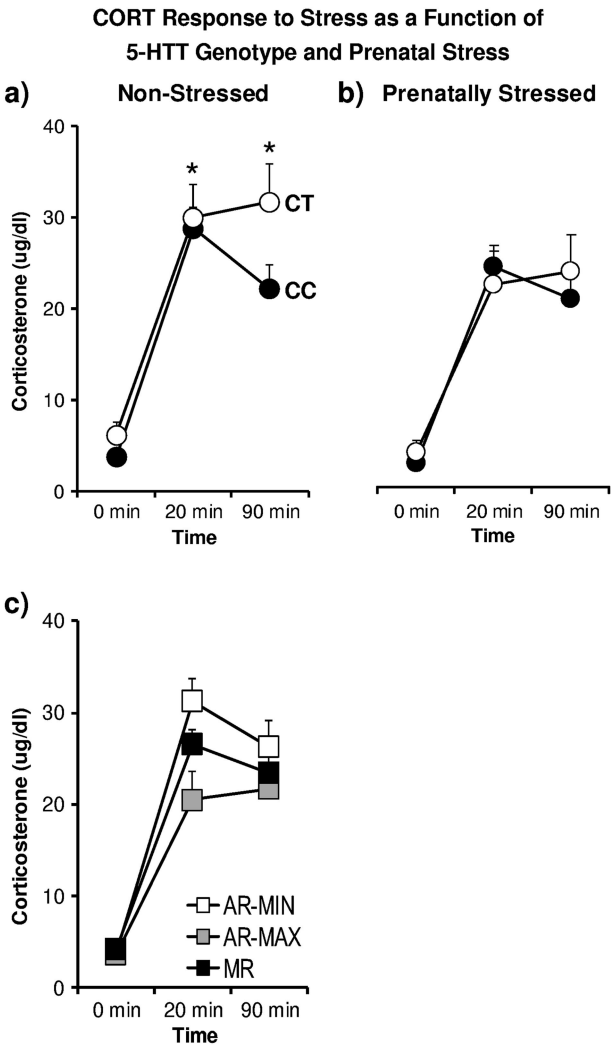


*Figure 3.* The figure depicts mean CORT levels (+sem) for nonstressed (NS; left-panel) and prenatally stressed (PS; right panel) groups and both 5-HTT genotypes (CC and CT). All groups showed an increase in CORT levels following restraint stress [$F(2, 86) = 102.43$, $p < .0001$]. There was a significant 5-HTT genotype x prenatal condition interaction [$F(1, 43) = 4.82$, $p < .05$]. While the CORT levels within the PS group were not significantly influenced by rats' 5-HTT genotype, the genotype significantly influenced CORT levels within the NS group. * (NS) CT rats showed greater CORT levels than (NS) CC rats ($p < .05$).

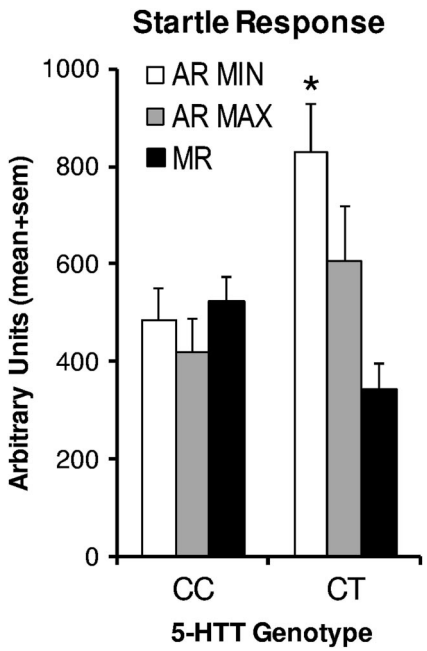


*Figure 4.* The figure shows mean (+sem) displacement of the startle platform (arbitrary units) for different postnatal treatment groups (AR-MIN, AR-MAX and MR) and for both 5-HTT genotypes (CC and CT). There was significant postnatal experience × 5-HTT genotype interaction [$F(2, 43) = 3.30$, $p < .05$]. * Post hoc tests indicated that CT AR-MIN rats showed significantly higher startle amplitudes compared to all groups with the exception of CT AR-MAX group ($p$'s $< .05$).

acted with 5-HTT genotype to affect markers of HPA function while the postnatal environment interacted with the 5-HTT genotype to affect adult behavior. These data suggest that the 5-HTT polymorphism may mediate vulnerability or resilience to early adversity.

## Gene × Environment Interactions

The effects of the 5-HTT polymorphism on markers of HPA function were dependent on the prenatal and not the postnatal environment. For example, hippocampal GR mRNA levels were consistently elevated in the nonstressed CT animals. In contrast, differences in GR mRNA expression as a function of genotype were absent among animals exposed to prenatal stress. Thus, our findings reveal clear evidence for an interaction between 5-HTT genotype and prenatal environment on hippocampal GR mRNA expression. A similar pattern of results was also reported for overall CORT levels. Nonstressed rats bearing the CT genotype showed greater CORT levels after the stressors compared to those carrying the CC genotype. In contrast CORT levels were consistently lower in prenatally stressed rats regardless of their genotype. No overall significant group differences were observed for baseline CORT.

The finding that prenatal stress decreased hippocampal GR expression, at least among animals bearing the CT genotype, is consistent with a number of studies that report early stress effects on the neuroendocrine regulation of the stress response in rodents (Henry, Kabbaj, Simon, Le Moal, & Maccari, 1994; Kapoor, Leen, & Matthews, 2008; Welberg, Seckl, & Holmes, 2001). Although we expected that prenatal stress would also enhance the corticosterone response in adulthood (Kapoor, Leen, & Matthews, 2008; Welberg, Seckl, & Holmes, 2001), an inhibitory early experience stress effect on CORT, as we reported, is also not without precedent. There are an increasing number of studies now that show in some species and for some paradigms a reduction, rather than an enhancement, in the stress response in adulthood following periods of early prenatal, preweaning, or postweaning "adversity" or stress (Fan, Chen, Jin, & Du, 2009; Glover et al., 2009; Jodi, Maxim, Jamie, & Gina, 2009; Weinstock, 2008).

Moreover, in the monkey there is evidence that postnatal adversity interacts with genetic influences. In the rhesus macaque Barr, Newman Shannon, et al. (2004) reported a separation × rearing × genotype (rh5-HTTLPR) interaction on stress-induced HPA activity. Peer-reared monkeys with the l/s genotype had higher ACTH levels during separation and lower baseline cortisol than did other animals studied. The authors concluded that the influence of rh5-HTTLPR 5-HTT polymorphism on the HPA responses during stress is modulated by early experience. With the exception of the timing of the early adversity on physiology (ours occurred prenatally, and theirs postnatally), our present results are consistent with these findings.

What is puzzling in the present data is the contrasting effects of prenatal stress on hippocampal GRs and HPA axis response to stress in the same animals, as previous reports show that the activation of the hippocampal GR would normally be expected to exert an inhibitory effect on stress-induced HPA activity (Liu et

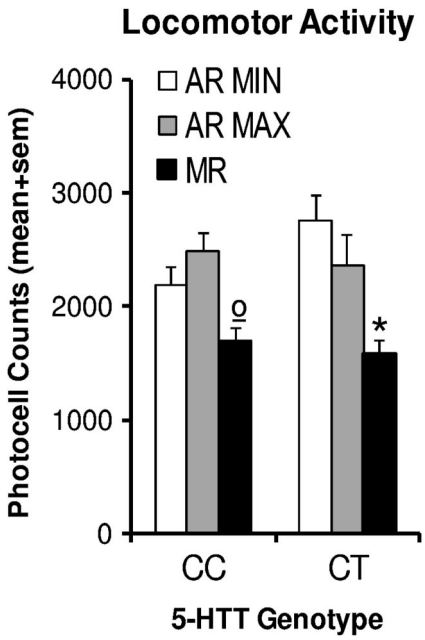


*Figure 5.* The figure depicts mean (+sem) number of photobeam interruptions for different postnatal treatment groups (AR-MIN, AR-MAX and MR) and for both 5-HTT genotypes (CC and CT). There was a significant postnatal experience × 5-HTT genotype interaction [$F(2, 43) = 4.1$, $p < .05$]. Post hoc analyses: * MR (CT) rats were significantly different form AR-MIN (CT) rats ($p < .05$); °MR (CC) rats were significantly different from AR-MIN (CC) and AR-MAX (CC) rats ($p < .05$).

al., 1997; Liu et al., 2000; Burton, Chatterjee, Chatterjee, Lovic . . . Fleming, 2007). In our data, CT animals that were prenatally stressed showed both reductions in GR and reductions in post-stress corticosterone levels. One possible explanation for this finding is that rats were sacrificed to examine GR mRNA expression when the rats were in a resting state and some time after the stress-response was measured. Also, this effect was apparent only among animals bearing the CT genotype, which may further underscore the importance of genotype in determining the influence of the prenatal stress. Clearly, the absence of a clear relationship in our animals between GR levels and the HPA stress response, while unexpected, requires further exploration of the negative feedback effects of corticosterone onto the GR receptors in animals with different genotypes and perinatal experiences. One approach to answer this question may be to infuse a glucocorticoid or a glucocorticoid agonist directly onto the hippocampal GR receptors followed by measurement of both circulating ACTH and endogenous corticosterone after application of a stressor.

In contrast to the genotypic effects on physiology, the effects of the 5-HTT genotype on behavior were dependent on the postnatal and not the prenatal environment. A clear gene × postnatal environment interaction was also observed for the acoustic startle response. Among animals bearing the CT genotype there was a clear effect of postnatal rearing with the most intense startle responses observed among rats deprived of their mothers with minimal stimulation (AR-MIN). In contrast, among CC rats there was no effect of postnatal rearing condition on startle responses. Among the CT animals, greater levels of maternal or maternal-like stimulation (stroking) were associated with progressively lower startle responsivity. These findings are consistent with previous studies showing increased startle in AR rats (Burton et al., 2006; Lovic & Fleming, 2004) and the demonstrated relation between startle and serotonin functioning (Swerdlow et al., 2006).

Our results showing a gene × postnatal environment effect on acoustic startle and locomotor activity are consistent with results reported by Kinnally and colleagues (2010), who found a gene × gene × environment effect on emotional behavior in the rhesus monkey. They simultaneously explored 2 genes possessing "high risk" 5-HTT genotypes and found that the interaction between the gene for the 5-HTT (rh5-HTTLPR) and monoamine oxidase A (rhMAOA-LPR) predicted emotional reactivity and had effects that were "exacerbated" by the experience of nursery as opposed to mother-rearing.

## The Rat 5-HTT Polymorphism: C3598T

These results suggest that the single nucleotide polymorphism, C3598T, found in the third exon of the rat 5-HTT gene, SLC6A4, plays a role in individual differences in response to early adverse environments (prenatal stress and complete maternal deprivation). Pups heterozygous for the T allele appear significantly more susceptible to perinatal adversities compared to pups homozygous for the C allele. The C3598T polymorphism is a synonymous SNP that does not affect the amino acid encoded by the respective codon. Although the change in the nucleotide does not affect the amino acid sequence, this DNA alteration can nevertheless influence regulation of 5-HTT mRNA. For example, genotype specific differences in RNA folding and structure can result in differences in subsequent RNA processing and regulation such as splicing and translational regulation of the RNA (Capon et al., 2004; Duan et al., 2003; Shen, Basilion, & Stanton, 1999). A disruption of the optimal secondary structure of mRNA due to a given polymorphic site that affects the base pairing in the secondary structure formation can affect the stability of that RNA, resulting in its premature or delayed degradation. This could affect the rate of translational properties of the gene which in turn could affect the timing of the cascade of events in the pathway in which the protein is involved (Akashi & Eyre-Walker, 1998; Capon et al., 2004; Ikemura, 1985; see review in Chamary, Parmley, & Hurst, 2006; Kimchi-Sarfaty et al., 2007). Alternatively, C3598T might simply exert its effect as a nearby marker to an as yet undescribed polymorphism or through interactions with other as yet unidentified polymorphic sites. However, until we undertake a full qt-PCR analysis in serotonin system brain sites of animals carrying the different alleles of this gene, we do not know precisely what is its physiological meaning.

There is now considerable evidence from studies of child development (Belsky & Pluess, 2009; Obradović & Boyce, 2009) that genotypes that were previously thought to enhance the risk for illness, now appear to determine differential sensitivity to early environmental influences (e.g., "plasticity" genes). In the rhesus monkey studies, maternally reared animals with the s/s 5-HTT genotype showed decreased aggressivity and more moderate emotional reactivity when compared to maternally reared l/L animals (Spinelli et al., 2007). The exact opposite developmental profile emerged among peer-reared animals. Likewise, genotype in humans is associated with differential sensitivity to environmental conditions, including treatment interventions (see Belsky & Pluess, 2009 for a review). The findings presented here suggest that the rat SLC6A4 polymorphism examined in these studies provides a model for the studies of the mechanism by which genetic variation might influence sensitivity to environmental influences.

In this paper we sought to understand how the effects of early "adverse" experiences either during prenatal life (stress exposure) or during postnatal life, (rearing without mother and nest) on later stress physiology and behavior are affected by an animal's genotype. This study indicates that the 5-HTT gene polymorphisms in the rat contributes to how an animal is affected by their earlier experiences, indicating once again that resilience in the face of adversity or susceptibility to the beneficial effects of healthy early environments, can be better understood by including genes in the complex equation. Overall, these findings suggest that rats with the 5-HTT CT polymorphism are more sensitive to early environmental perturbations than are animals with the CC polymorphism; the CC polymorphism may confer protection from prenatal and/or postnatal adversity.

## References

Akashi, H., & Eyre-Walker, A. (1998). Translational selection and molecular evolution. *Current Opinion in Genetics & Development, 8,* 688–693.

Barr, C. S., Newman, T. K., Lindell, S., Becker, M. L., Shannon, C., Champoux, M., . . . Higley, J. D. (2004). Early experience and sex interact to influence limbic-hypothalamic-pituitary-adrenal-axis function after acute alcohol administration in rhesus macaques (*Macaca mulatta*). *Alcoholism, Clinical and Experimental Research, 28,* 1114–1119.

Barr, C. S., Newman, T. K., Shannon, C., Parker, C., Dvoskin, R. L., Becker, M. L., . . . Higley, J. D. (2004). Rearing condition and rh5-

HTTLPR interact to influence limbic-hypothalamic-pituitary-adrenal axis response to stress in infant macaques. *Biological Psychiatry, 55,* 733–738.

Belsky, J., & Pluess, M. (2009). Beyond diathesis stress: Differential susceptibility to environmental influences. *Psychological Bulletin, 135,* 885–908.

Bennett, A. J., Lesch, K. P., Heils, A., Long, J. C., Lorenz, J. G., Shoaf, S. E., . . . Higley, J. D. (2002). Early experience and serotonin transporter gene variation interact to influence primate CNS function. *Molecular Psychiatry, 7,* 118–122.

Beydoun, H., & Saftlas, A. F. (2008). Physical and mental health outcomes of prenatal maternal stress in human and animal studies: A review of recent evidence. *Paediatric and Perinatal Epidemiology, 22,* 438–466.

Biagini, G., Pich, E. M., Carani, C., Marrama, P., & Agnati, L. F. (1998). Postnatal maternal separation during the stress hyporesponsive period enhances the adrenocortical response to novelty in adult rats by affecting feedback regulation in the CA1 hippocampal field. *International Journal of Developmental Neuroscience, 16,* 187–197.

Borrell, J., Vela, J. M., Arevalo-Martin, A., Molina-Holgado, E., & Guaza, C. (2002). Prenatal immune challenge disrupts sensorimotor gating in adult rats. Implications for the etiopathogenesis of schizophrenia. *Neuropsychopharmacology, 26,* 204–215.

Brake, W. G., Zhang, T. Y., Diorio, J., Meaney, M. J., & Gratton, A. (2004). Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *European Journal of Neuroscience, 19,* 1863–1874.

Burton, C., Lovic, V., & Fleming, A. S. (2006). Early adversity alters attention and locomotion in adult Sprague-Dawley rats. *Behavioral Neuroscience, 120,* 665–675.

Burton, C. L., Chatterjee, D., Chatterjee-Chakraborty, M., Lovic, V., Grella, S. L., Steiner, M., & Fleming, A. S. (2007). Prenatal restraint stress and motherless rearing disrupts expression of plasticity markers and stress-induced corticosterone release in adult female Sprague-Dawley rats. *Brain Research, 1158,* 28–38.

Caldji, C., Diorio, J., & Meaney, M. J. (2003). Variations in maternal care alter GABA(A) receptor subunit expression in brain regions associated with fear. *Neuropsychopharmacology, 28,* 1950–1959.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P. M., & Meaney, M. J. (1998). Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proceedings of the National Academy of Sciences of the United States of America, 95,* 5335–5340.

Cameron, N. M., Fish, E. W., & Meaney, M. J. (2008). Maternal influences on the sexual behavior and reproductive success of the female rat. *Hormornes and Behavior, 54,* 178–184.

Capon, F., Allen, M. H., Ameen, M., Burden, A. D., Tillman, D., Barker, J. N., & Trembath, R. C. (2004). A synonymous SNP of the corneodesmosin gene leads to increased mRNA stability and demonstrates association with psoriasis across diverse ethnic groups. *Human Molecular Genetics, 13,* 2361–2368.

Carneiro, A. M., Airey, D. C., Thompson, B., Zhu, C. B., Lu, L., Chesler, E. J., . . . Blakely, R. D. (2009). Functional coding variation in recombinant inbred mouse lines reveals multiple serotonin transporter-associated phenotypes. *Proceedings of the National Academy of Sciences of the United States of America, 106,* 2047–2052.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig, I. W., Harrington, H., . . . Poulton, R. (2003). Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science, 301,* 386–389.

Chamary, J. V., Parmley, J. L., & Hurst, L. D. (2006). Hearing silence: Non-neutral evolution at synonymous sites in mammals. *Nature Reviews Genetics, 7,* 98–108.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology, 29,* 386–397.

Champagne, F. A., Francis, D. D., Mar, A., & Meaney, M. J. (2003). Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiology & Behavior, 79,* 359–371.

Champoux, M., Bennett, A., Shannon, C., Higley, J. D., Lesch, K. P., & Suomi, S. J. (2002). Serotonin transporter gene polymorphism, differential early rearing, and behavior in rhesus monkey neonates. *Molecular Psychiatry, 7,* 1058–1063.

Chapillon, P., Patin, V., Roy, V., Vincent, A., & Caston, J. (2002). Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: A review. *Developmental Psychobiology, 41,* 373–387.

Deminiere, J. M., Piazza, P. V., Guegan, G., Abrous, N., Maccari, S., Le Moal, M., & Simon, H. (1992). Increased locomotor response to novelty and propensity to intravenous amphetamine self-administration in adult offspring of stressed mothers. *Brain Research, 586,* 135–139.

Duan, J. B., Wainwright, M. S., Comeron, J. M., Saitou, N., Sanders, A. R., Gelernter, J., & Gejman, P. V. (2003). Synonymous mutations in the human dopamine receptor D2 (DRD2) affect mRNA stability and synthesis of the receptor. *Human Molecular Genetics, 12,* 205–216.

Ellenbroek, B. A., van den Kroonenberg, P. T., & Cools, A. R. (1998). The effects of an early stressful life event on sensorimotor gating in adult rats. *Schizophrenia Research, 30,* 251–260.

Erickson, K., Gabry, K. E., Lindell, S., Champoux, M., Schulkin, J., Gold, P., . . . Higley, J. D. (2005). Social withdrawal behaviors in nonhuman primates and changes in neuroendocrine and monoamine concentrations during a separation paradigm. *Developmental Psychobiology, 46,* 331–339.

Fan, J. M., Chen, X. Q., Jin, H., & Du, J. Z. (2009). Gestational hypoxia alone or combined with restraint sensitizes the hypothalamic-pituitary-adrenal axis and induces anxiety-like behavior in adult male rat offspring. *Neuroscience, 159,* 1363–1373.

Farid, M., Martinez, Z. A., Geyer, M. A., & Swerdlow, N. R. (2000). Regulation of sensorimotor gating of the startle reflex by serotonin 2A receptors. Ontogeny and strain differences. *Neuropsychopharmacology, 23,* 623–632.

Fleming, A. S., Kraemer, G. W., Gonzalez, A., Lovic, V., Rees, S., & Melo, A. (2002). Mothering begets mothering: The transmission of behavior and its neurobiology across generations. *Pharmacology Biochemistry and Behavior, 73,* 61–75.

Francis, D. D., Diorio, J., Plotsky, P. M., & Meaney, M. J. (2002). Environmental enrichment reverses the effects of maternal separation on stress reactivity. *Journal of Neuroscience, 22,* 7840–7843.

Francis, D. D., & Meaney, M. J. (1999). Maternal care and the development of stress responses. *Current Opinions in Neurobiology, 9,* 128–134.

Fride, E., Dan, Y., Feldon, J., Halevy, G., & Weinstock, M. (1986). Effects of prenatal stress on vulnerability to stress in prepubertal and adult rats. *Physiology & Behavior, 37,* 681–687.

Fujioka, T., Sakata, Y., Yamaguchi, K., Shibasaki, T., Kato, H., & Nakamura, S. (1999). The effects of prenatal stress on the development of hypothalamic paraventricular neurons in fetal rats. *Neuroscience, 92,* 1079–1088.

Gerra, G., Zaimovic, A., Castaldini, L., Garofano, L., Manfredini, M., Somaini, L., . . . Donnini, C. (2010). Relevance of perceived childhood neglect, 5-HTT gene variants and hypothalamus-pituitary-adrenal axis dysregulation to substance abuse susceptibility. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics, 153*B, 715–722.

Glover, V., O'Connor, T. G., & O'Donnell, K. (2009). Prenatal stress and the programming of the HPA axis. *Neuroscience and Biobehavioral Reviews, 35,* 17–22.

Gonzalez, A., Lovic, V., Ward, G. R., Wainwright, P. E., & Fleming, A. S.

(2001). Intergenerational effects of complete maternal deprivation and replacement stimulation on maternal behavior and emotionality in female rats. *Developmental Psychobiology, 38,* 11–32.

Hashimoto, M., Watanabe, T., Fujioka, T., Tan, N., Yamashita, H., & Nakamura, S. (2001). Modulating effects of prenatal stress on hyperthermia induced in adult rat offspring by restraint or LPS-induced stress. *Physiology & Behavior, 73,* 125–132.

Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., & Lesch, K. P. (1996). Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry, 66,* 2621–2624.

Henry, C., Kabbaj, M., Simon, H., Le Moal, M., & Maccari, S. (1994). Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *Journal of Neuroendocrinology, 6,* 341–345.

Hofer, M. A. (1994). Early relationships as regulators of infant physiology and behavior. *Acta Paediatrica Suppl, 397,* 9–18.

Ikemura, T. (1985). Codon usage and tRNA content in unicellular and multicellular organisms. *Molecular Biology and Evolution, 2,* 13–34.

Jodi, L. L., Maxim, V. M., Jamie. L. S., & Gina, L. (2009). Foster adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and lathered hormonal stress responses. *Hormones and Behavior, 55,* 248–256.

Kalinichev, M., Easterling, K. W., Plotsky, P. M., & Holtzman, S. G. (2002). Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats. *Pharmacology Biochemisty and Behavior, 73,* 131–140.

Kapoor, A., Dunn, E., Kostaki, A., Andrews, M. H., & Matthews, S. G. (2006). Fetal programming of hypothalamo-pituitary-adrenal function: Prenatal stress and glucocorticoids. *Journal of Physiology, 572,* 31–44.

Kapoor, A., Leen, J., & Matthews, S. G. (2008). Molecular regulation of the hypothalamic-pituitary-adrenal axis in adult male guinea pigs after prenatal stress at different stages of gestation. *Journal of Physiology, 586,* 4317–4326.

Kimchi-Sarfaty, C., OH, J. M., Kim, I. W., Sauna, Z. E., Calcagno, A. M., Ambudkar, S. V., & Gottesman, M. M. (2007). A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science, 315,* 525–528.

Kinnally, E. L., Karere, G. M., Lyons, L. A., Mendoza, S. P., Mason, W. A., & Capitanio, J. P. (2010). Serotonin pathway gene-gene and gene-environment interactions influence behavioral stress response in infant rhesus macaques. *Developmental Psychopathology, 22,* 35–44.

Knackstedt, M. K., Hamelmann, E., & Arck, P. C. (2005). Mothers in stress: Consequences for the offspring. *American Journal of Reproductive Immunology, 54,* 63–69.

Koenig, J. I., Elmer, G. I., Shepard, P. D., Lee, P. R., Mayo, C., Joy, B., . . . Brady, D. L. (2005). Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: Potential relevance to schizophrenia. *Behavioural Brain Research, 156,* 251–261.

Kraemer, G. W., Moore, C. F., Newman, T. K., Barr, C. S., & Schneider, M. L. (2008). Moderate level fetal alcohol exposure and serotonin transporter gene promoter polymorphism affect neonatal temperament and limbic-hypothalamic-pituitary-adrenal axis regulation in monkeys. *Biological Psychiatry, 63,* 317–324.

Ladd, C. O., Owens, M. J., & Nemeroff, C. B. (1996). Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. *Endocrinology, 137,* 1212–1218.

Lee, P. R., Brady, D. L., Shapiro, R. A., Dorsa, D. M., & Koenig, J. I. (2007). Prenatal stress generates deficits in rat social behavior: Reversal by oxytocin. *Brain Research, 1156,* 152–167.

Lehmann, J., Stohr, T., & Feldon, J. (2000). Long-term effects of prenatal stress experiences and postnatal maternal separation on emotionality and attentional processes. *Behavioural Brain Research, 107,* 133–144.

Lemaire, V., Koehl, M., Le Moal, M., & Abrous, D. N. (2000). Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America, 97,* 11032–11037.

Levine, S. (1967). Maternal and environmental influences on the adrenocortical response to stress in weanling rats. *Science, 156,* 258–260.

Levine, S., Haltmeyer, G. C., Karas, G. G., & Denenberg, V. H. (1967). Physiological and behavioral effects of infantile stimulation. *Physiology & Behavior, 2,* 55–59.

Liu, D., Caldji, C., Sharma, S., Plotsky, P. M., & Meaney, M. J. (2000). Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinepherine release in the hypothalamic paraventricular nucleus. *Journal of Neuroendocrinology, 12,* 5–12.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., . . . Meaney, M. J. (1997). Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science, 277,* 1659–1662.

Louvart, H., Maccari, S., Vaiva, G., & Darnaudery, M. (2009). Prenatal stress exacerbates the impact of an aversive procedure on the corticosterone response to stress in female rats. *Psychoneuroendocrinology, 34,* 786–790.

Lovic, V., & Fleming, A. S. (2004). Artificially-reared female rats show reduced prepulse inhibition and deficits in the attentional set shifting task–reversal of effects with maternal-like licking stimulation. *Behavioural Brain Research, 148,* 209–219.

Maccari, S., Piazza, P. V., Kabbaj, M., Barbazanges, A., Simon, H., & Le Moal, M. (1995). Adoption reverses the long-term impairment in glucocorticoid feedback induced by prenatal stress. *Journal of Neuroscience, 15,* 110–116.

Macri, S., Mason, G. J., & Wurbel, H. (2004). Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. *European Journal of Neuroscience, 20,* 1017–1024.

McCormick, C. M., Smythe, J. W., Sharma, S., & Meaney, M. J. (1995). Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. *Brain Research Developmental Brain Research, 84,* 55–61.

Meaney, M. J., Aitken, D. H., Bodnoff, S. R., Iny, L. J., Tatarewicz, J. E., & Sapolsky, R. M. (1985). Early postnatal handling alters glucocorticoid receptor concentrations in selected brain regions. *Behavioral Neuroscience, 99,* 765–770.

Melo, A. I., Perez-Ledezma, M., Clapp, C., Arnold, E., Rivera, J. C., & Fleming, A. S. (2009). Effects of prolactin deficiency during the early postnatal period on the development of maternal behavior in female rats: Mother's milk makes the difference. *Hormones and Behavior, 56,* 281–291.

Mintz, M., Yovel, G., Gigi, A., & Myslobodsky, M. S. (1998). Dissociation between startle and prepulse inhibition in rats exposed to gamma radiation at day 15 of embryogeny. *Brain Research Bulletin, 45,* 289–296.

Moore, C. L., & Power, K. L. (1986). Prenatal stress affects mother-infant interaction in Norway rats. *Developmental Psychobiology, 19,* 235–245.

Novakov, M., & Fleming, A. S. (2005). The effects of early rearing environment on the hormonal induction of maternal behavior in virgin rats. *Hormornes and Behavior, 48,* 528–536.

Obradović, J., & Boyce, W. T. (2009). Individual differences in behavioral, physiological, and genetic sensitivities to contexts: Implications for development and adaptation. *Developmental Neuroscience, 31,* 300–308.

O'Donnell, K., O'Connor, T. G., & Glover, V. (2009). Prenatal stress and neurodevelopment of the child: Focus on the HPA axis and role of the placenta. *Developmental Neuroscience, 31,* 285–292.

Paxinos, G., & Watson, C. (1996). *The rat brain in stereotaxic coordinates.* San Diego: Academic Press.

Plotsky, P. M., Thrivikraman, K. V., Nemeroff, C. B., Caldji, C., Sharma, S., & Meaney, M. J. (2005). Long-term consequences of neonatal

rearing on central corticotropin-releasing factor systems in adult male rat offspring. *Neuropsychopharmacology, 30,* 2192–2204.

Roughton, E. C., Schneider, M. L., Bromley, L. J., & Coe, C. L. (1998). Maternal endocrine activation during pregnancy alters neurobehavioral state in primate infants. *American Journal of Occupational Therapy, 52,* 90–98.

Schanberg, S. N., Evoniuk, G., & Kuhn, C. M. (1984). Tactile and nutritional aspects of maternal care: Specific regulators of neuroendocrine function and cellular development. *Proceedings of the Society for Experimental Biology and Medicine, 175,* 135–146.

Seckl, J. R., & Meaney, M. J. (2004). Glucocorticoid programming. *Annals of the New York Academy of Sciences, 1032,* 63–84.

Shannon, C., Champoux, M., & Suomi, S. J. (1998). Rearing condition and plasma cortisol in rhesus monkey infants. *American Journal of Primatology, 46,* 311–321.

Shen, L. X., Basilion, J. P., & Stanton, V. P. (1999). Single-nucleotide polymorphisms can cause different structural folds of mRNA. *Proceedings of the National Academy of Sciences of the United States of America, 96,* 7871–7876.

Spinelli, S., Schwandt, M. L., Lindell, S. G., Newman, T. K., Heilig, M., Suomi, S. J., . . . Barr, C. S. (2007). Association between the recombinant human serotonin transporter linked promoter region polymorphism and behavior in rhesus macaques during a separation paradigm. *Development and Psychopathology, 19,* 977–987.

Stein, M. B., Schork, N. J., & Gelernter, J. (2008). Gene-by-environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. *Neuropsychopharmacology, 33,* 312–319.

Stone, E. A., Bonnet, K. A., & Hofer, M. A. (1976). Survival and development of maternally deprived rats: Role of body temperature. *Psychosomatic Medicine, 38,* 242–249.

Suomi, S. J. (1991). Early stress and adult emotional reactivity in rhesus monkeys. *Ciba Foundation Symposium, 156,* 171–183; discussion 183–178.

Suomi, S. J. (1997). Early determinants of behaviour: Evidence from primate studies. *British Medical Bulletin, 53,* 170–184.

Swerdlow, N. R., Geyer, M. A., Shoemaker, J. M., Light, G. A., Braff, D. L., Stevens, K. E., . . . Auerbach, P. P. (2006). Convergence and divergence in the neurochemical regulation of prepulse inhibition of startle and N40 suppression in rats. *Neuropsychopharmacology, 31,* 506–515.

Thomas, M. B., Hu, M., Lee, T. M., Bhatnagar, S., & Becker, J. B. (2009). Sex-specific susceptibility to cocaine in rats with a history of prenatal stress. *Physiology & Behavior, 97,* 270–277.

Thompson, W. R. (1957). Influence of prenatal maternal anxiety on emotionality in young rats. *Science, 125,* 698–699.

Vallee, M., MacCari, S., Dellu, F., Simon, H., Le Moal, M., & Mayo, W. (1999). Long-term effects of prenatal stress and postnatal handling on age-related glucocorticoid secretion and cognitive performance: A longitudinal study in the rat. *European Journal of Neuroscience, 11,* 2906–2916.

Vallee, M., Mayo, W., Dellu, F., Le Moal, M., Simon, H., & Maccari, S. (1997). Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: Correlation with stress-induced corticosterone secretion. *Journal of Neuroscience, 17,* 2626–2636.

Wakshlak, A., & Weinstock, M. (1990). Neonatal handling reverses behavioral abnormalities induced in rats by prenatal stress. *Physiology & Behavior, 48,* 289–292.

Ward, I. L. (1972). Prenatal stress feminizes and demasculinizes the behavior of males. *Science, 175*(17), 82–84.

Weaver, I. C., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., . . . Meaney, M. J. (2004). Epigenetic programming by maternal behaviour. *Nature Neuroscience, 7,* 847–854.

Weaver, I. C., Diorio, J., Seckl, J. R., Szyf, M., & Meaney, M. J. (2004). Early environmental regulation of hippocampal glucocorticoid receptor gene expression: Characterization of intracellular mediators and potential genomic target sites. *Annals of the New York Academy of Science, 1024,* 182–212.

Weinstock, M. (2005). The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain, Behavior, and Immunity, 19,* 296–308.

Weinstock, M. (2008). The long-term behavioural consequences of prenatal stress. *Neuroscience and Biobehavioral Reviews, 32,* 1073–1086.

Welberg, L. A., Seckl, J. R., & Holmes, M. C. (2001). Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: Possible implications for behaviour. *Neuroscience, 104,* 71–79.

Zhang, T. Y., & Meaney, M. J. (2010). Epigenetics and the environmental regulation of the genome and its function. *Annual Review of Psychology, 61,* 439–466, C431–433.

Zhang, T. Y., Parent, C., Weaver, I., & Meaney, M. J. (2004). Maternal programming of individual differences in defensive responses in the rat. *Annals of the New York Academy of Sciences, 1032,* 85–103.

Zimmerberg, B., & Shartrand, A. M. (1992). Temperature-dependent effects of maternal separation on growth, activity, and amphetamine sensitivity in the rat. *Developmental Psychobiology, 25,* 213–226.

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