Published in final edited form as:

Dev Psychobiol. 2012 November ; 54(7): 723–735. doi:10.1002/dev.21028.

Effects of brief stress exposure during early postnatal development in Balb/CByJ mice: II. Altered Cortical Morphology

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Nakia Beard, Patience Kari-Kari and Qiana Simmons were undergraduate students, and Natasha Jarvis was a graduate student in the Biology Department at Morgan State University when they conducted the work in this manuscript.

Abstract

Early life experience can significantly determine later mental health status and cognitive function. Neonatal stress, in particular, has been linked to the etiology of mental health disorders as divergent as mood disorder, schizophrenia and autism. Our study uses a Balb/CByJ mouse model to test the hypothesis, that neonatal stress will alter development and subsequent environmental modulation of neocortex. Using a split litter design, we generated stressed mice (STR) and within litter controls (LMC) along with age-matched, untreated animals (AMC), to serve as across litter controls. Short, daily exposure to a psychosocial/physical stressor, during the first week of life, resulted by adulthood in significant changes in neocortical thickness and architecture, which were further modulated by exposure to behavioral testing. Surprisingly, cortical size in LMC mice was also affected. These observations were compared to the effects of environmental enrichment in the same mouse strain. Our data indicate that LMC and STR males share with environmentally enriched males, an increase in thickness in infra-granular cortical layers, while STR also display a stress selective decrease in supragranular layers, in response to behavioral training as adults.

Keywords

early experience; stress; neocortex; rodent; sex differences

Introduction

The involvement of environmental stressors in the etiology of mental health disorders, such as schizophrenia, clinical depression and more recently, Autism Spectrum Disorder, has been well established (Anisman & Zacharko, 1982; de Kloet, Joels, & Holsboer, 2005; Heim & Nemeroff, 1999; Juul-Dam, Townsend, & Courchesne, 2001; Kinney, Munir, Crowley, & Miller, 2008; McEwen, 2004). The underlying concept of developmental programming suggests, that early life experiences predict subsequent patterns of gene expression, hormone release and behavioral outcomes, which may manifest in conjunction with secondary stress exposure in later life, to trigger psychiatric and or neurological disorders (Anisman &

Zacharko 1982; de Kloet et al., 2005; Holmes, le Guisquet, Vogel, Millstein, Leman, & Belzung, 2005; Seckl & Meaney, 2004).

To date, research on stress mediated developmental programming in animal models has focused predominantly on hormonal responses and behavioral parameters. In rat and mouse, neonatal maternal separation, typically removal of the pups from the dam for intervals of three to six hours/day, for a two to three week duration following birth, has been most frequently used to model early postnatal stress exposure (e.g. Anisman, Zaharia, Meaney, & Merali, 1998; Kaffman & Meaney, 2007; Sanchez, Ladd, & Plotsky, 2001). Such neonatal stress protocols result in HPA hyper-responsiveness to stressors in later life, significant alterations in adult behavior and also changes in hippocampal neuronal networks (Anisman et al., 1998; de Kloet et al., 2005; Avisheh-Eliner, Gilles, Eghbal-Ahmadi, Bar-El & Baram 95; Champagne, Bagot, VanHasselt, Ramakers, Meaney & de Kloet 08; Levine, 2002; Liu, Diorio, Tannenbaum, Caldji, Francis, Freedman, Sharma, Pearson, Plotsky, & Meaney, 1997; Meaney, Diorio, Francis, Widdowson, LaPlante, Caldji, Sharma, Seckl, & Plotsky, 1996; Zhang, Levine, Dent, Zhan, Xing, Okimoto 2002; Anderson & Teicher 2004). On the other hand, there has been surprisingly little attention directed towards neonatal stress effects on the functional development of neocortex, considering, that in most mental health disorders associated with stress, neocortical morphology is affected. Thus, a better understanding of the effects of early life stress on functional neocortical development and subsequent modulation by environmental effects is in order.

The present study is designed to test the hypothesis, that early stress will alter neocortical morphogenesis and the ability of functional neocortical networks to adapt to subsequent environmental challenges. The importance of early environmental influences on the functional morphology of rodent sensory neocortex is well illustrated by studies concerning the effects of early enrichment and training (Baroncelli, Braschi, Spolidoro, Begenisic, Sale, & Maffei, 2010; Diamond, 2001; Rosenzweig & Bennet, 1996; van Praag, Kempermann, & Gage, 2000; Walsh, 1981). Enrichment also has been shown to modulate behavioral, endocrine and physiological phenotypes associated with early life stressors (Bakos, Duncko, Makatsori, Pirnik, Kiss, & Jezova, 2004; Bredy, Humpartzoomian, Cain, & Meaney, 2003; Koo, Park, Choi, Kim, Kim, Choe, & Suh, 2003), suggesting, that enrichment and stress may be acting on similar structural and functional brain substrates.

The stress protocol we have developed to test our hypothesis differs from many currently used approaches for several reasons. Prolonged maternal separation has the confounding factor of metabolic deprivation (Schmidt, Levine, Alam, Harbich, Sterlemann, Ganea, de Kloet, Holsboer, & Muller, 2006; Suchecki, Rosenfeld, & Levine, 1993; van Oers, de Kloet, Whelan, & Levine, 1998). Malnutrition can substantially affect brain development (Del Angel-Meza, Ramirez-Cortes, Adame-Gonzalez, Gonzalez Burgos, & Beas-Zarate, 2002; Resnick, Miller, Forbes, Hall, Kemper, Bronzino, & Morgane, 1979) and we therefore chose to minimize the maternal separation time. To ensure, nevertheless, a sufficient stress effect in our mice, we introduced temperature as an additional, physical stressor, alternating warm and cold temperature exposures, on subsequent days, to minimize habituation to the cold stressor. We limited our stress exposure, furthermore, to the first postnatal week, since this time window represents the most dynamic developmental period in the ontogeny of

somatosensory cortex (Berger-Sweeney & Hohmann, 1997) and has shown itself highly effective in eliciting stress effects in the brain (Avishai-Eliner, Gilles, Eghbal-Ahmadi, Bar-El, & Baram, 2001; Enthoven et al., 2008; Bock, Gruss, Becker, & Braun, 2005; Gos et al., 2008; Knuth & Etgen, 2005). We also implemented a split litter design, raising maternally separated/temperature stressed (STR) and non-separated pups (LMC) in the same litters, with the goal of minimizing variability due to between-litter maternal care differences.

We selected the somatosensory, anterior parietal cortex as most appropriate neocortical area for our investigation for two reasons. First, substantial data suggest that somatosensory maternal interactions with the pups (e.g. licking/grooming), or the lack thereof, are important mediators/modulators of maternal separation stress (Caldji, Diorio, & Meaney, 2000; Garoflos, Stamatakis, Rafrogianni, Pondiki, & Stylianopoulou, 2008; Kaffman & Meaney, 2007; Levine, 2002; Liu et al. 1997, 2000). Second, the rodent somatosensory cortex, particularly, the “barrel field” area (Jeanmonod, Rice, & Van der Loos, 1981), provides a well-delineated sampling area for the study of development and plasticity in somatosensory neocortex, and has been used by us effectively, in other developmental studies, to characterize neuromodulatory effects on cortical thickness (Hohmann & Berger-Sweeney, 1998; Hohmann, Richardson, Pitts, & Berger-Sweeney, 2000; Krasnova, Betts, Dada, Jefferson, Ladenheim, Becker, Cadet, & Hohmann, 2007). We are using Balb/CByJ mice for our paradigm, because of their high stress responsiveness (Brinks, van der Mark, de Kloet, & Oitzl, 2007; Ducottet & Belzung, 2004; Shanks, Griffith & Anisman, 1994) and potential for developmental plasticity in response to environmental factors (Chapillon, Manneche, Belzung, & Caston, 1999; Priebe, Brake, Romeo, Sisti, Mueller, McEwen, & Francis, 2006; Zaharia, Kulczycki, Shanks, Meaney, & Anisman, 1996).

Materials and Methods

All studies were performed on offspring of Balb/CByJ mice, purchased from Jackson Laboratories and raised in our own breeding colony at Morgan State University (MSU). Mice were housed in clear polycarbonate cages, in temperature- and humidity-controlled rooms with a timed 12 light/dark cycle in accordance with to the guidelines of the American Association of Veterinary Medicine. Mice were behaviorally tested during the light portion of the light/dark cycle. All protocols were approved by the Institutional Animal Care and Use Committee at MSU and carried out in accordance with NIH Guide for the Care and Use of Laboratory Animals and the guidelines of the American Veterinary association.

Neonatal stress exposure and experimental design

Male and female Balb/CByJ pups were subjected to maternal separation for one hour daily between postnatal day [PND] two and seven. Pups were removed for stress exposure late morning, during the light cycle. We employed a split litter design, removing half the male and female pups daily from the dam, for 6 days, for one hour/day (STR group); the other half of the litter remained with the dam (LMC group). STR vs. LMC pups were marked by toe clip (digit 5, STR bilaterally, LMC digit 5 unilaterally). STR and LMC pups were marked with non-toxic paint on their backs during the first postnatal week, for easier identification of STR pups, for daily removals. During the stress procedure, pups were

exposed, on alternating days, to 30 min. of heat (37 degree C°) or cold stress (5 degree C°), allowed to re-acclimatize to room temperature for 30 minutes and then returned to the dam in the home cage. Following the stress exposure, pups remained with the dam until PND 30 when they were weaned. At weaning, mice were separated by sex, and group housed according to their litters of origin. Additional litters, born during the same time period as the STR/LMC mice, were used as age matched, colony-reared controls (AMC). With the exception of weekly cage changes, which did not commence until the pups were one week old, these mice were not handled at all, until weaning. At three months of age, a subset of STR, LMC and AMC mice were assigned to behavioral testing.

Behaviorally tested and yoked, non-tested mice for each condition, were transcardially perfused with 10% buffered formaldehyde solution, four to eight weeks after conclusion of behavioral testing, and brains were removed for quantitative histological assessments as described previously by us (e.g. Boylan, Blue, & Hohmann, 2007; Hohmann et al., 2000; Krasnova et al., 2007). Brain weights were taken at this time. The brains were blocked perpendicular to the cortical surface, with one cut taken through the base of the cerebellum, quick frozen in isopentane, and then stored at -70°C until the time of sectioning for histology. All experiments include mice from at least three different litters (usually 5-6 different litters) in each group. Sample sizes for each experiment are indicated in the results section.

Corticosterone levels

Separate groups of STR and LMC male and female mice were exsanguinated on PND 7, within 60-90 minutes following the last stress exposure of the STR group. For each litter, STR and LMC male and female mice were alternated during this procedure to avoid biased measurements based on exact time of blood collection. Blood was collected into 1.5ml Eppendorf tubes (containing 100 microliters of a 0.6% NaEDTA solution in physiological saline) and gently vortexed; serum was removed by centrifugation and stored at -70°C. Corticosterone content was assayed by radioimmunoassay (RIA) using the commercially available 125RIA kit for rats and mice from MP Biomedicals, LLC. Statistical analysis was performed using Student's t-test (StatView).

Behavioral Training/Testing

At three months of age all mice assigned to behavioral testing were first subjected to a **neurological test battery** as previously described (Hohmann, Walker, Boylan, & Blue, 2007; Picker, Yang, Ricceri, & Berger-Sweeney, 2006). Briefly, we assessed coordination, balance and strength by monitoring the mice' righting response, grip strength and balance on crossing a thin long rod towards the home cage. Mice were scored with the examiner blind to treatment group. All tests were performed in triplicate and average scores for each animal were used for analysis. This neurological testing took approximately one hour/mouse and also served as "handling" exposure for the mice, acquainting them to the individual conducting their subsequent behavioral testing. Subsequently a subset of mice performed the **Open Field Object Recognition Task (OFOR)**. This task has been adapted from the procedure used by Ricceri and colleagues (Ricceri, Usiello, Valanzano, Calamandrei, Frick, & Berger-Sweeney, 1999) and is performed in a round enclosure of approximately three feet

diameter, populated by multiple objects. A detailed description of tasks, including diagrams, is presented in (Hohmann et al., 2007; Krasnova et al., 2007) and also provided in our behavioral companion paper. Statistical analysis of the data was performed using repeated measures (one way) ANOVA with Tuckey's post hoc tests (GraphPad Prism).

Environmental Enrichment [EER] was carried out according to previously described paradigms (Baroncelli et al., 2010; Comeau et al.; Rosenzweig & Bennet, 1996). Briefly, at weaning (30 days postnatal), EER mice were housed in large (16 1/2" L × "8 1/2" D × "8" H) cages, in same sex groups of littermates. Rodent toys, purchased from a pet store (building block that allowed different 3D assemblies of tunnels, boxes and free standing objects), were added to the cage and the configuration of these toys was changed each week, until the mice were three months of age. Mice were perfused for histological assessment at this time.

Histology and Morphometry

Frozen brains were mounted, posterior surface down, on a frozen stage microtome (Microm) and sectioned in the coronal plane at 50 microns. Sections were collected free-floating, mounted on gelatin-coated slides and Nissl stained. All slides were coded to avoid experimenter bias during morphometric analysis. Cortical cytoarchitecture was assessed using the AIS Image system (ST. Cathrine's, Ontario) to measure individual cortical layer thickness in parietal cortex, as described previously by us, for developing and mature mice and rats (Byers, Irwin, Moss, Sumaya, & Hohmann, 2005; Hohmann & Berger-Sweeney, 1998; Hohmann et al., 2000; Krasnova et al., 2007; Ricceri, Hohmann, & Berger-Sweeney, 2002). Briefly, we sampled the anterior and posteromedial barrel field area of somatosensory cortex, bilaterally. As in prior studies, we used landmarks such as the anterior commissure, positioning/shape of the lateral ventricle and the Posteromedial Barrel Subfield boundaries for positioning of the sample area. Five individual measurements of a) total cortical thickness (including layer I) and b) of layers II/III, IV, V and VI individually, were made, bilaterally, in each section (see also Figure 1). Means of these individual measurements for each hemisphere/section were analyzed for group differences using factorial ANOVA with Fisher post-hoc tests (StatView).

Results

In the following sections we present a series of studies, conducted on several, separate cohorts of experimental and control mice. We thus have grouped these studies by cohort and assigned them designation as Experiment #1 through #4. Please note that the goal of our corticosterone measurements was to validate the "stressfulness" of our brief maternal separation/temperature stress paradigm. Therefore we selected to measure corticosterone levels only in pups, at postnatal day 7, immediately upon conclusion of the stress procedure. Meaningful corticosterone assessments in adulthood would require a re-stress protocol that may have interfered with the outcomes of our behavioral and morphological experiments. Experiments # 1 and # 2 utilized, exclusively, within litter controls (LMC) for the stressed mice (STR). However, after identifying altered morphology and behavior in these mice, we routinely included separate, yoked control litters as well.

Experiment # 1: Effects of maternal separation/temperature stress on corticosterone levels

Eight male and female STR and LMC mice each, from five different litters were used for corticosterone measurements at PND 7, immediately following the last stress exposure of the STR groups. Corticosterone was elevated in STR compared to LMC mice.

Measurements in several samples, equally distributed among males/females and STR/LMC conditions fell below the linear part of the standard curve for the assay and were eliminated from subsequent statistical analysis. Thus, males and females were analyzed jointly, using a t-test, to generate sufficient sample size. Although there was considerable variability among the STR mice, differences between the groups closely approached statistical significance ($P = 0.057$, $F=4.06$, DF_{20}) (see Table 1). This demonstrates that our maternal separation/temperature stress protocol is effective in acutely altering the HPA axis response in the pups during the stress hypo-responsive period.

Experiment # 2: Effects of neonatal stress and adult behavioral experience on cortical morphology

Twenty male and female Balb/CByJ mouse pups received stress as neonates (STR) and twenty littermates remained unstressed (LMC), as described in the materials and methods section. At weaning, these litters were further separated into mice slated for behavioral testing (STR-B, LMC-B) and mice kept behaviorally naïve (STR, LMC). The neurological test battery, performed at three month of age, on all mice (including behaviorally naïve litters) did not reveal any significant differences in coordination, balance and grip-strength between stressed and litter-mate mice, of either sex. Ten male and female STR and LMC mice were subsequently subjected to the Open Field Object Recognition test. Repeated measures (one way) ANOVA, of the overall exploratory behavioral patterns, showed significant group differences ($P = 0.03$, $F=3.792$, $DF = 12$); Tuckey's post-hoc analysis revealed that STR males displayed significantly ($P < 0.05$) less object exploration compared to other male groups (see supplemental Figure 1). The data suggest an accelerated habituation in STR males.

Following these behavioral assessments, all tested mice (STR-B, LMC-B) and their non-tested littermates (STR, LMC) were perfused transcardially and body and brain weights were assessed. No significant differences between STR and LMC mice, tested or untested, were observed in body or brain weight (see Table 2).

A total of $N=25$ male and $N=19$ female brains were selected based on quantifiable cortical histology from each group, STR (stressed), STR-B (stressed-behaviorally tested), LMC (litter mate controls) and LMC-B (behaviorally tested litter mate controls) and analyzed morphometrically. Measurements for each hemisphere were made separately and pooled for statistical analysis, using two-way, factorial ANOVA for sex and condition with Fisher post-hoc testing (StatView). Qualitative analysis of these brains revealed clear staining patterns with well-differentiated cortical layers (see Figure 1). ANOVA showed a significant main effect for condition, in overall cortical thickness ($P = 0.03$, $F = 3.0$, $DF = 88$), cortical layers II/III ($P = 0.02$, $F = 3.4$, $DF = 88$) and cortical layer IV ($P = 0.005$, $F = 4.5$, $DF = 88$). There was no significant difference for condition in layers V and VI (not shown), however sex by condition interaction did approach significance ($P = 0.06$, $F = 2.5$, $DF = 88$) in layer V. ANOVA

main effects for total cortical thickness were driven by a highly significant decrease of cortical thickness in stressed behaviorally tested males (STR-B) compared to STR males without behavioral testing (post-hoc $P=0.007$). Only in cortical layers II/III was there a significant decrease, post behavioral testing, in females ($P=0.03$, post hoc) as well as males ($P=0.04$). In layer IV, significant post-hoc differences between STR and STR-B mice ($P=0.008$) were augmented by a significant decrease in thickness in male LMC-B ($P=0.02$) compared to the un-tested LMC. In layer V, a significant post hoc difference emerged between STR and STR-B males, only. These data are illustrated in Figure 2.

A decrease in cortical thickness, as a result of behavioral testing, was entirely unexpected. Such decrease was inconsistent with prior reports of behavioral enrichment effects on cortical size (Comeau et al. 2010; Diamond et al., 1966; Greenough, Juraska, & Volkmar, 1979; Rosenzweig & Bennet, 1996) and also appeared to be in disagreement with previously reported “rescue” of the behavioral phenotype by enrichment (Bakos et al., 2004; Bredy et al., 2003; Koo et al. 2003; Moncek et al., 2004; Van Oers et al. 1998). In order to verify that our particular mouse strain could, indeed, mount a “normal” plastic response to environmental enrichment, we designed experiment # 3, below.

Experiment # 3: Effects of environmental enrichment on cortical plasticity

Three groups of eight each, normal Balb/CByJ males were exposed to one of the following conditions: 1) “Classical” enriched rearing from weaning to three month postnatal, as described in the methods above; 2) normal group housing in small mouse cages until three months postnatal, followed by OFOR testing as conducted for our initial experiment or 3) normal group housing in small mouse cages until three month postnatal (control). All three groups were perfused and processed for histological analysis of cortical thickness as described in the methods, using the same procedures as employed in experiments # 2 and # 4. Morphometric analysis (based on separate assessment for right and left hemisphere for each brain), revealed a significant ANOVA main effect for total cortex ($P=0.04$, $F=3.3$, $DF=44$), which derived mainly from an increase in thickness in the “classical enrichment paradigm” ($P=0.018$ post hoc) and augmented by a non-significant trend for increased thickness in males tested in the Open Field Object Recognition task (Figure 3 A). Interestingly as shown in Figure 3 B&C, enrichment related increases in cortical thickness were apparent only in infra-granular cortex layers V ($P=0.0008$, $F=8.4$, $DF=44$, ANOVA main effect; $P=0.0003$ post-hoc) and VI ($P=0.0085$, $F=5.3$, $DF=44$ ANOVA main effect; $P=0.0028$ post hoc), leaving layer IV unaffected (3D). Thus, environmental enrichment, in normal age matched Balb/CByJ mice, did conform to the previous literature, demonstrating increased cortical thickness (see introduction). Open Field Object Recognition test exposure, on the other hand, showed itself insufficient in inducing significant enrichment effects in normal male Balb/CbyJ mice, but nevertheless, led to a slight increase in cortical thickness, not a decrease, as observed in experiment # 2 with neonatally stressed mice. Thus, we designed experiment # 4, to directly compare the effects of OFOR testing on neonatal stress in STR and LMC mice, with a yoked cohort of age matched control mice from different litters

Experiment # 4: Interactions of neonatal stress, rearing conditions and behavioral experience on cortical cytoarchitecture

Due to our previously observed, minimal morphological effects on females, we excluded them here from further analysis. We used 12, behaviorally naïve, age matched (AMC) and behaviorally tested, AMC-B, males and doubled the sample size of STR, STR-B, LMC and LMC-B males to 16. These data revealed a more complex relationship between stress and behavioral effects than the original study had indicated, with significant one-way ANOVA main effects for overall cortical thickness ($P < 0.0001$, $F = 5.9$, $DF = 87$), as well as, layers II/III ($P = 0.0008$, $F = 4.6$, $DF = 87$), IV ($P = 0.001$, $F = 4.5$, $DF = 87$), V ($P = 0.002$, $F = 4.3$, $DF = 87$) and VI ($P = 0.005$, $F = 3.7$, $DF = 87$). As shown in Figure 4A, STR exposure, without subsequent behavioral testing, actually increased cortical thickness significantly, compared to AMC (Fisher post hoc $P < 0.0001$) and behavioral experience in STR males, subsequently, decreased cortical thickness in STR-B mice in a highly significant way (Fisher post-hoc $P = 0.0003$). However, STR-B mice did not differ in cortical thickness compared to AMC mice, thus, adult behavioral experience appeared to reduce overall cortical thickness back to “normal” levels. Behavioral testing in AMC, as also observed in experiment #3, increased overall cortical thickness slightly, though not significantly. On the other hand, both untested LMC ($P = 0.001$ post-hoc) as well as behaviorally tested LMC-B ($P = 0.005$ post hoc) did show significantly increased thickness, compared to behaviorally naïve AMC. Thus, LMC males experienced increased cortical thickness, like STR males, but unlike their STR littermates, did not show a significant decrease in overall cortical thickness as a result of behavioral testing.

Fisher post-hoc analysis of individual cortical layers revealed that this paradigm, in both STR and LMC, increased infra-granular cortical layer thickness, compared to AMC (see Fig 4 D, E). In fact, a significant increase in thickness was observed in layers V and VI for all conditions, including AMC-B (ranging from $P < 0.05$ to $P < 0.005$) compared to untested AMC. But in layer IV (4C), behaviorally tested STR-B mice showed a highly significant decrease compared to STR ($P = 0.002$) as well LMC-B compared to LMC ($P = 0.02$). STR-B, furthermore, were significantly thinner compared to AMC ($P < 0.0001$) and AMC-B ($P = 0.001$).

In contrast, in layers II/III, only STR-B mice displayed significantly diminished thickness after behavioral testing, compared to STR males ($P = 0.001$). STR-B also showed significantly thinning compared to AMC ($P < 0.0001$). Although both LMC and LMC-B were significantly thinner ($P < 0.05$) than AMC, there was no decrease induced by behavioral testing in LMC-B compared to LMC males.

Discussion

We show here, that a relatively short, combined psychosocial/physical stress protocol in neonatal Balb/CByJ mice, results in increased corticosterone levels during the stress hypo-responsive period (Sapolsky & Meaney 1986) and leads to significant changes in somatosensory cortical size and architecture in Balb/CByJ mice. Furthermore, the cortical changes, resulting from the stress exposure, are subject to modification by behavioral exposure in young adulthood. The results we present here support our hypothesis that early

stress will alter neocortical morphogenesis and the ability of functional neocortical networks to adapt to subsequent environmental challenges. To our knowledge, this is the first such account of early postnatal stress effects. While the magnitude of morphological changes we describe may be unexpectedly large, it is not out of proportion with other studies we have conducted using the same morphometric approach in Balb/CbyJ mice (Hohmann & Berger-Sweeney, 1998; Hohmann et al., 2000; Krasnova et al., 2007). Few other labs have previously conducted quantitative morphological assessments of cortex in mice, in response to early environmental exposure. We presume that our selection of a mouse strain with high reactivity to environmental factors is largely responsible for the magnitude of changes we report here.

Our data show a complex pattern of layer, sex and experience specific alteration in cortical thickness. Surprisingly, both LMC and STR males, in our split litter design, compared to AMC males, displayed increased cortical thickness, selectively, in infragranular cortical layers V and VI (see Fig. 4). These layer specific changes were similar to the cortical thickness increases we saw in the same cortical layers when our Balb/CByJ mice were exposed to “classic” environmental enrichment conditions (see Fig. 2). Changes in infragranular cortex are consistent with the literature. For example, Greenough and colleagues observed increased dendritic material in layer V of parietal cortex following Hebb-Williams maze training (Greenough et al., 1979) and Kolb and colleagues noted increased dendritic length and branching in forelimb cortex after learning of a motor task (Kolb, Cioe, & Cateau 2008).

Since infragranular cortical changes (layers V and VI) are not selective to STR males but also occur in LMC males, both STR and LMC male pups appear to be vulnerable to a shared environmental trigger. This could be some level of stress as consequence of the nest disruption. Interestingly, within this context, it has been proposed that environmental enrichment itself may actually be a mild stressor in mice (Moncek et al 2004). Alternatively, both LMC and STR pups might experience an early “enrichment” effect; daily “handling” of the pups may induce altered maternal behavior toward all pups which is regarded as an early enrichment effect (Kaffman & Meaney 2007; Ladd et al. 2005, Meerlo et al. 1999, Suchecki Rosenfeld & Levine 1993, Zaharia et al. 1996) which may in turn increase cortical size in all pups (STR and LMC) in the split litter. This is a hypothesis we are currently testing in our paradigm.

However, neither of the above interpretations can adequately explain why cortex in male LMC and STR mice is selectively affected, particularly, since males and females do not significantly differ in their corticosterone response as infants (Table 1). It is noteworthy, that females are also less affected behaviorally in our stress model (see: Supplemental Fig. 1 and Hohmann et al. 2012a). Future studies will have to investigate if and how changes in cortical morphology and plasticity may be related to specific endocrine alterations in our model and how they may become dimorphic by sex. The mechanisms associated with these cortical changes may have relevance for the further understanding of, and subsequent therapeutic approaches to a number of different mental health disorders. There is, for example, a preponderance of males with diagnosis of schizophrenia and autism spectrum disorder (Abel,

Drake & Goldstein 2010; Fombonne, 1999; Leung & Chue, 2000), both disorders with stress related etiologies and profound changes in cortical developmental.

As described in our companion paper (Hohmann et al. 2011a) and also illustrated in supplemental Figure 1, the behavioral profiles in STR and LMC males differ significantly from each other. Neither the behavioral profiles of STR nor LMC males are fully consistent with the enrichment literature, typically characterized by improved cognitive performance (compare: Hohmann et al. 2011a; Rosenzweig & Bennet, 1996). However, while we did not observe a stress induced improvements in learning/memory performance in either STR or LMC mice, neither did we see a decrease in cognitive performance, as reported by many previous studies utilizing longer duration stress protocols (Anisman et al., 1998; Levine, 2002; de Kloet et al., 2005; Meaney, Diorio, Francis, Widdowson, LaPlante, Caldji, Sharma, Seckl, & Plotsky, 1996; Liu et al. 2000). This could be due to the mice experiencing a mild “enrichment effect” as consequence of out maternal separation protocol and subsequent changes in maternal care, or, as adults, due to behavioral testing in the Open Field Object Recognition test. Interestingly, increased motor activity, such as our mice experience when performing the Open Field Object Recognition task, has been associated with enrichment effects in the past (van Praag et al., 2000) Increased cognitive performance, despite increased anxiety (indicative of stress) also was associated with enriched early life experiences, such as communal nesting (Branchi, D'Andrea, Sietzema, Fiore, Di Fausto, Aloe, & Alleva, 2006). It may be useful, in future, to explore the effects of our short duration stress paradigm on more complex assessments of cognitive function then the tests employed here and in our companion paper.

Our morphological findings, for both STR and LMC mice, most closely resemble observations by Gibb and Kolb (Gibb & Kolb, 2005) of significantly increased overall, parietal cortical thickness. In their study, Wistar rat pups, with and without medial frontal cortical lesions, were exposed to what was intended to be a “handling” paradigm, from postnatal day four to weaning. The rats were separated from the dam three times daily, for 15 minutes each, between postnatal days (PND) 4 and weaning at PND 21. Such daily separation intervals are similar to our maternal separation paradigm, since rat pups were separated from the dam for 45 min total/day, much longer then is typical for “handling” paradigms. Moreover, all rats were exposed to hypothermia anesthesia at PND 4. Unlike typically found for “handled” rat, the animals in the study by Gibbs and Kolb did not show behavioral improvements by adulthood.

The morphological effect that was selective to STR-B mice in our study and most closely corresponded to the baseline corticosterone levels at PND 7, was the significant decrease in cortical layers II/III thickness, following adult behavioral experience. This observation is consistent also with reports by Gibb and Kolb of decreased apical and basal dendritic length, in comparable cortical regions, as result of their altered rearing conditions (Gibb & Kolb, 2005). In contrast however, Bock and colleagues observed increased spine densities in layers II/III of parietal, somatosensory cortex (Bock et al., 2005), in Wistar rats, exposed to 1 hour daily separations, for periods ranging either from PND 1-3, 5-7 or 14-16. Increased spine densities, in were observed under all separation conditions in layers II/III, but were most pronounced following separation on PND 5-7 when significantly increased volume in

basal dendrites also was seen. The stress paradigm used by Bock and colleagues is closely comparable to our stress protocol. Thus, at first glance, increases in spine and dendritic volume in the Bock et al. study appears to be in conflict with the thickness decreases we observed in layer II/III in our STR-B mice, and certainly the opposite as seen by Gibbs and Kolb. However, the rats used by Brock et al. were behaviorally naïve and home cage raised, whereas our STR-B mice and the rats used for Golgi studies by Gibbs and Kolb, were behaviorally tested prior to euthanasia for histological processing. Thus, one could speculate, that early postnatal stress effects may increase synaptic complexity in supragranular cortex but also confer vulnerability to subsequent adult experience such as behavioral testing.

It is worth mentioning, that decreases in cortical thickness have previously been reported following cryo-anesthesia in newborn rats (Kolb & Cioe, 2001; Nunez, Kim, & Juraska, 1998). Nunez et al. studied the effects of exposing newborn Hooded rat pups to 5°C and measured a significant decrease in occipital cortical thickness, predominantly with 30-60 minutes of cold exposure; these effects were more pronounced in male compared to female rats. The cortical layers most affected in these studies were layers II/III, along with infragranular pyramidal layers. Kolb and Cioe subsequently confirmed these observations in Long Evans rat for parietal cortical areas closely corresponding to our sample area (Kolb & Cioe, 2001). In their study, layers II/III, also were prominently decreased in thickness. Hypothermia exposures of the pups in these studies, and the subsequent equilibration period, inevitably required maternal separation of the pups for periods of 60 minutes and above, and therewith closely resemble our stress paradigm. As with our STR-B males, the cryo-anesthesia exposed rats used by Kolb & Cioe were behaviorally tested prior to euthanasia for histological assessments. The post cryo-anesthesia life experiences of the rats used by Nunez et al. is not as clear. In light, of our current data, effects of cryo-anesthesia may be considered a consequence of neonatal stress. Clearly, future studies will need to investigate corticosterone levels under various rearing conditions, but within the same animal model, in order to more clearly establish the boundaries between rearing conditions currently designated as enrichment vs. stress. Likewise, the effects of maternal separation and temperature exposure in their respective impact on endocrine, morphological and behavioral changes will have to be differentiated.

In order to approach functional interpretations of our results in future, we will need to determine the cellular substrate for the rather dramatic change in cortical size we have observed in our mice; and we will need to further examine if there are correlates with specific behavioral changes. Of particularly interest will be behavioral changes and morphological alterations of relevance to mental health disorders such as depression, autism and schizophrenia (Courchesne, Pierce, Schumann, Redcay, Buckwalter, Kennedy, & Morgan, 2007; Eastwood, 2004; Kalia, 2005; Kinney et al. 2008; Rapoport, Addington, Frangou, & Psych, 2005). In the past, stress exposures of various durations have been associated with a decrease in synapses and neuropil in fronto-limbic cortical areas (Andersen & Teicher, 2004; Gos et al., 2008; Huot, Plotsky, Lenox, & McNamara, 2002). Reductions in the number of glia and glia-associated markers have been demonstrated in visual cortex and frontolimbic cortex in neonatal stress protocols similar to ours in duration (Musholt, Cirillo, Cavaliere, Rosaria Bianco, Bock, Helmeke, Braun, & Papa, 2009;). There

also are indications, that neuronal apoptosis is altered by stressful conditions (Zhang, Levine, Dent, Zhan, Xing, Okimoto, Kathleen Gordon, Post, & Smith, 2002). The studies by Bock and colleagues indicate that only short durations of maternal separation stress are necessary to induce dendritic and spine alterations in sensory cortex (Bock et al., 2005).

Finally, future studies will also have to investigate how changes in cortical morphology and plasticity may be related to specific endocrine alterations. Our long-term goal is to connect stress mediated changes in cortical morphology to specific molecular mechanisms involved in neuronal network formation and modulation. As indicated above, mechanisms associated with these cortical changes may have significance for the further understanding of and subsequent therapeutic approaches to a number of different mental health disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We want to express our appreciation to Dr. D. E. Carlson Department of Surgery, University of Maryland Baltimore, School of Medicine, for helping us to establish the radioimmunoassay procedure for CORT measurements in our mice. This work was supported by SO6GM51971 to Dr. Christine Hohmann and R25GM058904 training support to MSU undergraduate students N. Beard and P. Kari-Kari.

References Cited

- Abel KM, Drake R, Goldstein JM. Sex differences in Schizophrenia. *International Review of Psychiatry*. 2010; 22(5):417–428. [PubMed: 21047156]
- Altman J, Das GD. Autoradiographic examination of the effects of enriched environment on the rate of glial multiplication in the adult rat brain. *Nature*. 1964; 204:1161–1163. [PubMed: 14264369]
- Andersen SL, Teicher MH. Delayed effects of early stress on hippocampal development. *Neuropsychopharmacology*. 2004; 29(11):1988–1993. [PubMed: 15316569]
- Anisman H, Zacharko RM. Depression, the predisposing influence of stress. *The Behav. and Brain Sciences*. 1982; 5:89–137.
- Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early life events permanently alter behavioral and hormonal responses to stressors? *Int. J. Devel. Neurosci.* 1998; 16(3-4):149–164. [PubMed: 9785112]
- Avishai-Eliner S, Yi S-J, Newth CJL, Baram TZ. Effects of maternal and sibling deprivation on basal and stress induced hypothalamic-pituitary-adrenal components in the infant rat. *Neurosci Letters*. 1995; 192:49–52.
- Avishai-Eliner S, Gilles EE, Eghbal-Ahmadi M, Bar-El Y, Baram TZ. Altered regulation of gene and protein expression of hypothalamic-pituitary-adrenal axis components in an immature rat model of chronic stress. *J Neuroendocrinol*. 2001; 13(9):799–807. [PubMed: 11578530]
- Bagot RC, van Hasselt FN, Champagne DL, Meaney MJ, Krugers HJ, Joels M. Maternal care determines rapid effects of stress mediators on synaptic plasticity in adult rat hippocampal dentate gyrus. *Neurobiol Learn Mem*. 2009
- Bakos J, Duncko R, Makatsori A, Pirnik Z, Kiss A, Jezova D. Prenatal immune challenge affects growth, behavior, and brain dopamine in offspring. *Ann N Y Acad Sci*. 2004; 1018:281–287. [PubMed: 15240379]
- Baroncelli L, Braschi C, Spolidoro M, Begenisic T, Sale A, Maffei L. Nurturing brain plasticity: impact of environmental enrichment. *Cell Death Differ*. 2010; 17(7):1092–1103. [PubMed: 20019745]

- Berger-Sweeney J, Hohmann CF. Behavioral consequences of abnormal cortical development: insights into developmental disabilities. *Behav Brain Res.* 1997; 86:121–142. [PubMed: 9134147]
- Bock J, Gruss M, Becker S, Braun K. Experience-induced changes of dendritic spine densities in the prefrontal and sensory cortex: correlation with developmental time windows. *Cereb Cortex.* 2005; 15(6):802–808. [PubMed: 15371297]
- Boylan CB, Blue ME, Hohmann CF. Modeling early cortical serotonergic deficits in autism. *Behav Brain Res.* 2007; 176:94–108. [PubMed: 17034875]
- Branchi I, D'Andrea I, Sietzema J, Fiore M, Di Fausto V, Aloe L, et al. Early social enrichment augments adult hippocampal BDNF levels and survival of BrdU-positive cells while increasing anxiety- and “depression”-like behavior. *J Neurosci Res.* 2006; 83(6):965–973. [PubMed: 16477619]
- Braun K, Antemano R, Helmeke C, Buchner M, Poeggel G. Juvenile separation stress induces rapid region- and layer-specific changes in S100ss- and glial fibrillary acidic protein-immunoreactivity in astrocytes of the rodent medial prefrontal cortex. *Neuroscience.* 2009; 160(3):629–638. [PubMed: 19285122]
- Bredy TW, Humpartzoomian RA, Cain DP, Meaney MJ. Partial reversal of the effect of maternal care on cognitive function through environmental enrichment. *Neuroscience.* 2003; 118(2):571–576. [PubMed: 12699791]
- Bredy TW, Zhang TY, Grant RJ, Diorio J, Meaney MJ. Peripubertal environmental enrichment reverses the effects of maternal care on hippocampal development and glutamate receptor subunit expression. *Eur J Neurosci.* 2004; 20:1355–1362. [PubMed: 15341607]
- Brinks V, van der Mark M, de Kloet R, Oitzl M. Emotion and Cognition in High and Low Stress Sensitive Mouse Strains: A Combined Neuroendocrine and Behavioral Study in BALB/c and C57BL/6J Mice. *Front Behav Neurosci.* 2007; 1:8. [PubMed: 18958190]
- Byers DM, Irwin LN, Moss DE, Sumaya IC, Hohmann CF. Prenatal exposure to the acetylcholinesterase inhibitor methanesulfonyl fluoride alters forebrain morphology and gene expression. *Brain Res Dev Brain Res.* 2005; 158(1-2):13–22.
- Caldji C, Diorio J, Meaney MJ. Variations in maternal care in infancy regulate the development of stress reactivity. *Biol Psychiatry.* 2000; 48(12):1164–1174. [PubMed: 11137058]
- Champagne DL, Bagot RC, van Hasselt F, Ramakers G, Meaney MJ, de Kloet ER, et al. Maternal care and hippocampal plasticity: evidence for experience-dependent structural plasticity, altered synaptic functioning, and differential responsiveness to glucocorticoids and stress. *J Neurosci.* 2008; 28(23):6037–6045. [PubMed: 18524909]
- Chapillon P, Manneche C, Belzung C, Caston J. Rearing environmental enrichment in two inbred strains of mice. *Behav. Genetics.* 1999; 29:41–46.
- Courchesne E, Pierce K, Schumann CM, Redcay E, Buckwalter JA, Kennedy DP, et al. Mapping early brain development in autism. *Neuron.* 2007; 56(2):399–413. [PubMed: 17964254]
- Comeau WL, McDonald RJ, Kolb BE. Learning-induced alterations in prefrontal cortical dendritic morphology. *Behav Brain Res.* 2010; 214(1):91–101. [PubMed: 20433872]
- de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci.* 2005; 6(6):463–475. [PubMed: 15891777]
- Del Angel-Meza AR, Ramirez-Cortes L, Adame-Gonzalez IG, Gonzalez Burgos I, Beas-Zarate C. Cerebral GABA release and GAD activity in protein- and tryptophan-restricted rats during development. *Int J Dev Neurosci.* 2002; 20(1):47–54. [PubMed: 12008074]
- Diamond MC, Law F, Rhodes H, Lindner B, Rosenzweig MR, Krech D, et al. Increases in cortical depth and glia numbers in rats subjected to enriched environment. *J Comp Neurol.* 1966; 128(1): 117–126. [PubMed: 4165855]
- Diamond MC. Response of the brain to enrichment. *An Acad Bras Cienc.* 2001; 73(2):211–220. [PubMed: 11404783]
- Ducottet C, Belzung C. Behavior in the elevated plus-maze predicts coping after subchronic mild stress in mice. *Physiol Behav.* 2004; 81(3):417–426. [PubMed: 15135013]
- Eastwood SL. The synaptic pathology of schizophrenia: is aberrant neurodevelopment and plasticity to blame? *Int. Rev. Neurobiol.* 2004; 59:47–72. [PubMed: 15006484]

- Enthoven L, de Kloet ER, Oitzl MS. Differential development of stress system (re)activity at weaning dependent on time of disruption of maternal care. *Brain Res.* 2008; 1217:62–69. [PubMed: 18501879]
- Finlay BL. Cell death and the creation of regional differences in neuronal numbers. *J Neurobiol.* 1992; 23(9):1159–1171. [PubMed: 1469382]
- Fombonne E. The epidemiology of autism: a review. *Psychol Med.* 1999; 29(4):769–786. [PubMed: 10473304]
- Gibb R, Kolb B. Neonatal handling alters brain organization but does not influence recovery from perinatal cortical injury. *Behav Neurosci.* 2005; 119(5):1375–1383. [PubMed: 16300443]
- Gos T, Bock J, Poeggel G, Braun K. Stress-induced synaptic changes in the rat anterior cingulate cortex are dependent on endocrine developmental time windows. *Synapse.* 2008; 62(3):229–232. [PubMed: 18088062]
- Greenough WT, Juraska JM, Volkmar FR. Maze training effects on dendritic branching in occipital cortex of adult rats. *Behav. Neur. Biol.* 1979; 26:287–297.
- Heim C, Nemeroff CB. The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. *Biol Psychiatry.* 1999; 46(11):1509–1522. [PubMed: 10599479]
- Hohmann CF, Berger-Sweeney J. Sexually dimorphic responses to neonatal basal forebrain lesions in mice: II. Cortical morphology. *J Neurobiol.* 1998; 37(4):595–606. [PubMed: 9858261]
- Hohmann CF, Richardson C, Pitts E, Berger-Sweeney J. Neonatal 5,7-DHT lesions cause sex-specific changes in mouse cortical morphogenesis. *Neural Plast.* 2000; 7(4):213–232. [PubMed: 11486483]
- Hohmann CF, Walker EM, Boylan CB, Blue ME. Neonatal serotonin depletion alters behavioral responses to spatial change and novelty. *Brain Res.* 2007; 1139:163–177. [PubMed: 17296168]
- Holmes A, le Guisquet AM, Vogel E, Millstein RA, Leman S, Belzung C. Early life genetic, epigenetic and environmental factors shaping emotionality in rodents. *Neurosci Biobehav Rev.* 2005; 29(8):1335–46. [PubMed: 16095695]
- Huot RL, Plotsky PM, Lenox RH, McNamara RK. Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. *Brain Res.* 2002; 950(1-2):52–63. [PubMed: 12231228]
- Jacobson, M., editor. *Developmental Neurobiology.* 2nd ed.. Plenum Press; New York: 1978.
- Jeanmonod D, Rice FL, Van der Loos H. Mouse somatosensory cortex: alterations in the barrelfield following receptor injury at different early postnatal ages. *Neuroscience.* 1981; 6(8):1503–1535. [PubMed: 7266876]
- Juul-Dam N, Townsend J, Courchesne E. Prenatal, perinatal, and neonatal factors in autism, pervasive developmental disorder-not otherwise specified, and the general population. *Pediatrics.* 2001; 107(4):E63. [PubMed: 11335784]
- Kaffman A, Meaney MJ. Neurodevelopmental sequelae of postnatal maternal care in rodents: clinical and research implications of molecular insights. *J Child Psychol Psychiatry.* 2007; 48(3-4):224–244. [PubMed: 17355397]
- Kalia M. Neurobiological basis of depression: an update. *Metabolism.* 2005; 54(5 Suppl 1):24–27. [PubMed: 15877309]
- Kinney DK, Munir KM, Crowley DJ, Miller AM. Prenatal stress and risk for autism. *Neurosci Biobehav Rev.* 2008; 32(8):1519–1532. [PubMed: 18598714]
- Kolb B, Cioe J, Comeau W. Contrasting effects of motor and visual spatial learning tasks on dendritic arborization and spine density in rats. *Neurobiol Learn Mem.* 2008; 90(2):295–300. [PubMed: 18547826]
- Koo JW, Park CH, Choi SH, Kim NJ, Kim H, Choe JC, et al. The postnatal environment can counteract prenatal effects on cognitive ability, cell proliferation and synaptic protein expression. *Faseb J.* 2003; 17:1556–1582. [PubMed: 12824278]
- Knuth ED, Etgen AM. Corticosterone secretion induced by chronic isolation in neonatal rats is sexually dimorphic and accompanied by elevated ACTH. *Horm Behav.* 2005; 47(1):65–75. [PubMed: 15579267]
- Kolb B, Cioe J. Cryoanesthesia on postnatal day 1, but not day 10, affects adult behavior and cortical morphology in rats. *Brain Res Dev Brain Res.* 2001; 130(1):9–14.

- Kolb B, Gorny G, Soderpalm AH, Robinson TE. Environmental complexity has different effects on the structure of neurons in the prefrontal cortex versus the parietal cortex or nucleus accumbens. *Synapse*. 2003; 48(3):149–153. [PubMed: 12645040]
- Krasnova IN, Betts ES, Dada A, Jefferson A, Ladenheim B, Becker KG, et al. Neonatal dopamine depletion induces changes in morphogenesis and gene expression in the developing cortex. *Neurotox. Res*. 2007; 11(2):107–130. [PubMed: 17449454]
- Ladd CO, Thirivikraman KV, Huot RL, Plotsky PM. Differential neuroendocrine responses to chronic variable stress in adult Long Evans rats exposed to handling-maternal separation as neonates. *Psychoneuroendocrinology*. 2005; 30(6):520–533. [PubMed: 15808921]
- Leung A, Chue P. Sex differences in schizophrenia, review of the literature. *Acta Psychiat. Scand*. 2000; 401(Suppl.):3–38.
- Levine S. Regulation of the hypothalamic-pituitary-adrenal axis in the neonatal rat: the role of maternal behavior. *Neurotox Res*. 2002; 4(5-6):557–564. [PubMed: 12754166]
- Liu D, Diorio J, Francis D, Meaney JM. Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat. Neurosci*. 2000; 3(8):799–806. [PubMed: 10903573]
- Liu D, Diorio J, Tannenbaum B, Caldji C, Francis D, Freedman A, et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*. 1997; 277(5332):1659–1662. [PubMed: 9287218]
- McEwen BS. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci*. 2004; 1032:1–7. [PubMed: 15677391]
- Meaney MJ, Diorio J, Francis D, Widdowson J, LaPlante P, Caldji C, et al. Early environmental regulation of forebrain glucocorticoid receptor gene expression: implications for adrenocortical responses to stress. *Dev Neurosci*. 1996; 18(1-2):49–72. [PubMed: 8840086]
- Meerlo P, Horwath KM, Nagy GM, Bohus B, Koolhaas JM. The influence of postnatal handling on adult neuroendocrine and behavioral stress reactivity. *J. Neuroendocrinol*. 1999; 11(12):925–933. [PubMed: 10583727]
- Moncek F, Duncko R, Johansson BB, Jezova D. Effect of environmental enrichment on stress related systems in rats. *J Neuroendocrinol*. 2004; 16(5):423–431. [PubMed: 15117335]
- Musholt K, Cirillo G, Cavaliere C, Rosaria Bianco M, Bock J, Helmeke C, et al. Neonatal separation stress reduces glial fibrillary acidic protein- and S100beta-immunoreactive astrocytes in the rat medial precentral cortex. *Dev Neurobiol*. 2009; 69(4):203–211. [PubMed: 19137572]
- Nunez JL, Kim BY, Juraska JM. Neonatal cryoanesthesia affects the morphology of the visual cortex in the adult rat. *Dev. Brain Res*. 1998; 111(1):89–98. [PubMed: 9804905]
- Picker JD, Yang R, Ricceri L, Berger-Sweeney J. An altered neonatal behavioral phenotype in Mecp2 mutant mice. *Neuroreport*. 2006; 17(5):541–544. [PubMed: 16543822]
- Priebe K, Brake WG, Romeo RD, Sisti HM, Mueller A, McEwen BS, et al. Maternal influences on adult stress and anxiety-like behavior in C57BL/6J and BALB/CJ mice: A cross-fostering study. *Dev Psychobiol*. 2006; 48(1):95–96.
- Rapoport JL, Addington AM, Frangou S, Psych MR. The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry*. 2005; 10(5):434–449. [PubMed: 15700048]
- Resnick O, Miller M, Forbes W, Hall R, Kemper T, Bronzino J, et al. Developmental protein malnutrition: influences on the central nervous system of the rat. *Neurosci Biobehav Rev*. 1979; 3(4):233–246. [PubMed: 44355]
- Ricceri L, Hohmann C, Berger-Sweeney J. Early neonatal 192 IgG saporin induces learning impairments and disrupts cortical morphogenesis in rats. *Brain Res*. 2002; 954(2):160–172. [PubMed: 12414099]
- Ricceri L, Usiello A, Valanzano A, Calamandrei G, Frick K, Berger-Sweeney J. Neonatal 192 IgG-saporin lesions of basal forebrain cholinergic neurons selectively impair response to spatial novelty in adult rats. *Behav Neurosci*. 1999; 113(6):1204–1215. [PubMed: 10636299]
- Rosenfeld P, Suchecki D, Levine S. Multifactorial regulation of the hypothalamic-pituitary-adrenal axis during development. *Neurosci Biobehav Rev*. 1992; 16(4):553–568. [PubMed: 1480351]
- Rosenzweig MR, Bennet EL. Psychobiology of plasticity: effects of training and experience on brain and behavior. *Behav. Brain Res*. 1996; 78:57–65. [PubMed: 8793038]

- Sanchez MM, Ladd CO, Plotsky PM. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Dev Psychopathol.* 2001; 13(3):419–449. [PubMed: 11523842]
- Sapolsky RM, Meaney MJ. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Res.* 1986; 396(1):64–76. [PubMed: 3011218]
- Schmidt MV, Levine S, Alam S, Harbich D, Sterlemann V, Ganea K, et al. Metabolic signals modulate hypothalamic-pituitary-adrenal axis activation during maternal separation of the neonatal mouse. *J Neuroendocrinol.* 2006; 18(11):865–874. [PubMed: 17026536]
- Seckl JR, Meaney MJ. Glucocorticoid programming. *Ann N Y Acad Sci.* 2004; 1032:63–84. [PubMed: 15677396]
- Shanks N, Griffiths J, Anisman H. Norepinephrine and serotonin alterations following chronic stressor exposure: mouse strain differences. *Pharmacol Biochem Behav.* 1994; 49(1):57–65. [PubMed: 7816890]
- Suchecki D, Rosenfeld P, Levine S. Maternal regulation of the hypothalamic-pituitary-adrenal axis in the infant rat: the roles of feeding and stroking. *Brain Res Dev Brain Res.* 1993; 75(2):185–192.
- van Oers HJ, de Kloet ER, Whelan T, Levine S. Maternal deprivation effect on the infant's neural stress markers is reversed by tactile stimulation and feeding but not by suppressing corticosterone. *J Neurosci.* 1998; 18(23):10171–10179. [PubMed: 9822770]
- van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. *Nat Rev Neurosci.* 2000; 1(3):191–198. [PubMed: 11257907]
- Walsh RN. Effects of environmental complexity and deprivation on brain anatomy and histology: a review. *Int J Neurosci.* 1981; 12(1):33–51. [PubMed: 7009463]
- Yi S-J, Baram T. Corticotropin releasing hormone mediates the response to cold stress in neonatal rat without compensatory enhancement of the peptide's gene expression. *Endocrinology.* 1994; 135(6):2364–2368. [PubMed: 7988418]
- Zaharia MD, Kulczycki J, Shanks N, Meaney MJ, Anisman H. The effects of early postnatal stimulation on Morris water-maze acquisition in adult mice: genetic and maternal factors. *Psychopharmacology (Berl).* 1996; 128(3):227–239. [PubMed: 8972542]
- Zhang LX, Levine S, Dent G, Zhan Y, Xing G, Okimoto D, et al. Maternal deprivation increases cell death in the infant rat brain. *Brain Res Dev Brain Res.* 2002; 133(1):1–11.

FIGURE 1.

Histological images of coronal brain sections from a STR and STR-B male, as digitized for quantitative measurements of cortical thickness, using the AIS system. The boxes delineate the cortical areas that were sampled. Thin gray lines within the boxes illustrate individual samplings; the arrows are pointing at barrel shapes in Layer IV, which were used as landmarks for our sample area.

FIGURE 2.

Cortical thickness changes after stress exposure in male and female mice. There was a significant ANOVA main effect for condition for total cortex (A), layer IV (B) and layers II/III (D) and a nearly significant sex by condition interaction for Layer V (C) (see text for statistical details). Note, that cortical thickness decreased significantly in male STR-B and LMC-B mice in cortical layer IV, compared to untested STR and LMC, as is also reflected in experiment #4, Figure 4B. In contrast, the difference between STR and STR-B in layer V, is no longer apparent with the larger sample in Figure 4B. The significant decrease in layers II/III, for both males and females STR-B mice as shown here, is reflected in Figure 4, as significant difference from AMC. All P values in this Figure represent Fisher post-hoc tests (StatView).

FIGURE 3.

Environmental enrichment, from weaning to three months postnatal in normal male Balb/CByJ mice, significantly enlarged total cortical thickness (A). Significant thickness changes were apparent mainly in cortical layers V and VI (B, C). Normal males exposed to testing in the Open Field Object Recognition task, as young adults, also showed a small, non-significant, trend for a thickness increase. All P values shown here are Fisher post-hoc tests. (CTL = control, OFOR = Open Field Object Recognition tested, ENR = environmental enrichment).

FIGURE 4.

Total cortical thickness in neonatally stressed male mice (STR) was significantly larger, compared to STR males with behavioral experience (STR-B). STR mice also showed a highly significant increase (** $P < 0.0001$) in cortical thickness compared with AMC (age matched, behaviorally naïve males). In addition, as further marked by **, LMC behaviorally tested ($P = 0.005$) and behaviorally naïve mice ($P = 0.001$), also displayed significant thickness increases compared to the AMC. However, behavioral experience did not lead to significant differences between littermate control (LMC) and behaviorally tested littermate control (LMC-B) mice. All P values shown here represent Fisher post-hoc tests; see the text for ANOVA main effects.

FIGURE 4 B-E shows individual cortical layers of the mice depicted in Fig 4A. Although, the thickness of layers II/III for STR, LMC and LMC-B mice was decreased compared to all

AMC mice, note, in (B), that only STR-B was thinner than STR in this layer thus, constituting a selective effect in stressed mice only. In layer IV (C) both STR-B and LMC-B were thinner compared to their respective untested conditions and in layers V and VI, all conditions showed significantly increased thickness compared to AMC males (E). All P values shown represent Fisher post-hoc test; see the text for ANOVA main effects.

Table I
Corticosterone Levels at PND 7

Elevated corticosterone levels in serum of male and female STR pups at PND 7. The differences between the groups closely approached statistical significance ($P = 0.057$, $F=4.06$, DF_{20}) when male and female mice were analyzed jointly.

	Male	Female
STR	22.5 +/- 7.6 ng/ml	20.5 +/- 4.0 ng/ml
LMC	11.1 +/- 3.9 ng/ml	12.3 +/- 3.4 ng/ml

Table II**Brain Weight**

Brain weights for behaviorally tested (B) and naïve STR and LMC male and female mice as measured at the time of sacrifice for histology. Although female brains trended to be slightly larger in all groups, significant differences in brain weight were not observed between any of the groups.

	Male	Female
STR	.431 +/- .004 g	.432 +/- .013 g
STR – B	.428 +/- .015 g	.445 +/- .010 g
LMC	.419 +/- .012 g	.473 +/- .010 g
LMC – B	.434 +/- .011g	.445 +/- .018 g