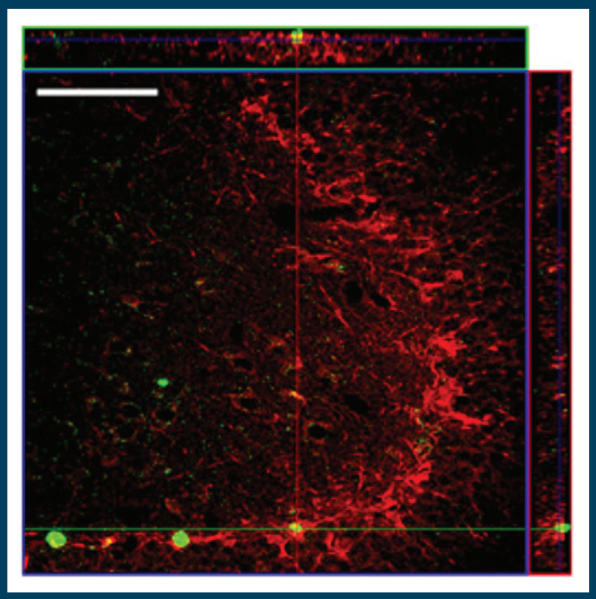
frontiers

RESEARCH TOPICS

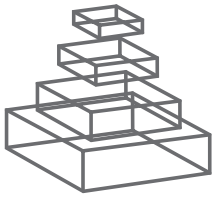


PROGRAMMING THE HPA-AXIS BY EARLY LIFE EXPERIENCE: MECHANISMS OF STRESS SUSCEPTIBILITY AND ADAPTATION

Topic Editors
Nikolaos P. Daskalakis and Rachel Yehuda

frontiers in ENDOCRINOLOGY

frontiers in NEUROSCIENCE



frontiers

ISSN 1664-8714
ISBN 978-2-88919-481-0
DOI 10.3389/978-2-88919-481-0

ABOUT FRONTIERS

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

FRONTIERS JOURNAL SERIES

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing.
All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

DEDICATION TO QUALITY

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view.
By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

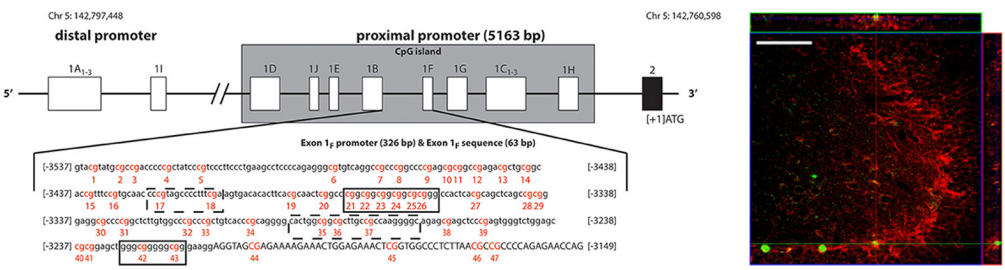
WHAT ARE FRONTIERS RESEARCH TOPICS?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area!
Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

PROGRAMMING THE HPA-AXIS BY EARLY LIFE EXPERIENCE: MECHANISMS OF STRESS SUSCEPTIBILITY AND ADAPTATION

Topic Editors:
Nikolaos P. Daskalakis, Icahn School of Medicine at Mount Sinai, USA
Rachel Yehuda, Icahn School of Medicine at Mount Sinai, USA

Experiences during early life program the central nervous- and endocrine-systems with consequences for susceptibility to physical and mental disorders. These programming effects depend on genetic and epigenetic factors, and their outcome leads to an adaptive or maladaptive phenotype to a given later environmental context. This Research Topic focused on the hypothalamus-pituitary-adrenal (HPA)-axis and stress-related phenotypes, and on how HPA-axis programming by the environment precisely occurs. We included original research, mini-review and review papers on a broad range of topics related to HPA-axis programming.



Left: Schematic representation of human glucocorticoid receptor gene (NR3C1) non-coding first exons and illustration of the exon 1F sequence (the CpG and NGFI-A–binding sites are depicted). For more details, see review by Daskalakis and Yehuda.
Right: Confocal microscope image of BrdU-DCX double-labeled cell (BrdU in green - DCX in red) in hippocampal dentate gyrus used to estimate the number of new cells undergoing a neuronal fate. For more details, see original research paper by Cinini et al.

Table of Contents

frontiers in
ENDOCRINOLOGY

EDITORIAL
published: 14 January 2015
doi: 10.3389/fendo.2014.00244

Early maternal influences on stress circuitry: implications for resilience and susceptibility to physical and mental disorders

***Nikolaos P. Daskalakis*[1,2,3]* and *Rachel Yehuda*[1,2,4]**

[1] *Traumatic Stress Studies Division, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
[2] *Mental Health Patient Care Center, James J. Peters Veterans Affairs Medical Center, Bronx, NY, USA*
[3] *Laboratory of Molecular Neuropsychiatry, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
[4] *Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
**Correspondence: nikolaos.daskalakis@mssm.edu*

Edited and reviewed by:
Hubert Vaudry, University of Rouen, France

Keywords: early life stress, HPA axis, vulnerability, resilience, maternal

Experiences in early life result in enduring and sometimes permanent differences in how the central nervous and endocrine systems function. These changes are referred to as developmental programing, and have numerous consequences for susceptibility to physical and mental disorders, which involve central nervous and endocrine systems. These effects may depend on genetic factors, but more importantly, involve epigenetic mechanisms, which can change the function of the gene independently of genotype. Environmentally induced epigenetic changes alter gene expression and function, and their outcome leads to adaptive or maladaptive expressions, depending on the specific environmental context of the individual (1).

In this Frontiers Research Topic "Programing the hypothalamic pituitary adrenal (HPA) axis by early life experience: mechanisms of stress susceptibility and adaptation," we have assembled a comprehensive selection of original research, mini reviews, and review articles that describe prenatal and postnatal maternal influences on stress response systems in offspring. This is an emerging and critical area in neuroendocrine science as it is becoming increasingly clear that much of the variance associated with biological alterations in physical and mental disorder may be accounted for by maternal history and experiences in the womb or in early postnatal life. While such influences have been broadly acknowledged, until recently, it has been difficult to measure the effects of these maternal influences on offspring. Epigenetic marks can be measured and provide a quantification of relevant early environmental influences. Carefully designed studies can then parse out the origin of early influences.

In the opening paper of the volume, Daskalakis and Yehuda systematically review the methodologies employed to study epigenetic effects of early adversity (2). They focus on studies of the most studied genomic region in human stress-related diseases, the exon 1_F promoter of the glucocorticoid receptor (GR) gene. The discussion is meant to present a series of issues that influence quantitative methylation analyses (method, tissue type, region of interest). It is important in an emerging field to discuss such issues since they can result in variable results from laboratory to laboratory. The authors make the important point that there may be site-specific accommodations of different adversity factors in different CpG sites within a promoter-based CpG island, leading to a greater complexity of data analysis and interpretation than is currently the standard. Finally, this review paper also includes unpublished data that extend the transgenerational effects previously observed in this region (3) by demonstrating site-specific effects based on maternal and/or paternal PTSD.

After this focused review, two review papers provide an overview first of the effects of early adversity on major depression, as an example of a critical psychiatric outcome, and second on the effects of early adversity on metabolic outcomes. Hornung and Heim (4) summarize recent human studies implicating gene by early life stress interactions in vulnerability and resilience for depression. The authors chose the term "intermediate phenotype" throughout the paper in order to emphasize the mediational character of some phenotypic features, which result from gene by environment interactions and may lead to depressive symptoms. Maniam et al. (5) using animal and human data support that early adversity induced maladaptation of the HPA axis will have negative impact on energy metabolism in the presence of chronic stress or unfavorable metabolic conditions in later-life leading to vulnerability to metabolic diseases. To this end, authors have attempted to review the relevant literature, with attention paid to findings dealing with metabolic/body weight measures. They further propose a mechanism whereby the cross-talk between HPA axis and altered glucocorticoid (GC) metabolism in the liver (by 11-beta hydroxysteroid dehydrogenase Type 1) may likely mediate the metabolic outcomes of early life stress.

Following these overviews, papers were divided according to the developmental time window in which adversity was present. Two original research articles describe the effects of prenatal maternal exposures to alcohol and GCs on stress-related neurocircuits. Raineki et al. (6) reveal complex sexual dimorphisms by providing a comprehensive (constrained principal component) analysis of the neural activity (by *c-fos* expression) in amygdala, hippocampus, medial prefrontal cortex, and paraventricular nucleus of hypothalamus in response to acute stress in male and female offspring of dams exposed to alcohol or two control conditions in gestation (gestation day 1–21), which where additionally exposed to a 10-day chronic mild stress paradigm in the peripubertal

period. Borges et al. (7) find that prenatal maternal exposure to dexamethasone (gestation days 18 and 19) increased the activity of two regions that belong to the mesopontine cholinergic system (laterodorsal and pedunculopontine tegmental nuclei) associated with enhanced behavioral stress vulnerability (anxiety behavior including fear-associated ultrasonic calls). The review by de Kloet et al. (8) complements the Borges paper by focusing on the impact of perinatal GC exposure on the developing HPA axis and the balance between GR and mineralocorticoid receptor. The authors review animal and also clinical research on perinatal disturbances by GC administration and possible intervention strategies.

Postnatal stress in rodents also exerts lasting effects on the stress system as well as on other brain systems. Daskalakis et al. (9) provide original data on strain-dependent differences in the immediate effects of maternal deprivation on the neonatal HPA axis response. While maternal deprivation causes a disruption of the neonatal stress hyporesponsive period in both mice strains studied, one strain was more affected calling for future studies on later-life consequences of early postnatal stress based on genetic background. Loi et al. (10) describe how hippocampal neurogenesis is affected by early life stress in rodents in an age- and sex-dependent manner, and provide novel data showing that normalization may be possible through GC-based interventions during critical stages of brain development. Brief treatment with mifepristone, a GR antagonist, on postnatal days 26–28 protected female rats against the effects of maternal deprivation (postnatal day 3) on neurogenesis markers in dentate gyrus. This finding is interesting because mifepristone is currently being tested in a clinical trial for post-traumatic stress disorder (11), a condition, which has also been linked with maternal PTSD (12), and childhood trauma (13). Loi et al. also speculate about the implications of findings in animal models of perinatal stress for the study of human brain development and vulnerability to psychopathology. Cinini et al. explore the effects of adversity in an early post-weaning period on hippocampal neurogenesis (14). The authors showed that social isolation in young (8–10 months old) non-human primates (marmosets) produced anxiety-like behaviors, elevated cortisol levels, and reduced neurogenesis.

Long-term effects of perinatal stress experiences are partially mediated by mother–pup interactions (15). How these interactions influence offspring development is summarized in the mini-review by Lucion and Bortolini (16) where the special role of genes, epigenetics, imprinting, neurohormones, and learning is outlined. The review by Rincon-Cortés and Sullivan continues on the same theme (17). It is explained that the development of rat odor aversion develops after postnatal day 10, when maternal absence can increase GCs and subsequently activate a fear-related amygdala-dependent neurocircuit. The effects of maternal contact are also explored in the mini-review by Stamatakis et al., who summarize findings from their new model of neonatal learning (18). In order to investigate the effects of the early maternal environment, rat pups either receive the expected reward of maternal contact or are denied this reward on postnatal days 10–13. The two training conditions have differential consequences on brain activity, HPA axis function, cognition, and stress-related behavior.

The final paper, a human study, by Bader et al. (19) explores transgenerational effects of parental trauma in offspring. The findings replicate and extend previous findings regarding urinary cortisol levels in Holocaust survivor offspring (20–22). Authors observed that the low cortisol levels in association with maternal PTSD are additionally associated with maternal age at Holocaust exposure (a parallel effect on cortisol in offspring that is not related to maternal PTSD).

To conclude, the articles of this Research Topic have highlighted the importance of investigating the biological effects of early stressful factors of maternal origin on stress circuitry in order to comprehend resilience and susceptibility to physical and mental disorders. The introduction of modern methodologies (e.g., epigenetic analyses) can give traditional neurobiological findings (e.g., neurogenesis markers) a molecular understanding.

ACKNOWLEDGMENTS

We wish to thank the authors for their excellent contributions, and the reviewers for their outstanding reports. We also wish to thank the Frontiers in Endocrinology editorial board, editorial assistants, and editors-in-chief for their work and advice, and for their trust in this project, particularly Dr. Hubert Vaudry, who was assigned to this Research Topic.

REFERENCES

1. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER. The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* (2013) **38**(9):1858–73. doi:10.1016/j.psyneuen.2013.06.008
2. Daskalakis NP, Yehuda R. Site-specific methylation changes in the glucocorticoid receptor exon 1F promoter in relation to life adversity: systematic review of contributing factors. *Front Neurosci* (2014) **8**:369. doi:10.3389/fnins.2014.00369
3. Yehuda R, Daskalakis NP, Lehrner A, Desarnaud F, Bader HN, Makotkine I, et al. Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring. *Am J Psychiatry* (2014) **171**(8):872–80. doi:10.1176/appi.ajp.2014.13121571
4. Hornung OP, Heim CM. Gene-environment interactions and intermediate phenotypes: early trauma and depression. *Front Endocrinol* (2014) **5**:14. doi:10.3389/fendo.2014.00014
5. Maniam J, Antoniadis C, Morris MJ. Early-life stress, HPA axis adaptation, and mechanisms contributing to later health outcomes. *Front Endocrinol* (2014) **5**:73. doi:10.3389/fendo.2014.00073
6. Raineki C, Hellemans KG, Bodnar T, Lavigne KM, Ellis L, Woodward TS, et al. Neurocircuitry underlying stress and emotional regulation in animals prenatally exposed to alcohol and subjected to chronic mild stress in adulthood. *Front Endocrinol* (2014) **5**:5. doi:10.3389/fendo.2014.00005
7. Borges S, Coimbra B, Soares-Cunha C, Ventura-Silva AP, Pinto L, Carvalho MM, et al. Glucocorticoid programing of the mesopontine cholinergic system. *Front Endocrinol* (2013) **4**:190. doi:10.3389/fendo.2013.00190
8. de Kloet ER, Claessens SEF, Kentrop J. Context modulates outcome of perinatal glucocorticoid action. *Front Endocrinol* (2014) **5**:100. doi:10.3389/fendo.2014.00100
9. Daskalakis NP, Enthoven L, Schoonheere E, de Kloet ER, Oitzl MS. Immediate effects of maternal deprivation on the (Re)activity of the HPA axis differ in CD1 and C57Bl/6J mouse pups. *Front Endocrinol* (2014) **5**:190. doi:10.3389/fendo.2014.00190
10. Loi M, Koricka S, Lucassen PJ, Joels M. Age- and sex-dependent effects of early life stress on hippocampal neurogenesis. *Front Endocrinol* (2014) **5**:13. doi:10.3389/fendo.2014.00013
11. Golier JA, Caramanica K, Demaria R, Yehuda R. A pilot study of mifepristone in combat-related PTSD. *Depress Res Treat* (2012) **2012**:393251. doi:10.1155/2012/393251

12. Yehuda R, Bell A, Bierer LM, Schmeidler J. Maternal, not paternal, PTSD is related to increased risk for PTSD in offspring of Holocaust survivors. *J Psychiatr Res* (2008) **42**(13):1104–11. doi:10.1016/j.jpsychires.2008.01.002
13. Breslau N, Chilcoat HD, Kessler RC, Davis GC. Previous exposure to trauma and PTSD effects of subsequent trauma: results from the Detroit area survey of trauma. *Am J Psychiatry* (1999) **156**(6):902–7. doi:10.1176/ajp.156.6.902
14. Cinini SM, Barnabe GF, Galvao-Coelho N, de Medeiros MA, Perez-Mendes P, Sousa MB, et al. Social isolation disrupts hippocampal neurogenesis in young non-human primates. *Front Neurosci* (2014) **8**:45. doi:10.3389/fnins.2014.00045
15. Claessens SE, Daskalakis NP, van der Veen R, Oitzl MS, de Kloet ER, Champagne DL. Development of individual differences in stress responsiveness: an overview of factors mediating the outcome of early life experiences. *Psychopharmacology (Berl)* (2011) **214**(1):141–54. doi:10.1007/s00213-010-2118-y
16. Lucion AB, Bortolini MC. Mother-pup interactions: rodents and humans. *Front Endocrinol* (2014) **5**:17. doi:10.3389/fendo.2014.00017
17. Rincon-Cortes M, Sullivan RM. Early life trauma and attachment: immediate and enduring effects on neurobehavioral and stress axis development. *Front Endocrinol* (2014) **5**:33. doi:10.3389/fendo.2014.00033
18. Stamatakis A, Diamantopoulou A, Panagiotaropoulos T, Raftogianni A, Stylianopoulou F. Effects of an early experience involving training in a T-maze under either denial or receipt of expected reward through maternal contact. *Front Endocrinol* (2013) **4**:178. doi:10.3389/fendo.2013.00178
19. Bader HN, Bierer LM, Lehrner A, Makotkine I, Daskalakis NP, Yehuda R. Maternal age at Holocaust exposure and maternal PTSD independently influence urinary cortisol levels in adult offspring. *Front Endocrinol* (2014) **5**:103. doi:10.3389/fendo.2014.00103
20. Bierer LM, Bader HN, Daskalakis NP, Lehrner AL, Makotkine I, Seckl JR, et al. Elevation of 11beta-hydroxysteroid dehydrogenase type 2 activity in Holocaust survivor offspring: evidence for an intergenerational effect of maternal trauma exposure. *Psychoneuroendocrinology* (2014) **48**:1–10. doi:10.1016/j.psyneuen.2014.06.001
21. Lehrner A, Bierer LM, Passarelli V, Pratchett LC, Flory JD, Bader HN, et al. Maternal PTSD associates with greater glucocorticoid sensitivity in offspring of Holocaust survivors. *Psychoneuroendocrinology* (2014) **40**(0):213–20. doi:10.1016/j.psyneuen.2013.11.019
22. Yehuda R, Bierer LM. Transgenerational transmission of cortisol and PTSD risk. *Prog Brain Res* (2008) **167**:121–35. doi:10.1016/S0079-6123(07)67009-5

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 02 December 2014; accepted: 22 December 2014; published online: 14 January 2015.

*Citation: Daskalakis NP and Yehuda R (2015) Early maternal influences on stress circuitry: implications for resilience and susceptibility to physical and mental disorders. Front. Endocrinol. **5**:244. doi: 10.3389/fendo.2014.00244*

This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
NEUROSCIENCE

REVIEW ARTICLE
published: 21 November 2014
doi: 10.3389/fnins.2014.00369

Site-specific methylation changes in the glucocorticoid receptor exon 1F promoter in relation to life adversity: systematic review of contributing factors

***Nikolaos P. Daskalakis*[1,2,3]* and *Rachel Yehuda*[1,2,4]**

[1] *Traumatic Stress Studies Division, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
[2] *Mental Health Patient Care Center, James J. Peters Veterans Affairs Medical Center, Bronx, New York, NY, USA*
[3] *Laboratory of Molecular Neuropsychiatry, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
[4] *Department of Neuroscience, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Edited by:
Billy K. C. Chow, University of Hong Kong, China

Reviewed by:
Bruno Bonaz, Grenoble Faculty of Medicine and Hospital, France
Leo T. O. Lee, The University of Hong Kong, Hong Kong

***Correspondence:**
Nikolaos P. Daskalakis, Department of Psychiatry, One Gustave Levy Place, Box 1668, Annenberg building - Room 22–38, New York, NY 10029, USA
e-mail: nikolaos.daskalakis@mssm.edu

There has been recent interest in epigenetics in psychiatry since it offers a means of understanding how stressful life experiences, in interaction with the genotype, result in epigenetic changes that result in altered gene expression, ultimately affecting the risk for mental disorders. Many studies focused on methylation of the glucocorticoid receptor exon 1F promoter following an initial observation that changes in this region could be modulated by the environment. This review examines all published studies that have attempted to measure methylation in this region using different techniques, several tissue types, populations at different behavioral state and stages of development. Methodological issues have been raised with the aim of attempting to understand methylation quantification and site of action. We propose that it is useful to examine whether methylation at specific sites within the promoter region may be particularly relevant to psychiatric vulnerability to stress-related outcomes.

Keywords: NR3C1, glucocorticoid receptor, promoter methylation, exon 1F, adversity, psychiatry, psychopathology, development

Epigenetic plasticity is a mechanism through which environmental exposures influence genetic predispositions resulting in persistent alterations in gene expression and protein synthesis (Zhang and Meaney, 2010; Feil and Fraga, 2011). There has been recent interest in epigenetics in psychiatry since it offers a means of understanding how stressful life experiences, in interaction with the genotype, result in epigenetic changes that result in altered gene expression, ultimately affecting the risk for mental disorders (Tsankova et al., 2007; Yehuda and Bierer, 2009; Nestler, 2014).

Among multiple epigenetic modifications, DNA cytosine methylation has been most reliably studied in experimental and clinical settings (Olkhov-Mitsel and Bapat, 2012; Klengel et al., 2014). Studies attempting to understand stress-dependent developmental programming, have largely focused on promoter methylation of stress-regulatory genes, such as the glucocorticoid receptor (GR) gene, in association with vulnerability and resilience to psychiatric disorders (Daskalakis et al., 2013). The first of these studies examined the rat hippocampal GR exon 1_7 promoter methylation showing an association with variation in maternal care the first week of life particularly at the nerve growth factor-inducible protein A (NGFI-A) binding sequence (Weaver et al., 2004). Soon, other methylation studies of the ortholog human GR promoter (GR exon 1_F promoter; GR-1_F promoter) emerged. This promoter also contains binding sequences for NGFI-A (two canonical and two non-canonical; **Figure 1**). In this paper, we present a systematic review of 16 studies that examined methylation in this region and reported methylation changes in the specific C—phosphate—G dinucleotides (i.e., CpG sites) in relation adverse experiences or adversity-related conditions (Oberlander et al., 2008; McGowan et al., 2009; Dammann et al., 2011; Perroud et al., 2011, 2014a,b; Tyrka et al., 2012; Conradt et al., 2013; Hompes et al., 2013; Melas et al., 2013; Martin-Blanco et al., 2014; Na et al., 2014; Romens et al., 2014; Van Der Knaap et al., 2014; Vukojevic et al., 2014; Yehuda et al., 2014b); **Figure 2**. More studies examined methylation in this region (Moser et al., 2007; Alt et al., 2010; Radtke et al., 2011; Mulligan et al., 2012; Steiger et al., 2013; Yehuda et al., 2013, 2014a; Rodney and Mulligan, 2014), but only the above 16 report methylation differences at a single CpG site resolution. It is already becoming clear that different studies use different methodologies, examine slightly different sub-regions, and accordingly, produce different findings with respect to directionality of the associations with stressful experience and stress-related illness (**Figure 2**). To date, most studies draw conclusions about whether the GR-1_F promoter is hyper- or hypo- methylated based on the average % methylation across several CpG sites. Upon careful review of the data we propose that it is equally useful to examine whether specific sites within the promoter region may be particularly relevant to psychiatric vulnerability to stress-related outcomes.

PROMOTER METHYLATION

Heightened promoter methylation is typically associated with downregulation of gene expression, whereas intragenic

methylation correlates with higher transcriptional activity (Jones, 2012; Moore et al., 2013; Yang et al., 2014). Methylation at promoter regions of highly expressed genes is often low contributing to the notion that promoter methylation is negatively associated with gene expression (Weber et al., 2007; Moore et al., 2013). Most of the studies in such hypomethylated genomic regions detect increases in methylation under pathologic conditions (e.g., Tan et al., 2013), but this could be also related to lack of methodological sensitivity to detect decreases in methylation. The transcriptional repression by methylation is thought to be mediated by blockade of transcription factor binding (Weber et al., 2007; Moore et al., 2013). The extent of promoter hypomethylation needed to enhance gene-expression, and conversely, the extent of hypermethylation required for reduction in gene expression is currently not known. In cancer research, and severe developmental disorders, large effects have been observed (Robertson and Wolffe, 2000; Bergman and Cedar, 2013). However, modest changes could have functional impact for example in psychiatric conditions if they are stable and produce meaningful changes in functional outcomes (Yehuda et al., 2013; Klengel et al., 2014). It is noteworthy, that even in cancer small percent changes in promoter methylation have been found to have great impact (Galetzka et al., 2012).

GR-1_F PROMOTER METHYLATION

The human GR gene (*NR3C1)* is localized on chromosome 5q31-q32, contains nine exons (1–9), with the start codon located 13 nucleotides downstream from the start of exon 2. The 5′untranslated region (5′UTR) has 14 exon 1 splice variants (**Figure 1**, Turner and Muller, 2005; Steiger et al., 2013), all of which bear unique splice donor sites and share a common exon 2 splice acceptor site (Turner and Muller, 2005). Four of these alternative first exons (A_{1-3} and I) and their promoters are forming the distal promoter region of the gene (30 kb upstream of the start codon), while the other 10 first exons (D, J, E, B, F, G, C_{1-3}, and H) and their promoters are forming the proximal promoter (5 kb upstream of the start codon, **Figure 1**), which comprises of a CpG island. The usage of the alternative exon 1 promoters, and the resulting participation of the alternative exon 1 splice variants in the mature GR mRNA, is tissue specific. The promoter usage is considered to eventually affect the expression of GR mature transcripts and protein isoforms, but the nature of this relationship is not known (Turner et al., 2010, 2014).

The GR-1_F is transcriptionally active in hippocampus, B lymphocytes and innate immune cells (but not in T lymphocytes or monocytes) (Turner and Muller, 2005). GR-1_F promoter (326 bp) and exon 1_F (63 bp) contain 47 CpG sites (**Figure 1**). Methylation of the GR-1_F promoter region associated, in neonatal cord blood, with maternal depression during gestation (Oberlander et al., 2008) and, in adult offspring hippocampus, with childhood abuse (McGowan et al., 2009). We have recently observed lower peripheral blood mononuclear cells' (PBMCs) methylation of the same promoter region in adult combat exposed veterans with PTSD compared with combat exposed controls (Yehuda et al., 2014b). The small % changes in GR-1_F promoter methylation had a functional impact since it was associated with endocrine functional outcomes (Yehuda et al., 2014b).

TECHNICAL CONSIDERATIONS ON METHYLATION ANALYSIS OF GR-1_F PROMOTER REGION

METHOD

DNA methylation analysis involves bisulfate treatment of the DNA as an initial step. Bisulfite treatment describes the conversion of all unmethylated cytosine residues to uracils (deamination) in the presence of NaOH and sodium bisulfite, but leaves the methylated cytosine residues intact. Thus, sequencing of the treated DNA after amplification of the target region allows the analysis of methylation, by determining the ratio of cytosine to thymine, at a single CpG site level (Tost and Gut, 2007; Zhang et al., 2009).

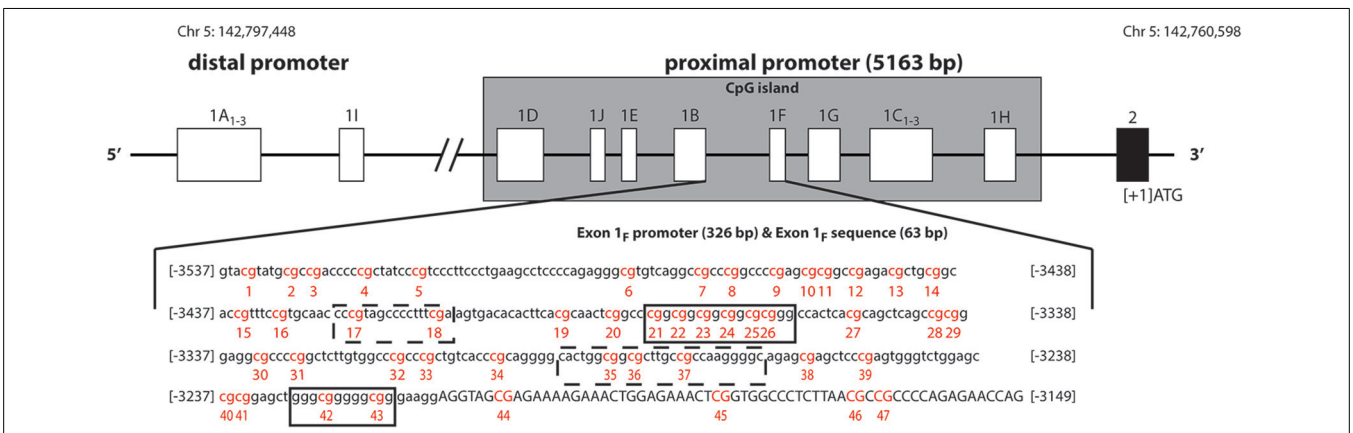


FIGURE 1 | Schematic representation of human glucocorticoid receptor gene (NR3C1) non-coding first exons according to Turner and Muller (2005), Sinclair et al. (2012), Steiger et al. (2013). The solid black line boxes with a number represent the different exons and the 5′–3′ orientation goes from left to right. The NR3C1 gene 5′ region is composed of multiple first exons: four in the distal promoter region (A_{1-3} and I) and ten (D, J, E, B, F, G, C_{1-3}, and H) in the proximal promoter region located in a C—phosphate—G (CpG) island. The exon 1_F promoter (lower case) and exon 1_F sequence (uppercase) is illustrated. The numbering is relative to the start codon (ATG: +1), which is located 13 nucleotides downstream from the start of exon 2. The 47 CpG sites are in red and numbered. Boxes represent known or putative canonical (solid-lined box) and non-canonical (broken-lined box) NGFI-A–binding sites according to McGowan et al. (2009).

Quantitative measures of DNA methylation patterns are essential in the context of disease. Given the heterogeneity in methylation between cells, Sanger sequencing of bisulfite-treated samples alone is not sufficient for quantitative analysis of methylation status of the target region. To overcome this, cloning of the region of interest (usually 300–500 bp) followed by sequencing of individual clones is needed (Olkhov-Mitsel and Bapat, 2012). Alternatively, the use of other sequencing methods with improved quantitative resolution does not require the expensive, time-consuming and laborious clone-based methylation analysis. Pyrosequencing gained popularity the recent years as an accurate and reliable approach for methylation analysis of short DNA stretches (usually <150 bp). Bisulfite treated DNA is first amplified and then, since one of the amplification primers used is biotinylated, a single strand is isolated. Finally, with the use of a pyrosequencing primer, the purified single strand is subjected to a pyrosequencing reaction where single nucleotides are incorporated sequentially and generate light that can be detected and quantified (Tost and Gut, 2007).

No direct comparisons have been made with respect to Sanger sequencing and pyrosequencing with respect to the GR-1_F promoter methylation analysis. Methylation in this region has been analyzed by some using clone-based Sanger sequencing (McGowan et al., 2009; Yehuda et al., 2014b) and by others using pyrosequencing (Oberlander et al., 2008; Dammann et al., 2011; Perroud et al., 2011, 2014a,b; Tyrka et al., 2012; Conradt et al., 2013; Martin-Blanco et al., 2014; Na et al., 2014; Romens et al., 2014; Vukojevic et al., 2014). More recently, the Matrix-assisted laser desorption ionization time of flight mass spectrometry (MassARRAY) provided another method for quantitative methylation analysis of long DNA sequences (usually >600 bp), based on fragmentation by base-specific cleavage and subsequent analysis of the cleavage products (Claus et al., 2012) and three studies in this review have utilized it (Hompes et al., 2013; Melas et al., 2013; Van Der Knaap et al., 2014).

TISSUE TYPE

Methylation patterns at transcriptional regulatory elements, which contain transcription factor binding sites, are tissue-specific and cell-type specific, apart from development-, individual-, or disease- specific (Zhang et al., 2013; Ziller et al., 2013). Tissue-type and cell-type specific methylation changes occur at evolutionary conserved sequences (Zhang et al., 2013). The tissue-specific methylation differences, in an order of magnitude, are larger than the cell-type specific differences (Ziller et al., 2013). Promoter methylation seems to participate more in tissue differentiation, whereas enhancer methylation in cell-type differentiation (Zhang et al., 2013). DNA in the under review studies was extracted from a wide range of tissue-types (blood, brain, placenta, saliva) involving many cell-types, suggesting that a big part of variance between studies could be related to this choice.

In psychiatry, there is special interest on peripheral tissues, more often blood, in methylation studies because peripheral tissue is more readily accessible. Using peripheral methylation as a surrogate to brain methylation has been supported by two types of data: first, causal biological mechanisms of the condition studied can affect peripheral methylation, in an independent way to brain methylation, providing useful peripheral biomarkers; second, condition-related methylation patterns in some loci can be the same in the periphery and brain revealing common condition-related epigenetic reprogramming giving clues on developmental origins of the condition (Aberg et al., 2013).

SUB-REGION OF INTEREST WITHIN THE GR-1_F PROMOTER

When observing the studies under review in chronological order, several trends in the field can be observed (**Figure 2**). Initial publications have an extremely high influence on the choices of regions of interest in subsequent studies. Oberlander et al. (2008) choice focused on methylation at one canonical NGFI-A binding site sequence was motivated by previous work (reviewed above) in rats by Weaver et al. (2004) on the rat ortholog sequence. The technique that Oberlander et al. chose (i.e., pyrosequencing) limited the sequence length (105 bp) which trucated the number of CpGs under investigation to eight, four in exon 1_F promoter and four in exon 1_F (another five sites were measured from the downstream region) from 47 potential sites. This first original human publication investigating the effects of prenatal maternal mood on newborn cord blood on methylation, affected the choice of technique and region for two studies examining similar prenatal factors (Conradt et al., 2013; Perroud et al., 2014b), but also seven studies that examined other questions entirely (Perroud et al., 2011, 2014a,b; Tyrka et al., 2012; Martin-Blanco et al., 2014; Romens et al., 2014; Vukojevic et al., 2014).

Similarly, the choice of McGowan et al. (2009) to study 39 CpG sites (38 in exon 1_F promoter, 1 in exon 1_F) containing 4 NGFI-A binding sites (2 canonical and 2 non-canonical, **Figure 2**) in association with childhood abuse on adult hippocampal methylation has influenced the choice of another study of early adversity (Van Der Knaap et al., 2014) and a study of combat-related PTSD (Yehuda et al., 2014b). Two recent studies using MassARRAYs reported methylation for even larger regions (Hompes et al., 2013; Melas et al., 2013).

STABILITY

There is an emerging dialectic between the idea that epigenetic changes are enduring enough to persist through gamete mitosis and meiosis and the idea that epigenetic marks may undergo observable changes in response to the environment throughout life (Bergman and Cedar, 2013). The apparent discrepancy between these two ideas is partly resolved by the idea that the nature of epigenetic changes may in part depend on regional specificity (gene promoter vs. gene body) and sub-regional specificity (site-specific effects).

GR-1_F PROMOTER SITE-SPECIFIC METHYLATION

From the 16 studies reviewed (Oberlander et al., 2008; McGowan et al., 2009; Dammann et al., 2011; Perroud et al., 2011, 2014a,b; Tyrka et al., 2012; Conradt et al., 2013; Hompes et al., 2013; Melas et al., 2013; Martin-Blanco et al., 2014; Na et al., 2014; Romens et al., 2014; Van Der Knaap et al., 2014; Vukojevic et al., 2014; Yehuda et al., 2014b), four studies showed site-specific effects in offspring based on maternal mood during gestation [anxiety (Hompes et al., 2013), depression (Oberlander et al., 2008;

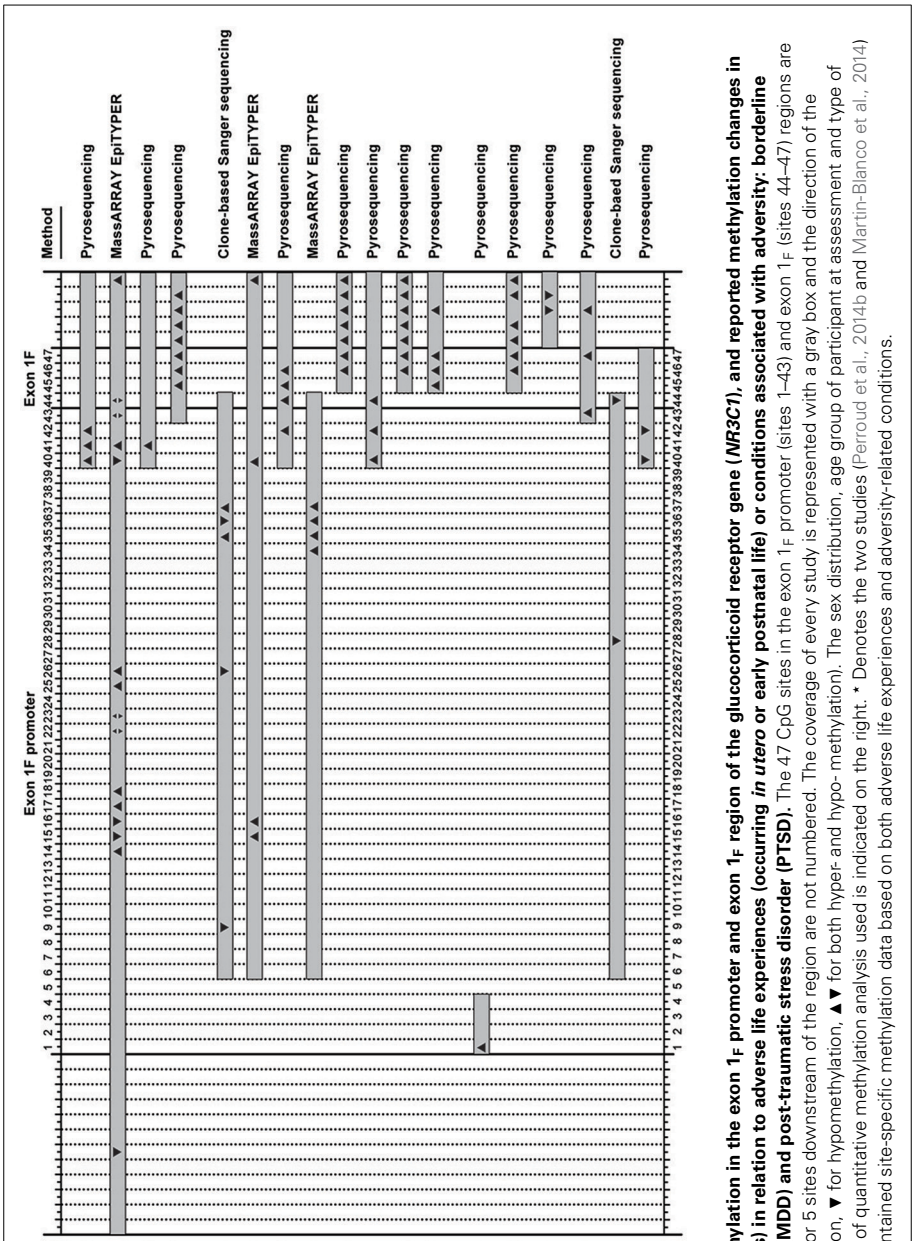


FIGURE 2 | Overview of 16 studies that examined methylation in the exon 1_F promoter and exon 1_F region of the glucocorticoid receptor gene (*NR3C1*), and reported methylation changes in specific C—phosphate—G dinucleotides (i.e., CpG sites) in relation to adverse life experiences (occurring *in utero* or early postnatal life) or conditions associated with adversity: borderline personality disorder (BPD), major depressive disorder (MDD) and post-traumatic stress disorder (PTSD). The 47 CpG sites in the exon 1_F promoter (sites 1–43) and exon 1_F (sites 44–47) regions are numbered according to **Figure 1**, while 12 sites upstream or 5 sites downstream of the region are not numbered. The coverage of every study is represented with a gray box and the direction of the site-specific findings is also depicted (▲ for hypermethylation, ▼ for hypomethylation, ▲▼ for both hyper- and hypo- methylation). The sex distribution, age group of participant at assessment and type of collected tissue are indicated in the left, while the method of quantitative methylation analysis used is indicated on the right. * Denotes the two studies (Perroud et al., 2014b and Martin-Blanco et al., 2014) that were included twice in this overview because they contained site-specific methylation data based on both adverse life experiences and adversity-related conditions.

Conradt et al., 2013; Hompes et al., 2013), PTSD (Perroud et al., 2014b)], six studies investigated the site-specific effects of adversity occurring in the period from birth to adolescence [abuse (McGowan et al., 2009; Perroud et al., 2011, 2014a; Tyrka et al., 2012; Martin-Blanco et al., 2014; Romens et al., 2014; Van Der Knaap et al., 2014), low parental care (Tyrka et al., 2012), parental death (Melas et al., 2013), parental loss (Tyrka et al., 2012) and other early stressful life events (Van Der Knaap et al., 2014)], and four studies detected differences in subjects meeting criteria for disorders associated with life adversity [BPD (Dammann et al., 2011; Martin-Blanco et al., 2014), MDD (Na et al., 2014), PTSD (Perroud et al., 2014b; Vukojevic et al., 2014; Yehuda et al., 2014b)]. There was further variation across studies based on the age (neonate, adolescent, adult) and sex distribution (male only, female only, mixed) of the sample used.

While the majority of studies detected hypermethylation in the specific CpG sites, there were sites where hypomethylation was detected demonstrating a general positive association of adversity with methylation of this region. However, the lower rate of detected hypomethylation in association with adversity might be also related to a "floor effect" due to low overall methylation levels in this region, which make difficult to statistically prove lowering of methylation. Potential false negative detection of hypomethylation needs to be investigated further in large studies especially since it can also have effects in downstream expression and functional endocrinology (Yehuda et al., 2014b) so as hypermethylation (McGowan et al., 2009).

If different adversity factors and associated conditions can affect the methylation of different CpG sites in opposing directions, it remains to be explored what is the outcome of the opposing methylation changes for gene expression. Only two of the 16 studies provided data on gene expression in relation to site-specific methylation in GR-1_F promoter. We have reported a negative correlation of GR-1_F expression with methylation at site 28 (Yehuda et al., 2014b), which was one of the two sites associated with PTSD. Vukojevic et al. observed an inverse correlation of GR expression with methylation at site 42 in healthy young adults, but they did not report expression data in the sample, where they observed the association with PTSD (Vukojevic et al., 2014).

Furthermore, while there were CpG sites for which multiple studies were in agreement (CpG 35, 37, 40, 41, 42, 43, 44, 45, 46, and 47), but there were also sites with disagreement (CpG 15, 16, 22, 23, 26, 36, 40, 42, 43, and 44). Factors that could potentially account for the disagreements are different time window in which adversity acted (1st -2nd - 3rd trimester of pregnancy, postnatal period and childhood, adulthood), differences in tissue type, subject's sex and age (at assessment).

POSSIBLE TRANGENERATIONAL EFFECTS OF PARENTAL TRAUMA EXPOSURE AND SYMPTOMS ON GR-1_F PROMOTER SITE-SPECIFIC METHYLATION

In a recent study in Holocaust survivor offspring and demographic controls (Yehuda et al., 2014a), we wanted to identify

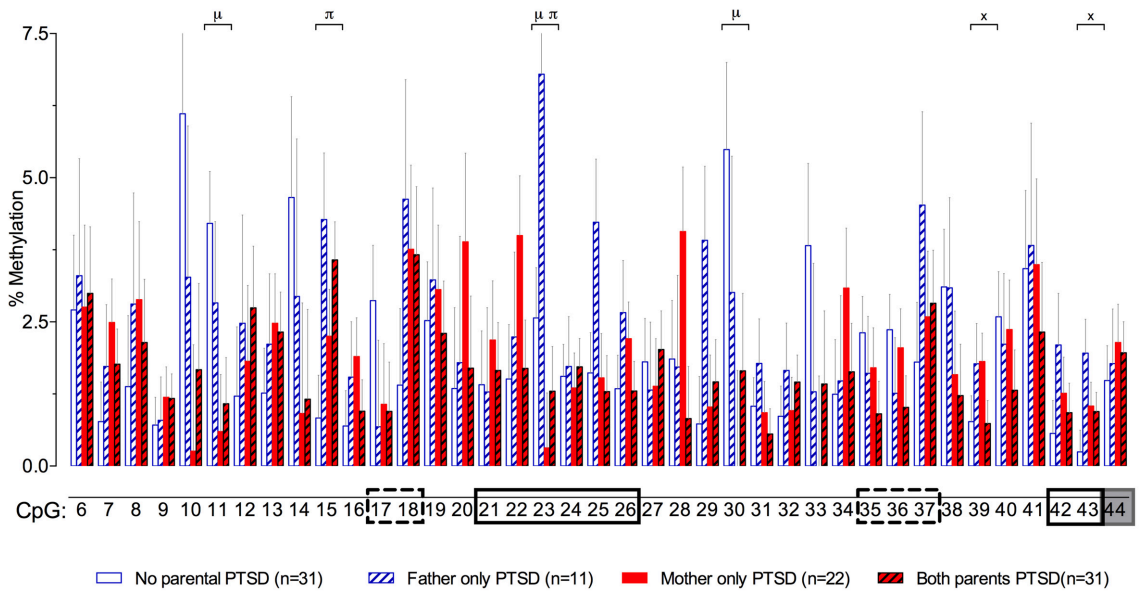


FIGURE 3 | Percent methylation at each of 39 C-phosphate-G dinucleotides (i.e., CpG sites) of the glucocorticoid receptor gene (*NR3C1)* exon 1_F promoter (sites 6–43) and exon 1_F region (site 44) analyzed by cytosine methylation bisulfite mapping (clone-based Sanger sequencing) using peripheral blood mononuclear cells (PBMCs) DNA, in a population of offspring described in our previous publications (Lehrner et al., 2014; Yehuda et al., 2014a). The 39 CpG sites accessed in the study are numbered as in **Figure 1**. The represented data (mean ± s.e.m.) are based on a multivariate Two-Way analysis of covariance with maternal and paternal post-traumatic stress disorder (PTSD) as fixed factors and parental Holocaust exposure, age, smoking history, and PBMC-type as covariates. μ, significant maternal PTSD effect. π, significant paternal PTSD effect. x, significant maternal by paternal PTSD interaction effect. Significance was set at $p < 0.05$. Boxes represent the location of known or putative canonical (solid-lined box) and non-canonical (broken-lined box) NGFI-A binding sites, according to McGowan et al. (2009), and a gray box represents the location of the beginning of exon 1_F.

effects of parental Holocaust exposure or exposure-related symptoms (i.e., PTSD) on GR-1_F promoter methylation in order to find epigenetic marks associated with glucocorticoid dysregulation (Bader et al., 2014; Bierer et al., 2014; Lehrner et al., 2014) in this population at risk for PTSD. Paternal PTSD, only in the absence of maternal PTSD, was associated with higher levels of GR-1_F promoter methylation, while offspring with both maternal and paternal PTSD displayed the lowest level of methylation. The relatively small differences in methylation were associated with differences in GR-1_F expression and functional endocrinology (Yehuda et al., 2014a). A site-specific analysis presented here (**Figure 3**), reveals that there are sites with significant effects of maternal PTSD (CpG 11, 23 and 30), paternal PTSD (CpG 15 and 23) or their interaction (CpG 39 and 43).

Exposure to trauma in parents has been also linked to an increased risk for child abuse and maltreatment in offspring especially in the presence of maternal or paternal PTSD (Yehuda et al., 2001; Yehuda and Bierer, 2008; Palosaari et al., 2013). It is difficult to disentangle effects that reflect trauma-related transgenerational inheritance from early rearing influences, including childhood traumatic events, experienced as a consequence of having trauma-exposed or symptomatic parents. We have previously suggested that part of the phenotype in Holocaust offspring is the recollection of perceived emotional abuse or neglect, whereas recollections of sexual abuse and physical abuse or neglect may be relatively independent risk factors for PTSD in Holocaust offspring, as in persons who develop PTSD without having traumatized parents (Yehuda et al., 2001). Phenotypic clustering of Holocaust offspring demonstrated an association of paternal, but not maternal, PTSD with childhood trauma and abuse and increased GR-1_F promoter methylation (Yehuda et al., 2014a).

CONCLUSIONS

Because the study of epigenetics in neuropsychiatry is relatively new, many fundamental questions are just beginning to be answered. It is not clear whether epigenetic marks are equally stable across all genes and all gene regions since some epigenetic marks have been shown to persist across generations (Gapp et al., 2014), while others have demonstrated change in response to psychotherapeutic interventions (Perroud et al., 2013; Yehuda et al., 2013). At the current time, complicating the discussion about the stability of epigenetic marks is that the reliability of the assessment of epigenetic marks such as methylation is not fully explored making difficult to identify the rate of potential stochastic epigenetic phenomena (Nestler, 2014). Thus, even in studies that assume epigenetic marks to be stable, there is value in performing multiple assessments of the same sample, or different samples from individuals within short periods of time. Such studies have been lacking, but will be very informative if performed in the near future.

ACKNOWLEDGMENTS

Supported by a grant from NIMH (1RC1MH088101-01) and by grant UL1TR000067 from the National Center for Advancing Translational Sciences (NCATS), a component of NIH. NIMH, NIH, and NCATS had no role in the study design, in the collection, analysis, and interpretation of the data, in writing the study, or in the decision to submit the study for publication.

REFERENCES

Aberg, K. A., Xie, L. Y., Mcclay, J. L., Nerella, S., Vunck, S., Snider, S., et al. (2013). Testing two models describing how methylome-wide studies in blood are informative for psychiatric conditions. *Epigenomics* 5, 367–377. doi: 10.2217/epi.13.36

Alt, S. R., Turner, J. D., Klok, M. D., Meijer, O. C., Lakke, E. A., Derijk, R. H., et al. (2010). Differential expression of glucocorticoid receptor transcripts in major depressive disorder is not epigenetically programmed. *Psychoneuroendocrinology* 35, 544–556. doi: 10.1016/j.psyneuen.2009.09.001

Bader, H. N., Bierer, L. M., Lehrner, A., Makotkine, I., Daskalakis, N. P., and Yehuda, R. (2014). Maternal age at holocaust exposure and maternal PTSD independently influence urinary cortisol cevels in adult offspring. *Front. Endocrinol.* 5:103. doi: 10.3389/fendo.2014.00103

Bergman, Y., and Cedar, H. (2013). DNA methylation dynamics in health and disease. *Nat. Struct. Mol. Biol.* 20, 274–281. doi: 10.1038/nsmb.2518

Bierer, L. M., Bader, H. N., Daskalakis, N. P., Lehrner, A., Makotkine, I., Seckl, J. R., et al. (2014). Elevation of 11β-hydroxysteroid dehydrogenase type 2 in Holocaust survivor offspring: evidence for an intergenerational effect of maternal trauma exposure. *Psychoneuroendocrinology* 48, 1–10. doi: 10.1016/j.psyneuen.2014.06.001

Claus, R., Wilop, S., Hielscher, T., Sonnet, M., Dahl, E., Galm, O., et al. (2012). A systematic comparison of quantitative high-resolution DNA methylation analysis and methylation-specific PCR. *Epigenetics* 7, 772–780. doi: 10.4161/epi.20299

Conradt, E., Lester, B. M., Appleton, A. A., Armstrong, D. A., and Marsit, C. J. (2013). The roles of DNA methylation of NR3C1 and 11beta-HSD2 and exposure to maternal mood disorder *in utero* on newborn neurobehavior. *Epigenetics* 8, 1321–1329. doi: 10.4161/epi.26634

Dammann, G., Teschler, S., Haag, T., Altmuller, F., Tuczek, F., and Dammann, R. H. (2011). Increased DNA methylation of neuropsychiatric genes occurs in borderline personality disorder. *Epigenetics* 6, 1454–1462. doi: 10.4161/epi.6.12.18363

Daskalakis, N. P., Bagot, R. C., Parker, K. J., Vinkers, C. H., and De Kloet, E. R. (2013). The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* 38, 1858–1873. doi: 10.1016/j.psyneuen.2013.06.008

Feil, R., and Fraga, M. F. (2011). Epigenetics and the environment: emerging patterns and implications. *Nat. Rev. Genet.* 13, 97–109. doi: 10.1038/nrg3142

Galetzka, D., Hansmann, T., El Hajj, N., Weis, E., Irmscher, B., Ludwig, M., et al. (2012). Monozygotic twins discordant for constitutive BRCA1 promoter methylation, childhood cancer and secondary cancer. *Epigenetics* 7, 47–54. doi: 10.4161/epi.7.1.18814

Gapp, K., Von Ziegler, L., Tweedie-Cullen, R. Y., and Mansuy, I. M. (2014). Early life epigenetic programming and transmission of stress-induced traits in mammals: how and when can environmental factors influence traits and their transgenerational inheritance? *Bioessays* 36, 491–502. doi: 10.1002/bies.201300116

Hompes, T., Izzi, B., Gellens, E., Morreels, M., Fieuws, S., Pexsters, A., et al. (2013). Investigating the influence of maternal cortisol and emotional state during pregnancy on the DNA methylation status of the glucocorticoid receptor gene (NR3C1) promoter region in cord blood. *J. Psychiatr. Res.* 47, 880–891. doi: 10.1016/j.jpsychires.2013.03.009

Jones, P. A. (2012). Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* 13, 484–492. doi: 10.1038/nrg3230

Klengel, T., Pape, J., Binder, E. B., and Mehta, D. (2014). The role of DNA methylation in stress-related psychiatric disorders. *Neuropharmacology* 80, 115–132. doi: 10.1016/j.neuropharm.2014.01.013

Lehrner, A., Bierer, L. M., Passarelli, V., Pratchett, L. C., Flory, J. D., Bader, H. N., et al. (2014). Maternal PTSD associates with greater glucocorticoid sensitivity in offspring of Holocaust survivors. *Psychoneuroendocrinology* 40, 213–220. doi: 10.1016/j.psyneuen.2013.11.019

Martin-Blanco, A., Ferrer, M., Soler, J., Salazar, J., Vega, D., Andion, O., et al. (2014). Association between methylation of the glucocorticoid receptor gene, childhood maltreatment, and clinical severity in borderline personality disorder. *J. Psychiatr. Res.* 57, 34–40. doi: 10.1016/j.jpsychires.2014.06.011

McGowan, P. O., Sasaki, A., D'alessio, A. C., Dymov, S., Labonte, B., Szyf, M., et al. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat. Neurosci.* 12, 342–348. doi: 10.1038/nn.2270

Melas, P. A., Wei, Y., Wong, C. C., Sjoholm, L. K., Aberg, E., Mill, J., et al. (2013). Genetic and epigenetic associations of MAOA and NR3C1 with depression and childhood adversities. *Int. J. Neuropsychopharmacol.* 16, 1513–1528. doi: 10.1017/S1461145713000102

Moore, L. D., Le, T., and Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology* 38, 23–38. doi: 10.1038/npp.2012.112

Moser, D., Molitor, A., Kumsta, R., Tatschner, T., Riederer, P., and Meyer, J. (2007). The glucocorticoid receptor gene exon 1-F promoter is not methylated at the NGFI-A binding site in human hippocampus. *World J. Biol. Psychiatry* 8, 262–268. doi: 10.1080/15622970701429862

Mulligan, C. J., D'errico, N. C., Stees, J., and Hughes, D. A. (2012). Methylation changes at NR3C1 in newborns associate with maternal prenatal stress exposure and newborn birth weight. *Epigenetics* 7, 853–857. doi: 10.4161/epi.21180

Na, K. S., Chang, H. S., Won, E., Han, K. M., Choi, S., Tae, W. S., et al. (2014). Association between glucocorticoid receptor methylation and hippocampal subfields in major depressive disorder. *PLoS ONE* 9:e85425. doi: 10.1371/journal.pone.0085425

Nestler, E. J. (2014). Epigenetic mechanisms of depression. *JAMA Psychiatry* 71, 454–456. doi: 10.1001/jamapsychiatry.2013.4291

Oberlander, T. F., Weinberg, J., Papsdorf, M., Grunau, R., Misri, S., and Devlin, A. M. (2008). Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics* 3, 97–106. doi: 10.4161/epi.3.2.6034

Olkhov-Mitsel, E., and Bapat, B. (2012). Strategies for discovery and validation of methylated and hydroxymethylated DNA biomarkers. *Cancer Med.* 1, 237–260. doi: 10.1002/cam4.22

Palosaari, E., Punamaki, R. L., Qouta, S., and Diab, M. (2013). Intergenerational effects of war trauma among Palestinian families mediated via psychological maltreatment. *Child Abuse Negl.* 37, 955–968. doi: 10.1016/j.chiabu.2013.04.006

Perroud, N., Dayer, A., Piguet, C., Nallet, A., Favre, S., Malafosse, A., et al. (2014a). Childhood maltreatment and methylation of the glucocorticoid receptor gene NR3C1 in bipolar disorder. *Br. J. Psychiatry* 204, 30–35. doi: 10.1192/bjp.bp.112.120055

Perroud, N., Paoloni-Giacobino, A., Prada, P., Olie, E., Salzmann, A., Nicastro, R., et al. (2011). Increased methylation of glucocorticoid receptor gene (NR3C1) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Transl. Psychiatry* 1, e59. doi: 10.1038/tp.2011.60

Perroud, N., Rutembesa, E., Paoloni-Giacobino, A., Mutabaruka, J., Mutesa, L., Stenz, L., et al. (2014b). The Tutsi genocide and transgenerational transmission of maternal stress: epigenetics and biology of the HPA axis. *World J. Biol. Psychiatry* 15, 334–345. doi: 10.3109/15622975.2013.866693

Perroud, N., Salzmann, A., Prada, P., Nicastro, R., Hoeppli, M. E., Furrer, S., et al. (2013). Response to psychotherapy in borderline personality disorder and methylation status of the BDNF gene. *Transl. Psychiatry* 3, e207. doi: 10.1038/tp.2012.140

Radtke, K. M., Ruf, M., Gunter, H. M., Dohrmann, K., Schauer, M., Meyer, A., et al. (2011). Transgenerational impact of intimate partner violence on methylation in the promoter of the glucocorticoid receptor. *Transl. Psychiatry* 1:e21. doi: 10.1038/tp.2011.21

Robertson, K. D., and Wolffe, A. P. (2000). DNA methylation in health and disease. *Nat. Rev. Genet.* 1, 11–19. doi: 10.1038/35049533

Rodney, N. C., and Mulligan, C. J. (2014). A biocultural study of the effects of maternal stress on mother and newborn health in the Democratic Republic of Congo. *Am. J. Phys. Anthropol.* 155, 200–209. doi: 10.1002/ajpa.22568

Romens, S. E., Mcdonald, J., Svaren, J., and Pollak, S. D. (2014). Associations between early life stress and gene methylation in children. *Child Dev*. doi: 10.1111/cdev.12270. [Epub ahead of print].

Sinclair, D., Fullerton, J. M., Webster, M. J., and Shannon Weickert, C. (2012). Glucocorticoid receptor 1B and 1C mRNA transcript alterations in schizophrenia and bipolar disorder, and their possible regulation by GR gene variants. *PLoS ONE* 7:e31720. doi: 10.1371/journal.pone.0031720

Steiger, H., Labonte, B., Groleau, P., Turecki, G., and Israel, M. (2013). Methylation of the glucocorticoid receptor gene promoter in bulimic women: associations with borderline personality disorder, suicidality, and exposure to childhood abuse. *Int. J. Eat Disord.* 46, 246–255. doi: 10.1002/eat.22113

Tan, J., Gu, Y., Zhang, X., You, S., Lu, X., Chen, S., et al. (2013). Hypermethylation of CpG islands is more prevalent than hypomethylation across the entire genome in breast carcinogenesis. *Clin. Exp. Med.* 13, 1–9. doi: 10.1007/s10238-011-0173-2

Tost, J., and Gut, I. G. (2007). DNA methylation analysis by pyrosequencing. *Nat. Protoc.* 2, 2265–2275. doi: 10.1038/nprot.2007.314

Tsankova, N., Renthal, W., Kumar, A., and Nestler, E. J. (2007). Epigenetic regulation in psychiatric disorders. *Nat. Rev. Neurosci.* 8, 355–367. doi: 10.1038/nrn2132

Turner, J. D., Alt, S. R., Cao, L., Vernocchi, S., Trifonova, S., Battello, N., et al. (2010). Transcriptional control of the glucocorticoid receptor: CpG islands, epigenetics and more. *Biochem. Pharmacol.* 80, 1860–1868. doi: 10.1016/j.bcp.2010.06.037

Turner, J. D., and Muller, C. P. (2005). Structure of the glucocorticoid receptor (NR3C1) gene 5′ untranslated region: identification, and tissue distribution of multiple new human exon 1. *J. Mol. Endocrinol.* 35, 283–292. doi: 10.1677/jme.1.01822

Turner, J. D., Vernocchi, S., Schmitz, S., and Muller, C. P. (2014). Role of the 5′-untranslated regions in post-transcriptional regulation of the human glucocorticoid receptor. *Biochim. Biophys. Acta* 1839, 1051–1061. doi: 10.1016/j.bbagrm.2014.08.010

Tyrka, A. R., Price, L. H., Marsit, C., Walters, O. C., and Carpenter, L. L. (2012). Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: preliminary findings in healthy adults. *PLoS ONE* 7:e30148. doi: 10.1371/journal.pone.0030148

Van Der Knaap, L. J., Riese, H., Hudziak, J. J., Verbiest, M. M., Verhulst, F. C., Oldehinkel, A. J., et al. (2014). Glucocorticoid receptor gene (NR3C1) methylation following stressful events between birth and adolescence. The TRAILS study. *Transl. Psychiatry* 4, e381. doi: 10.1038/tp.2014.22

Vukojevic, V., Kolassa, I. T., Fastenrath, M., Gschwind, L., Spalek, K., Milnik, A., et al. (2014). Epigenetic modification of the glucocorticoid receptor gene is linked to traumatic memory and post-traumatic stress disorder risk in genocide survivors. *J. Neurosci.* 34, 10274–10284. doi: 10.1523/JNEUROSCI.1526-14.2014

Weaver, I. C., Cervoni, N., Champagne, F. A., D'alessio, A. C., Sharma, S., Seckl, J. R., et al. (2004). Epigenetic programming by maternal behavior. *Nat. Neurosci.* 7, 847–854. doi: 10.1038/nn1276

Weber, M., Hellmann, I., Stadler, M. B., Ramos, L., Paabo, S., Rebhan, M., et al. (2007). Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat. Genet.* 39, 457–466. doi: 10.1038/ng1990

Yang, X., Han, H., De Carvalho, D. D., Lay, F. D., Jones, P. A., and Liang, G. (2014). Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell* 26, 577–590. doi: 10.1016/j.ccr.2014.07.028

Yehuda, R., and Bierer, L. M. (2008). Transgenerational transmission of cortisol and PTSD risk. *Prog. Brain Res.* 167, 121–135. doi: 10.1016/S0079-6123(07)67009-5

Yehuda, R., and Bierer, L. M. (2009). The relevance of epigenetics to PTSD: implications for the DSM-V. *J. Trauma. Stress* 22, 427–434. doi: 10.1002/jts.20448

Yehuda, R., Daskalakis, N. P., Desarnaud, F., Makotkine, I., Lehrner, A. L., Koch, E., et al. (2013). Epigenetic biomarkers as predictors and correlates of symptom improvement following psychotherapy in Combat Veterans with PTSD. *Front. Psychiatry* 4:118. doi: 10.3389/fpsyt.2013.00118

Yehuda, R., Daskalakis, N. P., Lehrner, A., Desarnaud, F., Bader, H. N., Makotkine, I., et al. (2014a). Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in holocaust survivor offspring. *Am. J. Psychiatry* 171, 872–880. doi: 10.1176/appi.ajp.2014.13121571

Yehuda, R., Flory, J. D., Bierer, L. M., Henn-Haase, C., Lehrner, A., Desarnaud, F., et al. (2014b). Lower methylation of glucocorticoid receptor gene promoter 1 in peripheral blood of veterans with posttraumatic stress disorder. *Biol. Psychiatry*. doi: 10.1016/j.biopsych.2014.02.006. [Epub ahead of print].

Yehuda, R., Halligan, S. L., and Grossman, R. (2001). Childhood trauma and risk for PTSD: relationship to intergenerational effects of trauma, parental PTSD, and cortisol excretion. *Dev. Psychopathol.* 13, 733–753. doi: 10.1017/S0954579401003170

Zhang, B., Zhou, Y., Lin, N., Lowdon, R. F., Hong, C., Nagarajan, R. P., et al. (2013). Functional DNA methylation differences between tissues, cell types, and across individuals discovered using the M&M algorithm. *Genome Res.* 23, 1522–1540. doi: 10.1101/gr.156539.113

Zhang, T. Y., and Meaney, M. J. (2010). Epigenetics and the environmental regulation of the genome and its function. *Annu. Rev. Psychol.* 61, 439–466, C431–C433. doi: 10.1146/annurev.psych.60.110707.163625

Zhang, Y., Rohde, C., Tierling, S., Stamerjohanns, H., Reinhardt, R., Walter, J., et al. (2009). DNA methylation analysis by bisulfite conversion, cloning, and sequencing of individual clones. *Methods Mol. Biol.* 507, 177–187. doi: 10.1007/978-1-59745-522-0_14

Ziller, M. J., Gu, H., Muller, F., Donaghey, J., Tsai, L. T., Kohlbacher, O., et al. (2013). Charting a dynamic DNA methylation landscape of the human genome. *Nature* 500, 477–481. doi: 10.1038/nature12433

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 21 August 2014; accepted: 28 October 2014; published online: 21 November 2014.

Citation: Daskalakis NP and Yehuda R (2014) Site-specific methylation changes in the glucocorticoid receptor exon 1F promoter in relation to life adversity: systematic review of contributing factors. Front. Neurosci. ***8****:369. doi: 10.3389/fnins.2014.00369*

This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Neuroscience.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 17 February 2014
doi: 10.3389/fendo.2014.00014

Gene–environment interactions and intermediate phenotypes: early trauma and depression

Orla P. Hornung*† and Christine M. Heim*†

Institute of Medical Psychology, Charité University Medicine Berlin, Berlin, Germany

Edited by:
Rachel Yehuda, Mount Sinai School of Medicine, USA

Reviewed by:
James A. Carr, Texas Tech University, USA
Tania L. Roth, University of Delaware, USA

****Correspondence:***
Christine M. Heim, Institute of Medical Psychology, Charité University Medicine Berlin, Luisenstraße 57, Berlin 10117, Germany
e-mail: christine.heim@charite.de

† Orla P. Hornung and Christine M. Heim have contributed equally to this work.

This review focuses on current research developments in the study of gene by early life stress (ELS) interactions and depression. ELS refers to aversive experiences during childhood and adolescence such as sexual, physical or emotional abuse, emotional or physical neglect as well as parental loss. Previous research has focused on investigating and characterizing the specific role of ELS within the pathogenesis of depression and linking these findings to neurobiological changes of the brain, especially the stress response system. The latest findings highlight the role of genetic factors that increase vulnerability or, likewise, promote resilience to depression after childhood trauma. Considering intermediate phenotypes has further increased our understanding of the complex relationship between early trauma and depression. Recent findings with regard to epigenetic changes resulting from adverse environmental events during childhood promote current endeavors to identify specific target areas for prevention and treatment schemes regarding the long-term impact of ELS. Taken together, the latest research findings have underscored the essential role of genotypes and epigenetic processes within the development of depression after childhood trauma, thereby building the basis for future research and clinical interventions.

Keywords: depression, stress, maltreatment, development, HPA axis, polymorphisms

INTRODUCTION

Mood disorders are among the most prevalent forms of mental illness. The lifetime prevalence of major depressive disorder (MDD) among US adults is 16.5%, with women being 70% more likely than men to develop depression. Stress and trauma exposure are major risk factors for the development of depression, particularly when experienced early in life. However, up to 40% of the risk to develop depression appears to be genetic. Additional risk factors of depression include family history of depression, past episodes of depression, and specific personality traits, such as neuroticism among others. The combined impact of genetic and environmental factors contributing to the pathogenesis and onset of depression is in line with the diathesis–stress model (1). According to this psychological model, the interaction between predispositional factors and stressors leads to disorders such as depression.

A wealth of studies document the prominent role of early life stress (ELS), such as sexual, physical or emotional abuse, emotional or physical neglect, or parental loss, in the pathogenesis of depression [see Ref. (2–5)]. During periods of heightened neural plasticity throughout development, brain regions involved in the regulation of emotion and the mediation of the stress response appear to be particularly sensitive to the effects of ELS. Such experience-dependent plasticity may produce altered neural circuits, and hence maladaptive responsiveness to the environment, that ultimately leads to elevated risk for depression. However, not all individuals who experience ELS develop depression in adulthood, underscoring the importance of investigating the underpinnings of such variability in the link between ELS and depression. Understanding the source of such outcome variability could aid in identifying pre-disease vulnerability tests and in devising targeted prevention strategies.

A major body of evidence has rapidly accumulated over the past years concerning interactive effects of genetic factors and ELS in determining depression risk (3). These studies have mostly focused on studying the moderating effects of variations in candidate genes acting in neurobiological systems that have been implicated in the pathophysiology of major depression in large-scale epidemiological samples. Outcome measures usually comprise lifetime or current diagnoses of major depression or dimensional ratings of depressive symptoms, respectively. More recently, however, this focus has shifted to consider Gene × ELS interaction effects on phenotypic features, such as neural structure or function, neuroendocrine function, or behavioral constructs, that may mediate the link between stress and clinical manifestations of depression and that may be affected by genetic variation [see Ref. (6)]. This so-called intermediate phenotype approach is thought to provide more potent and valid associations between genetic factors and a given disease, such as depression, because genes are more proximate to the pathophysiological pathways that lead to depression than to the diagnostic categorization as defined by DSM-IV. In the following, we provide an overview of studies identifying interactions between candidate genes and ELS in the prediction of the clinical manifestation of depression as well as corresponding intermediate phenotypes.

GENE × ELS INTERACTIONS IN THE PREDICTION OF DEPRESSION

A large body of research has focused on identifying candidate genetic variations that interact with ELS in predicting current or

lifetime diagnoses, or symptom severity, of MDD. Core symptoms of depression include sad mood, loss of pleasure, sleep disruption, loss of appetite, decreased libido, impaired cognition, feelings of worthlessness and guilt, and suicidal ideation. Diagnosis of an episode of MDD requires the presence of five or more symptoms over a 2-week period, with sad mood or loss of pleasure being one of the symptoms. Candidate genes studied to date have considered single nucleotide polymorphisms (SNPs) in neurobiological systems relevant to stress and depression, i.e., the monoaminergic neurotransmitter, corticotropin-releasing hormone (CRH), and glucocorticoid receptor (GR) and GR-chaperone systems, as well as neurotrophin, oxytocin, and endocannabinoid systems.

SEROTONIN TRANSPORTER

The serotonergic system is the most prominent target of antidepressant pharmacotherapy [see Ref. (1)]. The main mechanism of action of serotonergic drugs is based on the inhibition of serotonin reuptake. Serotonergic neurons originate in the nucleus raphé and project to the forebrain. Serotonin is thought to be critical for regulating emotional responses in cortical–limbic networks and has regulatory effects on neuroendocrine stress responses. Stress mediators, such as CRH, modulate serotonin release in the nucleus raphé and regulate serotonin release in the medial prefrontal cortex (PFC) (7). Based on this evidence, the serotonergic system is of interest in the study of Gene × ELS interactions in predicting MDD. At the synaptic level, serotonergic neurotransmission and reuptake is regulated by the serotonin transporter gene (*SLC6A4*). A common functional polymorphism (5-HTTLPR) in the promoter region of *SLC6A4* involves either short (s) or long (l) alleles, with the short allele variant being associated with lower transcriptional efficiency. In a seminal report, Caspi et al. (8) demonstrated in the prospective Dunedin Birth Cohort of New Zealand that 5-HTTLPR interacts with stressful life events, including child maltreatment, to predict current and lifetime diagnoses of MDD as well as suicide attempts. Specifically, the s-allele was found to confer risk for developing depression in the context of life stress in this study. The report by Caspi et al. (8) served as an impetus for a wealth of subsequent studies, providing both replications and failures to replicate the original finding [see Ref. (9)]. A meta-analysis failed to confirm the interaction effect (10). A critical debate over this topic identified potential sources of this heterogeneity, mainly stemming from methodological differences between studies (11). The method of assessing ELS, as well as the type and timing of ELS, appear to be important effect modifiers (12). In addition, the definition of the outcome variable seems to be of particular importance. Specifically, the 5-HTTLPR × ELS interaction appears to predict persistent depression (i.e., an episode of MDD diagnosed at least twice), but is not predictive of a single-episode of MDD (13). Furthermore, it has now been reported that the interaction between 5-HTTLPR and ELS in predicting depression is moderated by other polymorphisms (see below).

In the light of a female preponderance in the risk for MDD, sex differences in Gene × ELS interactions have been studied. An interaction of 5-HTTLPR and ELS in predicting depression was found to be more pronounced in females than in males (14, 15). Carli et al. (16) reported that the l-allele was associated with increased risk for depression in male subjects exposed to ELS. It remains unclear whether these Gene × ELS × Sex interactions are attributable to sex differences at a neurobiological level or to sex differences in exposure variables. In addition to sex differences, age may moderate the 5-HTTLPR × ELS interaction effect in the prediction of depression, e.g., the l-allele carrying genotype exhibits higher vulnerability to develop depression in interaction with ELS in elderly adults (17).

DOPAMINE RECEPTOR (DAT1)

Dopamine is assumed to play an important role in the pathogenesis of MDD through its function in regulating motivation, reward, and the ability to experience pleasure (18). Stress contributes to loss of pleasure or anhedonia, a cardinal symptom of depression, by impacting on dopaminergic pathways that are implicated in reward processing, such as the mesocorticolimbic pathways (19, 20). In a study of male adolescents, a polymorphism, rs40184, in the dopamine receptor gene (*DAT1*) interacted with perceived maternal rejection in predicting the presence of MDD and suicidal ideation (21). This Gene × ELS interaction effect was specific to the prediction of MDD and did not predict anxiety, perhaps in accordance with the behavioral effects of dopaminergic pathway dysfunction. Of note, polymorphisms in 5-HTTLPR and other genes appear to interact with stress in predicting reward learning, which is an important intermediate phenotype in the link between genes, stress, and depression (see below).

CORTICOTROPIN-RELEASING HORMONE RECEPTOR AND BINDING GLOBULIN

The central CRH system plays a pivotal role in the relationship between ELS and depression. CRH neurons in the paraventricular nucleus of the hypothalamus form the central component of the HPA axis, the major neuroendocrine stress response system in mammals. Cortisol, the end product of the HPA axis exerts stress-adaptive metabolic, immune-regulatory and behavioral effects, and controls HPA axis responsiveness via negative feedback inhibition. In addition, CRH acts in a widespread circuitry connecting cortical, limbic, and brainstem regions and is thought to integrate endocrine, autonomic, and behavioral responses to stress. Behavioral effects of CRH resemble the core signs and symptoms of stress, depression, and anxiety. These effects are mediated though CRHR1 receptors (22). CRH hyperactivity is a core biological feature of MDD (23). There is ample evidence from animal and human studies that ELS contributes to such hyperactivity (24). Hence, the CRHR1 gene has been studied as a candidate gene in moderating the relationship between ELS and MDD. Bradley et al. (25) demonstrated an interaction between self-reported childhood abuse and polymorphisms in the CRHR1 gene in predicting adult depression. Two haplotypes were identified that conferred protection against developing depression in persons who reported moderate–severe child abuse exposure, as measured by the Childhood Trauma Questionnaire (CTQ) (26). When exposed to moderate–severe childhood abuse, persons who were homozygous for the protective haplotypes showed significantly less symptoms of depression as compared to individuals with other haplotype combinations. Within the protective haplotypes, rs110402 was most potent in predicting depression after ELS, with A allele carriers being protected. These

findings were replicated in two independent cohorts (25, 27). In the Polanczyk et al. (27) study, the interaction was replicated in the UK Environmental Risk Longitudinal Twin Study using retrospective self-reports of childhood maltreatment, assessed by CTQ similar to the Bradley et al. (25) study. In contrast, these authors could not replicate the interaction effect in the Dunedin Birth Cohort that provides prospective multi-informant reports of childhood maltreatment. The authors, therefore, speculate that the CRHR1 gene moderates emotional memory, which in turn may be linked to risk versus resilience against depression (27). Other potential reasons for discordant results might be differences in features of ELS. Heim et al. (22) reported that the interaction between rs110402 and ELS in predicting depression was specific to physical abuse. This produced a sex difference in the interaction of rs110402 and ELS, because males were more frequently exposed to physical abuse than females in this sample. Of note, Grabe et al. (28) also report interactions between different SNPs in CRHR1 and specifically physical neglect, as measured by CTQ. CRHR1 polymorphisms also interact with physical assault during childhood or adolescence in predicting suicide risk particularly in males (29), further stressing the importance of this gene in interacting with physically abusive experiences. These results suggest that the environmental exposure must be carefully assessed in Gene × ELS studies [see Ref. (3)]. In addition, two recent studies have demonstrated that CRHR1 SNPs interact with 5-HTTLPR in predicting depression after ELS (30, 31). Such three-way Gene × Gene × ELS interaction effects may contribute to inconsistent results between studies considering only one gene. Beyond CRHR1 polymorphisms, an interaction of a polymorphism in the CRH binding protein (CRHBP) gene, which may modulate the availability of CRH at the synapse, and childhood trauma in predicting suicide attempts has been recently reported (32). Taken together, these studies support a pivotal role of the central CRH system in mediating the link between ELS and depression, and the genetic moderation of this link.

GLUCOCORTICOID RECEPTOR AND FKBP5

The regulating effects of cortisol on brain and behavior are mediated by binding of the hormone to specific receptors. High-affinity mineralocorticoid receptors (MR) are located in the hippocampus and exert tonic inhibitory effects on basal HPA axis activity, whereas high-affinity GR are widely distributed throughout the PFC, amygdala, hippocampus, and brainstem, and are critical to regulating responses during conditions of elevated cortisol secretion or stress. Upon binding of cortisol to the intracellular cytosolic GR, the GR–hormone complex becomes activated and translocates to the nucleus, where the complex binds to gene responsive elements which impact on transcription of proteins. Relative GR resistance is a core feature of MDD, perhaps leading to disinhibition of central CRH secretion and HPA axis hyperactivity. The GR gene has been studied as a candidate gene that may moderate the link between ELS and MDD in one study. Bet et al. (33) demonstrated in a longitudinal aging study conducted in the Netherlands in individuals aged 55–85 years that the 22/23EK and 9beta polymorphisms in the GR gene interacted with life events and adversities during youth, including war experiences, sexual abuse, parental loss, or physical illness in the prediction of clinically relevant depression.

The sensitivity of the GR is fine-tuned by FK506 binding protein 51 (FKBP5), a co-chaperone of hsp90. When bound to the GR-hsp90 complex, FKBP5 decreases GR affinity for cortisol and prevents translocation of the GR to the nucleus [see Ref. (34)]. Of note, glucocorticoids induce the expression of FKBP5, forming an ultra-short negative feedback loop for GR activity (34). Given the important role of GR in regulating stress responses and the evidence for GR resistance in depression, variation of the FKBP5 gene likely is a critical modulator of the relationship between ELS and depression (34). Variants in the FKBP5 gene have been shown to modulate risk of developing PTSD in relation to childhood trauma (35). In a recent population-representative sample of more than 2000 German people, Appel et al. (36) report an interaction between rs1360780 of the FKBP5 gene and ELS, assessed by CTQ, in predicting both depressive symptoms and DSM-IV diagnoses of MDD. Specifically, TT carriers who reported physical abuse were at greater risk for depression than CC/CT carriers exposed to physical abuse. This interaction effect was confirmed for individuals who experienced very severe forms of sexual and emotional abuse (36). Another recent study in 884 adolescent and young adult individuals confirmed an interaction of different FKBP5 variants, including rs1360780, and traumatic life events in predicting the onset of MDD in a 10-year prospective study (37). These latter findings were replicated in the UK Environmental Risk Longitudinal Twin Study (37). FKBP5 polymorphisms were further reported to interact with CTQ score in predicting suicide attempts in an adult African American sample (38). The same group reported an additive effect of FKBP5 polymorphism on the interaction of the CRHBP gene and ELS in predicting suicide attempts (32). These results, taken together, strongly implicate the FKBP5 gene in the pathogenesis of stress-related depression, likely mediated through determining individual level of GR resistance and, consequently, glucocorticoid signaling.

BRAIN-DERIVED NEUROTROPHIC FACTOR

Brain-derived neurotrophic factor (BDNF) is a widely expressed neurotrophin in the brain that is implicated in neuronal growth, synaptic plasticity, and neuronal survival, and therefore is thought to play an important role in structural brain abnormalities observed in depressed individuals, such as reduced hippocampal volume (1, 39). In animal models, prolonged stress exposure and elevated glucocorticoid levels down-regulate BDNF expression, whereas administration of antidepressant drugs induces BDNF expression; antidepressant treatment normalizes hippocampal volume in depressed patients (1). The BDNF gene contains a functional polymorphism (rs6265), which is associated with a valine to methionine substitution (Val66Met) and leads to reduced BDNF expression. Aguilera et al. (40) report an interaction of this polymorphism and childhood sexual abuse in the prediction of adult depression. Specifically, carriers of the Met allele (Met/Met, Met/Val) who reported childhood sexual abuse were at greater risk for developing depressive symptoms compared to carriers of the Val/Val genotype. Several studies have provided evidence that the 5-HTTLPR and BDNF Val66Met polymorphisms interact in predicting depression after ELS. In one study, maltreated

children carrying the Met allele of BDNF Val66Met and two short alleles of 5-HTTLPR exhibit highest depression scores (41). This BDNF Val66Met × 5-HTTLPR × ELS interaction in the prediction of depression was replicated in a study of adult female twins (42). However, two other studies failed to replicate this three-way interaction in the prediction of depression (40, 43), perhaps suggesting further moderating factors.

OXYTOCIN RECEPTOR

The central oxytocin system mediates social attachment, including mother–infant bonding, and buffers emotional and physiological responses to stress (44). For example, intranasal administration of oxytocin reduces amygdala activation in response to fearful visual stimuli in humans (45). In patients with MDD, plasma oxytocin concentrations are inversely correlated with symptom severity of depression and anxiety (46). Women reporting exposure to childhood maltreatment exhibit deceases in cerebrospinal fluid oxytocin concentrations, which in turn were associated with increased anxiety (22). In a mouse model, oxytocin exerted anxiolytic effects by regulating serotonin release though activation of oxytocin receptors (OXTR) in serotonergic neurons (47). Therefore, the OXTR receptor gene is a candidate gene that may moderate the link between ELS and depression. One study reported an interaction of OXTR rs2254298 and adverse parental environment in predicting symptoms of depression in 9–14 years old girls (48). Girls who were heterozygous (A/G) for the *OXTR* rs2254298 polymorphism and reported high levels of adversity exhibited the most severe depression as well as physical symptoms and social anxiety. Of note, the level of parental adversity in this study was operationalized as a history of recurrent MDD in the mother, hence measured associations between adversity and depression in the girls may also reflect genetic risk. Other studies report associations of another OXTR polymorphism, rs53576, on several intermediate phenotypes that are relevant in the link between ELS and depression, which are reviewed below.

ENDOCANNABINOID RECEPTOR

The endocannabinoid system, consisting of endocannabinoid receptors and their endogenous ligands appear to play a role in the adaptation to stress and emotional responses (49). Endocannabinoid receptor 1 receptor knockout in mice modulates the effects of chronic unpredictable stress on the development of anhedonia in mice (50). In a recent study Agrawal et al. (51) evaluated the role of a polymorphism in the human endocannabinoid receptor (CNR1), rs1049353, in moderating the association between childhood physical abuse and anhedonia or MDD in young adult US women. Those women who carried two copies of the minor allele (G/G) exhibited less anhedonia when exposed to childhood physical abuse compared to carriers of the A allele (A/A or A/G). This protective effect was also seen for MDD, but was largely attributable to anhedonic MDD. The effect was replicated in an independent Australian sample (51). Of note, the interaction effect was specific to physical abuse and was not observed in relation to sexual abuse.

SUMMARY

In summary, a number of genes operating in neurobiological systems that mediate stress responses, social–emotional regulation, and/or synaptic plasticity have been identified to date as significant moderators of the relationship between ELS and depression. While several of these interaction effects have been replicated across independent studies, there is heterogeneity in the field. It becomes evident that multiple factors, both on the genetic and on the environmental side of the equation, must be considered in the prediction of depression. First, it is unlikely that a single gene is implicated in the relationship between ELS and depression and it appears that multiple polymorphisms in different genes interact with the environment in determining depression risk. Second, early environmental exposures such as ELS are complex in nature and there is increasing evidence for specificity of Gene × ELS interaction effects in depression, inasmuch as effects were reported for one type of maltreatment but not another. In addition, the method of assessment of ELS (i.e., retrospective self-report versus prospective informant reports) influences results. Additional sources of variability concern gender, ethnic background, and resources such as social support, among others. Future studies should scrutinize these complex interactions. In addition, genome-wide association studies (GWAS) of Gene × ELS interactions in depression have the potential to confirm candidate gene effects and/or to reveal novel genes that might be critical in the link between ELS and depression that were not selected as candidates based on theoretical reasoning [for detailed discussion, see Ref. (3)]. Finally, the functional significance of many of the identified polymorphisms remains unknown and, hence, the biological mechanisms that mediate genetically determined risk or resilience to depression, in the context of ELS, are poorly understood. Toward that end, there is an increasing body of studies adopting the so-called intermediate phenotype approach to further our understanding of the association between ELS and depression, as discussed in the following.

GENE × ELS INTERACTIONS IN DEPRESSION: CONCEPT OF INTERMEDIATE PHENOTYPES

Genetic disposition and environmental exposures are linked to the manifestation of a psychiatric syndrome through a number of intermediate steps that span from altered cells to altered neural circuits to altered behavioral domains, such as cognition or emotion processing. The intermediate phenotype approach in psychiatry seeks to identify genetic effects on each level of these intermediate phenotypes, which are more proximal to the etiological process than the diagnosis itself that is derived from disputable classification criteria. By mapping neurobiological effects of a risk gene on known brain phenotypes of a disorder, this approach also allows for deciding whether a risk gene indeed is relevant for a pathophysiological mechanism involved in the given disorder or whether a found association between a gene and a disorder is a pure epiphenomenon of another causal association. Hence, the intermediate phenotype approach increases power and validity of detecting a genetic effect on a psychiatric disorder (6, 52). While a number of intermediate phenotype studies have been conducted to test for genetic main effects on phenotypes linked to a given disorder, only a few studies have applied this approach to elucidate Gene × ELS interactions in depression. In order to study Gene × ELS effects on intermediate phenotypes that might be located on the pathway to the manifestation of depression, it must be decided which

phenotypes are indeed relevant for mediating the link between ELS and depression risk. In other words, some of the known biological features of depression might be linked to ELS, whereas others are not. Therefore, the selection of the appropriate intermediate phenotype for Gene × ELS studies needs to be informed by research on the contribution of ELS to the neurobiology of MDD.

INTERMEDIATE PHENOTYPES LINKING ELS AND MDD

A number of clinical studies have now elucidated effects of ELS on the neurobiology of MDD in humans [see Ref. (3, 53)]. Adult women with histories of childhood sexual or physical abuse exhibit markedly increased neuroendocrine and autonomic responses to psychosocial laboratory stress, particularly those with depression (54). Other alterations in humans with ELS experiences altered glucocorticoid feedback, increased levels of inflammation, increased central CRH activity, and decreased activity of the prosocial neuropeptide, oxytocin (22, 55–59). A small hippocampus has also been linked to ELS in humans (60–65). ELS also has been linked to reduced volumes of the insula (61), orbitofrontal cortex (61, 66), anterior cingulate (61, 67), caudate (61), and medial PFC (62, 68). Moreover, increased amygdala volume and reactivity (61, 69–71) as well as left basal ganglia dysfunction in response to reward cues (72) have been reported as a function of ELS. Furthermore, ELS is associated with altered cortical affective processing in patients with various psychiatric disorders (73). It appears that ELS produces changes in a connected neural network that fails to adapt or compensate in response to additional challenge, thereby leading to the behavioral and physiological changes that ultimately form the syndrome of clinical depression (59). More recently, epigenetic marks that occur as a function of ELS have been reported, such as hyper-methylation of the GR gene, resulting in reduced GR expression (74, 75). In a genome-wide post-mortem study of hippocampal tissue obtained from suicides, ELS was associated with differential methylation of 362 genes, particularly affecting genes involved in neuronal plasticity (76). In several of the above studies, neurobiological changes clearly distinguished between depressed persons or suicide victims with and without ELS experiences, including HPA axis reactivity (54, 59), hippocampal volume (65), PFC volume (62), amygdala reactivity (69), and methylation pattern (74, 76), suggesting that these phenotypes mediate the link between ELS and MDD and, therefore, might be appropriate targets for studying Gene × ELS interactions in depression. ELS further modulates behavioral domains, including social–emotional processing (71, 77), conditioned fear (78), or reward anticipation (72), all of which represent relevant intermediate phenotypes for Gene × ELS studies in depression.

GENE × ELS INTERACTION EFFECTS ON INTERMEDIATE PHENOTYPES RELATED TO DEPRESSION

Only a small, but rapidly increasing number of studies to date have assessed the combined impact of genetic variation and ELS exposure on intermediate phenotypes of interest for the development of depression. In addition to studies in humans, several studies in non-human primates provide additional insight into Gene × ELS interactions in predicting intermediate phenotypes relevant to depression.

GENE × ELS INTERACTION EFFECTS ON THE HPA AXIS

Due to the central role of the HPA axis activity in the pathogenesis of depression, it is of interest to investigate Gene × ELS interaction effects on HPA axis activity as intermediate phenotype of depression. The 5-HTTLPR polymorphism of the serotonin transporter gene (SLC6A4) has been of prime interest in this context with the s-allele being associated with an elevated risk for depression following critical life stress compared to the l-allele (8). Several studies indicate that there is an interaction between 5-HTTLPR genotype and ELS on HPA axis activity.

In a laboratory setting with a standardized stress task (stress induced by a subtraction task and semi-structured interview) girls aged between 9 and 14 years showed higher and prolonged levels of cortisol if they were s-allele carriers compared to l-allele carriers (79). These findings support the idea that HPA axis activity is differentially affected by an interaction between 5-HTTLPR genotype and stress. Adding to these findings, s-allele carriers with a history of stressful life events showed increased cortisol reactivity to laboratory stress (public speaking) in a sample of healthy male adults (80). Genotype and life stressor history, therefore, both seem to modulate endocrine reactivity to acute stress. Young adults who had taken part in the Trier Social Stress Test (TSST) showed distinct cortisol reactivity depending on the evaluative context of the condition (critical, supportive, or none) (81). Only in the negative audience condition an interaction with the 5-HTTLPR genotype emerged indicating that the s-allele carriers are particularly sensitive to social threat and therefore show heightened endocrine stress responses.

An age-dependent pattern of results was found in a recent study (82). HPA axis activity was investigated by measuring cortisol responses and stressful life events in children, young and old adults who were subjected to the TSST. An interaction between 5-HTTLPR genotype and life stress on endocrine stress responses was found only in young adults and only if the life stressors had occurred in the first 5 years of life suggesting that the first years of life are particularly sensitive. An interactive effect between 5-HTTLPR genotype and stress was also found in cortisol responses of newborn babies exposed to painful stimulation (83). Heightened sensitivity of s-allele carriers toward stress exposure therefore seems to be present at an early developmental stage.

There is evidence for a Gene × Gene × ELS effect; an interaction between 5-HTTLPR and acute stress (TSST) on cortisol response emerged in a non-clinical sample only when at least one copy of the D4 dopamine receptor gene (DRD4) 7R allele was present (84). Another study found that BDNF Val66Met polymorphisms moderate the interactive effects between 5-HTTLPR and stress on HPA axis reactivity in a sample of preschool children (85). Therefore, it seems necessary to include several genetic polymorphisms within crucial pathways of stress regulation and investigate their interactive effects in more detail.

In a seminal study, Tyrka et al. (86) evaluated whether the functional polymorphisms rs110402 and rs242924 in the CRHR1 gene, which previously had been identified as an important moderator of depression risk after ELS [see above; (25)], interacts with child maltreatment to predict HPA axis reactivity. For each polymorphism, subjects who carried the GG genotype and who reported a history of child maltreatment exhibited elevated cortisol responses

in the dexamethasone/CRH test. In accordance with the clinical outcome study (25), A allele carriers (AA, AG) with child maltreatment experience were protected and showed responses similar to persons with no child maltreatment experience. In persons with no child maltreatment experience, CRHR1 polymorphisms did not modify HPA axis reactivity. These results suggest an interaction effect, in which the CRHR1 gene and childhood maltreatment impact on glucocorticoid-mediated inhibition of the HPA axis under challenge conditions, as tested by the specific test. Increased responsiveness in the dexamethasone/CRH test has long been known to be a potent risk marker for depression (87). Hence, this study provides important insight into the potential mechanism for a Gene × ELS interaction in depression.

A study published in the same year by Heim et al. (22) replicated and extended this finding. In this study, it was shown that the effect of CRHR1 rs110402 in moderating depression risk in relation to ELS exposure was limited to men, but could not be observed in women; this apparent sex difference was attributable to differential exposure types between the sexes, with men experiencing more physical abuse than women (see above). Of note, in response to the same challenge test used in the study reported in Ref. (86), there was a graded effect of the protective A allele on HPA axis reactivity (GG > AG > AA) that was observed only in male participants, whereas the polymorphism had no effect on cortisol response in women. Accordingly, women with maltreatment showed enhanced cortisol responses to the dexamethasone/CRH test, whereas maltreated men did not. These results demonstrate that the complex interplay of abuse type, sex, and genetic variation is reflected at the level of an intermediate phenotype that is a relevant risk marker for depression. Cicchetti et al. (30) recently extended to this line of evidence showing that the CRHR1 TAT haplotype interacts with 5-HTTLPR to predict diurnal cortisol levels in maltreated children.

The GR is critically implicated in regulating HPA axis reactivity and, as noted above, a polymorphism in the GR gene moderates the link between ELS and depression (33). In the same study, it was shown that the 22/23EK polymorphism in the GR gene interacts with ELS in predicting a free cortisol index, calculated as ratio between total cortisol and cortisol binding globulin levels. In the light of evidence that the FKBP5 gene moderates risk for affective disorders in relation to ELS [see above; (35–37)], several studies investigated interactions of FKBP5 gene variants and stress in determining indices of HPA axis function. Ising et al. (88) report that three FKBP5 polymorphisms are associated with prolonged cortisol elevations after standardized psychosocial stress (rs4713916: GG > GT,TT; rs1360780: TT > CT,CC; rs3800737: AA > AG,GG), although ELS was not assessed in this study. Effects of FKBP5 polymorphisms on dexamethasone-induced suppression of cortisol have been reported in interaction with child abuse-related PTSD, inasmuch as risk variants in various FKBP5 polymorphisms interacted with PTSD status in predicting hypersuppression of cortisol (75, 89). These findings show that risk genes may interact with a clinical diagnosis that is in turn related to ELS; however, similar results for depression are lacking. In a recent seminal study (90), the same group provides strong evidence for an FKBP5 × ELS interaction in determining de-methylation of functional glucocorticoid response elements (GREs) in the FKBP5 gene. This de-methylation was associated with an increased transcription of the gene, leading to enhanced feedback between FKBP5 and GR and, consequently to GR resistance. Effects of this risk allele-specific and ELS-dependent de-methylation were shown at the level of *in vitro* glucocorticoid sensitivity, glucocorticoid-regulated metabolic and immune gene expression, as well as brain structure (90). This remarkable study is the first to map in detail a molecular to neural pathway that underlies a Gene × ELS interaction effect in the prediction of depression risk.

Other studies have investigated interactions of ELS and candidate genes implicated in stress or regulation, although these candidate genes have not been considered in Gene × ELS studies predicting clinical depression outcomes. Neuropeptide Y (NPY) is a peptide that is known to exert anxiolytic and stress-buffering effects in the central nervous system. A polymorphism in the NPY gene promoter, rs16147, was found to interact with ELS in predicting neuroendocrine response to psychosocial laboratory stress in a prospective cohort study of children at risk conducted in Germany (91). A study by Armbruster et al. (92) focused on the enzyme, catechol-*O*-methyltransferase (COMT), which catabolizes catecholamines. Results revealed main effects for COMT and stressful life events, but no interaction effect between the two factors, suggesting that the COMT gene operates independently from stress exposure and is likely not involved in mediating the link between ELS and depression.

GENE × ELS INTERACTION EFFECTS ON REGIONAL BRAIN STRUCTURE AND FUNCTION

The study of the impact of specific serotonin genotypes on structural and functional characteristics of the brain related to depression has become of great interest in recent years. In this context, the prefrontal–amygdala interaction seems to be of particular importance in affective processing and was found to be differentially affected by 5-HTTLPR genotype in depressive patients and healthy controls (93). These findings were further specified in a study on healthy adults (94). In this study, carriers of the risk s-allele displayed increased amygdala activation. Further statistical analysis revealed that this genotype effect was based on the amygdala structure, which proved to be smaller in the s-allele carriers compared to the LL genotype. These findings indicate that genetic effects during neurodevelopment play a role in this context.

At a structural level, serotonin 3A receptor genotype and its interaction with ELS were investigated with regard to frontolimbic gray matter in a non-clinical sample (95). Interestingly, the HTR3A CC genotype group displayed gray matter loss in hippocampal structures compared to the TT genotype group, with CC carriers plus ELS showing additional gray matter loss in frontal cortices. In line with this, another study found that carriers of 5-HTTLPR risk s-allele displayed smaller hippocampal volumes following a history of emotional neglect in depressive patients compared to patients without ELS or genetic risk genotype (62). Independent of genotype, hippocampal white matter alterations were predicted by childhood stress in patients. Compared to healthy controls, the PFC was smaller in patients. Of note, patients with ELS and non-risk l-allele genotype displayed a larger PFC, indicating compensatory or protective characteristics. A recent study in children further highlights the potential mechanisms of

Gene × ELS interactions (96). Study participants who were carriers of the 5-HTTLPR s-allele showed greater attentional bias to subliminally presented fearful faces than carriers of the non-risk l-allele. The former also showed increased neural activations to fearful and angry faces in regions of the association cortex linked to attention-control in adults. The s-allele genotype, therefore, seems to be related to hypervigilant behavioral and neural activation in face of negative stimuli. In the same line, the effects of acute stress were investigated in a group of healthy women (97). Women with the SS genotype displayed increased neural activation during threat anticipation in a large corticolimbic network compared with l-allele carriers, potentially reflecting increased sensitivity to develop psychopathological symptoms when faced with stressful events. In healthy men, similar Gene × ELS interactions were found regarding neural response patterns and functional amygdala–hypothalamus connectivity (98). S-allele carriers with a history of ELS showed heightened sensitivity toward negative, fearful stimuli in this context. These findings were further supported by a study, which focused on fear conditioning in a similar experimental setting (99). Another recent study found an interaction between recent stressful life events and SNPs within the HTR1A and HTR1B genes regarding symptom scores of depression (100). More specifically, the interactive effect was observed with regard to HTR1A rs878567 T alleles and rs6295 G alleles as well as HTR1B rs6296 C and recent stressful life events. An experimental face emotion processing task was presented to a subgroup of the population sample to further investigate threat-related information processing. Notably, healthy HTR1A rs6295 GG carriers did not display an affective bias to perceive more negative emotions but showed reduced reaction times when presented with fearful faces. This modulation of threat-related information processing by genotype may predispose certain individuals to develop depression throughout the life course.

As mentioned earlier, the CRH system plays an important role in neuroendocrine and behavioral responses to stress and is regarded as a central component within the pathogenesis of depression. Especially polymorphisms of the CRHR1 gene seem to play an important role in moderating the effects of stress. A recent study found that variations in the CRHR1 gene, more specifically SNP rs110402, moderate neural responses to emotional stimuli (101). A differential pattern of results emerged between A allele carriers and GG homozygotes suggesting that vulnerability to depression is associated with a specific CRHR1 genotype.

Reward learning was in the focus of a study regarding the effects of the CRHR1 SNP rs12938031 and acute stress (102). Reward learning is regarded as a crucial component of anhedonia, which is closely related to stress-related psychopathology. The authors investigated response bias as a function of reward in healthy female participants under no-stress and acute stress conditions. The authors found that acute stress led to reduced response bias, which indicates impaired reward learning under stressful conditions. Notably, an interaction between rs12938031 and acute stress was also observed, with A homozygotes displaying stress-related impairment in reward learning. Behavioral findings were also supported by neural activation patterns. The modulation of reward learning by genotype may lead to increased vulnerability to depression in some individuals.

Another recent study investigating this issue used the personality trait neuroticism as proxy for depression given that it reflects a tendency to experience negative affect and that it is a major risk factor for developing depression (103). As childhood maltreatment is also regarded as major risk factor for depression, the authors were interested in investigating the interaction of childhood maltreatment with a specific genotype regarding the personality trait of neuroticism. Based on previous research findings, variation in the three CRHR1 SNPs rs110402, rs2452924, and rs7209436 was investigated in maltreated as well as in non-maltreated children who participated in a day camp research program. Number and types of stressors were also taken into account in the analysis. As expected by the authors, a significant interactive effect between childhood maltreatment and CRHR1 genotype was found in the prediction of neuroticism. Maltreated children with two copies of the TAT haplotype of CRHR1 showed higher levels of neuroticism than non-maltreated children with the same genotype. Notably, this effect was not displayed in children with multiple types of maltreatment or sexual abuse, who seemed to be protected from increased neuroticism by the same genotype. This remarkable finding needs to be explored in further detail and highlights the importance of distinguishing between different types and numbers of stressors.

The BDNF gene polymorphism Val66Met has been found to be related to anxiety and mood disorders (104). More specifically, the interaction between BDNF Val66Met polymorphism and ELS was investigated with regard to brain and arousal pathways to syndromal depression and anxiety in a non-clinical sample. Significant interactions between BDNF genotype and ELS emerged with BDNF Met-carriers exposed to greater ELS showing smaller hippocampal and amygdala volumes, heart rate elevations, and a decline in working memory. The relationship between this polymorphism and specific, emotion-related brain circuitries has also been studied in anxious and depressed adolescents compared to healthy controls (105). In the patient group, a significant interaction between genotype and emotional stimulus presentation was found. Met-carriers showed greater neural responses than Val/Val homozygotes when presented with emotional faces.

The MR iso/val polymorphism (rs5522) has recently been found to moderate the association between childhood emotional neglect and amygdala reactivity in children and adolescents (106). The interactive effects of childhood maltreatment and the OXTR SNP rs53576 on adult emotional dysregulation and attachment style were investigated in a sample of low-income, African American men and women (107). Significant interactive effects were found between childhood maltreatment and OXTR rs53576 in predicting levels of adult emotional dysregulation and attachment style. While G/G allele carriers appeared to be particularly vulnerable to severe childhood maltreatment, A/A and A/G allele carriers displayed resilience. Distinguishing between numbers and types of stressors was crucial in this study. Other findings indicate that NPY genetic variation may predispose to low NPY expression, thereby increasing neural responsivity to negative stimuli in emotion-related brain circuitries (108).

Gene × Gene × ELS effects also need to be considered in this context as recent studies have found interactive effects for 5-HTTLPR and BDNF, CREB1, BDNF and NTRK2, as well as

BDNF and HTR3A genotypes (109–111). CREB1 (cyclic adenosine monophosphate response element-binding protein 1), BDNF and NTRK2 (neurotrophic tyrosine kinase receptor, type 2) form a neuroplastic pathway, which plays a crucial role in brain adaptation to stress (110).

CONCLUSION

One of the most promising pathways of future research is the investigation of epigenetic processes modulating the relationship between Gene × ELS interactions and depression. In this context, quality of maternal care was found to influence hypothalamic–pituitary–adrenal function in rats through epigenetic programing of GR expression (112). Low levels of maternal care were associated with increased methylation of the promoter region of the GR gene as well as enhanced hormonal and behavioral responses to stress. These findings could be translated to the brains of human suicide victims who had experienced childhood abuse compared to those victims who had not (74) and were further reflected in a study on childhood adversity and epigenetic modulation of the leukocyte GR (75). Adding to these findings, differential maternal rearing conditions led to differential DNA methylation in PFC and T cells of rhesus macaques (113). These findings could be further translated and expanded by evidence suggesting that the trajectory of male physical aggression is associated with differential DNA methylation in cytokines and their regulators in T cells and monocytes (114). The seminal findings with regard to epigenetic processes and FKBP5 following childhood trauma have substantially added to this knowledge and will encourage future research endeavors (90).

It is important to note that past studies on Gene × ELS interactions and depression have been subject to a large amount of heterogeneity due to differences in experimental procedures. An important difference concerns the measurement of ELS, with critical issues concerning timing of measurement, type, and context of ELS studied as well as gender-specific considerations (115). In addition, timing of onset of depression and chronicity of the disorder may be an important factor in this context and should be considered in future research efforts.

Previous findings with regard to Gene × ELS interactions and depression suggest that genes and environment have significant interactive effects in predicting depression, in the absence of strong main genetic effects (3). While previous research has been hypotheses-driven focusing on candidate gene approaches, future studies will move beyond this confinement to genome-wide hypotheses-free studies, which require much larger study samples. By this means, new patterns of gene classes or transcription factor binding sites, which are altered by ELS, may be detected, thereby supporting the current strive for adequate prevention and treatment schemes in sufferers from childhood trauma.

Future research will further investigate the differential role of certain genotypes as a function of adverse and beneficial environments. Current models on childhood development outline the concept of a differential susceptibility to environment, i.e., some individuals are more susceptible than others to both negative (risk-promoting) and positive (development-enhancing) environmental conditions (116). Recent findings on Gene × ELS interactions seem to confirm the concept of differential susceptibility (107, 117), which questions the classic diathesis–stress framework as conceptual basis of past research. In this context, the identification of developmentally sensitive periods of brain maturation will be of particular importance when it comes to prevention and early intervention (118).

The existence of biologically distinguishable subtypes of depression as a function of ELS is of importance in the response to differential treatment (59). When comparing the effectiveness of pharmacotherapy and psychotherapy in the treatment of depressed patients with and without childhood trauma distinct patterns of treatment success were observed (119). In absence of early trauma, combination treatment proved to be the most effective treatment option, whereas in case of early trauma, remission rates were higher for psychotherapy than for pharmacological treatment and combination therapy did not lead to any advantage over the effects of psychotherapy alone. These differential effects may be based on differences in neurobiological pathways to depression, which need to be further elucidated (120).

Taken together, the latest research findings have underscored the essential role of genotypes and epigenetic processes within the development of depression after childhood trauma, thereby building the basis for future research and clinical interventions.

ACKNOWLEDGMENTS

Principal source of funding: Charité.

REFERENCES

1. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron* (2002) **34**(1):13–25. doi:10.1016/S0896-6273(02)00653-0
2. Davidson RJ, McEwen BS. Social influences on neuroplasticity: stress and interventions to promote well-being. *Nat Neurosci* (2012) **15**(5):689–95. doi:10.1038/nn.3093
3. Heim C, Binder EB. Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Exp Neurol* (2011) **233**(1):102–11. doi:10.1016/j.expneurol.2011.10.032
4. Meyer-Lindenberg A, Tost H. Neural mechanisms of social risk for psychiatric disorders. *Nat Neurosci* (2012) **15**(5):663–8. doi:10.1038/nn.3083
5. Pechtel P, Pizzagalli DA. Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology (Berl)* (2011) **214**(1):55–70. doi:10.1007/s00213-010-2009-2
6. Meyer-Lindenberg A. Intermediate or brainless phenotypes for psychiatric research? *Psychol Med* (2010) **40**(7):1057–62. doi:10.1017/S0033291709991929
7. Forster GL, Pringle RB, Mouw NJ, Vuong SM, Watt MJ, Burke AR, et al. Corticotropin-releasing factor in the dorsal raphe nucleus increases medial prefrontal cortical serotonin via type 2 receptors and median raphe nucleus activity. *Eur J Neurosci* (2008) **28**(2):299–310. doi:10.1111/j.1460-9568.2008.06333.x
8. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* (2003) **301**(5631):386–9. doi:10.1126/science.1083968
9. Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *Am J Psychiatry* (2010) **167**(5):509–27. doi:10.1176/appi.ajp.2010.09101452
10. Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, et al. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: a meta-analysis. *JAMA* (2009) **301**(23):2462–71. doi:10.1001/jama.2009.878
11. Wankerl M, Wüst S, Otte C. Current developments and controversies: does the serotonin transporter gene-linked polymorphic region (5-HTTLPR) modulate the association between stress and depression? *Curr Opin Psychiatry* (2010) **23**(6):582–7. doi:10.1097/YCO.0b013e32833f0e3a

12. Karg K, Burmeister M, Shedden K, Sen S. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. *Arch Gen Psychiatry* (2011) **68**(5):444–54. doi:10.1001/archgenpsychiatry.2010.189
13. Uher R, Caspi A, Houts R, Sugden K, Williams B, Poulton R, et al. Serotonin transporter gene moderates childhood maltreatment's effects on persistent but not single-episode depression: replications and implications for resolving inconsistent results. *J Affect Disord* (2011) **135**(1–3):56–65. doi:10.1016/j.jad.2011.03.010
14. Aslund C, Leppert J, Comasco E, Nordquist N, Oreland L, Nilsson KW. Impact of the interaction between the 5HTTLPR polymorphism and maltreatment on adolescent depression. A population-based study. *Behav Genet* (2009) **39**(5):524–31. doi:10.1007/s10519-009-9285-9
15. Hammen C, Brennan PA, Keenan-Miller D, Hazel NA, Najman JM. Chronic and acute stress, gender, and serotonin transporter gene-environment interactions predicting depression symptoms in youth. *J Child Psychol Psychiatry* (2010) **51**(2):180–7. doi:10.1111/j.1469-7610.2009.02177.x
16. Carli V, Mandelli L, Zaninotto L, Roy A, Recchia L, Stoppia L, et al. A protective genetic variant for adverse environments? The role of childhood traumas and serotonin transporter gene on resilience and depressive severity in a high-risk population. *Eur Psychiatry* (2011) **26**(8):471–8. doi:10.1016/j.eurpsy.2011.04.008
17. Ritchie K, Jaussent I, Stewart R, Dupuy AM, Courtet P, Ancelin ML, et al. Association of adverse childhood environment and 5-HTTLPR Genotype with late-life depression. *J Clin Psychiatry* (2009) **70**(9):1281–8. doi:10.4088/JCP.08m04510
18. Dunlop BW, Nemeroff CB. The role of dopamine in the pathophysiology of depression. *Arch Gen Psychiatry* (2007) **64**(3):327–37. doi:10.1001/archpsyc.64.3.327
19. Bogdan R, Pizzagalli DA. Acute stress reduces reward responsiveness: implications for depression. *Biol Psychiatry* (2006) **60**(10):1147–54. doi:10.1016/j.biopsych.2006.03.037
20. Dillon DG, Deveney CM, Pizzagalli DA. From basic processes to real-world problems: how research on emotion and emotion regulation can inform understanding of psychopathology, and vice versa. *Emot Rev* (2011) **3**(1):74–82. doi:10.1177/1754073910380973
21. Haeffel GJ, Getchell M, Koposov RA, Yrigollen CM, Deyoung CG, Klinteberg BA, et al. Association between polymorphisms in the dopamine transporter gene and depression: evidence for a gene-environment interaction in a sample of juvenile detainees. *Psychol Sci* (2008) **19**(1):62–9. doi:10.1111/j.1467-9280.2008.02047.x
22. Heim C, Bradley B, Mletzko TC, Deveau TC, Musselman DL, Nemeroff CB, et al. Effect of childhood trauma on adult depression and neuroendocrine function: sex-specific moderation by CRH receptor 1 gene. *Front Behav Neurosci* (2009) **3**:41. doi:10.3389/neuro.08.041.2009
23. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol* (1999) **160**(1):1–12. doi:10.1677/joe.0.1600001
24. Heim C, Plotsky PM, Nemeroff CB. Importance of studying the contributions of early adverse experience to neurobiological findings in depression. *Neuropsychopharmacology* (2004) **29**(4):641–8. doi:10.1038/sj.npp.1300397
25. Bradley RG, Binder EB, Epstein MP, Tang Y, Nair HP, Liu W, et al. Influence of child abuse on adult depression: moderation by the corticotropin-releasing hormone receptor gene. *Arch Gen Psychiatry* (2008) **65**(2):190–200. doi:10.1001/archgenpsychiatry.2007.26
26. Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, et al. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse Negl* (2003) **27**(2):169–90. doi:10.1016/S0145-2134(02)00541-0
27. Polanczyk G, Caspi A, Williams B, Price TS, Danese A, Sugden K, et al. Protective effect of CRHR1 gene variants on the development of adult depression following childhood maltreatment: replication and extension. *Arch Gen Psychiatry* (2009) **66**(9):978–85. doi:10.1001/archgenpsychiatry.2009.114
28. Grabe HJ, Schwahn C, Appel K, Mahler J, Schulz A, Spitzer C, et al. Childhood maltreatment, the corticotropin-releasing hormone receptor gene and adult depression in the general population. *Am J Med Genet B Neuropsychiatr Genet* (2010) **153B**(8):1483–93. doi:10.1002/ajmg.b.31131
29. Ben-Efraim YJ, Wasserman D, Wasserman J, Sokolowski M. Gene-environment interactions between CRHR1 variants and physical assault in suicide attempts. *Genes Brain Behav* (2011) **10**(6):663–72. doi:10.1111/j.1601-183X.2011.00703.x
30. Cicchetti D, Rogosch FA, Oshri A. Interactive effects of corticotropin releasing hormone receptor 1, serotonin transporter linked polymorphic region, and child maltreatment on diurnal cortisol regulation and internalizing symptomatology. *Dev Psychopathol* (2011) **23**(4):1125–38. doi:10.1017/S0954579411000599
31. Ressler KJ, Bradley B, Mercer KB, Deveau TC, Smith AK, Gillespie CF, et al. Polymorphisms in CRHR1 and the serotonin transporter loci: gene x gene x environment interactions on depressive symptoms. *Am J Med Genet B Neuropsychiatr Genet* (2010) **153B**(3):812–24. doi:10.1002/ajmg.b.31052
32. Roy A, Hodgkinson CA, Deluca V, Goldman D, Enoch MA. Two HPA axis genes, CRHBP and FKBP5, interact with childhood trauma to increase the risk for suicidal behavior. *J Psychiatr Res* (2012) **46**(1):72–9. doi:10.1016/j.jpsychires.2011.09.009
33. Bet PM, Penninx BW, Bochdanovits Z, Uitterlinden AG, Beekman AT, van Schoor NM, et al. Glucocorticoid receptor gene polymorphisms and childhood adversity are associated with depression: new evidence for a gene-environment interaction. *Am J Med Genet B Neuropsychiatr Genet* (2009) **150B**(5):660–9. doi:10.1002/ajmg.b.30886
34. Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology* (2009) **34**(Suppl 1):S186–95. doi:10.1016/j.psyneuen.2009.05.021
35. Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, et al. Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA* (2008) **299**(11):1291–305. doi:10.1001/jama.299.11.1291
36. Appel K, Schwahn C, Mahler J, Schulz A, Spitzer C, Fenske K, et al. Moderation of adult depression by a polymorphism in the FKBP5 gene and childhood physical abuse in the general population. *Neuropsychopharmacology* (2011) **36**(10):1982–91. doi:10.1038/npp.2011.81
37. Zimmermann P, Brückl T, Nocon A, Pfister H, Binder EB, Uhr M, et al. Interaction of FKBP5 gene variants and adverse life events in predicting depression onset: results from a 10-year prospective community study. *Am J Psychiatry* (2011) **168**(10):1107–16. doi:10.1176/appi.ajp.2011.10111577
38. Roy A, Gorodetsky E, Yuan Q, Goldman D, Enoch MA. Interaction of FKBP5, a stress-related gene, with childhood trauma increases the risk for attempting suicide. *Neuropsychopharmacology* (2010) **35**(8):1674–83. doi:10.1038/npp.2009.236
39. Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. *Nat Neurosci* (2007) **10**(9):1089–93. doi:10.1038/nn1971
40. Aguilera M, Arias B, Wichers M, Barrantes-Vidal N, Moya J, Villa H, et al. Early adversity and 5-HTT/BDNF genes: new evidence of gene-environment interactions on depressive symptoms in a general population. *Psychol Med* (2009) **39**(9):1425–32. doi:10.1017/S0033291709005248
41. Kaufman J, Yang BZ, Douglas-Palumberi H, Grasso D, Lipschitz D, Houshyar S, et al. Brain-derived neurotrophic factor-5-HTTLPR gene interactions and environmental modifiers of depression in children. *Biol Psychiatry* (2006) **59**(8):673–80. doi:10.1016/j.biopsych.2005.10.026
42. Wichers M, Kenis G, Jacobs N, Mengelers R, Derom C, Vlietinck R, et al. The BDNF Val(66)Met x 5-HTTLPR x child adversity interaction and depressive symptoms: an attempt at replication. *Am J Med Genet B Neuropsychiatr Genet* (2008) **147B**(1):120–3. doi:10.1002/ajmg.b.30576
43. Nederhof E, Bouma EM, Oldehinkel AJ, Ormel J. Interaction between childhood adversity, brain-derived neurotrophic factor val/met and serotonin transporter promoter polymorphism on depression: the TRAILS study. *Biol Psychiatry* (2010) **68**(2):209–12. doi:10.1016/j.biopsych.2010.04.006
44. Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat Rev Neurosci* (2011) **12**(9):524–38. doi:10.1038/nrn3044
45. Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, et al. Oxytocin modulates neural circuitry for social cognition and fear in humans. *J Neurosci* (2005) **25**(49):11489–93. doi:10.1523/JNEUROSCI.3984-05.2005
46. Scantamburlo G, Hansenne M, Fuchs S, Pitchot W, Maréchal P, Pequeux C, et al. Plasma oxytocin levels and anxiety in patients with major

depression. *Psychoneuroendocrinology* (2007) **32**(4):407–10. doi:10.1016/j.psyneuen.2007.01.009
47. Yoshida M, Takayanagi Y, Inoue K, Kimura T, Young LJ, Onaka T, et al. Evidence that oxytocin exerts anxiolytic effects via oxytocin receptor expressed in serotonergic neurons in mice. *J Neurosci* (2009) **29**(7):2259–71. doi:10.1523/JNEUROSCI.5593-08.2009
48. Thompson RJ, Parker KJ, Hallmayer JF, Waugh CE, Gotlib IH. Oxytocin receptor gene polymorphism (rs2254298) interacts with familial risk for psychopathology to predict symptoms of depression and anxiety in adolescent girls. *Psychoneuroendocrinology* (2011) **36**(1):144–7. doi:10.1016/j.psyneuen.2010.07.003
49. Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TT, Gray JM, et al. Endogenous cannabinoid signaling is essential for stress adaptation. *Proc Natl Acad Sci U S A* (2010) **107**(20):9406–11. doi:10.1073/pnas.0914661107
50. Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology (Berl)* (2002) **159**(4):379–87. doi:10.1007/s00213-001-0946-5
51. Agrawal A, Nelson EC, Littlefield AK, Bucholz KK, Degenhardt L, Henders AK, et al. Cannabinoid receptor genotype moderation of the effects of childhood physical abuse on anhedonia and depression. *Arch Gen Psychiatry* (2012) **69**(7):732–40. doi:10.1001/archgenpsychiatry.2011.2273
52. Rasetti R, Weinberger DR. Intermediate phenotypes in psychiatric disorders. *Curr Opin Genet Dev* (2011) **21**(3):340–8. doi:10.1016/j.gde.2011.02.003
53. Heim C, Shugart M, Craighead WE, Nemeroff CB. Neurobiological and psychiatric consequences of child abuse and neglect. *Dev Psychobiol* (2010) **52**(7):671–90. doi:10.1002/dev.20494
54. Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, et al. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA* (2000) **284**(5):592–7. doi:10.1001/jama.284.5.592
55. Carpenter LL, Tyrka AR, McDougle CJ, Malison RT, Owens MJ, Nemeroff CB, et al. Cerebrospinal fluid corticotropin-releasing factor and perceived early-life stress in depressed patients and healthy control subjects. *Neuropsychopharmacology* (2004) **29**(4):777–84. doi:10.1038/sj.npp.1300375
56. Carpenter LL, Gawuga CE, Tyrka AR, Lee JK, Anderson GM, Price LH. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology* (2010) **35**(13):2617–23. doi:10.1038/npp.2010.159
57. Danese A, Caspi A, Williams B, Ambler A, Sugden K, Mika J, et al. Biological embedding of stress through inflammation processes in childhood. *Mol Psychiatry* (2011) **16**(3):244–6. doi:10.1038/mp.2010.5
58. Danese A, Pariante CM, Caspi A, Taylor A, Poulton R. Childhood maltreatment predicts adult inflammation in a life-course study. *Proc Natl Acad Sci U S A* (2007) **104**(4):1319–24. doi:10.1073/pnas.0610362104
59. Heim C, Newport DJ, Mletzko T, Miller AH, Nemeroff CB. The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology* (2008) **33**(6):693–710. doi:10.1016/j.psyneuen.2008.03.008
60. Buss C, Lord C, Wadiwalla M, Hellhammer DH, Lupien SJ, Meaney MJ, et al. Maternal care modulates the relationship between prenatal risk and hippocampal volume in women but not in men. *J Neurosci* (2007) **27**(10):2592–5. doi:10.1523/JNEUROSCI.3252-06.2007
61. Dannlowski U, Stuhrmann A, Beutelmann V, Zwanzger P, Lenzen T, Grotegerd D, et al. Limbic scars: long-term consequences of childhood maltreatment revealed by functional and structural magnetic resonance imaging. *Biol Psychiatry* (2012) **71**(4):286–93. doi:10.1016/j.biopsych.2011.10.021
62. Frodl T, Reinhold E, Koutsouleris N, Donohoe G, Bondy B, Reiser M, et al. Childhood stress, serotonin transporter gene and brain structures in major depression. *Neuropsychopharmacology* (2010) **35**(6):1383–90. doi:10.1038/npp.2010.8
63. Stein MB, Koverola C, Hanna C, Torchia MG, McClarty B. Hippocampal volume in women victimized by childhood sexual abuse. *Psychol Med* (1997) **27**(4):951–9. doi:10.1017/S0033291797005242
64. Teicher MH, Anderson CM, Polcari A. Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum. *Proc Natl Acad Sci U S A* (2012) **109**(9):E563–72. doi:10.1073/pnas.1115396109
65. Vythilingam M, Heim C, Newport J, Miller AH, Anderson E, Bronen R, et al. Childhood trauma associated with smaller hippocampal volume in women with major depression. *Am J Psychiatry* (2002) **159**(12):2072–80. doi:10.1176/appi.ajp.159.12.2072
66. Pollak DD, Rogan MT, Egner T, Perez DL, Yanagihara TK, Hirsch J. A translational bridge between mouse and human models of learned safety. *Ann Med* (2010) **42**(2):115–22. doi:10.3109/07853890903583666
67. Treadway MT, Grant MM, Ding Z, Hollon SD, Gore JC, Shelton RC. Early adverse events, HPA activity and rostral anterior cingulate volume in MDD. *PLoS One* (2009) **4**(3):e4887. doi:10.1371/journal.pone.0004887
68. van Harmelen AL, van Tol MJ, van der Wee NJ, Veltman DJ, Aleman A, Spinhoven P, et al. Reduced medial prefrontal cortex volume in adults reporting childhood emotional maltreatment. *Biol Psychiatry* (2010) **68**(9):832–8. doi:10.1016/j.biopsych.2010.06.011
69. Grant MM, Cannistraci C, Hollon SD, Gore J, Shelton R. Childhood trauma history differentiates amygdala response to sad faces within MDD. *J Psychiatr Res* (2011) **45**(7):886–95. doi:10.1016/j.jpsychires.2010.12.004
70. Tottenham N, Hare TA, Quinn BT, McCarry TW, Nurse M, Gilhooly T, et al. Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Dev Sci* (2010) **13**(1):46–61. doi:10.1111/j.1467-7687.2009.00852.x
71. Tottenham N, Hare TA, Millner A, Gilhooly T, Zevin JD, Casey BJ. Elevated amygdala response to faces following early deprivation. *Dev Sci* (2011) **14**(2):190–204. doi:10.1111/j.1467-7687.2010.00971.x
72. Dillon DG, Holmes AJ, Birk JL, Brooks N, Lyons-Ruth K, Pizzagalli DA. Childhood adversity is associated with left basal ganglia dysfunction during reward anticipation in adulthood. *Biol Psychiatry* (2009) **66**(3):206–13. doi:10.1016/j.biopsych.2009.02.019
73. Weber K, Miller GA, Schupp HT, Borgelt J, Awiszus B, Popov T, et al. Early life stress and psychiatric disorder modulate cortical responses to affective stimuli. *Psychophysiology* (2009) **46**(6):1234–43. doi:10.1111/j.1469-8986.2009.00871.x
74. McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* (2009) **12**(3):342–8. doi:10.1038/nn.2270
75. Tyrka AR, Price LH, Marsit C, Walters OC, Carpenter LL. Childhood adversity and epigenetic modulation of the leukocyte glucocorticoid receptor: preliminary findings in healthy adults. *PLoS One* (2012) **7**(1):e30148. doi:10.1371/journal.pone.0030148
76. Labonte B, Yerko V, Gross J, Mechawar N, Meaney MJ, Szyf M, et al. Differential glucocorticoid receptor exon 1(B), 1(C), and 1(H) expression and methylation in suicide completers with a history of childhood abuse. *Biol Psychiatry* (2012) **72**(1):41–8. doi:10.1016/j.biopsych.2012.01.034
77. Pollak SD, Kistler DJ. Early experience is associated with the development of categorical representations for facial expressions of emotion. *Proc Natl Acad Sci U S A* (2002) **99**(13):9072–6. doi:10.1073/pnas.142165999
78. Jovanovic T, Blanding NQ, Norrholm SD, Duncan E, Bradley B, Ressler KJ. Childhood abuse is associated with increased startle reactivity in adulthood. *Depress Anxiety* (2009) **26**(11):1018–26. doi:10.1002/da.20599
79. Gotlib IH, Joormann J, Minor KL, Hallmayer J. HPA axis reactivity: a mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biol Psychiatry* (2008) **63**(9):847–51. doi:10.1016/j.biopsych.2007.10.008
80. Alexander N, Kuepper Y, Schmitz A, Osinsky R, Kozyra E, Hennig J. Gene-environment interactions predict cortisol responses after acute stress: implications for the etiology of depression. *Psychoneuroendocrinology* (2009) **34**(9):1294–303. doi:10.1016/j.psyneuen.2009.03.017
81. Way BM, Taylor SE. The serotonin transporter promoter polymorphism is associated with cortisol response to psychosocial stress. *Biol Psychiatry* (2010) **67**(5):487–92. doi:10.1016/j.biopsych.2009.10.021
82. Mueller A, Armbruster D, Moser DA, Canli T, Lesch KP, Brocke B, et al. Interaction of serotonin transporter gene-linked polymorphic region and stressful life events predicts cortisol stress response. *Neuropsychopharmacology* (2011) **36**(7):1332–9. doi:10.1038/npp.2011.11
83. Mueller A, Brocke B, Fries E, Lesch KP, Kirschbaum C. The role of the serotonin transporter polymorphism for the endocrine stress response in newborns. *Psychoneuroendocrinology* (2010) **35**(2):289–96. doi:10.1016/j.psyneuen.2009.07.002
84. Armbruster D, Mueller A, Moser DA, Lesch KP, Brocke B, Kirschbaum C. Interaction effect of D4 dopamine receptor gene and serotonin transporter

promoter polymorphism on the cortisol stress response. *Behav Neurosci* (2009) **123**(6):1288–95. doi:10.1037/a0017615
85. Dougherty LR, Klein DN, Congdon E, Canli T, Hayden EP. Interaction between 5-HTTLPR and BDNF Val66Met polymorphisms on HPA axis reactivity in preschoolers. *Biol Psychol* (2010) **83**(2):93–100. doi:10.1016/j.biopsycho.2009.10.009
86. Tyrka AR, Price LH, Gelernter J, Schepker C, Anderson GM, Carpenter LL. Interaction of childhood maltreatment with the corticotropin-releasing hormone receptor gene: effects on hypothalamic-pituitary-adrenal axis reactivity. *Biol Psychiatry* (2009) **66**(7):681–5. doi:10.1016/j.biopsych.2009.05.012
87. Heuser I, Yassouridis A, Holsboer F. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. *J Psychiatr Res* (1994) **28**(4):341–56. doi:10.1016/0022-3956(94)90017-5
88. Ising M, Depping AM, Siebertz A, Lucae S, Unschuld PG, Kloiber S, et al. Polymorphisms in the FKBP5 gene region modulate recovery from psychosocial stress in healthy controls. *Eur J Neurosci* (2008) **28**(2):389–98. doi:10.1111/j.1460-9568.2008.06332.x
89. Mehta D, Gonik M, Klengel T, Rex-Haffner M, Menke A, Rubel J, et al. Using polymorphisms in FKBP5 to define biologically distinct subtypes of posttraumatic stress disorder: evidence from endocrine and gene expression studies. *Arch Gen Psychiatry* (2011) **68**(9):901–10. doi:10.1001/archgenpsychiatry.2011.50
90. Klengel T, Mehta D, Anacker C, Rex-Haffner M, Pruessner JC, Pariante CM, et al. Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat Neurosci* (2013) **16**(1):33–41. doi:10.1038/nn.3275
91. Witt SH, Buchmann AF, Blomeyer D, Nieratschker V, Treutlein J, Esser G, et al. An interaction between a neuropeptide Y gene polymorphism and early adversity modulates endocrine stress responses. *Psychoneuroendocrinology* (2011) **36**(7):1010–20. doi:10.1016/j.psyneuen.2010.12.015
92. Armbruster D, Mueller A, Strobel A, Lesch KP, Brocke B, Kirschbaum C. Children under stress – COMT genotype and stressful life events predict cortisol increase in an acute social stress paradigm. *Int J Neuropsychopharmacol* (2012) **15**(9):1229–39. doi:10.1017/S1461145711001763
93. Friedel E, Schlagenhauf F, Sterzer P, Park SQ, Bermpohl F, Ströhle A, et al. 5-HTT genotype effect on prefrontal-amygdala coupling differs between major depression and controls. *Psychopharmacology (Berl)* (2009) **205**(2):261–71. doi:10.1007/s00213-009-1536-1
94. Kobiella A, Reimold M, Ulshöfer DE, Ikonomidou VN, Vollmert C, Vollstädt-Klein S, et al. How the serotonin transporter 5-HTTLPR polymorphism influences amygdala function: the roles of in vivo serotonin transporter expression and amygdala structure. *Transl Psychiatry* (2011) **1**:e37. doi:10.1038/tp.2011.29
95. Gatt JM, Williams LM, Schofield PR, Dobson-Stone C, Paul RH, Grieve SM, et al. Impact of the HTR3A gene with early life trauma on emotional brain networks and depressed mood. *Depress Anxiety* (2010) **27**(8):752–9. doi:10.1002/da.20726
96. Thomason ME, Henry ML, Paul Hamilton J, Joormann J, Pine DS, Ernst M, et al. Neural and behavioral responses to threatening emotion faces in children as a function of the short allele of the serotonin transporter gene. *Biol Psychol* (2010) **85**(1):38–44. doi:10.1016/j.biopsycho.2010.04.009
97. Drabant EM, Ramel W, Edge MD, Hyde LW, Kuo JR, Goldin PR, et al. Neural mechanisms underlying 5-HTTLPR-related sensitivity to acute stress. *Am J Psychiatry* (2012) **169**(4):397–405. doi:10.1176/appi.ajp.2011.10111699
98. Alexander N, Klucken T, Koppe G, Osinsky R, Walter B, Vaitl D, et al. Interaction of the serotonin transporter-linked polymorphic region and environmental adversity: increased amygdala-hypothalamus connectivity as a potential mechanism linking neural and endocrine hyperreactivity. *Biol Psychiatry* (2012) **72**(1):49–56. doi:10.1016/j.biopsych.2012.01.030
99. Klucken T, Alexander N, Schweckendiek J, Merz CJ, Kagerer S, Osinsky R, et al. Individual differences in neural correlates of fear conditioning as a function of 5-HTTLPR and stressful life events. *Soc Cogn Affect Neurosci* (2013) **8**(3):318–25. doi:10.1093/scan/nss005
100. Mekli K, Payton A, Miyajima F, Platt H, Thomas E, Downey D, et al. The HTR1A and HTR1B receptor genes influence stress-related information processing. *Eur Neuropsychopharmacol* (2011) **21**(1):129–39. doi:10.1016/j.euroneuro.2010.06.013
101. Hsu DT, Mickey BJ, Langenecker SA, Heitzeg MM, Love TM, Wang H, et al. Variation in the corticotropin-releasing hormone receptor 1 (CRHR1) gene influences fMRI signal responses during emotional stimulus processing. *J Neurosci* (2012) **32**(9):3253–60. doi:10.1523/JNEUROSCI.5533-11.2012
102. Bogdan R, Santesso DL, Fagerness J, Perlis RH, Pizzagalli DA. Corticotropin-releasing hormone receptor type 1 (CRHR1) genetic variation and stress interact to influence reward learning. *J Neurosci* (2011) **31**(37):13246–54. doi:10.1523/JNEUROSCI.2661-11.2011
103. DeYoung CG, Cicchetti D, Rogosch FA. Moderation of the association between childhood maltreatment and neuroticism by the corticotropin-releasing hormone receptor 1 gene. *J Child Psychol Psychiatry* (2011) **52**(8):898–906. doi:10.1111/j.1469-7610.2011.02404.x
104. Gatt JM, Nemeroff CB, Dobson-Stone C, Paul RH, Bryant RA, Schofield PR, et al. Interactions between BDNF Val66Met polymorphism and early life stress predict brain and arousal pathways to syndromal depression and anxiety. *Mol Psychiatry* (2009) **14**(7):681–95. doi:10.1038/mp.2008.143
105. Lau JY, Goldman D, Buzas B, Hodgkinson C, Leibenluft E, Nelson E, et al. BDNF gene polymorphism (Val66Met) predicts amygdala and anterior hippocampus responses to emotional faces in anxious and depressed adolescents. *Neuroimage* (2010) **53**(3):952–61. doi:10.1016/j.neuroimage.2009.11.026
106. Bogdan R, Williamson DE, Hariri AR. Mineralocorticoid receptor Iso/Val (rs5522) genotype moderates the association between previous childhood emotional neglect and amygdala reactivity. *Am J Psychiatry* (2012) **169**(5):515–22. doi:10.1176/appi.ajp.2011.11060855
107. Bradley B, Westen D, Mercer KB, Binder EB, Jovanovic T, Crain D, et al. Association between childhood maltreatment and adult emotional dysregulation in a low-income, urban, African American sample: moderation by oxytocin receptor gene. *Dev Psychopathol* (2011) **23**:87–100. doi:10.1017/S0954579411000162
108. Mickey BJ, Zhou Z, Heitzeg MM, Heinz E, Hodgkinson CA, Hsu DT, et al. Emotion processing, major depression, and functional genetic variation of neuropeptide Y. *Arch Gen Psychiatry* (2011) **68**(2):158–66. doi:10.1001/archgenpsychiatry.2010.197
109. Nederhof E, Bouma EM, Riese H, Laceulle OM, Ormel J, Oldehinkel AJ. Evidence for plasticity genotypes in a gene-gene-environment interaction: the TRAILS study. *Genes Brain Behav* (2010) **9**(8):968–73. doi:10.1111/j.1601-183X.2010.00637.x
110. Juhasz G, Dunham JS, McKie S, Thomas E, Downey D, Chase D, et al. The CREB1-BDNF-NTRK2 pathway in depression: multiple gene-cognition-environment interactions. *Biol Psychiatry* (2011) **69**(8):762–71. doi:10.1016/j.biopsych.2010.11.019
111. Gatt JM, Nemeroff CB, Schofield PR, Paul RH, Clark CR, Gordon E, et al. Early life stress combined with serotonin 3A receptor and brain-derived neurotrophic factor valine 66 to methionine genotypes impacts emotional brain and arousal correlates of risk for depression. *Biol Psychiatry* (2010) **68**(9):818–24. doi:10.1016/j.biopsych.2010.06.025
112. Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. *Annu Rev Neurosci* (2001) **24**:1161–92. doi:10.1146/annurev.neuro.24.1.1161
113. Provençal N, Suderman MJ, Guillemin C, Massart R, Ruggiero A, Wang D, et al. The signature of maternal rearing in the methylome in rhesus macaque prefrontal cortex and T cells. *J Neurosci* (2012) **32**(44):15626–42. doi:10.1523/JNEUROSCI.1470-12.2012
114. Provençal N, Suderman MJ, Caramaschi D, Wang D, Hallett M, Vitaro F, et al. Differential DNA methylation regions in cytokine and transcription factor genomic Loci associate with childhood physical aggression. *PLoS One* (2013) **8**(8):e71691. doi:10.1371/journal.pone.0071691
115. Nugent NR, Tyrka AR, Carpenter LL, Price LH. Gene-environment interactions: early life stress and risk for depressive and anxiety disorders. *Psychopharmacology* (2011) **214**(1):175–96. doi:10.1007/s00213-010-2151-x
116. Ellis BJ, Boyce WT, Belsky J, Bakermans-Kranenburg MJ, van Ijzendoorn MH. Differential susceptibility to the environment: an evolutionary-neurodevelopmental theory. *Dev Psychopathol* (2011) **23**(1):7–28. doi:10.1017/S0954579410000611
117. Dick DM, Meyers JL, Latendresse SJ, Creemers HE, Lansford JE, Pettit GS, et al. CHRM2, parental monitoring, and adolescent externalizing behavior: evidence for gene-environment interaction. *Psychol Sci* (2011) **22**(4):481–9. doi:10.1177/0956797611403318
118. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* (2009) **10**(6):434–45. doi:10.1038/nrn2639

119. Nemeroff CB, Heim CM, Thase ME, Klein DN, Rush AJ, Schatzberg AF, et al. Differential responses to psychotherapy versus pharmacotherapy in patients with chronic forms of major depression and childhood trauma. *Proc Natl Acad Sci U S A* (2003) **100**(24):14293–6. doi:10.1073/pnas.2336126100
120. Mayberg HS. Modulating dysfunctional limbic-cortical circuits in depression: towards development of brain-based algorithms for diagnosis and optimised treatment. *Br Med Bull* (2003) **65**:193–207. doi:10.1093/bmb/65.1.193

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 21 October 2013; paper pending published: 19 November 2013; accepted: 01 February 2014; published online: 17 February 2014.
*Citation: Hornung OP and Heim CM (2014) Gene–environment interactions and intermediate phenotypes: early trauma and depression. Front. Endocrinol. **5**:14. doi: 10.3389/fendo.2014.00014*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 13 May 2014
doi: 10.3389/fendo.2014.00073

Early-life stress, HPA axis adaptation, and mechanisms contributing to later health outcomes

Jayanthi Maniam, Christopher Antoniadis and Margaret J. Morris*

Department of Pharmacology, School of Medical Sciences, UNSW Australia, Sydney, NSW, Australia

Edited by:
Rachel Yehuda, Icahn School of Medicine at Mount Sinai, USA

Reviewed by:
Aldo Lucion, Universidade Federal do Rio Grande do Sul, Brazil
Gábor B. Makara, Hungarian Academy of Sciences, Hungary

***Correspondence:**
Margaret J. Morris, Department of Pharmacology, School of Medical Sciences, UNSW Australia, Sydney, NSW 2052, Australia
e-mail: m.morris@unsw.edu.au

Stress activates the hypothalamic–pituitary–adrenal (HPA) axis, which then modulates the degree of adaptation and response to a later stressor. It is known that early-life stress can impact on later health but less is known about how early-life stress impairs HPA axis activity, contributing to maladaptation of the stress–response system. Early-life stress exposure (either prenatally or in the early postnatal period) can impact developmental pathways resulting in lasting structural and regulatory changes that predispose to adulthood disease. Epidemiological, clinical, and experimental studies have demonstrated that early-life stress produces long term hyper-responsiveness to stress with exaggerated circulating glucocorticoids, and enhanced anxiety and depression-like behaviors. Recently, evidence has emerged on early-life stress-induced metabolic derangements, for example hyperinsulinemia and altered insulin sensitivity on exposure to a high energy diet later in life. This draws our attention to the contribution of later environment to disease vulnerability. Early-life stress can alter the expression of genes in peripheral tissues, such as the glucocorticoid receptor and 11-beta hydroxysteroid dehydrogenase (11β-HSD1). We propose that interactions between altered HPA axis activity and liver 11β-HSD1 modulates both tissue and circulating glucocorticoid availability, with adverse metabolic consequences. This review discusses the potential mechanisms underlying early-life stress-induced maladaptation of the HPA axis, and its subsequent effects on energy utilization and expenditure. The effects of positive later environments as a means of ameliorating early-life stress-induced health deficits, and proposed mechanisms underpinning the interaction between early-life stress and subsequent detrimental environmental exposures on metabolic risk will be outlined. Limitations in current methodology linking early-life stress and later health outcomes will also be addressed.

Keywords: early-life stress, metabolic disorders, 11-beta hydroxysteroid dehydrogenase 1, hyperinsulinemia, liver, insulin signaling, glucocorticoids

INTRODUCTION

Stress can be defined as any condition including an adverse environment, experience, or perceived threat to alter an organism's homeostasis, which elicits a physiological response involving both peripheral and central systems via the release of glucocorticoids from the adrenal cortex through activation of the hypothalamic–pituitary–adrenal (HPA) axis (1). Glucocorticoids serve as the critical end product of the HPA axis and negative feedback through glucocorticoid–glucocorticoid receptor binding in the hippocampus promotes adaptation and recovery from stress (2). Activity of the HPA axis plays a critical role in restoring homeostasis following imminent or acute stressor exposure (1). In contrast, recurrent or persistent activation of the HPA axis and the autonomic nervous system are associated with adverse health outcomes (3). Individuals respond differently to stressors, which can reflect a wide range of adversities in life from major events to daily conflicts and pressures. Increased levels of glucocorticoids interfere with energy utilization and modify metabolic hormones such as insulin and glucose, which are key regulators of energy metabolism (4–6). The elicited response affects multiple physiological systems including neuroendocrine, autonomic, and the immune system and is an established risk factor for the development of disease (7–10).

There is longstanding recognition of the impact of stress during critical early developmental periods such as childhood and the consequential association with adverse mental health outcomes and changes in brain development (11–13), however, less is known regarding how this dysfunction may confer increased metabolic disease risk. Emerging epidemiological evidence demonstrates that adverse early-life stress-induced dysregulation of the HPA axis and increases vulnerability for metabolic disorders. In particular, exposure to an adverse environment during prenatal and postnatal periods, such as lack of nutrition or starvation during war, traumatic experiences including childhood physical or sexual abuse, neglect, adverse parenting or medical trauma has been demonstrated to be one of the major risk factors contributing to the development metabolic disorders including insulin resistance, type 2 diabetes mellitus (T2DM), and hyperlipidemia (14–16).

While studies aimed at exploring underlying mechanisms are difficult to achieve in humans, animal studies have shed some light into the effects of early-life stress induced during the prenatal and

postnatal periods on metabolic hormones and on peripheral tissues involved in glucose/insulin and lipid metabolism (17–24). In addition, the morphology of the pancreas is also affected by prenatal stress, with reduced beta-cell numbers (25). Thus, stress during early development may incur permanent alterations in morphology and function of key peripheral organs involved in metabolism of insulin, glucose and lipids, and these changes suggest programing effects of early-life stress (26).

This review will unravel how early-life stress induces metabolic derangements; an area that is less well explored. Despite the emergence of human data in the field, mechanisms elucidating the association between stress and adverse metabolic outcomes are lacking. Animal studies permit closer investigation of the stress system and through various manipulations, allow the mechanisms underlying effects of stress on metabolism to be explored. Of particular focus is exploring outcomes of stress during the perinatal and early-life period for later life metabolic outcomes.

DOES EARLY-LIFE STRESS AFFECT RISK FOR LATER LIFE METABOLIC DEFICIT?

MODELING EARLY-LIFE STRESS IN ANIMALS

As direct examination of the prospective effects of early-life stress is not feasible in humans this had led to the development of numerous animal models to explore the question of whether early-life stress can affect risk for later life metabolic deficit. Animal models of early-life stress allow for controlled environmental manipulation throughout developmental periods and later life. With necessary caution these models can assist our understanding of the link between developmental and environment experiences and the conferred later life metabolic disease vulnerabilities. Inherent differences in human and rodent biological maturation and neuroendocrine development must be considered in study design and translation to human health. Humans give birth to mature young; with the final trimester of pregnancy being a period of rapid brain development (27, 28). Rodent offspring are born relatively immature with maximal growth phase initiated early in the postnatal period. Despite greater maturity of human offspring at the time of birth, development is far from complete with changes in neurological processes, synaptogenesis, synaptic pruning, and plastic changes in key functional areas including the hippocampus occurring until late adolescence (29, 30). The environment has been shown to impact this ongoing development beyond gestation. Children physically healthy at the time of birth who were abused in early life were shown to have reduced brain volume, correlated to age of abuse onset, and duration of the stress (31). Imposed stressors during rodent gestational or early postnatal life are suggested to model the period of gestation, early postnatal, or infancy in humans.

Humans and rodents have a vital dependence on adequate nourishment and care to ensure normal development. This altricial nature and vulnerable perinatal period across both species means that models of maternal care can provide insight into how early support and sensitivity to offspring needs can impact development. Changes in maternal care have been shown to impact rodent development with mother–offspring interactions such as licking and feeding providing critical input for normal neurobiological development and HPA axis function (32, 33). Adequate maternal contact during this period assists in maintaining rodents in their early-life hypo-responsive stress state and adverse experience through physical or psychological means or synthetic glucocorticoid administration can permanently alter HPA axis function (34, 35).

Three popular paradigms of postnatal early-life stress are maternal separation for varied periods of time from 15 min to 8 h, maternal deprivation (absence of the dam for a more extended period) and provision of only limiting nesting material. The maternal separation model has been studied for more than five decades, and is demonstrated in both mice and rats to affect the HPA axis, and behavioral responses in mothers and offspring in a sex dependant manner (36–39). Maternal deprivation is another common form of early-life stress which has been studied over decades (40–44). Twenty-four hours of maternal deprivation in neonatal rodents induced marked elevations in plasma corticosterone and decreases in plasma glucose and leptin, amongst other hormonal and neurotrophic factor changes (40, 43). This model, however appears to reflect a severe nutritional insult rather than psychological disturbances. Therefore, whether maternal deprivation represents adverse early experience in the human context is debatable. The limiting nesting paradigm, a more recently developed model of early-life stress, has been described elsewhere (35) but, briefly, involves limiting the dam's available material for nest building, resulting in rudimentary and inadequate housing for offspring, a chronic stressor for the dam and pups. Limited nesting (LN) material, attempts to enhance commonalities to the human condition of childhood neglect and maternal stress in which the mother is present, yet care is abnormal and fragmented. Notably, the LN model has been demonstrated to impair HPA axis activity and induce behavioral deficits both in the dam and pups (35, 45, 46).

Recently, early-life stress models in non-human primates have demonstrated a lasting health impact following adverse early experiences. Altering secure attachment relationships during early life in Rhesus macaques significantly elevated prevalence and frequency of illness and increased bodyweight trajectory (47). Stress induced via variable foraging demand in bonnet macaques during lactation affected the metabolic profile of their offspring; a decreased glucose disposal rate was observed during hyperinsulinemic-euglycemic clamps in those exposed to early-life stress (48).

OBSERVATIONS IN HUMAN STUDIES

Retrospective and observational studies in humans demonstrate that early-life experiences can influence later life metabolic outcome. Environmental changes during gestation and the early postnatal period may impact development and predict metabolic health outcomes. Manipulations of the early environment can affect the developing nervous system, shaping individual differences in physiological and behavioral responses to environmental insults. For example, disruption of the mother–infant relationship during early life contributes to neuroendocrine, neurochemical, and behavioral changes in the adult organism (49). Experience of adversity during early life and adolescence in the form of parental conflict or parental separation increased the risk of later life obesity (50). Similarly, experience of a range of early-life stressors

was positively correlated with increased adult BMI in men, independently of mental health condition (51). These adverse early-life experiences are associated with persisting changes in HPA axis function in adult life with changes in the normal dynamics of the stress system and its end-point hormone cortisol (52). Commonly, a flattened cortisol circadian rhythm and hypo- or hyper-responsiveness to future novel stressors is observed (53, 54).

Barker's theory postulates that low birth weight predicts increased disease risk later in life, including metabolic vulnerability (55). Given this association, identifying factors that influence gestational growth, and ultimately determine birth weight is important. Reduced birth weight and preterm birth have both been associated with psychosocial stress exposure during pregnancy (56–58). Having a low birth weight baby (<2500 g) was associated with stress-related psychiatric illness in pregnant mothers, such as melancholic depression (59). Prenatal psychological stress such as experiencing bereavement during pregnancy led to an increased risk of developing T2DM in their children during adulthood (60). Degree of social support during pregnancy has also been associated with birth weight (61). Other psychological stressors such as financial, relationship problems, and illness during pregnancy led to elevated glucose, insulin, and C-peptide levels during glucose tolerance test in their children aged 25 (62). Nutritional stress during the neonatal period has also been shown to adversely impact offspring health outcome, as explored below.

Famine exposure during pregnancy is a chronic early-life stress that is also known to affect birth weight and increase risk for metabolic disorders later in life. A well-documented period providing epidemiological evidence linking early-life adversity and health outcomes is the 1944–1945 Dutch Famine (see **Table 1**). A cohort of 741 subjects exposed to the Dutch Famine prenatally had a reduced birth weight, yet at adulthood these subjects had increased body weight, BMI, fasting proinsulin levels, and glucose intolerance (63). A report of 7557 women exposed to the Dutch famine showed increased risk for T2DM development in their offspring (64). Exposure to the Chinese famine during the 1960s showed similar adverse outcomes for offspring, with women having higher prevalence of metabolic disorders such as diabetes, hypertriglyceridemia, and hypertension (65). A cross-sectional study of subjects exposed to the Biafran famine during the Nigerian Civil War showed derangements in their metabolic profile during adulthood with increased risk for diabetes in both middle-aged men and non-pregnant women (66) (see **Table 1**). These data highlight that adverse early experiences, whether psychological or nutritional in nature during vulnerable developmental periods can impact offspring insulin and glucose metabolism during adulthood (**Table 1**).

Despite increasingly available epidemiological evidence, the mechanisms driving stress effects to lower birth weight remain largely unknown. Changes in HPA axis function in individuals of low birth weight have been identified. A group of adult men born with low birth weight had increased HPA axis responsiveness, which was shown to be associated with metabolic risk factors including blood pressure and increased triglycerides (80). Thus epidemiological evidence generally suggests early-life stress induces perturbed HPA axis function and alters neuroendocrine axis responsiveness. The increasing evidence demonstrating the risk of early-life stress and later metabolic disorders emphasizes the need to explore the mechanisms underlying this association. Targets for intervention, whether through pharmacological means or through lifestyle modification, need to be identified to reduce this identified risk. It is important to note that not all individuals exposed to adverse early environments develop metabolic deficits later in life; these differences may relate to genetic makeup and the environment to which the individual is exposed throughout life.

THE EFFECT OF LATER ENVIRONMENT ON HEALTH OUTCOMES FOLLOWING EARLY-LIFE STRESS

Early-life stress in combination with a sub-optimal later environment, such as a sedentary lifestyle, increased consumption of high energy food or persistent adulthood stress may alter the risk for developing metabolic disorders throughout life. Humans and rodents are able to habituate and adapt to the environmental conditions to which they are exposed (81–83). This adaptation occurs in prediction of exposure to similar situations in the future, and under normal conditions is of significant value, improving future resilience and coping in these situations (see **Figure 2**). However, it is suggested that if adaptation is inadequate, maladaptive, or future environment differs from the programed phenotype, there is increased disease susceptibility (84–87). Determining the long term consequences of early-life stress-induced changes in neuronal structure, and hormonal and nutritional status across different environments is an important public health concern. Although prevention or mitigation of early-life stress is the ideal, if through modulating the later environment (e.g., providing a positive environment) disease risk can be attenuated, important targets for intervention can be identified.

Stress throughout adulthood negatively influences lifestyle choices that are risk factors for metabolic disease, such as alterations in eating behavior, intake of high-fat food (88), drug addiction (89, 90), and reduced physical activity levels (91). Given this, it is critical to determine whether early-life stress can have a lasting influence on adult behavioral choices. Indeed, parental care factors play a key role in developing health behaviors and outcomes in children (92, 93). Parental behaviors are often imitated by children, thus a push to improve attitudes in parents, whether through reducing stress, improving eating attitudes or increasing physical activity may foster improved health status in successive generations. Recent research showed a direct association between activity levels of parents and their preschool-aged children (94). Additionally, the behavioral profile that results from early-life stress heightens the risk for impaired psychosocial function and psychiatric disorders, and this may independently influence metabolic disease risk. Future human studies must focus on these lifestyle factors during critical exposure periods and throughout adult life.

PRENATAL STRESS AND IMPACT OF LATER ENVIRONMENT: HUMAN STUDIES

Prenatal exposure to adverse environments such as famine have been associated with poorer lifestyle choices including smoking incidence (66), HFD consumption, and reduced physical activity (95, 96). As explored, a possible consequence of gestational stress is preterm birth or low birth weight. Total physical activity levels (97) and non-conditioning leisure time physical activity levels

Table 1 | Human early-life stress studies exploring metabolic outcomes.

Early-life stressor	Participants	Offspring age	Exclusion criteria	Metabolic impact on offspring	Reference
Prenatal: maternal stress, holocaust exposure	137 adults, 74% reported parental holocaust exposure. Remainder considered unexposed controls	Middle-aged men and women	Psychosis, bipolar disorder, substance dependence Organic mental disorder Dementia; oral corticosteroids	↑ Reported use of medications, including psychotropic, antihypertensives, dyslipidemia medication ↑ Association with having two or more metabolic syndrome components, e.g., T2DM, hypertension, dyslipidemia or increased BMI	Flory et al. (67)
Prenatal: maternal stress, psychosocial	58 offspring, of whom 36 exposed to maternal stress. Remaining 22 considered unexposed controls	Young adults	Pregnancy complication Smoker Acute or chronic health problems	↑ BMI ↑ Very low-density lipoprotein (138%) ↓ High-density lipoprotein (16%) and low-density lipoprotein (33%) *OGTT: offspring of mothers whom experienced psychosocial stress compared to control* ↑ Fasting plasma insulin levels (58%) ↑ Plasma insulin 2-h post-oral glucose load (59%) ↑ C-peptide 2-h post-oral glucose load (40%)	Entringer et al. (62)
Prenatal: maternal stress, natural disaster exposure	111 Women pregnant during or conceived within 3 months of the Quebec ice storm	Children, 5.5 years of age		↑ Obesity risk of offspring at 5.5 years old, associated with severity of objective maternal stress Controlled for SES, pregnancy complications, breastfeeding, smoking, psychological function, and BMI	Dancause et al. (68)
Prenatal: maternal stress, natural disaster exposure	176 women pregnant during or conceived within 1 month of 1998 Quebec ice storm and their children	Children, mean age 13.5 years		Objective hardship positively correlated with insulin secretion ($P < 0.01$) and BMI ($P < 0.02$)	Dancause et al. (69)
Prenatal: maternal stress, bereavement	1,878,246 people, of whom 45,302 were exposed to stress. Remaining considered unexposed controls	Offspring followed for 2–32 years		↑ Risk for T2DM Second trimester identified as the most sensitive	Li et al. (60)
Prenatal and postnatal: maternal stress, famine	741 people born in Amsterdam before, during or after Dutch famine	Middle-aged men and women	Missing birth records Preterm birth (<37 weeks) Deceased Emigrated	↑ Bodyweight, BMI and waist circumference in women 50 years of age exposed to early gestation famine vs. non-exposed controls	Ravelli et al. (70)
Prenatal and postnatal: maternal stress, famine	702 people born in Amsterdam before, during or after Dutch famine	Middle-aged men and women	Missing birth records Preterm birth (<37 weeks) Diabetes Deceased Emigrated	*OGTT: offspring exposed to famine compared to control* ↑ Fasting proinsulin levels and 2-h glucose concentrations More pronounced if famine occurred during late gestation or with later life obesity	Ravelli et al. (63)

(Continued)

Table 1 | Continued

Early-life stressor	Participants	Offspring age	Exclusion criteria	Metabolic impact on offspring	Reference
Prenatal and postnatal: maternal perceived stress	152 women surveyed during pregnancy/first year of offspring life, predominantly low-income population	Infants		↑ Risk of infant being overweight ($P = 0.020$) Correlation with consumption of sugar-sweetened beverages ($P = 0.004$) and with feeding infants sugar-sweetened beverages ($P = 0.031$)	Watt et al. (71)
Prenatal and postnatal: maternal stress, depression	1249 women, depressive symptoms assessed during pregnancy and postpartum	Children 3 years of age	Multiple gestation Issues with English Move prior to delivery Gestational age greater than 22 weeks at first prenatal visit	*Antenatal depression* Smaller body size ↑ Central adiposity *Postpartum depression* ↑ Overall adiposity Independent of SES, BMI, and health condition during pregnancy	Ertel et al. (72)
Postnatal: childhood stress, death of a parent	135 bariatric surgery candidates	Middle-aged men and women	Substance abuse Severe personality disorder Mental retardation	↑ Risk of metabolic syndrome following childhood parental loss ($P = 0.012$)	Alciati et al. (73)
Postnatal: maternal stress (mental, physical, financial family structure) and altered food security	841 Children across 425 low-income households	Children, 3–17 years old	Households above 200% of poverty line	↑ Risk of offspring 3–10 years old being overweight or obese in food secure environments compared to periods of food insecurity (43.7%)	Gundersen et al. (74)
Postnatal: childhood maltreatment	67,853 women in Nurses Health Study II	25–42		Dose–response association between child physical and sexual abuse with adult T2DM. Hazard ratio for diabetes in child exposed to mild, moderate and severe are 1.03, 1.26 and 1.54 respectively	Rich-Edwards et al. (75)
Postnatal: childhood maltreatment	$n = 972$, born in between April 1972 and March 1973	32 years	Individuals with plasma c-reactive protein >10 mg/l	↑ Inflammation assessed by c-reactive protein	Danese et al. (76)
Postnatal: childhood maltreatment	342 from study of women health across the nation (SWAN)	45.7 year (mean age)		Physical abuse was associated with increased plasma triglyceride and blood pressure	Midei et al. (77)
Postnatal: childhood maltreatment	756 from population based study	Young adult (19–20 years)		↑ BMI in those exposed to neglect during childhood Odds ratio 9.8 CI 1.35–28.2	Lissau and Sorensen (78)
Postnatal: childhood maltreatment	9310 of 1958 British birth cohort	45 years		↑ BMI ↑ HbA1C ≥6 ↑ Central obesity	Thomas et al. (79)

(98) were not influenced by low birth weight. Despite this, adults born preterm with very low birth weight (i.e., less than 1500 g) had reduced smoking rates, yet were less likely to engage in leisure time physical activity with reduced energy expenditure than normal gestational birth controls (98). Further, healthy children aged 5–8 years old who were born prematurely were shown to have reduced physical ability to normal gestational age controls (99). Physical activity has been shown to improve health outcomes following premature birth, with 4 weeks of passive range of motion and compression exercises in premature infants shown to increase bone mineral density. The authors suggested that early exercise programs may improve physical fitness in later life (100). The unequivocal benefits of physical activity should be considered as a therapeutic tool, with activity levels inversely associated with

metabolic syndrome (101). In low birth weight individuals, physical activity has been shown to modestly attenuate the association between low birth weight and insulin resistance, as assessed by HOMA-IR (102). Although this finding has not been replicated by other studies (97, 103), physical activity was identified as a better predictor of HOMA-IR comparative to birth weight a possible target for intervention (103).

POSTNATAL STRESS AND IMPACT OF LATER ENVIRONMENT: HUMAN STUDIES

There are limited data exploring the metabolic outcomes of postnatal stress and environment interaction. Early-life stress has been associated with later life addiction problems, including drug and food addiction (104). Adult women who were abused as children were significantly heavier and had a marked increase in food addiction risk (105). Early-life maternal stress has been positively correlated with the risk of the infant being overweight in an environment of high food security (see **Table 1**). Infants exposed to maternal stress during periods of food insecurity showed no significant overweight risk (74) (see **Figure 2**). One limitation of this study is the low socioeconomic status of the participants, as this is an individual risk factor for the development of childhood and adult obesity, amongst other health issues. Similar to these findings, the number of parental stressors and degree of perceived stress were positively associated with child obesity and increased fast-food consumption (106). Children and adolescents are susceptible to stress, and given the association between perceived stress and increased food consumption frequency and palatability (107–109), environmental factors such as palatable food access and physical activity may play a mediating factor between human early-life stress and poor metabolic outcomes.

Physical activity may facilitate a protective role against childhood stressors, with more active children showing reduced salivary cortisol response to stressful situations, reflective of lower HPA axis reactivity (110). In addition to alterations in stress mediators, childhood maltreatment has been independently associated with elevated levels of inflammatory markers in adulthood (76, 111) (see **Table 1**). Increased inflammation following early-life stress is clinically relevant and may provide an important causal link between adverse early-life experience and adulthood metabolic risk. The benefits of physical activity on immune function and inflammation are well-established, with reductions in inflammatory biomarkers associated with disease (112).

PRENATAL STRESS AND IMPACT OF LATER ENVIRONMENT: ANIMAL STUDIES

Given the global epidemic of metabolic disease, examining the growing body of animal evidence linking prenatal stress with impaired metabolic profile when exposed to energy dense, palatable diets is imperative. The effects of stress during pregnancy on metabolic consequences in later life are well characterized relative to stress during the early postnatal period (22). Identifying how programed changes during early life adversely impact susceptibility to later life nutrition is critical and could provide targets for intervention (see **Figure 2**). Two common models of prenatal stress exposure involve subjecting dams to a single stressor during gestation, for instance restraint stress. Alternatively, dams can be exposed to a combination of variable stressors such as restraint, air puff startle, forced swimming, starvation, and bright light exposure (113).

Evidence to support a programed resilience to later life metabolic deficits following prenatal early-life stress has been observed in some animal studies. For example, independent of body weight, adult female offspring of stressed dams consuming standard laboratory chow, had a lower insulin area under the curve (AUC) during an oral glucose tolerance test (OGTT) (114), reduced plasma insulin, and improvements in HOMA-IR (24) compared to offspring of non-stressed dams. This improvement in insulin sensitivity is suggestive of a prenatal early-life stress programing of glucose handling in insulin sensitive tissues. However, it is unknown if these improvements in insulin sensitivity following prenatal early-life stress will be sustained as animals age. Exposure of offspring to a later negative environment following prenatal stress, such as an HFD, induces maladaptation, rather than resilience (see **Figure 2**). Offspring of stressed dams weaned onto an HFD showed alterations in metabolic parameters with female rats shown to have an elevated glucose AUC during an OGTT, relative to control and stress-chow groups, whilst HFD fed males of stressed dams required a greater amount of insulin to clear a given glucose load relative to control (114). In a similar study, prenatal stress did not alter glucose clearance as assessed through intraperitoneal glucose tolerance test (IPGTT), yet, female offspring exposed to stress had increased visceral and retroperitoneal fat depot mass after 10 weeks on a high-fat sucrose diet relative to unstressed controls consuming the same diet (24).

The mechanisms by which prenatal stress alters later life metabolic outcome remain unknown. Stress during pregnancy alters maternal hormones, including circulating glucocorticoid levels. Normal physiological levels of glucocorticoids during development are essential for tissue growth and maturation, however, excess levels of glucocorticoids, e.g., through pharmacological interventions, have been shown to affect maturation (115, 116). Elevated maternal glucocorticoid can cross the placenta (117), and this has been shown to affect growth, morphology, and function of brain and peripheral tissues during fetal development (118). Seckl (119) has demonstrated a stress-mediated mechanism that underpins the low birth weight and increased risk for adulthood health deficits; exposure to increased level of glucocorticoids either synthetically (dexamethasone) during late gestation or via stress (malnutrition, adverse environment exposures) reduces birth weight and impacts maturation of major organs (119, 120). Dams exposed to prenatal variable stress were shown to have heavier adrenal mass and lower fecal corticosterone secretion vs. non-stressed controls (121). This is suggestive of a reduced corticosterone clearance in these stressed dams during gestation. Excess glucocorticoid exposure during pregnancy has also been shown to affect glucose and insulin metabolism (17, 122, 123).

To model periods of nutritional stress in humans, such as famine, reduced nutritional availability during gestation can be used, enabling investigation of the developmental and programing outcomes on offspring (124–126). Restricting dams to 30% of normal food intake, led to low birth weight offspring; pups who were undernourished then weaned onto HFD were found to have significantly elevated leptin, C-peptide, insulin, and body fat

compared to control-HFD pups. Notably, injection with leptin for 10 days during lactation (PND3–13) completely normalized these markers (127), suggesting normal maternal levels of leptin are important during development. Food intake and activity levels are also influenced by nutritional insult during gestation. Offspring of female Wistar rats undernourished during gestation (30% of *ad libitum*) showed significantly reduced locomotor activity with marked hyperphagia and hyperleptinemia when consuming either standard chow or a high caloric diet (128). Leptin is a critical adipocyte derived hormone known to play an essential role in the regulation of feeding and in the maintenance of energy homeostasis. Adult rats exposed to a single 24-h deprivation period during the lactation period demonstrated marked reductions in leptin, although this is difficult to interpret due to the nutritional insult that would have accompanied 24 h of starvation (40, 129, 130).

POSTNATAL STRESS AND IMPACT OF LATER ENVIRONMENT: ANIMAL STUDIES

Various models of postnatal early-life stress have explored the influence of later life environmental insults on metabolic function, each of which seem to elicit different outcomes and sex-specific effects (see **Table 2**). Work in our lab demonstrated HFD fed male rats previously exposed to maternal separation, have marked elevations in plasma insulin, and decreased total white adipose tissue mass, independent of body weight vs. HFD controls (131, 132). In agreement, maternal deprivation induced early adulthood hyperinsulinemia and impairments in insulin sensitivity, measured through HOMA-IR, in male offspring fed with an HFD relative to HFD controls (130). Similar metabolic changes are observed in maternal deprivation exposed female offspring, with HFD shown to cause early adulthood hyperinsulinemia, at PND35 compared to PND102 in control rats consuming an HFD (130). Further, maternally deprived female rats consuming a high-fat sucrose diet had a trend for decreased fat depot mass vs. control (121). Less work has examined the metabolic profile arising subsequent to early-life stress induced by LN material. A recent study explored for the first time the metabolic profile in Wistar female rats; showing reduced body weight at weaning, and reduced food intake, suggesting altered energy utilization and storage. Interestingly, these pups had exaggerated HPA axis activity with delayed clearance of corticosterone from the circulation, and taken together these data further suggest an early-life stress-induced interaction between the HPA axis and metabolic profile (133) (see **Table 2**).

Few studies have explored biological characteristics of peripheral tissues following early-life stress. Recently, maternally deprived female rats were shown to have significantly reduced brown adipose tissue β3-adrenergic receptor mRNA expression and increased white adipose tissue prohibitin mRNA relative to control, with no change in UCP-1 (136). The authors concluded these results may facilitate adipose tissue proliferation later in life. An altered response to nutritional challenge following early-life stress is not only observed in high caloric fed states. Maternally separated rats consuming an omega-3 deficient diet demonstrated marked elevations in plasma insulin and impaired insulin sensitivity, as assessed by HOMA-IR, relative to control animals consuming the same diet (134). Functional studies performed by our lab have demonstrated attenuated adulthood outcomes of early-life stress when siblings are provided with a positive environment of voluntary running wheel exercise (see **Figure 2**). Male rats exposed to maternal separation and weaned onto a standard chow diet demonstrated a hyper-responsive corticosterone response to novel restraint stress, which was dampened with exercise and HFD. Impaired changes in metabolic parameters of insulin and diet-induced obesity were also attenuated with exercise, reversing maternal separation-induced hyperinsulinemia and increased body weight relative to HFD controls and stressed sedentary counterparts (132).

A common aspect across early-life stress models centers around glucocorticoid exposure and HPA axis activation during the stress-hypo-responsive period, a stage of neonatal resilience to mild stressors suggested to likely trigger corticosterone secretion in adult life (84). Synthetic glucocorticoid administration attempts to replicate stress models, as this elicits an HPA axis response during the early postnatal period in rodents (41, 138). This also allows for controlled dose administration and with necessary caution can improve understanding of the impact of glucocorticoid exposure during these critical periods. Early-life corticosterone administration reduced adult female rat body weight and decreased fat depot mass relative to control (139). Studies of glucocorticoid administration highlight the marked influence of combining stress hormones and additional metabolic insults. In young male Sprague-Dawley rats, glucocorticoid administration alone did not alter insulin sensitivity, assessed by HOMA-IR, or glucose disposal following an OGTT (140). Conversely, consumption of a palatable HFD in combination with glucocorticoid exposure is known to induce a marked increase in fasting plasma glucose and insulin, with impaired glucose clearance following OGTT (140, 141). Further investigation is required to determine whether the unaltered or improved insulin sensitivity with chow consumption would prove deleterious in the long term (as shown in **Figure 2**).

EARLY-LIFE STRESS AND PROGRAMING OF PERIPHERAL TISSUES

MECHANISMS UNDERLYING EARLY-LIFE STRESS-INDUCED METABOLIC DEFICITS: ROLE OF THE HPA AXIS, GLUCOCORTICOID, AND TISSUE 11β-HSD1

To uncover the mechanisms underlying early-life stress-induced metabolic derangements, it is first essential to understand the action of glucocorticoids at different concentrations on glucose and insulin homeostasis and lipid metabolism. At pharmacological doses, glucocorticoids act as potent anti-inflammatory agents but high levels of circulating glucocorticoids result in metabolic derangements including increased visceral adiposity, dyslipidemia (increased levels of triglycerides), increased non-esterified fatty acids (NEFA) (142–144), and impaired glucose and insulin tolerance (145–147). In contrast to the anabolic actions of insulin, glucocorticoids are predominantly catabolic, decreasing glucose utilization and insulin sensitivity with both human and animal data revealing insulin intolerance with excess exposure (145, 148).

Glucocorticoids exert tissue-specific metabolic effects, directly targeting tissues for insulin metabolism, and they regulate skeletal muscle, liver, and adipocyte insulin signaling (149). Glucocorticoids alter glucose and protein metabolism; increased levels of

Table 2 | Postnatal early-life stress and metabolic consequences in rodents.

Offspring	Stress protocol	Other interventions	Metabolic consequences	Reference
Male Wistar rats	*MS*: separation for 240 min daily from PND1 to 10 *Control*: non-handled	*Diet*: PND21–35: standard chow PND35 to cull: *n*-3 polyunsaturated fatty acid adequate or deficient diet	*MS vs. control* ↑ Food intake and bodyweight at weaning ↑ Gonadal and retroperitoneal WAT ↑ Plasma triglycerides *MS-deficient diet vs. control-adequate and control-deficient* ↑ Plasma leptin *MS-deficient vs. MS-adequate and control-deficient* ↑ Fasting plasma insulin ↑ HOMA-IR index	Bernardi et al. (134)
Male Sprague-Dawley rats	*MS*: separation for 180 min daily from PND1 to 14 *Control*: non-handled	*Social isolation*: weaned into group housing ($n = 3$ per cage) or isolation (single rat)	*MS vs. control* ↑ Bodyweight at weaning *MS-isolation vs. MS-group* ↑ Bodyweight from PND42 ↑ Food intake at PND42 and 56 *Control-group vs. control-isolation* No significant effect on weight gain with isolation	Ryu et al. (135)
Female Sprague-Dawley rats	*MS*: separation for 180 min daily from PND10 to 15 *Control*: non-handled	*Diet*: high-fat diet (HFD)	No change in bodyweight *Retroperitoneal WAT at 10 weeks of age* ↑ Prohibitin mRNA in MS rats compared to control ($P < 0.001$) *Interscapular BAT at 10 weeks of age* ↓ β3-Adrenergic receptor mRNA in MS rats compared to control ($P < 0.001$) No change in UCP-1 mRNA across groups	Miki et al. (136)
Male and female Sprague-Dawley rats	*MS*: separation for 180 min daily from PND2 to 14 *Control*: 15 min daily from PND2 to 14	*Diet*: weaning to cull: standard chow or cafeteria style HFD *Exercise*: weaning to cull: exercise (voluntary running wheels) or sedentary (locked running wheels)	*MS-chow vs. control* ↑ Plasma corticosterone following restraint stress ↓ Hippocampal GR mRNA expression Reversed with HFD or exercise *MS-HFD vs. control-HFD* ↑ Plasma insulin ↓ Total WAT per gram bodyweight *MS-chow-exercise vs. MS-chow-sedentary* ↓ Plasma corticosterone following restraint stress *MS-HFD-exercise vs. MS-HFD-sedentary* ↓ Plasma insulin	Maniam and Morris (131, 132)
Male and female Wistar rats	*MD*: 24 h maternal deprivation from PND9 to 10 *Control*: non-handled	*Diet*: weaning to cull: standard chow or HFD	*MD-chow vs. control* ↓ Plasma leptin Reversed by HFD consumption *MD-HFD vs. MD-chow and control-HFD* ↑ Hypothalamic IL-1β and TNF-α mRNA *MD-HFD males vs. MD-chow and control males* ↑ HOMA-IR	Mela et al. (130)
Male and female Wistar rats	*MD*: 24 h maternal deprivation from PND9 to 10 *Control*: non-handled	*Diet*: standard chow	*MD vs. control* ↓ Bodyweight until 40–50 days of age ↓ Plasma leptin at PND75 *MD males vs. control males* ↓ Plasma testosterone ↓ PPAR-α mRNA in perirenal adipose tissue at PND35 *MD females vs. control females* ↓ Plasma adiponectin at PND75	Viveros et al. (40)

(Continued)

Table 2 | Continued

Offspring	Stress protocol	Other interventions	Metabolic consequences	Reference
Male and female Sprague-Dawley rats	*LN*: dams and pups subject to limited nesting material from PND2 to 9 *Control*: normal bedding	*Diet*: standard chow	*LN vs. control at PND9* ↓ Bodyweight ↑ Plasma corticosterone and adrenal weight ↓ CRH mRNA in hypothalamic paraventricular nucleus ↓ GR mRNA in hypothalamic paraventricular nucleus and frontal cortex	Avishai-Eliner et al. (137)
Female Wistar rats	*LN*: dams and pups subject to limited nesting material from PND2 to 9 *Control*: normal bedding	*Diet*: weaning to PND111: standard chow PND111–141: standard chow or chow plus HFD Following which rats underwent a 24-h food preference test	*LN vs. control* ↓ Bodyweight ↑ Consumption of palatable HFD as a percentage of total food intake Prior chronic exposure to HFD did not decrease preference for palatable food in LN rats, whereas control demonstrated reduced preference for HFD	Machado et al. (133)
Male and female C56BL/6J mice	*LN*: dams and pups subject to limited nesting material from PND2 to 9 *Control*: normal bedding	*Diet*: standard chow	*LN vs. control at PND9* ↓ Bodyweight, positively correlated to amount of nesting material ↑ Plasma corticosterone ↓ CRH mRNA in hypothalamic paraventricular nucleus *LN vs. control at adulthood* Restored bodyweight ↑ Plasma corticosterone ↓ CRH mRNA in hypothalamic paraventricular nucleus	Rice et al. (45)

glucocorticoids induced during stress increase protein degradation, which results in the generation of amino acids that serve as precursor for glucose synthesis in the liver. In addition, excess glucocorticoids inhibit glucose uptake into muscle by inhibiting translocation of glucose transporter-4 (150–152). Chronically increased circulating or tissue glucocorticoid levels may also lead to insulin resistance, hypertriglyceridemia, and hepatic steatosis (see **Figure 1**). Circulating glucocorticoid concentrations are tightly controlled by activation of the HPA axis, however, tissue-specific availability is regulated by multiple means including glucocorticoid receptor expression, receptor affinity, and alterations in glucocorticoid metabolism and clearance.

Mechanisms regulating intracellular glucocorticoid concentrations are critical to understand the impact of stress on energy metabolism including energy expenditure, storage, and utilization. Intracellular levels of glucocorticoids are influenced by 11β-HSD1 with the type-1 isoform predominantly expressed in the liver (153) and to a lesser degree in adipose and skeletal muscle (154). Evidence shows that tissue glucocorticoid levels are regulated by 11-beta hydroxysteroid dehydrogenase (11β-HSD1) in target tissues (155) as 11β-HSD1 converts inactive cortisone to biologically active cortisol (156, 157). The liver is a major site of glucocorticoid metabolism where 11β-HSD1 regulates the access of glucocorticoid to the glucocorticoid receptor, leading to glucocorticoid metabolism which, is regulated by 5-alpha/beta reductase levels (155, 158). Fat, liver, and muscle-specific increases in 11β-HSD1 are known to increase the risk for metabolic disorders such as insulin resistance, hyperglycemia, and hyperlipidemia (159, 160). Tissue glucocorticoid amplifies the action of insulin to promote lipogenesis within hepatocytes (161). 11β-HSD1 in the liver increases glucocorticoid action in liver to stimulate gluconeogenesis and inhibit beta-oxidation of fat, thus promoting lipid synthesis (162–164). A very recent study demonstrated reduced 5-alpha reductase was associated with fatty liver (165). Thus liver-specific glucocorticoid synthesis and clearance regulated by 11β-HSD1 and 5-alpha reductase appear to affect hepatic lipid accumulation. Interestingly, animal studies demonstrate a link between hepatic glucocorticoid metabolism with regulation of HPA axis activity and lipid synthesis in the liver. For example, transgenic overexpression of 11β-HSD1 in liver of null mice normalized the exaggerated HPA axis activity in response to stress, and led to fatty liver (166, 167). On the other hand, hepatic deletion of 11β-HSD1 led to hyperactivity of the HPA axis (168). These studies suggest liver 11β-HSD1 greatly contributes to amplify circulating glucocorticoid levels, and thus likely mediates the negative feedback activity to dampen HPA axis activity, a concept previously proposed by Chapman et al. (169). Several studies have demonstrated a blunted activity of the HPA axis in response to novel stress following high energy diets such as high sugar or HFD either after adulthood chronic stress or chronic stress exposure during early life (131, 132, 170). As these studies did not report measures of liver 11β-HSD1, it is not clear whether under-activity of the HPA axis following early-life stress and postnatal high energy diet is modulated by liver 11β-HSD1 levels.

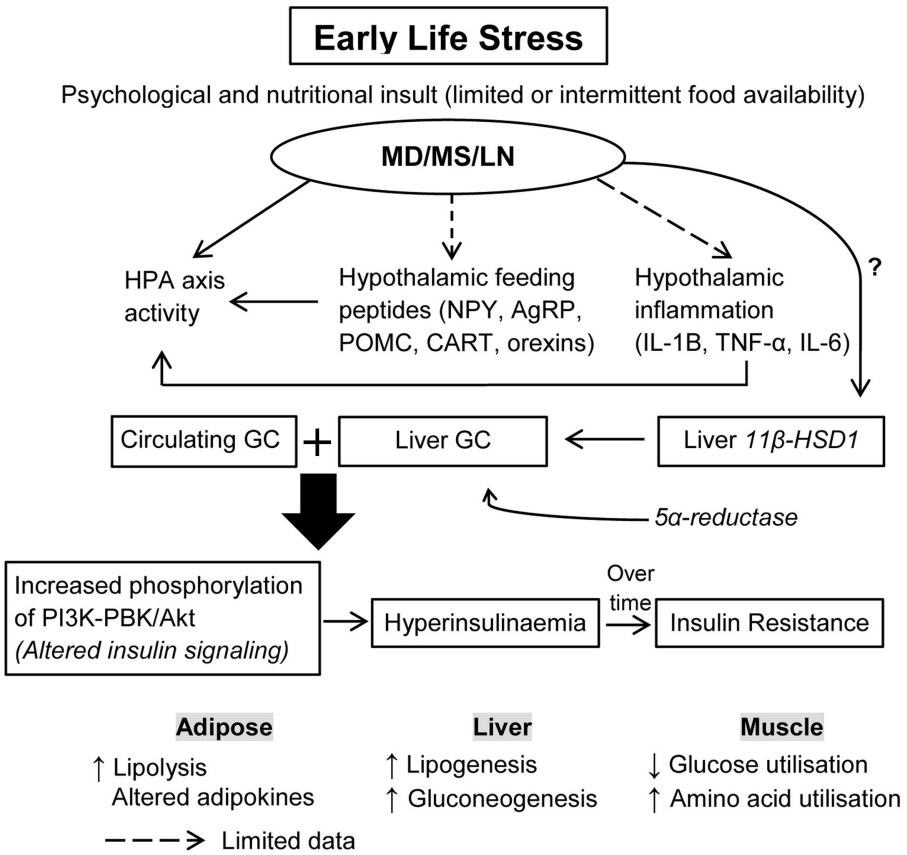


FIGURE 1 | How does ELS increase the risk for insulin resistance and hyperglycemia? Early-life stress (ELS) induced by three different paradigms including maternal deprivation, maternal separation, and limited nesting material are known to dysregulate HPA axis activity, with limited data on the effects of ELS on hypothalamic feeding neuropeptides and inflammation. It is proposed that ELS disturbs circulating glucocorticoids (GC) through a combined action of HPA axis activity, hypothalamic feeding neuropeptides, and inflammatory changes. The effects of ELS on liver 11β-HSD1, an enzyme that converts inactive GC to active GC, and 5α-reductase, an enzyme involved in GC metabolism, is less known. It is proposed that during the maladaptation period, ELS affects tissue levels of these enzymes, thus increasing exposure of peripheral tissues to GC. Excess GC availability can alter insulin signaling, leading to hyperinsulinemia and insulin resistance over time. Thus, increases in circulating and tissue GC induced by ELS act synergistically to exacerbate insulin resistance in peripheral tissues and alter energy expenditure and utilization. ELS, early-life stress; MD, maternal deprivation; MS, maternal separation; LN, limited nesting material; GC, glucocorticoids; TNF-α, tumor necrosis factor alpha; IL-6, interleukin 6; IL-1β, interleukin-1 beta; 11β-HSD1, 11-beta hydroxysteroid dehydrogenase.

Thus, liver 11β-HSD1-HPA axis is a potential pathway in early-life stress-mediated metabolic disturbances, particularly insulin sensitivity, glucose metabolism and lipid synthesis, and mobilization as outlined in **Box 1**. This hypothesis, however, needs systematic examination in the future. Early-life stress, modeled through prenatal dexamethasone treatment, has been shown to upregulate 11β-HSD1 in peripheral tissues such as liver, pancreas, and subcutaneous fat in rat offspring at 4 months with persistent increases at 1 year of age (171). Interestingly, it was previously shown that early-life stress alters the expression of liver 5-alpha reductase mRNA (172, 173). Thus, this suggests a programing effect of early-life stress on tissue 11β-HSD1 expression, glucocorticoid metabolism, and glucocorticoid signaling. There has been limited evidence regarding early postnatal stress effects on 11β-HSD1 expression and glucocorticoid metabolism of peripheral tissues, which needs to be explored in future studies (**Figure 1**).

We propose that early-life stress may alter availability of tissue glucocorticoids and glucocorticoid signaling. Specifically, we propose that during the maladaptation period (see **Figure 2**), early-life stress enhances availability of tissue glucocorticoids via increases in liver 11β-HSD1. The tissue glucocorticoid-induced insulin resistance may involve glucocorticoids altering insulin signaling via increasing phosphorylation of PKB/akt, which stimulates insulin secretion, that is, glucocorticoids work synergistically with insulin which increases adipocyte lipolysis and liver lipogenesis (see **Figure 1**). Another possibility is that early-life stress impairs glucocorticoid signaling involving glucocorticoid–glucocorticoid receptor binding and phosphorylation of the glucocorticoid receptor. Impairment in glucocorticoid signaling leads to alteration in glucocorticoid targeted genes that regulate hepatic glucose production, and hepatic lipogenesis including peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1-alpha), phosphoenolpyruvate carboxykinase

Box 1 | Outstanding research questions/proposals.

1. Does chronic exposure to negative postnatal environments such as HFD and adulthood stressors lead to maladaptation following early-life stress?
2. Is the stress-induced perturbation of metabolic profile mediated by the liver 11β-HSD1-HPA axis interaction?
3. How can positive postnatal environments reverse the early-life stress-induced metabolic damage?
4. What are the mechanism(s) underlying early-life stress-induced improvement of insulin sensitivity and improved glucose metabolism if rodents are maintained on chow diet?

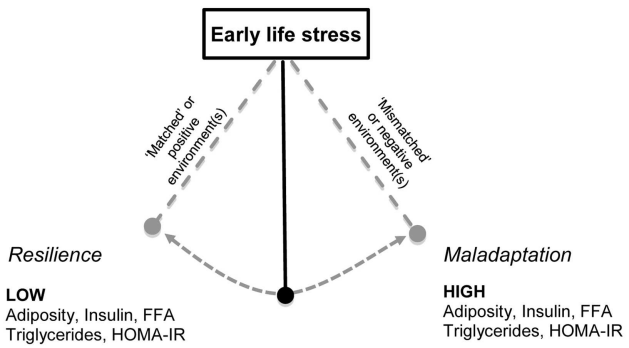


FIGURE 2 | The combination of early-life stress exposure with altered later environment may determine metabolic outcomes. Early-life stress (ELS) exposure during gestation or the postnatal period is hypothesized to influence an offspring's response to later environments (85, 87). This programing occurs in an attempt to facilitate habituation and resilience to future similar situations. Offspring exposed to environments that do not differ to that to which they were exposed during early life, i.e., "matched" or positive environments, such as exposure to exercise have been shown to adapt and demonstrate resilience (74, 114, 131, 132). Conversely, exposure to a negative environment, i.e., "mismatched," such as a sub-optimal diet (131, 132, 134) or chronic stress following ELS may lead to maladaptation, and metabolic deficits, with increased levels of triglycerides, free fatty acids, adiposity, and insulin resistance as measured by HOMA-IR. Thus, there is a pendulum of vulnerability and the trajectory following ELS is influenced by the later life environment.

(PEPCK), glucose 6-phosphate (G6P), and diacylglycerol acyltransferase (DGAT). Genes known to mediate hepatic lipid accumulation such as PGC1-alpha and adipose DGAT1 were altered in rat pups from dams that had been subjected to prenatal stress (174). PGC1-alpha plays an essential role in fatty acid oxidation while increased DGAT1 in adipose tissue increases lipogenesis. DGAT1 transgenic mice fed with an HFD demonstrated a 300% increase in liver triglycerides suggesting a redistribution of the fat from adipose tissue to liver via re-esterification of fatty acid with glycerol (175). Another study showed that prenatal stress induced through manipulation of the availability of food, that is either by limiting intake, or exposing to a high energy food during pregnancy led to fatty liver in pups relative to controls (176).

In conclusion, the role of liver 11β-HSD1 in regulating HPA axis activity, and whether it is modulated by stress early in life, warrants investigation. We propose that early-life stress induces changes in glucocorticoid metabolism and signaling, likely mediating the metabolic consequences reported. The question of how these affect glucocorticoid-induced insulin dependent processes, including hyperinsulinemia and lipid metabolism following an early-life stress exposure will be addressed in the following section.

PROPOSAL OF HOW ELS MAY INCREASE RISK OF INSULIN RESISTANCE, AND HYPERGLYCEMIA

It has been known for decades that stress early in life in both humans and animals can affect HPA axis activity in later life (131, 132, 177–179), however, the effects of early-life stress on hypothalamic neuropeptides involved in feeding are less well known, with only a few studies exploring hypothalamic neuropeptides following maternal separation or deprivation (131, 180). Feeding regulation, which is tightly regulated by orexigenic and anorexigenic hypothalamic neuropeptides, is also influenced by HPA axis activity and circulating glucocorticoid concentrations (181, 182). Glucocorticoids stimulate feeding responses by increasing the release of neuropeptide Y (NPY) and inhibiting that of corticotrophin releasing hormone in the hypothalamus however the orexigenic effect of glucocorticoids may be counteracted by leptin (183).

Mela and colleagues explored the effect of 24 h of maternal deprivation on postnatal day 9 in rats on hypothalamic feeding neuropeptides measured at 14 weeks of age in rats fed with chow or HFD (130). No significant differences in orexigenic (NPY, agouti-related peptide (AgRP) and anorexigenic (pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript) neuropeptides were observed between the chow and HFD fed rats. An interesting finding in this study was that while HFD did not alter these neuropeptides in control females, rats that experienced maternal deprivation had significantly decreased hypothalamic NPY and AgRP mRNA expression and a significant increase in hypothalamic pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript relative to their chow counterparts. The increased caloric intake by HFD fed rats relative to their chow counterparts was similar between the maternally deprived and control rats. But an increased plasma leptin concentration in the maternally deprived HFD fed female rats suggests a programing effect of maternal deprivation which dysregulated feeding homeostasis. Whether this may be linked to either the HPA axis activity or tissue-specific availability of glucocorticoids needs to be explored in the future. The significantly reduced fasting triglycerides and lack of change in fasting insulin by HFD feeding is puzzling, and these may be related to either the strain or the diet used. Thus, use of appropriate controls and dietary conditions are critical to enable exploration of the underlying mechanisms linking combined early-life stress and high-fat feeding with later metabolic risk. Overall, limited studies have directly investigated whether early-life stress affects hypothalamic neuropeptides and inflammation.

The increased availability of glucocorticoids discussed above may play a role in altering tissue insulin signaling by increasing the phosphorylation of PKB/akt. Animals exposed to early-life stress may be resilient to the increased availability of tissue glucocorticoids and thus subsequently show a dampened secretion of insulin. While there are no systematic studies on early-life stress

and metabolic consequences, there is evidence in rodents showing early-life stress does not affect basal metabolic hormones if animals consume chow, including non-fasted plasma insulin and leptin (131, 132). Another study demonstrated improved insulin sensitivity following early-life stress when measured during adulthood (170). Taken together, we propose that early-life stress may lead to resilience, and thus the organism potentially may adapt to any changes in the postnatal environment, such as exposure to high energy diet, or later stressors through enhanced negative feedback sensitivity of the HPA axis activity and reprograming of the peripheral tissue sensitivity to glucocorticoid exposure (see **Figure 2**). However, if an organism exposed to early-life stress is chronically exposed to these negative postnatal environments (high energy diet and later stressors), the enhanced negative feedback sensitivity maybe dysregulated, resulting in perturbed HPA axis activity, leading to a phase known as maladaptation (see **Figure 2**). Indeed there is evidence that such maladaptations can influence glucose/insulin homeostasis, resulting in the manifestation of metabolic disorders including insulin resistance, hyperglycemia, hyperlipidemia, as suggested in **Figure 2**.

During periods of maladaptation, increased tissue glucocorticoids and circulating corticosterone will impair the insulin signaling pathway, leading to hypersecretion of insulin; a condition leading to insulin resistance (see **Figure 2**). This will mediate tissue specific effects, that is, increased adipose lipolysis through beta-oxidation which releases free fatty acids into the circulation and re-esterification in the liver to promote lipogenesis. In addition, increased tissue glucocorticoid levels via insulin stimulation increases hepatic glucose production. Prenatal stress has been shown to increase hepatic PEPCK mRNA and this was enhanced with high energy feeding (174). There are no data thus far on the impact of early postnatal stress on hepatic glucose production, which is an important measure to be considered for future studies to improve understanding of the link between postnatal stress and risk of pre- or diabetes. In addition, increased glucocorticoid-induced hyperinsulinemia also alters the muscle glucose utilization via affecting the glucose transporter gene and expression, including the GLUT4-transporter.

CONCLUSION AND FUTURE DIRECTIONS

Despite significant progress in the field of early life programing and metabolic disease risk many challenges exist. Human studies are vital in providing evidence of the association between early-life adversity and disease incidence but data must be interpreted with necessary caution. A majority of human evidence is based on parental or offspring self-report raising the possibility of confounding due to issues with information recall, lack of accuracy, and the potential for bias.

Future studies in humans should seek to better quantify stress exposure during the early-life period and at the time of assessment to improve knowledge of how different stress types may alter disease risk. Given both environment and genetic predisposition determine health outcome, studies should not only consider stressor experience during early life but also control for socioeconomic status, food and education availability, ethnicity, and lifestyle factors such as nutritional status, smoking, and physical activity throughout their lifetime.

According to the hypothesis explored throughout this review, programed adaptation during early life occurs in an attempt to adapt to the predicted later life environment and hence even seemingly trivial variations in stressors during these periods may vary the observed outcomes. Inconsistency in findings across experiments could be due to the marked differences in study design. Procedural variations in maternal separation have been reviewed elsewhere (177). In the literature, there is no consistent procedure, rather multiple experimental conditions fall under the broad term of maternal separation. Thus, duration, and age at separation, temperature in which pups are separated, whether pups are isolated from litter mates during separation and whether pups are removed or remain in their home cage have all been shown to influence behavioral outcomes and brain function. Animal models must consider the influence of maternal care on long term outcomes, which is a major aspect the novel model of limiting nesting material attempts to explore. Work exploring prenatal or gestational stress exposure could benefit from cross-fostering to better delineate effects of maternal stress and the influence of received postnatal care. Offspring of low care dams that were cross-fostered by high-licking high arched back nursing dams were resilient in terms of the decline in hippocampal synaptogenesis and spatial learning seen in offspring reared by low care dams (184).

Animal research should ultimately aim to improve public health outcomes. To ensure this, analysis of behavioral, physiological, and molecular parameters is required. The current literature lacks assessment of whole body insulin sensitivity measures or assessment of β-cell structural changes – key factors that influence metabolic outcome. Models need to reflect the human condition upon which they are based, which brings the need for valid controls. Given the complex heterogeneity of both the stress system and metabolic disease the phenotype of experimental animals would ideally be comprehensively assessed, rather than examining single factors. Species and strain differences must be considered and these animals must be valid models for the environmental conditions that the study aims to investigate.

Investigation of the environmental influence provides us with an opportunity to better identify factors determining vulnerability and resilience to early-life experience. A better understanding of factors driving the association between genetic predisposition with both early and adult environment will help with the identification of targets for intervention with the hope of minimizing disease incidence. As known, high energy feeding impairs glucose and insulin tolerance and affects lipid metabolism, thus chronic HFD could serve as a good model to study the altered effects of early-life stress on metabolism. Our lab and others have shown early evidence that HFD or altered nutrition lead to altered insulin levels in early-life stressed animals relative to control (131, 132, 134, 170). Together these studies appear to suggest that both short term diet and long term diet exaggerate plasma insulin. Despite this, a single marker or circulating hormone levels limit ability to draw conclusions of how early-life stress may exert negative impact on insulin sensitivity and overall metabolic risk. Future studies need to adopt a mechanistic approach, examining animals using appropriate metabolic tests that will provide answers to the outstanding questions outlined in **Box 1**. An alternate model of early-life stress, variable foraging demand, demonstrated impaired insulin

resistance in non-human primates as measured by hyperglycemic-insulin clamp (185). Functional studies in rodent early-life stress models, such as glucose or insulin tolerance tests, or preferably glucose clamp, would be useful to explore this further.

In conclusion, the mechanisms whereby adverse early-life events accelerate metabolic deficits have received little attention to date. When combined with a sub-optimal subsequent environment (e.g., poor diet, stress, physical inactivity) early-life stress may exacerbate the risk of metabolic disease. One potential mechanism underlying early-life stress-induced metabolic deficits is the interaction between the HPA axis and liver 11β-HSD1. Positive later environments may modulate the negative impact of early-life stress not only on behavioral outcomes, but also on metabolism. Given the burgeoning issues of metabolic and mental health disorders, the question of how early-life stress impacts subsequent disease risk warrants further investigation.

REFERENCES

1. Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol* (2009) **5**:374–81. doi:10.1038/nrendo.2009.106
2. De Kloet ER, Derijk R. Signaling pathways in brain involved in predisposition and pathogenesis of stress-related disease: genetic and kinetic factors affecting the MR/GR balance. *Ann N Y Acad Sci* (2004) **1032**:14–34. doi:10.1196/annals.1314.003
3. McEwen BS, Gianaros PJ. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. *Ann N Y Acad Sci* (2010) **1186**:190–222. doi:10.1111/j.1749-6632.2009.05331.x
4. Dallman MF, Akana SF, Laugero KD, Gomez F, Manalo S, Bell ME, et al. A spoonful of sugar: feedback signals of energy stores and corticosterone regulate responses to chronic stress. *Physiol Behav* (2003) **79**:3–12. doi:10.1016/S0031-9384(03)00100-8
5. Dallman MF, La Fleur SE, Pecoraro NC, Gomez F, Houshyar H, Akana SF. Minireview: glucocorticoids – food intake, abdominal obesity, and wealthy nations in 2004. *Endocrinology* (2004) **145**:2633–8. doi:10.1210/en.2004-0037
6. Dallman MF, Akana SF, Pecoraro NC, Warne JP, La Fleur SE, Foster MT. Glucocorticoids, the etiology of obesity and the metabolic syndrome. *Curr Alzheimer Res* (2007) **4**:199–204. doi:10.2174/156720507780362236
7. Oda N, Nakai A, Mokuno T, Sawai Y, Nishida Y, Mano T, et al. Dexamethasone-induced changes in glucose transporter 4 in rat heart muscle, skeletal muscle and adipocytes. *Eur J Endocrinol* (1995) **133**:121–6. doi:10.1530/eje.0.1330121
8. McEwen BS. Steroid hormones: effect on brain development and function. *Horm Res* (1992) **37**(Suppl 3):1–10. doi:10.1159/000182393
9. Munck A, Koritz SB. Studies on the mode of action of glucocorticoids in rats. I. Early effects of cortisol on blood glucose and on glucose entry into muscle, liver and adipose tissue. *Biochim Biophys Acta* (1962) **57**:310–7. doi:10.1016/0006-3002(62)91124-1
10. Issekutz B Jr, Shaw WA. Glucose turnover in the exercising dog with chemically induced diabetes and the effect of methylprednisolone. *Diabetes* (1975) **24**:915–21. doi:10.2337/diab.24.10.915
11. Calabrese F, Molteni R, Racagni G, Riva MA. Neuronal plasticity: a link between stress and mood disorders. *Psychoneuroendocrinology* (2009) **34**(Suppl 1):S208–16. doi:10.1016/j.psyneuen.2009.05.014
12. Pruessner JC, Dedovic K, Pruessner M, Lord C, Buss C, Collins L, et al. Stress regulation in the central nervous system: evidence from structural and functional neuroimaging studies in human populations – 2008 Curt Richter Award Winner. *Psychoneuroendocrinology* (2010) **35**:179–91. doi:10.1016/j.psyneuen.2009.02.016
13. Heim C, Binder EB. Current research trends in early life stress and depression: review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Exp Neurol* (2012) **233**:102–11. doi:10.1016/j.expneurol.2011.10.032
14. Bjorntorp P, Holm G, Rosmond R. Hypothalamic arousal, insulin resistance and type 2 diabetes mellitus. *Diabet Med* (1999) **16**:373–83. doi:10.1046/j.1464-5491.1999.00067.x
15. Bjorntorp P, Rosmond R. Hypothalamic origin of the metabolic syndrome X. *Ann N Y Acad Sci* (1999) **892**:297–307. doi:10.1111/j.1749-6632.1999.tb07803.x
16. Buchmann AF, Kopf D, Westphal S, Lederbogen F, Banaschewski T, Esser G, et al. Impact of early parental child-rearing behavior on young adults' cardiometabolic risk profile: a prospective study. *Psychosom Med* (2010) **72**:156–62. doi:10.1097/PSY.0b013e3181c88343
17. Lesage J, Del-Favero F, Leonhardt M, Louvart H, Maccari S, Vieau D, et al. Prenatal stress induces intrauterine growth restriction and programmes glucose intolerance and feeding behaviour disturbances in the aged rat. *J Endocrinol* (2004) **181**:291–6. doi:10.1677/joe.0.1810291
18. Nemeroff CB. Neurobiological consequences of childhood trauma. *J Clin Psychiatry* (2004) **65**(Suppl 1):18–28.
19. Cohen RA, Grieve S, Hoth KF, Paul RH, Sweet L, Tate D, et al. Early life stress and morphometry of the adult anterior cingulate cortex and caudate nuclei. *Biol Psychiatry* (2006) **59**:975–82. doi:10.1016/j.biopsych.2005.12.016
20. Foscolo DR, Foscolo RB, Marubayashi U, Reis AM, Coimbra CC. Neonatal maternal separation affects endocrine and metabolic stress responses to ether exposure but not to restraint exposure in adult rats. *Metab Brain Dis* (2008) **23**:375–85. doi:10.1007/s11011-008-9102-9
21. Carpenter LL, Tyrka AR, Ross NS, Khoury L, Anderson GM, Price LH. Effect of childhood emotional abuse and age on cortisol responsivity in adulthood. *Biol Psychiatry* (2009) **66**:69–75. doi:10.1016/j.biopsych.2009.02.030
22. Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. *Front Behav Neurosci* (2009) **3**:19. doi:10.3389/neuro.08.019.2009
23. Brunton PJ, Sullivan KM, Kerrigan D, Russell JA, Seckl JR, Drake AJ. Sex-specific effects of prenatal stress on glucose homoeostasis and peripheral metabolism in rats. *J Endocrinol* (2013) **217**:161–73. doi:10.1530/JOE-12-0540
24. Paternain L, de la Garza AL, Batlle MA, Milagro FI, Martinez JA, Campion J. Prenatal stress increases the obesogenic effects of a high-fat-sucrose diet in adult rats in a sex-specific manner. *Stress* (2013) **16**:220–32. doi:10.3109/10253890.2012.707708
25. de Vries A, Holmes MC, Heijnis A, Seier JV, Heerden J, Louw J, et al. Prenatal dexamethasone exposure induces changes in nonhuman primate offspring cardiometabolic and hypothalamic-pituitary-adrenal axis function. *J Clin Invest* (2007) **117**:1058–67. doi:10.1172/JCI30982
26. Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal "programming" of adult pathophysiology. *Nat Clin Pract Endocrinol Metab* (2007) **3**:479–88. doi:10.1038/ncpendmet0515
27. Dobbing J. The later growth of the brain and its vulnerability. *Pediatrics* (1974) **53**:2–6.
28. Giedd JN, Snell JW, Lange N, Rajapakse JC, Casey BJ, Kozuch PL, et al. Quantitative magnetic resonance imaging of human brain development: ages 4-18. *Cereb Cortex* (1996) **6**:551–60. doi:10.1093/cercor/6.4.551
29. Lenroot RK, Giedd JN. Brain development in children and adolescents: insights from anatomical magnetic resonance imaging. *Neurosci Biobehav Rev* (2006) **30**:718–29. doi:10.1016/j.neubiorev.2006.06.001
30. Tau GZ, Peterson BS. Normal development of brain circuits. *Neuropsychopharmacology* (2010) **35**:147–68. doi:10.1038/npp.2009.115
31. De Bellis MD, Keshavan MS, Clark DB, Casey BJ, Giedd JN, Boring AM, et al. A.E. Bennett Research Award. Developmental traumatology. Part II: brain development. *Biol Psychiatry* (1999) **45**:1271–84. doi:10.1016/S0006-3223(99)00045-1
32. Suchecki D, Nelson DY, van Oers H, Levine S. Activation and inhibition of the hypothalamic-pituitary-adrenal axis of the neonatal rat: effects of maternal deprivation. *Psychoneuroendocrinology* (1995) **20**:169–82. doi:10.1016/0306-4530(94)00051-B
33. 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**:10171–9.
34. Sapolsky RM, Meaney MJ. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Res* (1986) **396**:64–76. doi:10.1016/0165-0173(86)90010-X
35. Ivy AS, Brunson KL, Sandman C, Baram TZ. Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. *Neuroscience* (2008) **154**:1132–42. doi:10.1016/j.neuroscience.2008.04.019
36. Hofer MA. Maternal separation affects infant rats' behavior. *Behav Biol* (1973) **9**:629–33. doi:10.1016/S0091-6773(73)80057-4

37. Meaney MJ, Aitken DH. The effects of early postnatal handling on hippocampal glucocorticoid receptor concentrations: temporal parameters. *Brain Res* (1985) **22**:301–4. doi:10.1016/0165-3806(85)90183-X
38. 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**:49–72. doi:10.1159/000111395
39. Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinepherine release in the hypothalamic paraventricular nucleus. *J Neuroendocrinol* (2000) **12**:5–12. doi:10.1046/j.1365-2826.2000.00422.x
40. Viveros MP, Diaz F, Mateos B, Rodriguez N, Chowen JA. Maternal deprivation induces a rapid decline in circulating leptin levels and sexually dimorphic modifications in hypothalamic trophic factors and cell turnover. *Horm Behav* (2010) **57**:405–14. doi:10.1016/j.yhbeh.2010.01.009
41. Schmidt MV, Enthoven L, van der Mark M, Levine S, de Kloet ER, Oitzl MS. The postnatal development of the hypothalamic-pituitary-adrenal axis in the mouse. *Int J Dev Neurosci* (2003) **21**:125–32. doi:10.1016/S0736-5748(03)00030-3
42. Schmidt M, Enthoven L, van Woezik JH, Levine S, de Kloet ER, Oitzl MS. The dynamics of the hypothalamic-pituitary-adrenal axis during maternal deprivation. *J Neuroendocrinol* (2004) **16**:52–7. doi:10.1111/j.1365-2826.2004.01123.x
43. 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**:865–74. doi:10.1111/j.1365-2826.2006.01482.x
44. Viveros MP, Llorente R, Lopez-Gallardo M, Suarez J, Bermudez-Silva F, De la Fuente M, et al. Sex-dependent alterations in response to maternal deprivation in rats. *Psychoneuroendocrinology* (2009) **34**(Suppl 1):S217–26. doi:10.1016/j.psyneuen.2009.05.015
45. Rice CJ, Sandman CA, Lenjavi MR, Baram TZ. A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology* (2008) **149**:4892–900. doi:10.1210/en.2008-0633
46. Baram TZ, Davis EP, Obenaus A, Sandman CA, Small SL, Solodkin A, et al. Fragmentation and unpredictability of early-life experience in mental disorders. *Am J Psychiatry* (2012) **169**:907–15. doi:10.1176/appi.ajp.2012.11091347
47. Conti G, Hansman C, Heckman JJ, Novak MF, Ruggiero A, Suomi SJ. Primate evidence on the late health effects of early-life adversity. *Proc Natl Acad Sci U S A* (2012) **109**:8866–71. doi:10.1073/pnas.1205340109
48. Kaufman D, Banerji MA, Shorman I, Smith ELP, Coplan JD, Rosenblum LA, et al. Early-life stress and the development of obesity and insulin resistance in juvenile bonnet macaques. *Diabetes* (2007) **56**:1382–6. doi:10.2337/db06-1409
49. Cirulli F, Berry A, Alleva E. Early disruption of the mother-infant relationship: effects on brain plasticity and implications for psychopathology. *Neurosci Biobehav Rev* (2003) **27**:73–82. doi:10.1016/S0149-7634(03)00010-1
50. D'Argenio A, Mazzi C, Pecchioli L, Di Lorenzo G, Siracusano A, Troisi A. Early trauma and adult obesity: is psychological dysfunction the mediating mechanism? *Physiol Behav* (2009) **98**:543–6. doi:10.1016/j.physbeh.2009.08.010
51. Gunstad J, Paul RH, Spitznagel MB, Cohen RA, Williams LM, Kohn M, et al. Exposure to early life trauma is associated with adult obesity. *Psychiatry Res* (2006) **142**:31–7. doi:10.1016/j.psychres.2005.11.007
52. Ward AM, Syddall HE, Wood PJ, Chrousos GP, Phillips DI. Fetal programming of the hypothalamic-pituitary-adrenal (HPA) axis: low birth weight and central HPA regulation. *J Clin Endocrinol Metab* (2004) **89**:1227–33. doi:10.1210/jc.2003-030978
53. Wust S, Entringer S, Federenko IS, Schlotz W, Hellhammer DH. Birth weight is associated with salivary cortisol responses to psychosocial stress in adult life. *Psychoneuroendocrinology* (2005) **30**:591–8. doi:10.1016/j.psyneuen.2005.01.008
54. Miller AL, Clifford C, Sturza J, Rosenblum K, Vazquez DM, Kaciroti N, et al. Blunted cortisol response to stress is associated with higher body mass index in low-income preschool-aged children. *Psychoneuroendocrinology* (2013) **38**:2611–7. doi:10.1016/j.psyneuen.2013.06.014
55. Mi J, Law C, Zhang KL, Osmond C, Stein C, Barker D. Effects of infant birthweight and maternal body mass index in pregnancy on components of the insulin resistance syndrome in China. *Ann Intern Med* (2000) **132**:253–60. doi:10.7326/0003-4819-132-4-200002150-00002
56. Class QA, Lichtenstein P, Langstrom N, D'Onofrio BM. Timing of prenatal maternal exposure to severe life events and adverse pregnancy outcomes: a population study of 2.6 million pregnancies. *Psychosom Med* (2011) **73**:234–41. doi:10.1097/PSY.0b013e31820a62ce
57. Rondo PH, Ferreira RF, Nogueira F, Ribeiro MC, Lobert H, Artes R. Maternal psychological stress and distress as predictors of low birth weight, prematurity and intrauterine growth retardation. *Eur J Clin Nutr* (2003) **57**:266–72. doi:10.1038/sj.ejcn.1601526
58. Wainstock T, Anteby E, Glasser S, Shoham-Vardi I, Lerner-Geva L. The association between prenatal maternal objective stress, perceived stress, preterm birth and low birthweight. *J Matern Fetal Neonatal Med* (2013) **26**:973–7. doi:10.3109/14767058.2013.766696
59. Kajantie E. Fetal origins of stress-related adult disease. *Ann N Y Acad Sci* (2006) **1083**:11–27. doi:10.1196/annals.1367.026
60. Li J, Olsen J, Vestergaard M, Obel C, Kristensen JK, Virk J. Prenatal exposure to bereavement and type-2 diabetes: a Danish longitudinal population based study. *PLoS One* (2012) **7**:e43508. doi:10.1371/journal.pone.0043508
61. Feldman PJ, Dunkel-Schetter C, Sandman CA, Wadhwa PD. Maternal social support predicts birth weight and fetal growth in human pregnancy. *Psychosom Med* (2000) **62**:715–25.
62. Entringer S, Wüst S, Kumsta R, Layes IM, Nelson EL, Hellhammer DH, et al. Prenatal psychosocial stress exposure is associated with insulin resistance in young adults. *Am J Obstet Gynecol* (2008) **199**:498.e1–7. doi:10.1016/j.ajog.2008.03.006
63. Ravelli AC, van der Meulen JH, Michels RP, Osmond C, Barker DJ, Hales CN, et al. Glucose tolerance in adults after prenatal exposure to famine. *Lancet* (1998) **351**:173–7. doi:10.1016/S0140-6736(05)79096-6
64. van Abeelen AFM, Elias SG, Bossuyt PMM, Grobbee DE, van der Schouw YT, Roseboom TJ, et al. Famine exposure in the young and the risk of type 2 diabetes in adulthood. *Diabetes* (2012) **61**:2255–60. doi:10.2337/db11-1559
65. Zheng X, Wang Y, Ren W, Luo R, Zhang S, Zhang JH, et al. Risk of metabolic syndrome in adults exposed to the great Chinese famine during the fetal life and early childhood. *Eur J Clin Nutr* (2012) **66**:231–6. doi:10.1038/ejcn.2011.161
66. Hult M, Tornhammar P, Ueda P, Chima C, Bonamy AK, Ozumba B, et al. Hypertension, diabetes and overweight: looming legacies of the Biafran famine. *PLoS One* (2010) **5**:e13582. doi:10.1371/journal.pone.0013582
67. Flory JD, Bierer LM, Yehuda R. Maternal exposure to the holocaust and health complaints in offspring. *Dis Markers* (2011) **30**:133–9. doi:10.3233/DMA-2011-0748
68. Dancause KN, Laplante DP, Fraser S, Brunet A, Ciampi A, Schmitz N, et al. Prenatal exposure to a natural disaster increases risk for obesity in 5(1/2)-year-old children. *Pediatr Res* (2012) **71**:126–31. doi:10.1038/pr.2011.18
69. Dancause KN, Veru F, Andersen RE, Laplante DP, King S. Prenatal stress due to a natural disaster predicts insulin secretion in adolescence. *Early Hum Dev* (2013) **89**:773–6. doi:10.1016/j.earlhumdev.2013.06.006
70. Ravelli AC, van der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr* (1999) **70**:811–6.
71. Watt TT, Appel L, Roberts K, Flores B, Morris S. Sugar, stress, and the supplemental nutrition assistance program: early childhood obesity risks among a clinic-based sample of low-income Hispanics. *J Community Health* (2013) **38**:513–20. doi:10.1007/s10900-012-9641-1
72. Ertel KA, Koenen KC, Rich-Edwards JW, Gillman MW. Antenatal and postpartum depressive symptoms are differentially associated with early childhood weight and adiposity. *Paediatr Perinat Epidemiol* (2010) **24**:179–89. doi:10.1111/j.1365-3016.2010.01098.x
73. Alciati A, Gesuele F, Casazza G, Foschi D. The relationship between childhood parental loss and metabolic syndrome in obese subjects. *Stress Health* (2013) **29**:5–13. doi:10.1002/smi.1435
74. Gundersen C, Lohman BJ, Garasky S, Stewart S, Eisenmann J. Food security, maternal stressors, and overweight among low-income US children: results from the National Health and Nutrition Examination Survey (1999-2002). *Pediatrics* (2008) **122**:e529–40. doi:10.1542/peds.2008-0556
75. Rich-Edwards JW, Spiegelman D, Lividoti Hibert EN, Jun HJ, Todd TJ, Kawachi I, et al. Abuse in childhood and adolescence as a predictor of type 2 diabetes in adult women. *Am J Prev Med* (2010) **39**:529–36. doi:10.1016/j.amepre.2010.09.007

76. Danese A, Pariante CM, Caspi A, Taylor A, Poulton R. Childhood maltreatment predicts adult inflammation in a life-course study. *Proc Natl Acad Sci U S A* (2007) **104**:1319–24. doi:10.1073/pnas.0610362104
77. Midei AJ, Matthews KA, Chang YF, Bromberger JT. Childhood physical abuse is associated with incident metabolic syndrome in mid-life women. *Health Psychol* (2013) **32**:121–7. doi:10.1037/a0027891
78. Lissau I, Sorensen TI. Parental neglect during childhood and increased risk of obesity in young adulthood. *Lancet* (1994) **343**:324–7. doi:10.1016/S0140-6736(94)91163-0
79. Thomas C, Hypponen E, Power C. Obesity and type 2 diabetes risk in midadult life: the role of childhood adversity. *Pediatrics* (2008) **121**:e1240–9. doi:10.1542/peds.2007-2403
80. Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Whorwood CB, et al. Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. *J Clin Endocrinol Metab* (2001) **86**:245–50. doi:10.1210/jc.86.1.245
81. Kant GJ, Eggleston T, Landman-Roberts L, Kenion CC, Driver GC, Meyerhoff JL. Habituation to repeated stress is stressor specific. *Pharmacol Biochem Behav* (1985) **22**:631–4. doi:10.1016/0091-3057(85)90286-2
82. Chrousos GP. Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. *Ann N Y Acad Sci* (1998) **851**:311–35. doi:10.1111/j.1749-6632.1998.tb09006.x
83. Grissom N, Bhatnagar S. Habituation to repeated stress: get used to it. *Neurobiol Learn Mem* (2009) **92**:215–24. doi:10.1016/j.nlm.2008.07.001
84. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER. The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* (2013) **38**:1858–73. doi:10.1016/j.psyneuen.2013.06.008
85. Gluckman PD, Hanson MA, Beedle AS. Early life events and their consequences for later disease: a life history and evolutionary perspective. *Am J Hum Biol* (2007) **19**:1–19. doi:10.1002/ajhb.20590
86. McEwen BS. Stress, adaptation, and disease. Allostasis and allostatic load. *Ann N Y Acad Sci* (1998) **840**:33–44. doi:10.1111/j.1749-6632.1998.tb09546.x
87. Schmidt MV. Animal models for depression and the mismatch hypothesis of disease. *Psychoneuroendocrinology* (2011) **36**:330–8. doi:10.1016/j.psyneuen.2010.07.001
88. Ng DM, Jeffery RW. Relationships between perceived stress and health behaviors in a sample of working adults. *Health Psychol* (2003) **22**:638–42. doi:10.1037/0278-6133.22.6.638
89. Keyes KM, Hatzenbuehler ML, Grant BF, Hasin DS. Stress and alcohol: epidemiologic evidence. *Alcohol Res* (2012) **34**:391–400.
90. Sinha R. Chronic stress, drug use, and vulnerability to addiction. *Ann N Y Acad Sci* (2008) **1141**:105–30. doi:10.1196/annals.1441.030
91. Stults-Kolehmainen MA, Sinha R. The effects of stress on physical activity and exercise. *Sports Med* (2014) **44**:81–121. doi:10.1007/s40279-013-0090-5
92. Brown R, Ogden J. Children's eating attitudes and behaviour: a study of the modelling and control theories of parental influence. *Health Educ Res* (2004) **19**:261–71. doi:10.1093/her/cyg040
93. Ornelas IJ, Perreira KM, Ayala GX. Parental influences on adolescent physical activity: a longitudinal study. *Int J Behav Nutr Phys Act* (2007) **4**:3. doi:10.1186/1479-5868-4-3
94. Hesketh KR, Goodfellow L, Ekelund U, McMinn AM, Godfrey KM, Inskip HM, et al. Activity levels in mothers and their preschool children. *Pediatrics* (2014) **133**:e973–80. doi:10.1542/peds.2013-3153
95. Lussana F, Painter RC, Ocke MC, Buller HR, Bossuyt PM, Roseboom TJ. Prenatal exposure to the Dutch famine is associated with a preference for fatty foods and a more atherogenic lipid profile. *Am J Clin Nutr* (2008) **88**:1648–52. doi:10.3945/ajcn.2008.26140
96. te Velde SJ, Twisk JW, van Mechelen W, Kemper HC. A birth-weight questionnaire indicated that life style modifies the birth weight and metabolic syndrome relationship at age 36. *J Clin Epidemiol* (2005) **58**:1172–9. doi:10.1016/j.jclinepi.2005.03.013
97. Ridgway CL, Brage S, Anderssen SA, Sardinha LB, Andersen LB, Ekelund U. Do physical activity and aerobic fitness moderate the association between birth weight and metabolic risk in youth? The European Youth Heart Study. *Diabetes Care* (2011) **34**:187–92. doi:10.2337/dc10-1178
98. Kaseva N, Wehkalampi K, Strang-Karlsson S, Salonen M, Pesonen AK, Raikkonen K, et al. Lower conditioning leisure-time physical activity in young adults born preterm at very low birth weight. *PLoS One* (2012) **7**:e32430. doi:10.1371/journal.pone.0032430
99. Falk B, Eliakim A, Dotan R, Liebermann DG, Regev R, Bar-Or O. Birth weight and physical ability in 5- to 8-yr-old healthy children born prematurely. *Med Sci Sports Exerc* (1997) **29**:1124–30. doi:10.1097/00005768-199709000-00002
100. Nemet D, Dolfin T, Litmanowitz I, Shainkin-Kestenbaum R, Lis M, Eliakim A. Evidence for exercise-induced bone formation in premature infants. *Int J Sports Med* (2002) **23**:82–5. doi:10.1055/s-2002-20134
101. Franks PW, Ekelund U, Brage S, Wong MY, Wareham NJ. Does the association of habitual physical activity with the metabolic syndrome differ by level of cardiorespiratory fitness? *Diabetes Care* (2004) **27**:1187–93. doi:10.2337/diacare.27.5.1187
102. Ortega FB, Ruiz JR, Hurtig-Wennlof A, Meirhaeghe A, Gonzalez-Gross M, Moreno LA, et al. Physical activity attenuates the effect of low birth weight on insulin resistance in adolescents: findings from two observational studies. *Diabetes* (2011) **60**:2295–9. doi:10.2337/db10-1670
103. Aoyama T, Tsushita K, Miyatake N, Numata T, Miyachi M, Tabata I, et al. Does cardiorespiratory fitness modify the association between birth weight and insulin resistance in adult life? *PLoS One* (2013) **8**:e73967. doi:10.1371/journal.pone.0073967
104. Volkow ND, Wise RA. How can drug addiction help us understand obesity? *Nat Neurosci* (2005) **8**:555–60. doi:10.1038/nn1452
105. Mason SM, Flint AJ, Field AE, Austin SB, Rich-Edwards JW. Abuse victimization in childhood or adolescence and risk of food addiction in adult women. *Obesity (Silver Spring)* (2013) **21**:E775–81. doi:10.1002/oby.20500
106. Parks EP, Kumanyika S, Moore RH, Stettler N, Wrotniak BH, Kazak A. Influence of stress in parents on child obesity and related behaviors. *Pediatrics* (2012) **130**:e1096–104. doi:10.1542/peds.2012-0895
107. Dallman MF, Pecoraro NC, La Fleur SE. Chronic stress and comfort foods: self-medication and abdominal obesity. *Brain Behav Immun* (2005) **19**:275–80. doi:10.1016/j.bbi.2004.11.004
108. Gibson EL. Emotional influences on food choice: sensory, physiological and psychological pathways. *Physiol Behav* (2006) **89**:53–61. doi:10.1016/j.physbeh.2006.01.024
109. Adam TC, Epel ES. Stress, eating and the reward system. *Physiol Behav* (2007) **91**:449–58. doi:10.1016/j.physbeh.2007.04.011
110. Martikainen S, Pesonen AK, Lahti J, Heinonen K, Feldt K, Pyhala R, et al. Higher levels of physical activity are associated with lower hypothalamic-pituitary-adrenocortical axis reactivity to psychosocial stress in children. *J Clin Endocrinol Metab* (2013) **98**:E619–27. doi:10.1210/jc.2012-3745
111. Matthews KA, Chang YF, Thurston RC, Bromberger JT. Child abuse is related to inflammation in mid-life women: role of obesity. *Brain Behav Immun* (2014) **36**:29–34. doi:10.1016/j.bbi.2013.09.013
112. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol* (2011) **11**:607–15. doi:10.1038/nri3041
113. Koenig JI, Elmer GI, Shepard PD, Lee PR, Mayo C, Joy B, et al. Prenatal exposure to a repeated variable stress paradigm elicits behavioral and neuroendocrinological changes in the adult offspring: potential relevance to schizophrenia. *Behav Brain Res* (2005) **156**:251–61. doi:10.1016/j.bbr.2004.05.030
114. Tamashiro KL, Terrillion CE, Hyun J, Koenig JI, Moran TH. Prenatal stress or high-fat diet increases susceptibility to diet-induced obesity in rat offspring. *Diabetes* (2009) **58**:1116–25. doi:10.2337/db08-1129
115. Bian XP, Seidler FJ, Slotkin TA. Promotional role for glucocorticoids in the development of intracellular signalling: enhanced cardiac and renal adenylate cyclase reactivity to beta-adrenergic and non-adrenergic stimuli after low-dose fetal dexamethasone exposure. *J Dev Physiol* (1992) **17**:289–97.
116. Fowden AL. Endocrine regulation of fetal growth. *Reprod Fertil Dev* (1995) **7**:351–63. doi:10.1071/RD9950351
117. Cottrell EC, Holmes MC, Livingstone DE, Kenyon CJ, Seckl JR. Reconciling the nutritional and glucocorticoid hypotheses of fetal programming. *FASEB J* (2012) **26**:1866–74. doi:10.1096/fj.12-203489
118. Duthie L, Reynolds RM. Changes in the maternal hypothalamic-pituitary-adrenal axis in pregnancy and postpartum: influences on maternal and fetal outcomes. *Neuroendocrinology* (2013) **98**:106–15. doi:10.1159/000354702
119. Seckl JR. Prenatal glucocorticoids and long-term programming. *Eur J Endocrinol* (2004) **151**(Suppl 3):U49–62. doi:10.1530/eje.0.151U049

120. Lesage J, Blondeau B, Grino M, Breant B, Dupouy JP. Maternal undernutrition during late gestation induces fetal overexposure to glucocorticoids and intrauterine growth retardation, and disturbs the hypothalamo-pituitary adrenal axis in the newborn rat. *Endocrinology* (2001) **142**:1692–702. doi:10.1210/endo.142.5.8139
121. Paternain L, Batlle MA, De la Garza AL, Milagro FI, Martinez JA, Campion J. Transcriptomic and epigenetic changes in the hypothalamus are involved in an increased susceptibility to a high-fat-sucrose diet in prenatally stressed female rats. *Neuroendocrinology* (2012) **96**:249–60. doi:10.1159/000341684
122. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenolpyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest* (1998) **101**:2174–81. doi:10.1172/JCI1567
123. Cleasby ME, Livingstone DE, Nyirenda MJ, Seckl JR, Walker BR. Is programming of glucocorticoid receptor expression by prenatal dexamethasone in the rat secondary to metabolic derangement in adulthood? *Eur J Endocrinol* (2003) **148**:129–38. doi:10.1530/eje.0.1480129
124. Jones AP, Simson EL, Friedman MI. Gestational undernutrition and the development of obesity in rats. *J Nutr* (1984) **114**:1484–92.
125. Bertram CE, Hanson MA. Animal models and programming of the metabolic syndrome. *Br Med Bull* (2001) **60**:103–21. doi:10.1093/bmb/60.1.103
126. Remacle C, Bieswal F, Bol V, Reusens B. Developmental programming of adult obesity and cardiovascular disease in rodents by maternal nutrition imbalance. *Am J Clin Nutr* (2011) **94**:1846S–52S. doi:10.3945/ajcn.110.001651
127. Vickers MH, Gluckman PD, Coveny AH, Hofman PL, Cutfield WS, Gertler A, et al. Neonatal leptin treatment reverses developmental programming. *Endocrinology* (2005) **146**:4211–6. doi:10.1210/en.2005-0581
128. Vickers MH, Breier BH, McCarthy D, Gluckman PD. Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. *Am J Physiol Regul Integr Comp Physiol* (2003) **285**:R271–3.
129. Llorente-Berzal A, Fuentes S, Gagliano H, Lopez-Gallardo M, Armario A, Viveros MP, et al. Sex-dependent effects of maternal deprivation and adolescent cannabinoid treatment on adult rat behaviour. *Addict Biol* (2011) **16**:624–37. doi:10.1111/j.1369-1600.2011.00318.x
130. Mela V, Llorente-Berzal A, Diaz F, Argente J, Viveros MP, Chowen JA. Maternal deprivation exacerbates the response to a high fat diet in a sexually dimorphic manner. *PLoS One* (2012) **7**(11):e48915. doi:10.1371/journal.pone.0048915
131. Maniam J, Morris MJ. Palatable cafeteria diet ameliorates anxiety and depression-like symptoms following an adverse early environment. *Psychoneuroendocrinology* (2010) **35**:717–28. doi:10.1016/j.psyneuen.2009.10.013
132. Maniam J, Morris MJ. Voluntary exercise and palatable high-fat diet both improve behavioural profile and stress responses in male rats exposed to early life stress: role of hippocampus. *Psychoneuroendocrinology* (2010) **35**:1553–64. doi:10.1016/j.psyneuen.2010.05.012
133. Machado TD, Molle RD, Laureano DP, Portella AK, Werlang ICR, Benetti CD, et al. Early life stress is associated with anxiety, increased stress responsivity and preference for "comfort foods" in adult female rats. *Stress* (2013) **16**:549–56. doi:10.3109/10253890.2013.816841
134. Bernardi JR, Ferreira CF, Senter G, Krolow R, de Aguiar BW, Portella AK, et al. Early life stress interacts with the diet deficiency of omega-3 fatty acids during the life course increasing the metabolic vulnerability in adult rats. *PLoS One* (2013) **8**(4):e62031. doi:10.1371/journal.pone.0062031
135. Ryu V, Yoo SB, Kang DW, Lee JH, Jahng JW. Post-weaning isolation promotes food intake and body weight gain in rats that experienced neonatal maternal separation. *Brain Res* (2009) **1295**:127–34. doi:10.1016/j.brainres.2009.08.006
136. Miki T, Liu JQ, Ohta K, Suzuki S, Kusaka T, Warita K, et al. Early postnatal maternal separation causes alterations in the expression of beta3-adrenergic receptor in rat adipose tissue suggesting long-term influence on obesity. *Biochem Biophys Res Commun* (2013) **442**:68–71. doi:10.1016/j.bbrc.2013.11.005
137. 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**:799–807. doi:10.1046/j.1365-2826.2001.00698.x
138. Pihoker C, Owens MJ, Kuhn CM, Schanberg SM, Nemeroff CB. Maternal separation in neonatal rats elicits activation of the hypothalamic-pituitary-adrenocortical axis: a putative role for corticotropin-releasing factor. *Psychoneuroendocrinology* (1993) **18**:485–93. doi:10.1016/0306-4530(93)90042-J
139. Nilsson C, Jennische E, Ho HP, Eriksson E, Bjorntorp P, Holmang A. Increased insulin sensitivity and decreased body weight in female rats after postnatal corticosterone exposure. *Eur J Endocrinol* (2002) **146**:847–54. doi:10.1530/eje.0.1460847
140. Shpilberg Y, Beaudry JL, D'Souza A, Campbell JE, Peckett A, Riddell MC. A rodent model of rapid-onset diabetes induced by glucocorticoids and high-fat feeding. *Dis Model Mech* (2012) **5**:671–80. doi:10.1242/dmm.008912
141. D'Souza AM, Beaudry JL, Szigiato AA, Trumble SJ, Snook LA, Bonen A, et al. Consumption of a high-fat diet rapidly exacerbates the development of fatty liver disease that occurs with chronically elevated glucocorticoids. *Am J Physiol Gastrointest Liver Physiol* (2012) **302**:G850–63. doi:10.1152/ajpgi.00378.2011
142. Taskinen MR, Nikkila EA, Pelkonen R, Sane T. Plasma lipoproteins, lipolytic enzymes, and very low density lipoprotein triglyceride turnover in Cushing's syndrome. *J Clin Endocrinol Metab* (1983) **57**:619–26. doi:10.1210/jcem-57-3-619
143. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* (2000) **21**:697–738. doi:10.1210/edrv.21.6.0415
144. Rockall AG, Sohaib SA, Evans D, Kaltsas G, Isidori AM, Monson JP, et al. Computed tomography assessment of fat distribution in male and female patients with Cushing's syndrome. *Eur J Endocrinol* (2003) **149**:561–7. doi:10.1530/eje.0.1490561
145. Buren J, Liu HX, Jensen J, Eriksson JW. Dexamethasone impairs insulin signalling and glucose transport by depletion of insulin receptor substrate-1, phosphatidylinositol 3-kinase and protein kinase B in primary cultured rat adipocytes. *Eur J Endocrinol* (2002) **146**:419–29. doi:10.1530/eje.0.1460419
146. Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther* (2002) **96**:23–43. doi:10.1016/S0163-7258(02)00297-8
147. Bernal-Mizrachi C, Weng S, Feng C, Finck BN, Knutsen RH, Leone TC, et al. Dexamethasone induction of hypertension and diabetes is PPAR-alpha dependent in LDL receptor-null mice. *Nat Med* (2003) **9**:1069–75. doi:10.1038/nm898
148. Reynolds RM, Walker BR. Human insulin resistance: the role of glucocorticoids. *Diabetes Obes Metab* (2003) **5**:5–12. doi:10.1046/j.1463-1326.2003.00221.x
149. Kuo T, Lew MJ, Mayba O, Harris CA, Speed TP, Wang JC. Genome-wide analysis of glucocorticoid receptor-binding sites in myotubes identifies gene networks modulating insulin signaling. *Proc Natl Acad Sci U S A* (2012) **109**:11160–5. doi:10.1073/pnas.1111334109
150. Hajduch E, Hainault I, Meunier C, Jardel C, Hainque B, Guerre-Millo M, et al. Regulation of glucose transporters in cultured rat adipocytes: synergistic effect of insulin and dexamethasone on GLUT4 gene expression through promoter activation. *Endocrinology* (1995) **136**:4782–9. doi:10.1210/endo.136.11.7588207
151. Dimitriadis G, Leighton B, Parry-Billings M, Sasson S, Young M, Krause U, et al. Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. *Biochem J* (1997) **321**(Pt 3):707–12.
152. Weinstein SP, Wilson CM, Pritsker A, Cushman SW. Dexamethasone inhibits insulin-stimulated recruitment of GLUT4 to the cell surface in rat skeletal muscle. *Metabolism* (1998) **47**:3–6. doi:10.1016/S0026-0495(98)90184-6
153. Moisan MP, Seckl JR, Edwards CR. 11 Beta-hydroxysteroid dehydrogenase bioactivity and messenger RNA expression in rat forebrain: localization in hypothalamus, hippocampus, and cortex. *Endocrinology* (1990) **127**:1450–5. doi:10.1210/endo-127-3-1450
154. Whorwood CB, Donovan SJ, Flanagan D, Phillips DI, Byrne CD. Increased glucocorticoid receptor expression in human skeletal muscle cells may contribute to the pathogenesis of the metabolic syndrome. *Diabetes* (2002) **51**:1066–75. doi:10.2337/diabetes.51.4.1066
155. Yehuda R, Seckl J. Minireview: stress-related psychiatric disorders with low cortisol levels: a metabolic hypothesis. *Endocrinology* (2011) **152**:4496–503. doi:10.1210/en.2011-1218

156. Jamieson PM, Chapman KE, Edwards CR, Seckl JR. 11 Beta-hydroxysteroid dehydrogenase is an exclusive 11 beta- reductase in primary cultures of rat hepatocytes: effect of physicochemical and hormonal manipulations. *Endocrinology* (1995) **136**:4754–61. doi:10.1210/en.136.11.4754
157. Gathercole LL, Lavery GG, Morgan SA, Cooper MS, Sinclair AJ, Tomlinson JW, et al. 11Beta-hydroxysteroid dehydrogenase 1: translational and therapeutic aspects. *Endocr Rev* (2013) **34**:525–55. doi:10.1210/er.2012-1050
158. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, et al. 11β-Hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev* (2004) **25**:831–66. doi:10.1210/er.2003-0031
159. Cleasby ME, Kelly PA, Walker BR, Seckl JR. Programming of rat muscle and fat metabolism by in utero overexposure to glucocorticoids. *Endocrinology* (2003) **144**:999–1007. doi:10.1210/en.2002-220559
160. Masuzaki H, Flier JS. Tissue-specific glucocorticoid reactivating enzyme, 11 beta-hydroxysteroid dehydrogenase type 1 (11 beta-HSD1) – a promising drug target for the treatment of metabolic syndrome. *Curr Drug Targets Immune Endocr Metabol Disord* (2003) **3**:255–62. doi:10.2174/1568008033340135
161. Amatruda JM, Danahy SA, Chang CL. The effects of glucocorticoids on insulin-stimulated lipogenesis in primary cultures of rat hepatocytes. *Biochem J* (1983) **212**:135–41.
162. Berdanier CD. Role of glucocorticoids in the regulation of lipogenesis. *FASEB J* (1989) **3**:2179–83.
163. Noguchi T, Iritani N, Tanaka T. Molecular mechanism of induction of key enzymes related to lipogenesis. *Proc Soc Exp Biol Med* (1992) **200**:206–9. doi:10.3181/00379727-200-43419
164. Vasiljevic A, Velickovic N, Bursac B, Djordjevic A, Milutinovic DV, Nestorovic N, et al. Enhanced prereceptor glucocorticoid metabolism and lipogenesis impair insulin signaling in the liver of fructose-fed rats. *J Nutr Biochem* (2013) **24**:1790–7. doi:10.1016/j.jnutbio.2013.04.001
165. Dowman JK, Hopkins LJ, Reynolds GM, Armstrong MJ, Nasiri M, Nikolaou N, et al. Loss of 5alpha-reductase type 1 accelerates the development of hepatic steatosis but protects against hepatocellular carcinoma in male mice. *Endocrinology* (2013) **154**(12):4536–47. doi:10.1210/en.2013-1592
166. Paterson JM, Morton NM, Fievet C, Kenyon CJ, Holmes MC, Staels B, et al. Metabolic syndrome without obesity: hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. *Proc Natl Acad Sci U S A* (2004) **101**:7088–93. doi:10.1073/pnas.0305524101
167. Paterson JM, Holmes MC, Kenyon CJ, Carter R, Mullins JJ, Seckl JR. Liver-selective transgene rescue of hypothalamic-pituitary-adrenal axis dysfunction in 11beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Endocrinology* (2007) **148**:961–6. doi:10.1210/en.2006-0603
168. Carter RN, Paterson JM, Tworowska U, Stenvers DJ, Mullins JJ, Seckl JR, et al. Hypothalamic-pituitary-adrenal axis abnormalities in response to deletion of 11beta-HSD1 is strain-dependent. *J Neuroendocrinol* (2009) **21**:879–87. doi:10.1111/j.1365-2826.2009.01899.x
169. Chapman K, Holmes M, Seckl J. 11Beta-hydroxysteroid dehydrogenases: intracellular gate-keepers of tissue glucocorticoid action. *Physiol Rev* (2013) **93**:1139–206. doi:10.1152/physrev.00020.2012
170. Paternain L, Martisova E, Milagro FI, Ramirez MJ, Martinez JA, Campion J. Postnatal maternal separation modifies the response to an obesogenic diet in adulthood in rats. *Dis Model Mech* (2012) **5**:691–7. doi:10.1242/dmm.009043
171. Nyirenda MJ, Carter R, Tang JI, de Vries A, Schlumbohm C, Hillier SG, et al. Prenatal programming of metabolic syndrome in the common marmoset is associated with increased expression of 11beta-hydroxysteroid dehydrogenase type 1. *Diabetes* (2009) **58**:2873–9. doi:10.2337/db09-0873
172. Gustafsson JA, Stenberg A. Irreversible androgenic programming at birth of microsomal and soluble rat liver enzymes active on androstene-3,17-dione and 5alpha-androstane-3alpha,17beta-diol. *J Biol Chem* (1974) **249**:711–8.
173. Stimson RH, Lobley GE, Maraki I, Morton NM, Andrew R, Walker BR. Effects of proportions of dietary macronutrients on glucocorticoid metabolism in diet-induced obesity in rats. *PLoS One* (2010) **5**:e8779. doi:10.1371/journal.pone.0008779
174. Drake AJ, Raubenheimer PJ, Kerrigan D, McInnes KJ, Seckl JR, Walker BR. Prenatal dexamethasone programs expression of genes in liver and adipose tissue and increased hepatic lipid accumulation but not obesity on a high-fat diet. *Endocrinology* (2010) **151**:1581–7. doi:10.1210/en.2009-1088
175. Chen N, Liu L, Zhang Y, Ginsberg HN, Yu Y-H. Whole-body insulin resistance in the absence of obesity in FVB mice with overexpression of Dgat1 in adipose tissue. *Diabetes* (2005) **54**:3379–86. doi:10.2337/diabetes.54.12.3379
176. Bruce KD, Cagampang FR, Argenton M, Zhang J, Ethirajan PL, Burdge GC, et al. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. *Hepatology* (2009) **50**:1796–808. doi:10.1002/hep.23205
177. Lehmann J, Feldon J. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? *Rev Neurosci* (2000) **11**:383–408. doi:10.1515/REVNEURO.2000.11.4.383
178. Lippmann M, Bress A, Nemeroff CB, Plotsky PM, Monteggia LM. Long-term behavioural and molecular alterations associated with maternal separation in rats. *Eur J Neurosci* (2007) **25**:3091–8. doi:10.1111/j.1460-9568.2007.05522.x
179. Macri S, Chiarotti F, Wurbel H. Maternal separation and maternal care act independently on the development of HPA responses in male rats. *Behav Brain Res* (2008) **191**:227–34. doi:10.1016/j.bbr.2008.03.031
180. Wortwein G, Husum H, Andersson W, Bolwig TG, Mathe AA. Effects of maternal separation on neuropetide Y and calcitonin gene-related peptide in "depressed" Flinders sensitive line rats: a study of gene-environment interactions. *Prog Neuropsychopharmacol Biol Psychiatry* (2006) **30**:684–93. doi:10.1016/j.pnpbp.2006.01.027
181. Krysiak R, Obuchowicz E, Herman Z. Interactions between the neuropeptide Y system and the hypothalamic-pituitary-adrenal axis. *Eur J Endocrinol* (1999) **140**:130–6. doi:10.1530/eje.0.1400130
182. Maniam J, Morris MJ. The link between stress and feeding behaviour. *Neuropharmacology* (2012) **63**:97–110. doi:10.1016/j.neuropharm.2012.04.017
183. Cavagnini F, Croci M, Putignano P, Petroni ML, Invitti C. Glucocorticoids and neuroendocrine function. *Int J Obes Relat Metab Disord* (2000) **24**(Suppl 2):S77–9. doi:10.1038/sj.ijo.0801284
184. Liu D, Diorio J, Day JC, Francis DD, Meaney MJ. Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat Neurosci* (2000) **3**:799–806. doi:10.1038/77702
185. Kauffman AS. Coming of age in the kisspeptin era: sex differences, development, and puberty. *Mol Cell Endocrinol* (2010) **324**:51–63. doi:10.1016/j.mce.2010.01.017

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 December 2013; accepted: 28 April 2014; published online: 13 May 2014.
*Citation: Maniam J, Antoniadis C and Morris MJ (2014) Early-life stress, HPA axis adaptation, and mechanisms contributing to later health outcomes. Front. Endocrinol. **5**:73. doi: 10.3389/fendo.2014.00073*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
ENDOCRINOLOGY

ORIGINAL RESEARCH ARTICLE
published: 13 February 2014
doi: 10.3389/fendo.2014.00005

Neurocircuitry underlying stress and emotional regulation in animals prenatally exposed to alcohol and subjected to chronic mild stress in adulthood

Charlis Raineki[1], Kim G. C. Hellemans[1,2], Tamara Bodnar[1], Katie M. Lavigne[3,4], Linda Ellis[1], Todd S. Woodward[3,4] and Joanne Weinberg[1]*

[1] *Department of Cellular and Physiological Sciences, The University of British Columbia, Vancouver, BC, Canada*
[2] *Department of Neuroscience, Carleton University, Ottawa, ON, Canada*
[3] *Department of Psychiatry, The University of British Columbia, Vancouver, BC, Canada*
[4] *BC Mental Health and Addictions Research Institute (BCMHARI), Vancouver, BC, Canada*

Edited by:
Nikolaos P. Daskalakis, Icahn School of Medicine at Mount Sinai, USA

Reviewed by:
Gábor B. Makara, Hungarian Academy of Sciences, Hungary
Ishwar Parhar, Monash University, Malaysia

****Correspondence:***
Joanne Weinberg, Department of Cellular and Physiological Sciences, The University of British Columbia, 2350 Health Sciences Mall, Vancouver, BC V6T 1Z3, Canada
e-mail: joanne.weinberg@ubc.ca

Individuals exposed to alcohol during gestation show higher rates of psychopathologies. The hyperresponsivity to stress induced by prenatal alcohol exposure (PAE) may be related to this increased rate of psychopathologies, especially because this population is more likely to be exposed to stressful environments throughout life. However, alcohol-induced changes in the overlapping neurocircuitries that underlie stress and the expression of psychopathologies are not fully understood. Here, we performed a comprehensive analysis of the neural activity within central areas known to play key roles in both emotional and stress regulation. Adult male and female offspring from PAE, pair-fed, and *ad libitum*-fed control conditions were exposed to chronic mild stress (CMS). Following CMS, the neural activity (*c-fos* mRNA) of the amygdala, ventral hippocampal formation, medial prefrontal cortex (mPFC), and paraventricular nucleus of hypothalamus (PVN) was assessed in response to an acute stress (elevated plus maze). Our results demonstrate that, overall, PAE decreased neural activity within the amygdala and hippocampal formation in males and increased neural activity within the amygdala and mPFC in females. CMS reduced neural activity within the mPFC and PVN in PAE males, but reduced activity in all areas analyzed in control males. By contrast, CMS reduced neural activity in the mPFC in PAE females and had no effects in control females. Furthermore, the constrained principal component analysis revealed that these patterns of neural activity resulted in differential activation of the functional neural networks in males compared to females, indicating sexually dimorphic effects of PAE and CMS. Importantly, the altered networks of brain activation in PAE animals may underlie the hyperresponsivity to stress and increased psychopathologies observed among individuals prenatally exposed to alcohol.

Keywords: prenatal alcohol exposure, chronic mild stress, amygdala, hippocampus, prefrontal cortex, paraventricular nucleus of hypothalamus, *c-fos*, constrained principal component analysis

INTRODUCTION

Fetal development is a dynamic process strongly influenced by the quality of the environment in which it occurs (1, 2). Adverse intrauterine environments can negatively alter the developmental trajectory, resulting in physical and mental health problems later in life (1, 3). In mammals, the mother plays a critical role in fetal development, not only by supplying nutrients and oxygen via the placenta, but also by modulating the environmental stimuli to which the fetus is exposed (4). Clinical and experimental studies have clearly demonstrated that alcohol consumed during pregnancy has pervasive and long-lasting negative effects on fetal development (5–9). Indeed, prenatal alcohol exposure (PAE) is strongly associated with a wide range of neural, behavioral, hormonal, and cognitive deficits in humans, non-human primates, and rodents (5–9). In addition, the rates of psychopathologies (e.g., anxiety, depression, other mood disorders, and substance use disorders) among individuals prenatally exposed to alcohol are disproportionately higher when compared to unexposed individuals (10–13). Unfortunately, little is known about the underlying neural processes that support this high incidence of psychopathologies following PAE.

Brain areas implicated in psychopathologies overlap to a large extent with areas that mediate responses to stress. The amygdala, hippocampus, and medial prefrontal cortex (mPFC) are intrinsically involved in the modulation of hypothalamic–pituitary–adrenal (HPA) axis activity (14, 15), and are also associated with several mental health disorders (16, 17). Abnormal function of any one of these highly interconnected areas may result in abnormal responses to stress and/or the emergence of psychopathologies. This is especially relevant for individuals exposed to alcohol during gestation, as neuroimaging studies have demonstrated structural and functional alterations in brain regions

involved in stress regulation, such as the hippocampus and PFC (18–21).

Not surprisingly, a striking deficit induced by PAE involves changes in how affected individuals process and/or respond to a stressful stimulus or situation. The human and animal literature clearly demonstrates that alcohol consumption during pregnancy results in offspring that are hyperresponsive to a variety of stressors (5, 9, 22–24). For example, children exposed to alcohol *in utero* show higher salivary cortisol levels following exposure to stressors such as blood draw and the still-face procedure when compared to unexposed counterparts (22, 23). Additionally, higher basal cortisol levels were also observed in children exposed to alcohol prenatally (23, 24). In parallel with these studies, rats prenatally exposed to alcohol show increased HPA activation and/or a delay in return to basal levels as compared to controls in response to a wide range of stressful stimuli including footshock, restraint, immune challenge, and exposure to novel environments (25–30). In addition, HPA dysregulation is observed not only following stress, but also under basal conditions, even in the face of similar basal hormone levels. Dysregulation is evident at multiple levels of the axis, and appears to reflect changes in both HPA drive and feedback regulation and/or in the balance between drive and feedback. Taken together, these findings suggest that for individuals prenatally exposed to alcohol, stressors occurring over the life span may be acting on an already dysregulated or sensitized stress neurocircuitry. Thus, stress hyperresponsivity and HPA dysregulation may be a crucial factor mediating the increased vulnerability of these individuals to develop later psychopathologies (5, 31, 32). An aggravating factor to this already unfavorable situation is that individuals prenatally exposed to alcohol are, in general, at a higher risk of encountering a more stressful environment throughout the lifespan (12, 13, 33).

Using a rodent model of PAE, our laboratory has been successful in demonstrating that the combination of PAE and chronic unpredictable stress in adulthood increases anxiety- and depressive-like behaviors in a sexually dimorphic manner (5, 31, 32). However, the neurocircuitry underlying both stress dysregulation and anxiety- and depressive-like behaviors of individuals prenatally exposed to alcohol is still not fully understood. In the present study, adult male and female rats that were prenatally exposed to alcohol underwent a 10-day chronic mild stress (CMS) regimen in adulthood, which was followed by a comprehensive analysis of the neural activity within brain areas known to play key roles in stress and emotional regulation. Specifically, we assessed *c-fos* mRNA expression as a measurement of neural activity in the medial parvocellular dorsal division of the paraventricular nucleus of hypothalamus (mpdPVN), ventral hippocampal formation (CA1, CA3, DG, and ventral subiculum), the amygdala (central, cortical, lateral, basal, and medial nuclei), and the mPFC [anterior cigulate cortex (ACC), prelimbic (PrL), and infralimbic (IL)] in response to an acute stressor – exposure to the elevated plus maze (34, 35). In order to identify networks of coordinated activity in these brain regions rather than simply to identify whether activity differed between experimental groups for each individual brain area, we employed constrained principal component analysis (CPCA) in addition to a traditional univariate analysis (ANOVAs followed by *post hoc* group comparisons). CPCA is a multivariate technique that combines multivariate multiple regression and principal component analysis (PCA) into a unified framework (36–41). In the current study, CPCA allowed for the identification of brain networks that were specifically related to our experimental conditions (i.e., PAE and CMS). Thus, we were able to identify the networks of brain regions associated with differences in neural activity between animals that were prenatally exposed to alcohol, with or without CMS exposure in adulthood, and their respective controls. Below, we present results from a traditional univariate technique (ANOVA) as well as CPCA to demonstrate how this multivariate technique can facilitate interpretation when group differences are observed in a variety of distinct, but interconnected, brain regions.

MATERIALS AND METHODS

ANIMALS AND BREEDING

Female Sprague-Dawley rats were obtained from Charles River Laboratories (St. Constant, QC, Canada) and male Sprague-Dawley rats were obtained from the UBC Animal Care Centre. Rats were pair-housed by sex and maintained at a constant temperature (21 ± 1°C) and on a 12-h light–dark cycle (lights on at 6 a.m.) with *ad libitum* access to water and standard laboratory chow (Jamieson's Pet Food Distributors Ltd., Canada). After a 10-day acclimation period, male and female pairs were placed together suspended in stainless steel cages with mesh front and floor (25 cm × 18 cm × 18 cm). Day 1 of gestation (G1) was indicated by the presence of a vaginal plug on the wax paper beneath the breeding cages, which were checked daily. All experiments were performed in accordance with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals and the Canadian Council on Animal Care guidelines and were approved by The University of British Columbia Animal Care Committee.

PRENATAL ALCOHOL EXPOSURE

On G1, females were single-housed and randomly assigned to one of the three prenatal treatment groups: PAE, Pair-Fed (PF), or *ad libitum*-fed Control (C). Dams in the PAE group were offered *ad libitum* liquid ethanol diet with 36% ethanol-derived calories (Dyets, Inc., Bethlehem, PA, USA). This diet is formulated to provide adequate nutrition to pregnant rats regardless of ethanol intake (42, 43). PF dams were offered a liquid control diet with maltose–dextrin isocalorically substituted for ethanol, in an amount matched to the consumption of a PAE partner according to gestation day (gram/kilogram body weight/day of gestation). The control dams were offered *ad libitum* access to standard laboratory chow (Jamieson's Pet Food Distributors Ltd.). All animals were provided with fresh diet daily within 1 h of lights off to prevent a shift in corticosterone circadian rhythms, which occurs in animals that are on a restricted feeding schedule, such as the PF dams (44, 45). Experimental diets were continued through G21. Beginning on G22, all animals were offered *ad libitum* access to standard laboratory chow and water, which they received throughout lactation. Pregnant dams were left undisturbed except for cage changing and weighing, which occurred on G1, G7, G14, and G21. On the day of birth [postnatal day 1 (PN1)], litters were weighed and culled to 10 pups with an attempt to preserve an equal number of males and females per litter. Dams and pups were weighed

on PN1, PN8, PN15, and PN22 [dam and pup body weight data were published in Ref. (31)]. On PN22, pups were weaned and group-housed by litter and sex.

CHRONIC MILD STRESS

On PN40, one male and one female rat from each litter were randomly assigned to either the CMS or non-CMS condition and were pair-housed with another animal of the same sex, prenatal treatment, and stress condition (CMS or non-CMS). In adulthood (PN60–90), CMS animals were subjected to 10 consecutive days of randomized stressors. Animals were exposed to two different stressors each day: the first at a randomized time in the morning (between 8:00 and 12:00 h) and the second at a randomized time in the afternoon (between 13:00 and 16:00 h), with a minimum of 2 h between stressors. On day 1 of CMS, all animals were weighed, handled, and moved to new colony rooms in a neighboring building. CMS and non-CMS animals were housed in separate colony rooms so that non-CMS animals were not exposed to the disturbance of either moving CMS animals between stressors, or stress odors and vocalizations produced by CMS animals. CMS exposure occurred in a room separate from the colony room. The order and type of stressor were randomized, but all animals received the same number of exposures to each stressor over the 10-day period. Stressors included: (1) platform: 5 min exposure to an elevated Plexiglass platform (20 cm × 20 cm) mounted on a 90-cm high post; (2) cage tilt: the home cage was tilted at a 30° angle for 2 h; (3) novel cage: exposure to a novel, small (18 cm × 25 cm × 15 cm), and opaque cage without food and water for 1 h; (4) soiled cage: exposure to the soiled cage from another sex-matched pair of animals for 1 h; (5) restraint: restraint in PVC tubes (15 cm × 6 cm for females and 19 cm × 7 cm for males) for 30 min; (6) social isolation: overnight isolation in hanging wire mesh cage (20 cm × 23 cm × 18 cm) without food and water for 12 h; (7) white noise: exposure to white noise (40 dB; Lafayette Instruments model no. 15800) for 2 h; and (8) tail nick: a cut was made 1 mm from the tip of the tail for blood collection from the tail vein [blood sample data was published in Ref. (31)]. Non-CMS animals remained undisturbed other than routine husbandry during this same period.

ACUTE STRESS (ELEVATED PLUS MAZE EXPOSURE) AND BRAIN COLLECTION

The day after the end of CMS (day 11), all animals from both CMS and non-CMS conditions were habituated to the behavioral testing room for 10 min. The following day (day 12), animals were exposed to an elevated plus maze for 5 min in a dimly lit room during the light phase (09:00 and 12:00 h) of the light–dark cycle [elevated plus maze data were published in Ref. (31)]. Exposure to the elevated plus maze is a stressor known to produce an increase in corticosterone levels (35). The elevated plus maze consisted of two closed arms (69 cm × 10.5 cm) and two open arms (69 cm × 10.5 cm) with a central platform (diameter 35 cm). Closed arms had walls of darkened Plexiglass 20 cm high along their length. Open arms had a 2-cm-high lip along the edges of the arms. Immediately following the elevated plus maze exposure, animals were individually housed and left undisturbed in a quiet holding room for 30 min. Animals were then decapitated, and brains were removed, frozen on dry ice, and stored at −80°C.

NEURAL ASSESSMENT OF *C-FOS* mRNA BY *IN SITU* HYBRIDIZATION

Probes and labeling

The ribonucleotide probe was prepared using a rat *c-fos* 2116 bp template provided by Dr. Victor Viau (Department Cellular and Physiological Sciences, The University of British Columbia, Canada). Probes were labeled with ^{35}S-UTP (Amersham Biosciences, NJ, USA) using Polymerase T^7 and Promega Riboprobe System (Promega Corporation, Madison, WI, USA). All probes were purified using Micro Bio-Spin 30 Columns (Bio-Rad, CA, USA). One molar of DTT was added to prevent oxidation.

In situ hybridization

Brains were sectioned coronally (20 μm) using a cryostat (−16°C) and stored at −80°C. Thawed sections were fixed in formalin for 30 min and then pre-hybridized as follows: 1× PBS twice for 10 min each, proteinase K (100 μg/L; at 37°C) for 9 min, 0.1 M triethanolamine-hydrochloride (TEA) for 10 min, 0.1 M TEA with 0.25% acetic anhydride for 10 min, 2× SSC twice for 10 min each, dehydration by a graded series of ethanol, chloroform for 5 min, and finally 100% ethanol before being air dried. Hybridization buffer (75% formamide, 3× SSC, 1× Denhardt's solution, 200 μg/mL yeast tRNA, 50 mM sodium phosphate buffer (pH 7.4), 10% dextran sulfate, and 10 mM DTT) was applied (1×10^6 cpm/slide) and covered with HybriSlips (Sigma-Aldrich, ON, Canada). Sections were incubated overnight at 55°C in chambers humidified with 75% formamide. HybriSlips were removed and the slides were rinsed as follows: 2× SSC twice for 20 min, 2× SSC for 30 min, 50 μg/L RNAse A solution (at 37°C) for 60 min, 2× SSC with 0.01 M DTT for 10 min, 1× SSC for 10 min, 0.5× SSC with 0.01 M DTT for 10 min, 0.1× SSC with 0.01 M DTT (at 60°C) for 60 min, and 0.1× SSC for 5 min. Sections were dehydrated by a graded series of ethanol and air dried overnight.

Kodak BioMax autoradiography film was exposed to hybridized slides of the ventral hippocampus for 28 days, and then developed using Kodak GBX developer and fixer. For all other areas, the hybridized slides were dipped in Kodak NTB2 autoradiography emulsion (diluted 50:50 with distilled water) and exposed in desiccated sealed, light tight boxes (4°C) for 49 days for mPFC, 70 days for mpdPVN, and 92 days for the amygdala. Slides were developed using Kodak D19 developer and standard Kodak fixer, counterstained with Toluidine Blue, and coverslipped with Permount (Fisher Scientific Ltd.). For all brain areas analyzed, $n = 4$–5 with the exception of the ventral hippocampal formation (CA1, CA3, DG, and ventral subiculum) where $n = 3$ for PF non-CMS males.

Densitometric analysis

The autoradiographic films for the ventral hippocampal formation were scanned and analyzed with Scion Image 4.0.3.2 (National Institutes of Health, USA). The left and right DG, CA1, and CA3, and ventral subiculum were traced freehand according to a stereotaxic rat brain atlas (46) in two sections per animal to determine mean gray density levels. Background was measured from the corpus callosum, and corrected gray levels were obtained by subtracting the background level from each of the four measurements. Left and right levels in each measured area were averaged together for analysis. For the emulsion dipped slides (amygdala, mPFC, and

mpdPVN), *in situ* signals were visualized with a Q-imaging monochrome 12-bit camera attached to a Zeiss Axioskop 2 motorized plus microscope. Images were captured under dark field illumination using Northern Elite 6.0v (Empix Imaging, Inc., Mississauga, ON, Canada) and analyzed with ImageJ 10.3 software (National Institutes of Health, USA).

STATISTICAL ANALYSIS

Data were first analyzed using two-way analysis of variance (ANOVA for the factors of prenatal treatment and CMS exposure) followed by Newman–Keuls *post hoc* tests for each brain region (presented in **Figures 1–4**). Differences were considered significant at $p \leq 0.05$. Further analyses utilized planned comparisons to test the *a priori* hypotheses that: (1) PAE will alter the response to acute stress, i.e., non-CMS PAE animals will show differential neural activity in response to acute stress (exposure to the elevated plus maze) compared to non-CMS control animals; and (2) CMS will differentially alter neural activity in response to acute stress in PAE compared to control animals.

In order to identify networks of brain regions altered by our experimental conditions, we employed CPCA, which is performed in two steps, referred to as the external analysis and the internal analysis. The external analysis consists of multivariate least-squares multiple regression, which serves to separate the overall variance (i.e., *c-fos* mRNA activity in the 13 brain regions of interest) into the variance that can (and cannot) be predicted by the experimental conditions (i.e., prenatal treatment and CMS exposure). This first step of CPCA simply separates the predicted and residual scores through multivariate multiple regression, using dummy-coded groupings as the independent variables [1 for group membership, 0 for not; (36)]. This first step produces a matrix of predicted scores that reflects the portion of variance in brain neural activity that is attributable to the experimental conditions (i.e., the predictable variance). A matrix of residual scores is also produced, which reflects the portion of variance in brain neural activity that is not attributable to experimental conditions (i.e., the residual variance). This residual variance was not further analyzed for the present study, as we were interested in brain networks associated with our experimental conditions. The second step in CPCA, the internal analysis, simply consists of a PCA on the predictable variance (i.e., the predicted scores from the multivariate regression in the first step). PCA is a data reduction technique that serves to combine large sets of related variables by reducing them to a smaller number of components (referred to as networks when the dependent variables consist of brain regions) that best explain the variance, while losing as little information as possible. The components that emerge from the PCA on the predicted scores reflect the brain networks that characterize the differences in brain activity between the experimental conditions (i.e., the brain networks that show differing degrees of activity across the different prenatal treatment and CMS groups). All PCA solutions were separately rotated using Varimax with Kaiser normalization, and the number of the components extracted was determined by inspection of scree plots (47, 48). In order to examine specifically how the groups differ in terms of brain activity in each of the resulting networks, correlations were computed between the experimental groups and the component scores from each of the extracted components. A more detailed description of the theory

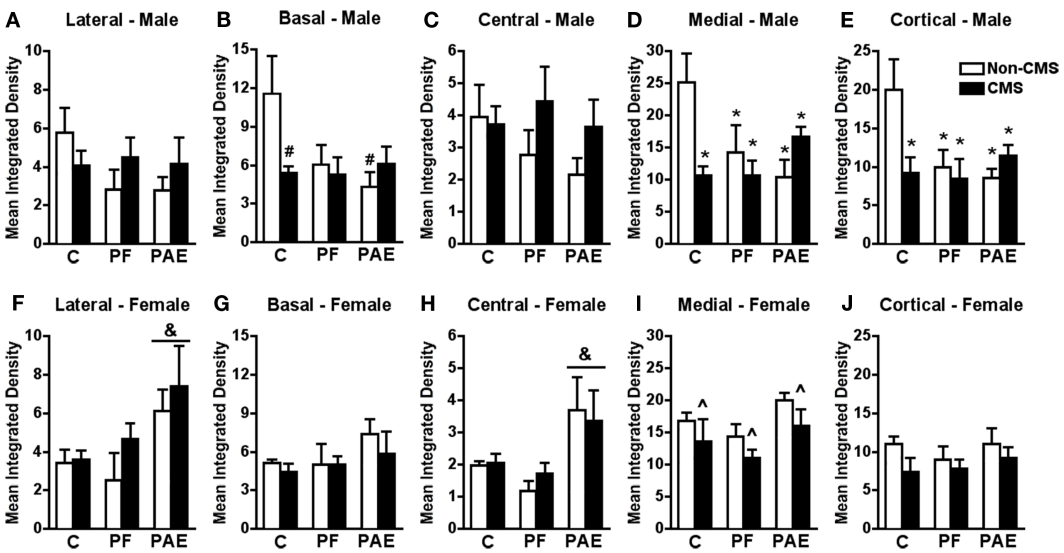


FIGURE 1 | Amygdala *c-fos* mRNA expression in response to the elevated plus maze test in male and female control (C), pair-fed (PF), and prenatal alcohol exposed (PAE) rats, with or without exposure to chronic mild stress (CMS) in adulthood. Bars represent the integrated density (mean ± SEM) of *c-fos* mRNA expression in the lateral **(A,F)**, basal **(B,G)**, central **(C,H)**, medial **(D,I)**, and cortical **(E,J)** amygdala nuclei. *Indicates a significant interaction between prenatal treatment and CMS exposure where all groups are different from C non-CMS; &indicates a significant main effect of prenatal treatment where the *post hoc* test shows that PAE is different from C and PF, independent of CMS; ^indicates a significant main effect of CMS exposure where all animals exposed to CMS are different from animals not exposed to CMS; #indicates that control CMS and PAE non-CMS are different from control non-CMS based on *a priori* comparisons ($n = 4$–5 for all groups).

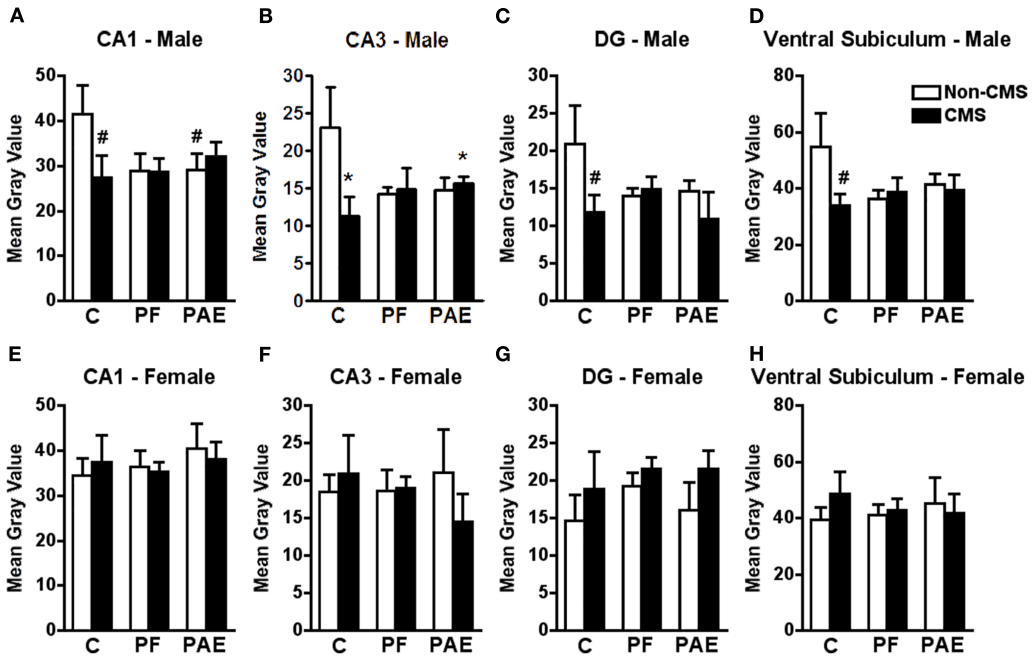


FIGURE 2 | Hippocampal formation *c-fos* mRNA expression in response to the elevated plus maze test in male and female control (C), pair-fed (PF), and prenatal alcohol exposed (PAE) rats, with or without exposure to chronic mild stress (CMS) in adulthood. Bars represent the gray value (mean ± SEM) of *c-fos* mRNA expression in the CA1 **(A,E)**, CA3 **(B,F)**, DG **(C,G)**, and ventral subiculum **(D,H)**. *Indicates a significant interaction between prenatal treatment and CMS exposure, where the control non-CMS group is different from control CMS and PAE CMS groups; #indicates that control CMS and PAE non-CMS are different from control non-CMS based on *a priori* comparisons ($n = 3$–5 for all groups).

and methodology of CPCA can be found in previously published manuscripts (38–41, 49, 50). CPCA was carried out using SPSS.

RESULTS

AMYGDALA

Males

c-fos mRNA expression within the medial and cortical amygdala nuclei in response to acute stress was significantly reduced in non-CMS PAE and PF compared to non-CMS control males (**Figures 1D,E**). Moreover, while CMS reduced *c-fos* mRNA expression within the medial and cortical amygdala nuclei in control males compared to that in their non-CMS counterparts, *c-fos* mRNA expression of PAE and PF rats was similar under CMS and non-CMS conditions following acute stress {significant interaction between prenatal treatment and CMS exposure for medial [$F_{(1,23)} = 6.578$; $p < 0.007$] and cortical [$F_{(1,23)} = 4.196$; $p < 0.03$] amygdala nuclei}.

Similarly, *c-fos* mRNA expression within the basal amygdala in response to acute stress was significantly reduced in non-CMS PAE compared to non-CMS control males (**Figure 1B**). Moreover, CMS control males showed reduced *c-fos* mRNA expression within the basal nucleus compared to that in their non-CMS counterparts, whereas PAE rats were similar in *c-fos* mRNA expression following acute stress under both CMS and non-CMS conditions {no significant interaction between prenatal treatment and CMS interaction [$F_{(1,23)} = 3.129$; $p = 0.06$]; *a priori* analysis indicated that PAE non-CMS ($p < 0.005$) and control CMS ($p < 0.02$) showed lower *c-fos* mRNA expression than the control non-CMS}.

Neither prenatal treatment nor CMS significantly altered *c-fos* mRNA expression within the central or lateral amygdala nuclei following acute stress {**Figures 1A,C**; no significant interaction between prenatal treatment and CMS exposure for central [$F_{(1,22)} = 0.803$] and lateral [$F_{(1,23)} = 1.499$] amygdala nuclei}.

Females

Among females, a different pattern of neural activity within the amygdala was observed. PAE, independent of CMS exposure, increased *c-fos* mRNA expression within the central and lateral amygdala nuclei in response to acute stress when compared to that in control and PF animals {**Figures 1F,H**; no significant interaction between prenatal treatment and CMS exposure for central [$F_{(1,23)} = 0.448$] or lateral [$F_{(1,23)} = 0.387$] amygdala nuclei, but significant main effect of prenatal treatment for central [$F_{(2,23)} = 7.198$; $p < 0.005$] and lateral [$F_{(2,23)} = 4.910$; $p < 0.02$] nuclei in response to acute stress}.

In the medial amygdala, CMS reduced *c-fos* mRNA expression in response to acute stress independent of prenatal treatment group {**Figure 1I**; no significant interaction between prenatal treatment and CMS exposure [$F_{(1,23)} = 0.015$], but significant main effect for CMS [$F_{(1,23)} = 4.176$; $p = 0.05$]}.

Neither prenatal treatment nor CMS significantly altered *c-fos* mRNA expression in response to acute stress within the cortical and basal amygdala nuclei {**Figures 1G,J**; no significant interaction between prenatal treatment and CMS exposure for cortical [$F_{(1,23)} = 0.312$] or basal [$F_{(1,23)} = 0.257$] amygdala nuclei}.

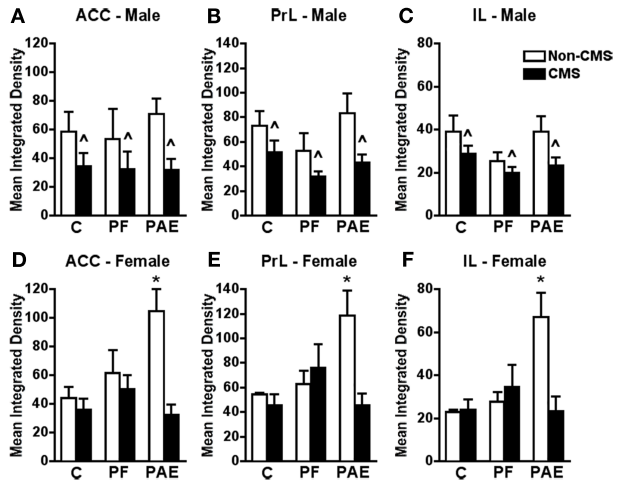


FIGURE 3 | Medial prefrontal cortex *c-fos* mRNA expression in response to the elevated plus maze test in male and female control (C), pair-fed (PF), and prenatal alcohol exposed (PAE) rats, with or without exposure to chronic mild stress (CMS) in adulthood. Bars represent the integrated density (mean ± SEM) of *c-fos* mRNA expression in the ACC **(A,D)**, PrL **(B,E)**, and IL **(C,F)**. *Indicates a significant interaction between prenatal treatment and CMS exposure where the PAE non-CMS group is different from all other groups; ^indicates a significant main effect of CMS exposure ($n = 4$–5 for all groups).

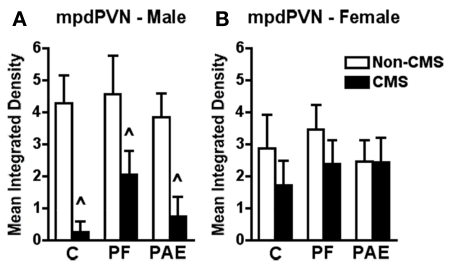


FIGURE 4 | Medial parvocellular dorsal division of the paraventricular nucleus of hypothalamus *c-fos* mRNA expression in response to the elevated plus maze test in male (A) and female (B) control (C), pair-fed (PF), or prenatal alcohol exposed (PAE) rats, with or without exposure to chronic mild stress (CMS) in adulthood. Bars represent the integrated density (mean ± SEM) of *c-fos* mRNA expression in the mpdPVN. ^Indicates a significant main effect of CMS exposure ($n = 4$–5 for all groups).

HIPPOCAMPAL FORMATION

Males

c-fos mRNA expression within the CA3 subregion of the ventral hippocampal formation in response to acute stress was significantly reduced in CMS PAE compared to non-CMS control males (**Figure 2B**). Moreover, similar to the medial and cortical amygdala, while CMS reduced *c-fos* mRNA expression within the CA3 region in control males compared to their non-CMS counterparts, *c-fos* mRNA expression of PAE and PF rats was similar under CMS and non-CMS conditions in response to acute stress {significant interaction between prenatal treatment and CMS exposure $[F_{(1,19)} = 3.714; p < 0.05]$}.

Consistent with these findings, *c-fos* mRNA expression in response to acute stress within the CA1 subregion of the ventral hippocampal formation was significantly reduced in non-CMS PAE compared to non-CMS control males (**Figure 2A**). Moreover, while *c-fos* mRNA expression was reduced within the CA1, DG, and ventral subiculum subregions in CMS control compared to non-CMS control males, *c-fos* mRNA expression of PAE rats was similar under CMS and non-CMS conditions in response to acute stress {**Figures 2A,C,D**; no significant interaction between prenatal treatment and CMS interaction for CA1 $[F_{(1,20)} = 2.257]$, DG $[F_{(1,20)} = 1.472]$, or ventral subiculum $[F_{(1,19)} = 1.839]$; *a priori* analysis indicates that non-CMS PAE showed a significantly lower *c-fos* mRNA expression within the CA1 ($p = 0.05$) than non-CMS controls, and that CMS controls ($p < 0.03$) showed lower *c-fos* mRNA expression than non-CMS control within CA1, DG, and ventral subiculum}.

Females

Among females, neither prenatal treatment nor CMS significantly altered *c-fos* mRNA expression in response to acute stress within any subregion of the hippocampal formation {**Figures 2E–H**; no significant interaction between prenatal treatment and CMS exposure for CA1 $[F_{(1,21)} = 0.193]$, CA3 $[F_{(1,21)} = 0.399]$, DG $[F_{(1,21)} = 0.130]$, or ventral subiculum $[F_{(1,21)} = 0.490]$}.

MEDIAL PREFRONTAL CORTEX

Males

In all mPFC subregions (ACC, PrL, and IL), CMS reduced *c-fos* mRNA expression in response to acute stress independent of prenatal treatment {**Figures 3A–C**; no significant interaction between prenatal treatment and CMS exposure for ACC $[F_{(1,22)} = 0.299]$, PrL $[F_{(1,22)} = 0.474]$, or IL $[F_{(1,22)} = 0.421]$, but significant main effect for CMS for ACC $[F_{(1,22)} = 7.426; p < 0.02]$, PrL $[F_{(1,22)} = 8.936; p < 0.008]$, and IL $[F_{(1,22)} = 5.365; p < 0.03]$}.

Females

Similar to findings for the amygdala, *c-fos* mRNA expression in response to acute stress within all areas of the mPFC was significantly increased in non-CMS PAE females compared to non-CMS control and PF females (**Figures 3D–F**). Moreover, while CMS PAE females showed reduced *c-fos* mRNA expression within the ACC, PrL, and IL compared to non-CMS PAE females, *c-fos* mRNA expression of control and PF rats was similar under CMS and non-CMS conditions in response to acute stress {significant interaction between prenatal treatment and CMS exposure for ACC $[F_{(1,23)} = 4.614; p < 0.03]$, PrL $[F_{(1,23)} = 5.139; p < 0.02]$, and IL $[F_{(1,23)} = 6.525; p < 0.007]$}.

MEDIAL PARVOCELLULAR DORSAL DIVISION OF THE PARAVENTRICULAR NUCLEUS OF HYPOTHALAMUS

Males

In the mpdPVN, CMS reduced *c-fos* mRNA expression in response to acute stress independent of prenatal treatment {**Figure 4A**; no significant interaction between prenatal treatment and CMS exposure $[F_{(1,23)} = 0.257]$, but significant main effect for CMS $[F_{(1,23)} = 15.541; p < 0.001]$}.

Females

By contrast to males, neither prenatal treatment nor CMS significantly altered *c-fos* mRNA expression among females within mpdPVN in response to acute stress {**Figure 4B**; no significant interaction between prenatal treatment and CMS exposure [$F_{(1,23)} = 0.783$]}.

CONSTRAINED PRINCIPAL COMPONENT ANALYSIS

Males

The experimental conditions (i.e., prenatal treatment and CMS exposure) accounted for 34.17% of the total variance in *c-fos* mRNA expression in males. PCA on this constrained variance (i.e., the predictable variance) revealed a two-component solution; **Table 1** displays the component loadings for each of the 13 brain regions on each component. The first component explained 19.40% of the total variance and 56.78% of the predictable variance and was defined as *Amygdala + Hippocampal Formation* because the medial, cortical, and basal amygdala nuclei, and CA3 and CA1 hippocampal regions showed the highest loadings, with lesser contributions from the ventral subiculum, DG, and lateral amygdala (**Figure 5B**). The second component explained 11.26% of the total variance and 32.97% of the predictable variance and was defined as *Prefrontal Cortex + Paraventricular Nucleus* because the PrL, mpdPVN, and ACC showed the highest loadings, with lesser contributions from the IL and central amygdala (**Figure 5D**).

Correlations between the subjects' component scores and their experimental condition membership are displayed in **Figure 5A** for the *Amygdala + Hippocampal Formation network* and in **Figure 5C** for the *Prefrontal Cortex + Paraventricular Nucleus network*. The *Amygdala + Hippocampal Formation network* showed a significant positive correlation with the control non-CMS condition ($r = 0.96$, $p < 0.0001$), but a significant negative correlation with the PAE non-CMS condition ($r = -0.39$, $p < 0.05$). Furthermore, although not reaching statistical significance, the *Amygdala + Hippocampal Formation network* was also negatively correlated with the control CMS condition. As seen in **Figure 5C**, the *Prefrontal Cortex + Paraventricular Nucleus network* showed positive correlations with all non-CMS conditions; however only PAE non-CMS ($r = 0.79$, $p < 0.0001$) reached significance. Conversely, the *Prefrontal Cortex + Paraventricular Nucleus network* showed negative correlations with all CMS groups, with PAE ($r = -0.44$, $p < 0.05$) and PF ($r = -0.45$, $p < 0.05$) CMS conditions showing significant correlations.

Females

The experimental conditions accounted for 25.72% of the total variance in *c-fos* expression in females. PCA on this constrained (i.e., predictable) variance revealed a two-component solution (see **Table 2** for component loadings). The first component explained 12.04% of the total variance and 46.81% of the predictable variance and was defined as *Prefrontal Cortex* because all mPFC subregions (ACC, IL, and PrL) contributed with the highest loadings to this component (**Table 2**; **Figure 6B**). The second component explained 9.14% of the total variance and 35.56% of the predictable variance and was defined as *Amygdala* because the majority of amygdala nuclei (central, lateral, medial, and basal) contributed with the highest loadings to this component; however, the IL also contributed to this component (**Table 2**; **Figure 6D**).

Table 1 | Component loadings for the predicted solution in males.

Variables	*Amygdala + Hippocampal Formation*	*Prefrontal Cortex + Paraventricular Nucleus*
Medial amygdala	**0.65**	0.06
Cortical amygdala	**0.63**	0.09
CA3	**0.61**	0.17
Basal amygdala	**0.58**	0.04
CA1	**0.53**	0.09
Ventral subiculum	**0.48**	0.20
DG	**0.44**	0.24
Lateral amygdala	**0.37**	−0.17
Prelimbic	0.14	**0.59**
Paraventricular nucleus	0.12	**0.54**
Anterior cingulate	0.07	**0.54**
Infralimbic	0.22	**0.48**
Central amygdala	0.21	−**0.36**

Values ≥0.30 are set in bold.

Correlations between the subjects' component scores and their experimental condition membership are displayed graphically in **Figure 6A** for the *Prefrontal Cortex network* and in **Figure 6C** for the *Amygdala network*. The *Prefrontal Cortex network* showed a significant positive correlation with the PAE non-CMS condition ($r = 0.82$, $p < 0.0001$). In contrast, the *Prefrontal Cortex network* showed a significant negative correlation with the PAE CMS condition ($r = -0.64$, $p < 0.0001$). Additionally, as seen in **Figure 6C**, the *Amygdala network* showed a significant positive correlation with the PAE group, independent of CMS condition ($r = 0.56$, $p < 0.01$ for PAE non-CMS and $r = 0.67$, $p < 0.0001$ for PAE CMS); however, this network was negatively correlated with the control group, again, independent of CMS condition. Furthermore, the *Amygdala network* showed a significant negative correlation with the PF non-CMS condition ($r = -0.55$, $p < 0.01$).

DISCUSSION

Stress and emotional regulation are dynamic and complex processes achieved by the fine coordination of interconnected brain regions, including, but not limited to, the amygdala, mPFC, hippocampus, and paraventricular nucleus of hypothalamus (PVN) (14–17, 51). The majority of these brain areas have dual functions, playing major roles in both the stress response and emotional regulation. Because of these dual roles and the intrinsic interconnectivity among these brain regions, dysfunction within or among structures in this neurocircuitry could result in dysregulation of the stress response and/or lead to mood and anxiety disorders. The present results indicate that, regardless of chronic stress later in life, PAE produces widespread alterations in neural activity within and between several brain areas of the stress/emotional neurocircuitry analyzed. Notably, male and female rats in the current study were differentially affected by PAE. Overall, PAE decreased neural activity within the amygdala and hippocampal formation in males and increased neural activity

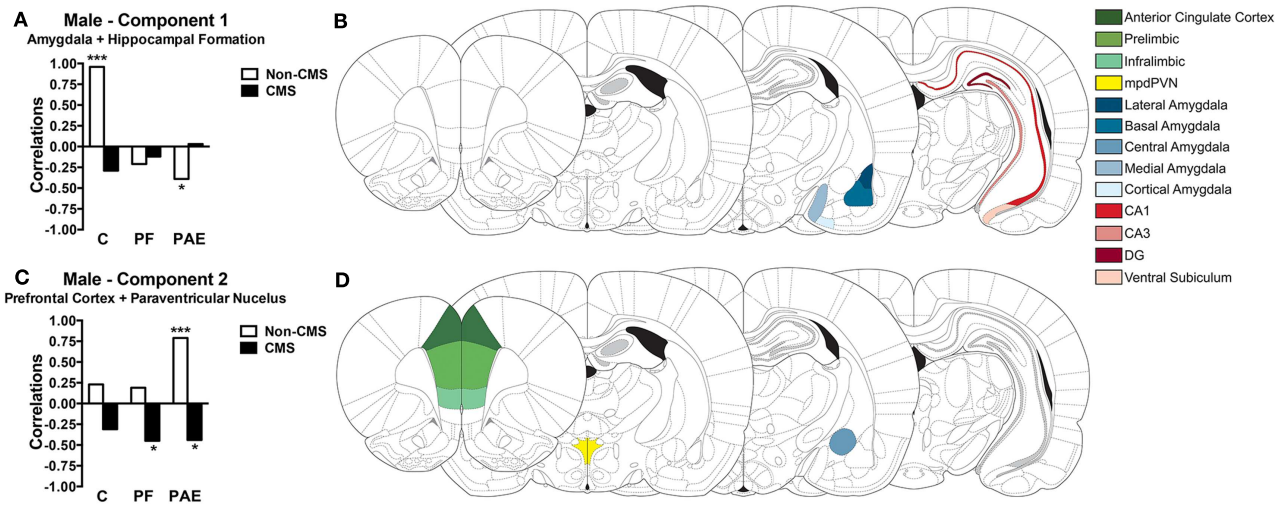


FIGURE 5 | Bars represent the correlation between the experimental group and the component scores for components 1 (A) and 2 (C) extracted from the CPCA for males. Schematic figure highlighting the areas that contributes to each neural network **(B,D)**. Images were adapted from Ref. (46), $***p < 0.0001$; $*p < 0.05$. C, control; PF, pair-fed; and PAE, prenatal alcohol exposed.

Table 2 | Component loadings for the predicted solution in females.

Variables	*Prefrontal Cortex*	*Amygdala*
Anterior cingulate	**0.69**	0.20
Infralimbic	**0.62**	**0.35**
Prelimbic	**0.61**	0.24
Cortical amygdala	0.21	0.15
CA3	0.20	−0.09
DG	−0.23	0.01
Central amygdala	0.12	**0.60**
Lateral amygdala	−0.06	**0.56**
Medial amygdala	0.29	**0.34**
Basal amygdala	0.26	**0.31**
CA1	0.10	0.17
Paraventricular nucleus	0.00	−0.15
Ventral subiculum	0.03	0.03

Values ≥0.30 are set in bold.

within the amygdala and mPFC in females. Exposure to CMS also resulted in a sexually dimorphic effect, as it reduced neural activity within the mPFC and PVN in PAE males, but reduced activity in all areas analyzed in controls. Conversely, CMS reduced neural activity only in the mPFC of PAE females and had no effects in control females. Importantly, these patterns of neural activity were reflected in the differential networks of brain activation for PAE and control male and female rats. Indeed, the CPCA revealed that, contrary to non-CMS PAE males, non-CMS control males showed a strongly active *Amygdala + Hippocampal Formation network*. Conversely, non-CMS PAE males showed a strongly active *Prefrontal Cortex + Paraventricular Nucleus network*. Among females, non-CMS PAE animals showed strongly active *Prefrontal Cortex* and *Amygdala networks*, which contrasts with the network activity observed in non-CMS controls. Of note, CMS also altered the networks of brain activation in PAE animals, as the pattern of activity within the *Prefrontal Cortex + Paraventricular Nucleus network* in PAE males and the *Prefrontal Cortex network* in PAE females switched from being active in the non-CMS animals to showing decreased activation in CMS animals. Overall, the changes in the network of brain areas following PAE could be implicated in the hyperresponsivity to stress and increased vulnerability to the development of psychopathologies observed among individuals prenatally exposed to alcohol (5, 9, 11–13, 22, 23).

AMYGDALA

Clinical studies have consistently demonstrated that amygdala dysfunction is associated with mood and anxiety disorders (16, 17, 52, 53). These disorders are among the most prevalent mental health problems in children and adults exposed to alcohol during gestation (11–13). Animal models have supported the relationship between amygdala dysfunction and anxiety- and depressive-like behaviors (52–55). Specifically, the basal and lateral amygdala nuclei have been defined as part of the neural circuitry activated in animals exhibiting depressive-like behaviors (56, 57). Additionally, the central, lateral, and basal amygdala nuclei have a large population of neurons that express corticotropin-releasing hormone (CRH) and its receptors (58, 59); hyperfunction of these neurons can result in increased levels of anxiety-like behaviors (60, 61).

The present results indicate sexually dimorphic effects of PAE and/or CMS on neural activity of the amygdala nuclei. PAE females showed an increase in neural activity within the central and lateral amygdala nuclei in response to acute stress, regardless of whether they were exposed to CMS. Increased neural activity in the amygdala is consistent with the increased stress responsiveness as well as anxiety-/depressive-like behavior often seen in PAE females (5, 31, 32). However, vulnerability to anxiety-/depressive-like behavior is often greater in PAE animals following CMS than in their non-CMS counterparts, and under some conditions, non-CMS

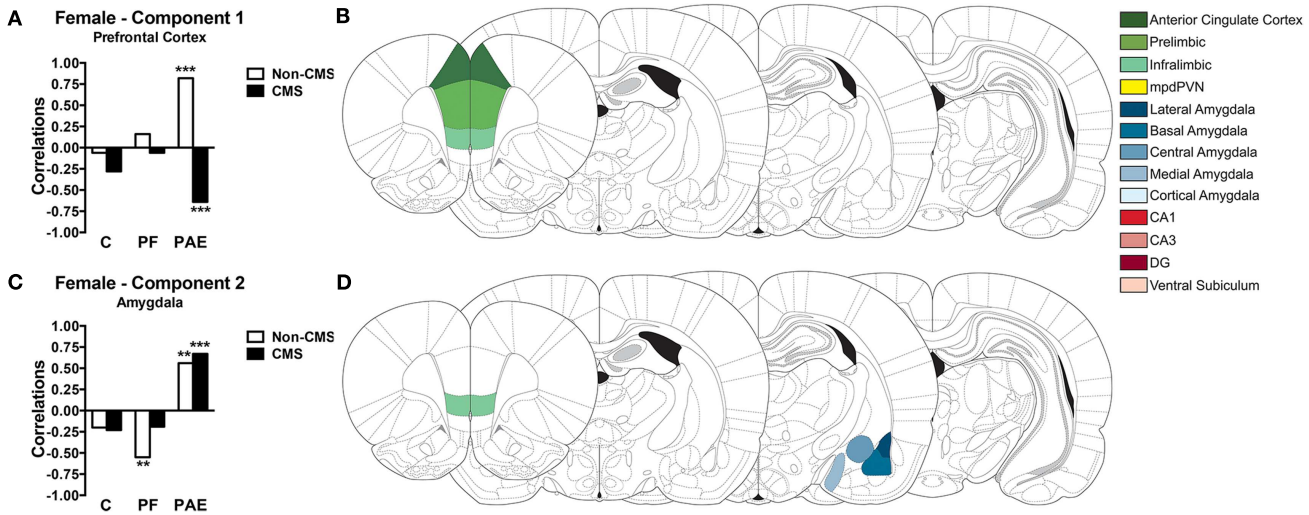


FIGURE 6 | Bars represent the correlation between the experimental group and the component scores for components 1 (A) and 2 (C) extracted from the CPCA for females. Schematic figure highlighting the areas that contributes to each neural network **(B,D)**. Images were adapted from Ref. (46), $***p < 0.0001$; $**p < 0.01$. C, control; PF, pair-fed; and PAE, prenatal alcohol exposed.

PAE animals may not differ from non-CMS controls. We found, for example, that while non-CMS PAE females were similar to non-CMS controls in time spent on the open arms of an elevated plus maze, exposure to CMS significantly reduced frequency of total open arm entries for PAE but not control females (31). These discrepancies in neural activity and behavior suggest that other brain areas besides the amygdala may be modulating anxiety-like behavior among PAE females. In males, PAE and pair-feeding prevented the increase in neural activity within the medial, cortical, and basal amygdala nuclei shown by control non-CMS animals. However, compared to controls, neither PAE nor CMS changed the neural activity of central and lateral amygdala nuclei in response to acute stress. These data are interesting in light of the finding that PAE males exposed to CMS show more robust increases in anxiety-like behavior in the elevated plus maze than PAE females (31), suggesting that the neural mechanisms involved in mediating the anxiety response among males may be dissociable from those in females. This is supported by our finding of networks of brain regions that were differentially associated with PAE and/or CMS in males and females in the current study, as discussed further below.

In addition to its well-characterized role in emotional processing, the amygdala also regulates different aspects of the neuroendocrine stress response (15, 62). In general, the central nucleus of the amygdala regulates the HPA response to systemic stressors, and integrates autonomic responses to psychogenic stressors; the basal, lateral, and the medial amygdala nuclei regulate the HPA response to psychological stressors (62). The increased neural activity within the lateral amygdala of PAE females reported here may, at least partially, underlie the hyperresponsivity to stress observed in these animals (5, 9, 22, 23).

HIPPOCAMPAL FORMATION

The hippocampal formation is well known for its role in learning and memory, stress, and emotional regulation (63). However, the dorsal and ventral components of the hippocampal formation have different functions: the dorsal hippocampus is primarily involved in cognitive functions while the ventral hippocampus is more essential for stress and emotional regulation (63). For example, lesions to the ventral hippocampus result in decreased anxiety-like behaviors (63, 64), whereas increased activity in the ventral hippocampus is positively correlated with anxiety-like behavior (65, 66). Additionally, the hippocampal formation is consistently associated with an overall inhibition of the stress response (14, 15). Indeed, hippocampal lesions result in increased stress responses and, in some cases, increased basal levels of corticosterone (67). Stimulation of the CA3, DG, and ventral subiculum subfields, on the other hand, results in reduced glucocorticoid secretion (68). Interestingly, PAE is known to induce morphological changes that can lead to functional deficits in the hippocampus: studies in the clinical literature reveal reduced hippocampal volume in individuals exposed to alcohol during gestation (20, 21). In rats, PAE results in hypertrophy of mossy fibers (69), reduced numbers of dendritic spines (70), as well as impaired long-term potentiation (71, 72) and adult neurogenesis – primarily in males (73, 74). In agreement with these findings, our results indicate that PAE males show reduced neural activity in the hippocampal formation in response to acute stress when compared to controls. This deficit in neural activity suggests that the inhibitory action of the hippocampal formation may be reduced, which could contribute to the hyperresponsivity to stress, and ultimately to the increase in anxiety-like behaviors observed in PAE animals (31).

Interestingly, PAE results in no apparent changes in basal expression of glucocorticoid and mineralocorticoid receptors in the hippocampal formation of male animals (75–77), whereas in females, some studies indicate a reduction (77), whereas others report no change (75, 76) in basal expression of glucocorticoid and mineralocorticoid receptor mRNA. However, removal of the inhibitory feedback effects of corticosterone on the HPA axis by

adrenalectomy unmasked alterations in basal HPA regulation not apparent in intact animals. Glucocorticoid receptor expression was differentially increased in the hippocampus of PAE males (75) whereas mineralocorticoid receptor expression was differentially increased in the hippocampus of PAE females compared to their control counterparts (75). Moreover, corticosterone replacement was shown to be ineffective at normalizing the adrenalectomy-induced increase in hippocampal mineralocorticoid mRNA levels in PAE males. Together, these results indicate that altered basal regulation of the HPA axis may play a critical role in the hyperresponsivity to stress observed in individuals exposed to alcohol during the prenatal period.

Our results also indicate that CMS reduced the neural activity in the hippocampal formation of control males. This is not surprising given the previous finding that CMS can reduce the number of hippocampal granule cells (78), which could be related to reduced survival of newborn cells (79).

MEDIAL PREFRONTAL CORTEX

The mPFC also plays a dual role in modulating stress and emotional responses. Abnormal volume and activation of the PFC are commonly observed in individuals with anxiety and mood disorders (16, 52). Importantly, functional neuroimaging studies indicate that individuals exposed to alcohol during gestation show greater blood oxygen level-dependent (BOLD) responses in the PFC when performing a behavioral inhibition task (18), suggesting that the PFC could be responding differentially when challenged. In rodents, reduced function of the mPFC, induced by electrolytic lesions or temporary deactivation, results in variable effects: some authors report decreased anxiety-like behaviors (80–82) while others report increased anxiety-like behaviors (83, 84). These discrepancies on the effects of the mPFC on anxiety-like behavior may be due to the extent of the lesions/deactivation and on the heterogeneous functions of the mPFC subregions. However, overall dysregulation of the mPFC can lead to an increased predisposition to psychopathologies. Our results show that PAE and control females exhibit different mPFC responses to the acute stress. Despite the fact that mPFC neural activity is increased in PAE non-CMS females, their behavioral performance was not different from controls in the elevated plus maze (31). Conversely, the neural activity within the mPFC of PAE males was not different from controls; however, PAE increased the expression of anxiety-like behaviors in male rats (31). This dissociation between behavioral and mPFC neural outcomes likely reflects the complex nature of anxiety- and depressive-like disorders, and suggests that other brain areas besides the mPFC may play a role in supporting increased anxiety-like behaviors observed in PAE animals.

The mPFC also plays a major role in modulating the HPA response to stressful events. However, its role is complex due to the fact that the PrL and ACC subregions are involved in inhibition while the IL is involved in excitation of the HPA axis (15, 85–87). Regardless of these opposing roles, our results show that the neural activity within the PrL and ACC in PAE and control animals follows a pattern similar to that within the IL. Specifically, PAE non-CMS females showed a higher response to acute stress in all mPFC areas than their female counterparts, with CMS reducing this neural activity. For the PrL, it is possible that the reduced neural activity in PAE females exposed to CMS may be associated with a reduction in CRH mRNA expression within this area (77), suggesting that the inhibitory action of the PrL may be reduced following CMS, contributing to the hyperresponsivity to stress in PAE females. Similar to the PrL, the ACC is also associated with inhibition of the HPA axis (15, 85–87) and the reduced neural activity of the ACC in PAE females exposed to CMS could also contribute to the hyperresponsivity to stress in those animals. Despite an opposite role, the IL – which is involved in excitation of the HPA axis (15, 85–87) – also showed reduced neural activity in PAE females exposed to CMS, which suggests a possible alteration in the excitatory/inhibitory balance within the mPFC of PAE females.

MEDIAL PARVOCELLULAR DORSAL DIVISION OF THE PARAVENTRICULAR NUCLEUS OF HYPOTHALAMUS

The mpdPVN is composed of a discrete set of neurons that integrate all direct and indirect stress-related inputs from the amygdala, mPFC, and hippocampal formation in order to launch an appropriate response to stress (15, 62). These neurons synthesize and secrete CRH, the primary regulator of the pituitary–adrenal axis (88). Our results indicate that, independent of sex, PAE, and control rats showed similar neural activity in the mpdPVN in response to the acute stress of exposure to the elevated plus maze. However, when facing a noxious stressor, such as shock, or a major challenge to HPA regulation, such as adrenalectomy, PAE has been shown to induce enhanced neural activity within the PVN (75, 89). Studies have demonstrated that exposure to the elevated plus maze induces small increases in neural activity within the PVN of control animals when compared to the neural activity of animals exposed to shock (34). These results suggest that when PAE animals are tested under basal conditions, or face a mild stressor such as the elevated plus maze, the PVN is capable of responding appropriately; however, when faced with a more severe stressor such as shock, the PVN launches an inappropriately large response.

CONSTRAINED PRINCIPAL COMPONENT ANALYSIS

The univariate results above indicate that PAE is associated with changes in neural activity across a wide range of brain regions; however, it is important to examine not only individual brain regions, but also how these regions interact to form networks and whether these networks might be differentially altered by PAE and/or CMS. In order to examine this possibility, we employed CPCA, which allowed for the identification of combinations of brain regions that were affected by PAE. CPCA is an ideal statistical technique for examining networks of brain regions that are related to one or more variables of interest (in the current study, prenatal treatment and CMS exposure) because networks are defined only from the portion of the overall variance that is predictable from these independent variables. As such, it was possible to identify networks of brain regions that were associated with PAE (and CMS) in the current study. Comparison between the univariate and multivariate results shows several similarities in terms of the brain regions affected by PAE and CMS; however, the aggregation of these brain regions into networks through CPCA facilitates interpretation.

Networks regulating stress and emotion in males

In males, CPCA revealed two functional networks that were collectively responsible for 34.17% of the total variation. The first network included the majority of the amygdala nuclei (medial, cortical, basal, and lateral) and all of the hippocampal formation areas analyzed (CA1, CA3, DG, and ventral subiculum). This *Amygdala + Hippocampal Formation network* was strongly active in control animals that were not exposed to CMS, but showed decreased activation in controls subjected to CMS. These findings suggest that the *Amygdala–Hippocampal Formation network* is critically involved in stress and emotional regulation in control males, and that exposure to chronic unpredictable stress induces functional changes in this network by preventing typical neural activation of the amygdala and hippocampal formation in response to acute stress. Dysregulation in this network may partially explain the increased susceptibility to the development of psychopathologies and abnormal stress responses in animals subjected to CMS (90–93). Importantly, in contrast to controls, the *Amygdala + Hippocampal Formation network* showed decreased activation for PAE non-CMS animals, indicating that PAE may alter the typical neural activity in these areas such that PAE animals in the non-CMS conditions look similar to controls in the CMS condition.

The second network extracted from the CPCA included all of the mPFC areas analyzed (PrL, ACC, and IL) and the mpdPVN. This *Prefrontal Cortex + Paraventricular Nucleus network* was activated to some degree in all prenatal groups in the non-CMS condition, although activation of this network was statistically significant only in the PAE non-CMS group. These data suggest that, independent of prenatal treatment, the mPFC and the mpdPVN form a network of structures that work together in the regulation of stress and emotion in males, but that PAE animals engage this network to a greater degree than control animals. This phenomenon could be a compensatory mechanism for the reduced involvement of the *Amygdala + Hippocampal Formation network*, as indicated by the negative correlation for PAE non-CMS males with this network. Additionally, all prenatal groups subjected to CMS in adulthood showed reduced activity in the *Prefrontal Cortex + Paraventricular Nucleus network* following acute stress. This switch between positive and negative associations with this network following exposure to CMS highlights the vulnerability of the mPFC and mpdPVN to chronic and unpredictable stress in adulthood. Indeed, the preclinical literature has demonstrated that the PFC is extremely susceptible to the effects of CMS, as chronic stress results in morphological alterations to mPFC neurons, including retraction of the apical dendritic branches and spine loss in layer II/III neurons (94–96). Additionally, stress-related morphological changes in the mPFC have been associated with increased anxiety- and depressive-like behaviors (90, 97) as well as with dysfunction in attentional set shifting and working memory (98, 99).

Networks regulating stress and emotion in females

Similar to males, CPCA indicated two functional neural networks in females. However, these networks were more restricted and only included major subdivisions of the same brain regions. The first network (*Prefrontal Cortex*) included all areas of the mPFC (ACC, IL, and PrL); the second network (*Amygdala*) included four nuclei of the amygdala (central, lateral, medial, and basal) and the IL. Together, these two functional neural networks in females accounted for 25.72% of the total variation, in contrast to 34.17% accounted for by the two functional networks in males.

The *Prefrontal Cortex network* was strongly activated in PAE non-CMS females, which contrasts with the decreased activity observed among control non-CMS females. Moreover, exposure to CMS resulted in a negative association for PAE females with the *Prefrontal Cortex network*, whereas there was no significant change in activation of this network in control females. These results indicate that PAE females rely on the *Prefrontal Cortex network* more than control females for stress and emotional regulation. This abnormal engagement of the *Prefrontal Cortex network* in response to acute stress is also observed in PAE males and indicates that alcohol exposure during the gestational period results in dysregulation of mPFC function in both sexes. Additionally, our results suggest that the mPFC of PAE females is more susceptible to the effects of CMS when compared to controls. The switch between positive and negative associations for the *Prefrontal Cortex network* following exposure to CMS highlights the vulnerability of the mPFC to chronic and unpredictable stress.

Similarly, the *Amygdala network*, which also contains the IL region, showed decreased activity in controls, independent of CMS condition, but was significantly positively activated in PAE females independent of CMS. As the amygdala and the IL subregion of the mPFC activate the HPA axis (14, 15), this abnormal increased activity of the *Amygdala network* following PAE may underlie, at least in part, the hyperresponsivity to stress observed in those animals. Additionally, the activity of the *Amygdala network* in response to acute stress in control and PAE females was not altered by CMS, indicating that, regardless of the level of activity, the amygdala of controls and PAE females is more resistant to chronic and unpredictable stress.

PAIR-FEEDING

The PF group is used to control the reduced food intake typically observed in alcohol-consuming dams. However, pair-feeding is an imperfect control procedure as it cannot control for the effects of alcohol on absorption and utilization of nutrients. Additionally, despite receiving optimal nutrition during pregnancy (43), the PF dams are underfed compared to the *ad libitum*-controls, as they receive a reduced ration matched to that of an alcohol-consuming partner. As a result, they are hungry, and typically consume their entire daily food ration within a few hours of presentation, remaining food deprived for the rest of the day. This pair-feeding regimen is thus a mild prenatal stressor, and its effects on offspring behavioral and physiological responsiveness represent, at least partially, an effect of stress above and beyond the nutritional aspect of receiving a reduced food ration. Our results suggest that PF males showed a pattern of neural activity within the amygdala and hippocampal formation similar to PAE males, while the pattern of neural activity within the mPFC and mdpPVN was similar to both PAE and controls. For females, on the other hand, the pattern of neural activity for PF animals was similar to the pattern displayed by the control group. Taken together, these results suggest that the males may be more susceptible to the effects of pair-feeding than females. However, the only effect exclusively observed in PF animals was a significant negative correlation of the *Amygdala network* with the non-CMS PF female condition,

which suggests that stress and emotional regulation rely less on the amygdala in PF than PAE or control females.

SUMMARY AND CONCLUSION

The results presented here build on and expand our knowledge of the differential neural regulation of stress and emotion in individuals exposed to alcohol during gestation. Importantly, despite a relatively low number of animals in one of the experimental groups, the use of CPCA provided a powerful approach for assessing global changes in functional neural networks in PAE compared to control animals, allowing us to go beyond simple assessments of changes in individual brain areas. Indeed, our results highlight how PAE and control males and females recruit different brain networks in the regulation of stress responses and emotion. Specifically, CPCA indicates that while control males rely more on the amygdala and hippocampal formation, PAE males rely more on the mPFC and mpdPVN when facing a stressful situation. In contrast, the functional neural networks underlying stress and emotional regulation in females are more restricted, as PAE females rely primarily on the mPFC and amygdala. Additionally, our results indicate that CMS differentially affected the neural networks regulating stress and emotion in PAE and control animals. Indeed, exposure to CMS reduced the activity of the *Amygdala + Hippocampal Formation network* in control males, but reduced the activity of the *Prefrontal Cortex + Paraventricular Nucleus network* in PAE males. For females, CMS only reduced the activity of the *Prefrontal Cortex network* in PAE animals. Together, our results suggest that PAE, regardless of its association with CMS, results in a sexually dimorphic dysregulation of the neurocircuitry that underlies stress and emotional regulation, which may be implicated in the stress hyperresponsivity and increased vulnerability to anxiety and depressive disorders observed among individuals exposed to alcohol during gestation (5, 9, 22, 23, 31). Finally, understanding the underlying neural mechanisms of deficits induced by PAE is a crucial step toward the establishment of specific strategies for treating resultant psychopathologies in these individuals.

ACKNOWLEDGMENTS

This research was funded by NIH/NIAAA grants R37 AA007789 and R01 AA022460, and NeuroDevNet (Canadian Networks of Centers of Excellence) grant 20R64153 to Joanne Weinberg, and Canadian Foundation of Fetal Alcohol Research (CFFAR) grant to Charlis Raineki and Joanne Weinberg. Kim G. C. Hellemans was funded by awards from the Michael Smith Foundation for Health Research and IMPART (Canadian Institute if Health Research STIHR). Tamara Bodnar was funded by Natural Sciences and Engineering Research Council of Canada (NSERC) graduate scholarship. Todd S. Woodward was funded by career investigator awards from CIHR and the Michael Smith Foundation. Katie M. Lavigne was funded by a CIHR award in partnership with the British Columbia Schizophrenia Society.

REFERENCES

1. Davidson RJ, McEwen BS. Social influences on neuroplasticity: stress and intervention to promote well-being. *Nat Neurosci* (2012) **15**:689–95. doi:10.1038/nn.3093
2. Levine S. Primary social relationships influence the development of the hypothalamic-pituitary-adrenal axis in the rat. *Physiol Behav* (2001) **73**:255–60. doi:10.1016/S0031-9384(01)00496-6
3. Cirulli F, Francia N, Berry A, Aloe L, Alleva E, Suomi AJ. Early life stress as a risk for mental health: role of neurotrophins from rodents to non-human primates. *Neurosci Biobehav Rev* (2009) **33**:573–85. doi:10.1016/j.neubiorev.2008.09.001
4. Murphy VE, Smith R, Giles WB, Clifton VL. Endocrine regulation of human fetal growth: the role of the mother, placenta, and fetus. *Endocr Rev* (2006) **27**:141–69. doi:10.1210/er.2005-0011
5. Hellemans KG, Sliwowska JH, Verma P, Weinberg J. Prenatal alcohol exposure: fetal programming and later life vulnerability to stress, depression and anxiety disorders. *Neurosci Biobehav Rev* (2010) **34**:791–807. doi:10.1016/j.neubiorev.2009.06.004
6. Riley EP, Infante MA, Warren KR. Fetal alcohol spectrum disorders: an overview. *Neurophychol Rev* (2011) **21**:73–80. doi:10.1007/s11065-011-9166-x
7. Schneider ML, Moore CF, Adkins MM. The effects of prenatal alcohol exposure on behavior: rodent and primate studies. *Neuropsychol Rev* (2011) **21**:186–203. doi:10.1007/s11065-011-9168-8
8. Valenzuela CF, Morton RA, Diaz MR, Topper L. Does moderate drinking harm the fetal brain? Insights from animal models. *Trends Neurosci* (2012) **35**:284–92. doi:10.1016/j.tins.2012.01.006
9. Weinberg J, Silwowska JH, Lan N, Hellemans KG. Prenatal alcohol exposure: foetal programming, the hypothalamic-pituitary-adrenal axis and sex differences in outcome. *J Neuroencodrinol* (2008) **20**:470–88. doi:10.1111/j.1365-2826.2008.01669.x
10. O'Connor MJ, Shah B, Whaley S, Cronin P, Gunderson B, Graham J. Psychiatric illness in clinical sample of children with prenatal alcohol exposure. *Am J Drug Alcohol Abuse* (2002) **28**:743–54. doi:10.1081/ADA-120015880
11. Pei J, Denys K, Hughes J, Rasmussen C. Mental health issues in fetal alcohol spectrum disorders. *J Ment Health* (2011) **20**:473–83. doi:10.3109/09638237.2011.577113
12. Streissguth AP, Aase JM, Clarren SK, Randels SP, LaDue RA, Smith DF. Fetal alcohol syndrome in adolescent and adults. *JAMA* (1991) **265**:1961–7. doi:10.1001/jama.265.15.1961
13. Streissguth AP, Bookstein FL, Barr HM, Sampson PD, O'Malley K, Young JK. Risk factors for adverse life outcomes in fetal alcohol syndrome and fetal alcohol effects. *J Dev Behav Pediatr* (2004) **25**:228–38. doi:10.1097/00004703-200408000-00002
14. Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry* (2005) **29**:1201–13. doi:10.1016/j.pnpbp.2005.08.006
15. Myers B, McKlveen JM, Herman JP. Neural regulation of the stress response: the many faces of feedback. *Cell Mol Neurobiol* (2012) **32**:683–94. doi:10.1007/s10571-012-9801-y
16. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct* (2008) **213**:93–118. doi:10.1007/s00429-008-0189-x
17. Krishnan V, Nestler EJ. Linking molecules to mood: new insight into the biology of depression. *Am J Psychiatry* (2010) **167**:1305–20. doi:10.1176/appi.ajp.2009.10030434
18. Fryer SL, Tapert SF, Mattson SN, Paulus MP, Spadonim AD, Riley SP. Prenatal alcohol exposure affects frontal-striatal BOLD response during inhibitory control. *Alcohol Clin Exp Res* (2007) **31**:1415–24. doi:10.1111/j.1530-0277.2007.00443.x
19. Norman AL, Crocker N, Mattson SN, Riley EP. Neuroimaging and fetal alcohol spectrum disorders. *Dev Disabil Res Rev* (2009) **15**:209–17. doi:10.1002/ddrr.72
20. Riikonen R, Salonen I, Partanen K, Verho S. Brain perfusion SPECT and MRI in foetal alcohol syndrome. *Dev Med Child Neurol* (1999) **41**:652–9. doi:10.1017/S0012162299001358
21. Willoughby KA, Sheard ED, Nash K, Rovet J. Effects of prenatal alcohol exposure on hippocampal volume, verbal learning, and verbal and spatial recall in late childhood. *J Int Neuopsychol Soc* (2008) **14**:1022–33. doi:10.1017/S1355617708081368
22. Haley DW, Handmaker NS, Lowe J. Infant stress reactivity and prenatal alcohol exposure. *Alcohol Clin Exp Res* (2006) **30**:2055–64. doi:10.1111/j.1530-0277.2006.00251.x

23. Jacobson SW, Bihun JT, Chiodo LM. Effects of prenatal alcohol and cocaine exposure on infant cortisol levels. *Dev Psychopathol* (1999) **11**:195–208. doi:10.1017/S0954579499002011
24. Ramsay DS, Bendersky MI, Lewis M. Effect of prenatal alcohol and cigarette exposure on two- and six-month-old infants' adrenocortical reactivity to stress. *J Pediatr Psychol* (1996) **21**:833–40. doi:10.1093/jpepsy/21.6.833
25. Lee S, Imaki T, Vale W, Rivier C. Effects of prenatal ethanol exposure to ethanol on the activity of the hypothalamic-pituitary-adrenal axis of the offspring: importance of the time of exposure to ethanol and possible modulating mechanisms. *Mol Cell Neurosci* (1990) **1**:168–77. doi:10.1016/1044-7431(90)90022-V
26. Lee S, Rivier C. Gender differences in the effects of prenatal alcohol exposure on the hypothalamic-pituitary-adrenal axis response to immune signals. *Psychoneuroendocrinology* (1996) **21**:145–55. doi:10.1016/0306-4530(95)00038-0
27. Nelson LR, Taylor AN, Lewis JW, Poland RE, Redei E, Branch BJ. Pituitary-adrenal responses to morphine and footshock stress are enhanced following prenatal alcohol exposure. *Alcohol Clin Exp Res* (1986) **10**:397–402. doi:10.1111/j.1530-0277.1986.tb05112.x
28. Redei E, Halasz I, Li LF, Prystowsky MB, Aird F. Maternal adrenalectomy alters the immune and endocrine functions of fetal alcohol-exposed male offspring. *Endocrinology* (1993) **133**:452–60. doi:10.1210/endo.133.2.8344191
29. Taylor AN, Branch BJ, Liu SH, Kokka N. Long-term effects of fetal ethanol exposure on pituitary-adrenal response to stress. *Pharmacol Biochem Behav* (1982) **16**:585–9. doi:10.1016/0091-3057(82)90420-8
30. Weinberg J. Hyperresonsiveness to stress: differential effects of prenatal ethanol on males and females. *Alcohol Clin Exp Res* (1988) **12**:647–52. doi:10.1111/j.1530-0277.1988.tb00258.x
31. Hellemans KG, Verma P, Yoon E, Yu WK, Weinberg J. Prenatal alcohol exposure increases vulnerability to stress and anxiety-like disorders in adulthood. *Ann N Y Acad Sci* (2008) **1144**:154–75. doi:10.1196/annals.1418.016
32. Hellemans KG, Verma P, Yoon E, Yu WK, Young AH, Weinberg J. Prenatal alcohol exposure and chronic mild stress differentially alter depressive- and anxiety-like behaviors in male and female offspring. *Alcohol Clin Exp Res* (2010) **34**:633–45. doi:10.1111/j.1530-0277.2009.01132.x
33. O'Connor MJ, Paley B. The relationship of prenatal alcohol exposure and postnatal environment to child depressive symptoms. *J Pediatr Psychol* (2006) **31**:50–64. doi:10.1093/jpepsy/jsj021
34. Duncan GE, Knapp DJ, Breese GR. Neuroanatomical characterization of Fos induction in rat behavior models of anxiety. *Brain Res* (1996) **713**:79–91. doi:10.1016/0006-8993(95)01486-1
35. File SE, Zangrossi H Jr, Sanders FL, Mabbutt PS. Raised corticosterone in the rat after exposure to the elevated plus-maze. *Psychopharmacology* (1994) **113**:543–6. doi:10.1007/BF02245237
36. Hunter MA, Takane Y. Constrained principal component analysis: various application. *J Educ Behav Stat* (2002) **22**:105–45. doi:10.3102/10769986027002105
37. Hyman JM, Whitman J, Emberly E, Woodward TS, Seamans JK. Action and outcome activity state patterns in the anterior cingulate cortex. *Cereb Cortex* (2013) **23**:1257–68. doi:10.1093/cercor/bhs104
38. Lavigne KM, Hofman S, Ring AJ, Ryder AG, Woodward TS. The personality of meaning in life: associations between dimensions of life meaning and the big five. *J Posit Psychol* (2013) **8**:34–43. doi:10.1080/17439760.2012.736527
39. Metzak P, Feredoes E, Takane Y, Wang L, Weinstein S, Cairo T, et al. Constrained principal component analysis reveals functionally connected load-dependent networks involved in multiple stages of working memory. *Hum Brain Mapp* (2011) **32**:856–71. doi:10.1002/hbm.21072
40. Takane Y, Shibayama T. Principal component analysis with external information on both subjects and variables. *Psychometrika* (1991) **56**:97–120. doi:10.1007/BF02294589
41. Woodward TS, Feredoes E, Metzak PD, Takane Y, Manoach DS. Epoch-specific functional networks involved in working memory. *Neuroimage* (2013) **65**:529–39. doi:10.1016/j.neuroimage.2012.09.070
42. Lan N, Yamashita F, Halpert AG, Ellis L, Yu WK, Viau V, et al. Prenatal ethanol exposure alters the effects of gonadectomy on hypothalamic-pituitary-adrenal activity in male rats. *J Neuroendocrinol* (2006) **18**:672–84. doi:10.1111/j.1365-2826.2006.01462.x
43. Weinberg J. Effects of ethanol and maternal nutritional status on fetal development. *Alcohol Clin Exp Res* (1985) **9**:49–55. doi:10.1111/j.1530-0277.1985.tb05049.x
44. Gallo PV, Weinberg J. Corticosterone rhythmicity in the rat: interactive effects of dietary restriction and schedule of feeding. *J Nutr* (1981) **111**:208–18.
45. Krieger DT. Food and water restriction shifts corticosterone, temperature, activity, and brain amine periodically. *Endocrinology* (1974) **95**:1195–202. doi:10.1210/endo-95-5-1195
46. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. San Diego: Academic Press (2005).
47. Cattell RB. The scree test for the number of factors. *Multivar Behav Res* (1966) **1**:245–76. doi:10.1207/s15327906mbr0102_10
48. Cattell RB, Vogelmann S. A comprehensive trial of the scree and KG criteria for determining the number of factors. *Multivar Behav Res* (1966) **12**:289–325. doi:10.1207/s15327906mbr1203_2
49. Takane Y. *Constrained Principal Component Analysis and Related Techniques*. Boca Raton: CRC Press (2013).
50. Takane Y, Hunter MA. Constrained principal component analysis: a comprehensive theory. *Appl Algebr Eng Comm* (2001) **12**:391–419. doi:10.1073/pnas.1306309110
51. Mairesse J, Viltart O, Salomé N, Giuliani A, Catalani A, Casolini P, et al. Prenatal stress alters the negative correlation between neuronal activation in limbic regions and behavioral responses in rats exposed to high and low anxiogenic environments. *Psychoneuroendocrinoligy* (2007) **32**:765–76. doi:10.1016/j.psyneuen.2007.03.013
52. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron* (2002) **34**:13–25. doi:10.1016/S0896-6273(02)00653-0
53. Ressler KJ, Mayberg HS. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nat Neurosci* (2007) **10**:1116–24. doi:10.1038/nn1944
54. Raineki C, Cortés MR, Belnoue L, Sullivan RM. Effects of early-life abuse differ across development: infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala. *J Neurosci* (2012) **32**:7758–65. doi:10.1523/JNEUROSCI.5843-11.2012
55. Shekhar A, Truitt W, Rainnie D, Sajdyk T. Role of stress, corticotrophin releasing factor (CRF) and amygdala plasticity in chronic anxiety. *Stress* (2005) **8**:209–19. doi:10.1080/10253890500504557
56. Coryell MW, Wunsch AM, Haenfler JM, Allen JE, Schinzler M, Ziemann AE, et al. Acid-sensing ion channel-1a in the amygdala, a novel therapeutic target in depression related behavior. *J Neurosci* (2009) **29**:5381–8. doi:10.1523/JNEUROSCI.0360-09.2009
57. Duncan GE, Breese GR, Criswell H, Stumpf WE, Mueller RE, Cavey JB. Effects of antidepressant drugs injected into the amygdala on behavioral responses of rats in the forced swim test. *J Pharmacol Exp Ther* (1986) **238**:758–62.
58. de Souza EB, Perrin MH, Insel TR, Rivier J, Vale WW, Kuhar MJ. Corticotropin-releasing factor receptors in rat forebrain: autoradiographic identification. *Science* (1984) **224**:1449–51. doi:10.1126/science.6328656
59. Sakanaka M, Shibasaki T, Lederis K. Distribution and efferent projections of corticotropin-releasing factor-like immunoreactivity in the rat amygdaloid complex. *Brain Res* (1986) **382**:213–38. doi:10.1016/0006-8993(86)91332-6
60. Rainnie DG, Bergeron R, Sajdky TJ, Patil M, Gehlert DR, Shekhar A. Corticotrophin releasing factor-induced synaptic plasticity in the amygdala translates stress into emotional disorders. *J Neurosci* (2004) **24**:3471–9. doi:10.1523/JNEUROSCI.5740-03.2004
61. Sajdyk TJ, Schober DA, Gehlert DR, Shekhar A. Role of corticotropin-releasing factor and urocortin within the basolateral amygdala of rats in anxiety and panic responses. *Behav Brain Res* (1999) **100**:207–15. doi:10.1016/S0166-4328(98)00132-6
62. Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress response. *Nat Rev Neurosci* (2009) **10**:397–409. doi:10.1038/nrn2647
63. Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* (2010) **65**:7–19. doi:10.1016/j.neuron.2009.11.031
64. Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, et al. Regional dissociations within the hippocampus-memory and anxiety. *Neurosci Biobehav Rev* (2004) **28**:273–83. doi:10.1016/j.neubiorev.2004.03.004
65. Adhikari A, Topiwala MA, Gordon JA. Synchronized activity between the ventral hippocampus and medial prefrontal cortex during anxiety. *Neuron* (2010) **65**:257–69. doi:10.1016/j.neuron.2009.12.002
66. Adhikari A, Topiwala MA, Gordon JA. Single units in the medial prefrontal cortex with anxiety-related firing patterns are preferentially influenced by ventral

hippocampus activity. *Neuron* (2011) **71**:898–910. doi:10.1016/j.neuron.2011.07.027
67. Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, et al. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol* (2003) **24**:151–80. doi:10.1016/j.yfrne.2003.07.001
68. Dunn JD, Orr SE. Differential plasma corticosterone responses to hippocampal stimulation. *Exp Brain Res* (1984) **54**:1–6. doi:10.1007/BF00235813
69. West JR, Hodges CA, Black AC. Prenatal exposure to ethanol alters the organization of hippocampal mossy fibres in rats. *Science* (1981) **211**:957–9. doi:10.1126/science.7466371
70. Abel EL, Jacobson S, Sherwin BT. In utero alcohol exposure: functional and structural brain damage. *Neurobehav Toxicol Teratol* (1983) **5**:363–6.
71. Christie BR, Swann SE, Fox CJ, Froc D, Lieblich SE, Redila C, et al. Voluntary exercise rescues deficits in spatial memory and long-term potentiation in prenatal ethanol-exposed male rats. *Eur J Neurosci* (2005) **21**:1719–26. doi:10.1111/j.1460-9568.2005.04004.x
72. Sutherland RJ, McDonald RJ, Savage DD. Prenatal exposure to moderate levels of ethanol can have long-lasting effects on hippocampal synaptic plasticity in adult offspring. *Hippocampus* (1997) **7**:232–8. doi:10.1002/(SICI)1098-1063(1997)7:2<232::AID-HIPO9>3.3.CO;2-O
73. Redila VA, Olson AK, Swann SE, Mohades G, Webber AJ, Weinberg J, et al. Hippocampal cell proliferation is reduced following prenatal ethanol exposure but can be rescued with voluntary exercise. *Hippocampus* (2006) **16**:305–11. doi:10.1002/hipo.20164
74. Sliwowska JH, Barker JM, Barha CK, Lan N, Weinberg J, Galea LA. Stress-induced suppression of hippocampal neurogenesis in adult male rats is altered by prenatal ethanol exposure. *Stress* (2010) **13**:302–14. doi:10.3109/10253890903531582
75. Glavas MM, Ellis L, Yu WK, Weinberg J. Effects of prenatal ethanol exposure on basal limbic-hypothalamic-pituitary-adrenal regulation: role of corticosterone. *Alcohol Clin Exp Res* (2007) **31**:1598–610. doi:10.1111/j.1530-0277.2007.00460.x
76. Kim CK, Yu W, Edin G, Ellis L, Osborn JA, Weinberg J. Chronic intermittent stress does not differentially alter brain corticosteroid receptor densities in rats prenatally exposed to ethanol. *Psychoneuroendocrinology* (1999) **24**:585–611. doi:10.1016/S0306-4530(99)00015-3
77. Uban KA, Comeau WL, Ellis LA, Galea LA, Weinberg J. Basal regulation of HPA and dopamine systems is altered differentially in males and females by prenatal alcohol exposure and chronic variable stress. *Psychoneuroendocrinology* (2013) **38**:1953–66. doi:10.1016/j.psyneuen.2013.02.017
78. Jayatissa MN, Bisgaard CF, West MJ, Wiborg O. The number of granule cells in rat hippocampus is reduced after chronic mild stress and re-established after chronic escitalopran treatment. *Neuropharmacology* (2008) **54**:530–41. doi:10.1016/j.neuropharm.2007.11.009
79. Lee KL, Kim SJ, Kim SJ, Choi SH, Shin YC, Park SH, et al. Chronic mild stress decreases survival, but nor proliferation, of new-born cells in adult rat hippocampus. *Exp Mol Med* (2006) **38**:44–54. doi:10.1038/emm.2006.6
80. Lacroix L, Spinelli S, Heidbreader CA, Feldon J. Differential role of the medial and lateral prefrontal cortices in fear and anxiety. *Behav Neurosci* (2000) **114**:1119–30. doi:10.1037/0735-7044.114.6.1119
81. Resstel LB, Souza RF, Guimarães FS. Anxiolytic-like effects induced by medial prefrontal cortex inhibition in rats submitted to the Vogel conflict test. *Physiol Behav* (2008) **93**:200–5. doi:10.1016/j.physbeh.2007.08.009
82. Shah AA, Treit D. Excitotoxic lesions of the medial prefrontal cortex attenuates fear responses in the elevated-plus maze, social interaction and shock probe burying tests. *Brain Res* (2003) **969**:183–94. doi:10.1016/S0006-8993(03)02299-6
83. Jinks AL, McGregor IS. Modulation of anxiety-related behaviours following lesions of the prelimbic or infralimbic cortex in the rat. *Brain Res* (2007) **772**:181–90. doi:10.1016/S0006-8993(97)00810-X
84. de Visser L, Baars AM, van't Klooster J, van den Bos R. Transient inactivation of the medial prefrontal cortex affects both anxiety and decision-making in male Wistar rats. *Front Neurosci* (2011) **5**:102. doi:10.3389/fnins.2011.00102
85. Figueiredo HF, Bruestle A, Bodie B, Doglas CM, Herman JP. The medial prefrontal cortex differentially regulates stress induced *c-fos* expression in the forebrain depending on type of stressor. *Eur J Neurosci* (2003) **18**:2357–64. doi:10.1046/j.1460-9568.2003.02932.x
86. Radley JJ, Arias CM, Sawchenko PE. Regional differentiation of the medial prefrontal cortex in regulating adaptive responses to acute emotional stress. *J Neurosci* (2006) **26**:12967–76. doi:10.1523/JNEUROSCI.4297-06.2006
87. Radley JJ, Gosselink KL, Sawchenko PE. A discrete GABAergic relay mediated medial prefrontal cortical inhibition of the neuroendocrine stress response. *J Neurosci* (2009) **29**:7330–40. doi:10.1523/JNEUROSCI.5924-08.2009
88. Charmandari E, Tsigos C, Chrousos G. Endocrinology of the stress response. *Annu Rev Physiol* (2005) **67**:259–84. doi:10.1146/annurev.physiol.67.040403.120816
89. Lee S, Schmidt D, Tilders F, Rivier C. Increased activity of the hypothalamic-pituitary-adrenal axis of rats exposed to alcohol *in utero*: role of altered pituitary and hypothalamic function. *Mol Cell Neurosci* (2000) **16**:515–28. doi:10.1006/mcne.2000.0890
90. Bondi CO, Rodriguez G, Gould GG, Frazer A, Morilak DA. Chronic unpredictable stress induces cognitive deficit and anxiety-like behavior in rats that is prevented by chronic antidepressant drug treatment. *Neuropsychopharmacology* (2008) **33**:320–31. doi:10.1038/sj.npp.1301410
91. Christiansen S, Bouzinova EV, Palme R, Wiborg O. Circadian activity of the hypothalamic-pituitary-adrenal axis is differentially affected in the rat chronic mild stress model of depression. *Stress* (2012) **15**:647–57. doi:10.3109/10253890.2011.654370
92. Hill MN, Hellemans KG, Verma P, Gorzalka BB, Weinberg J. Neurobiology of chronic mild stress: parallels to major depression. *Neurosci Biobehav Rev* (2012) **36**:2085–117. doi:10.1016/j.neubiorev.2012.07.001
93. Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by tricyclic antidepressant. *Psychopharmacology* (1987) **93**:358–64. doi:10.1007/BF00187257
94. Michelsen KA, van der Hove DL, Schmitz C, Segers O, Prickaerts J, Steinbusch HW. Prenatal stress and subsequent exposure to chronic mild stress influence dendritic spine density and morphology in the rat medial prefrontal cortex. *BMC Neurosci* (2007) **8**:107. doi:10.1186/1471-2202-8-107
95. Radley JJ, Rocher AB, Janssen WG, Hof PR, McEwen BC, Morrison JH. Reversibility of apical dendritic retraction in the rat medial prefrontal cortex following repeated stress. *Exp Neurol* (2005) **196**:199–203. doi:10.1016/j.expneurol.2005.07.008
96. Radley JJ, Rocher AB, Rodriguez A, Ehlenberger DB, Dammann M, McEwen BS, et al. Repeated stress alters dendritic spine morphology in the rat medial prefrontal cortex. *J Comp Neurol* (2008) **507**:1141–50. doi:10.1002/cne.21588
97. Banasr M, Duman RS. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. *Biol Psychiatry* (2008) **64**:863–70. doi:10.1016/j.biopsych.2008.06.008
98. Arnsten AF. Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci* (2009) **10**:410–22. doi:10.1038/nrn2648
99. Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, et al. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *J Neurosci* (2006) **26**:7870–4. doi:10.1523/JNEUROSCI.1184-06.2006

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 October 2013; accepted: 10 January 2014; published online: 13 February 2014.

*Citation: Raineki C, Hellemans KGC, Bodnar T, Lavigne KM, Ellis L, Woodward TS and Weinberg J (2014) Neurocircuitry underlying stress and emotional regulation in animals prenatally exposed to alcohol and subjected to chronic mild stress in adulthood. Front. Endocrinol. **5**:5. doi: 10.3389/fendo.2014.00005*

This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
ENDOCRINOLOGY

ORIGINAL RESEARCH ARTICLE
published: 13 December 2013
doi: 10.3389/fendo.2013.00190

Glucocorticoid programing of the mesopontine cholinergic system

Sónia Borges[1,2], Bárbara Coimbra[1,2], Carina Soares-Cunha[1,2], Ana P. Ventura-Silva[1,2], Luisa Pinto[1,2], Miguel M. Carvalho[1,2], José-Miguel Pêgo[1,2], Ana João Rodrigues[1,2]* and Nuno Sousa[1,2]*

[1] *Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Braga, Portugal*
[2] *ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal*

Edited by:
Nikolaos P. Daskalakis, Icahn School of Medicine at Mount Sinai, USA

Reviewed by:
Therese Jay, INSERM U894, France
Li-Tung Haung, Chang Gung University College of Medicine, Taiwan

***Correspondence:**
Ana João Rodrigues and Nuno Sousa, Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal
e-mail: ajrodrigues@ecsaude.uminho.pt; njcsousa@ecsaude.uminho.pt

Stress perception, response, adaptation, and coping strategies are individually distinct, and the sequel of stress and/or glucocorticoids (GCs) is also distinct between subjects. In the last years, it has become clear that early life stress is a powerful modulator of neuroendocrine stress-responsive circuits, programing intrinsic susceptibility to stress, and potentiating the appearance of stress-related disorders such as depression, anxiety, and addiction. Herein we were interested in understanding how early life experiences reset the normal processing of negative stimuli, leading to emotional dysfunction. Animals prenatally exposed to GCs (*in utero* glucocorticoid exposure, iuGC) present hyperanxiety, increased fear behavior, and hyper-reactivity to negative stimuli. In parallel, we found a remarkable increase in the number of aversive 22 kHz ultrasonic vocalizations in response to an aversive cue. Considering the suggested role of the mesopontine tegmentum cholinergic pathway, arising from the laterodorsal tegmental nucleus (LDT) and pedunculopontine tegmental nucleus (PPT), in the initiation of 22 kHz vocalizations and hypothetically in the control of emotional arousal and tone, we decided to evaluate the condition of this circuit in iuGC animals. Notably, in a basal situation, iuGC animals present increased choline acetyltransferase (ChAT) expression in the LDT and PPT, but not in other cholinergic nuclei, namely in the nucleus basalis of Meynert. In addition, and in accordance with the amplified response to an adverse stimulus of iuGC animals, we found marked changes in the cholinergic activation pattern of LDT and PPT regions. Altogether, our results suggest a specific cholinergic pathway programing by prenatal GC, and hint that this may be of relevance in setting individual stress vulnerability threshold.

Keywords: glucocorticoids, stress, acetylcholine, anxiety, fear, pedunculopontine tegmental nucleus, laterodorsal tegmental nucleus, ultrasonic vocalizations

INTRODUCTION

Exposure to stressful events or synthetic glucocorticoids (GCs), such as dexamethasone, early in life, are a risk factors for the development of different neuropsychiatric disorders in adulthood, namely depression and anxiety (1). Such effects are partially mediated by de-regulation of the hypothalamic-pituitary-adrenal (HPA) axis, leading to altered GC secretion (2, 3), which can induce long-term molecular and functional changes in GC-sensitive nuclei. Importantly, several recent studies showed that high levels of GCs have a deleterious effect in the developing brain, inducing prominent neurochemical, structural, and molecular changes in several brain regions (4–8) culminating in the development of anxious and depressive-like traits (9–13).

GC programing effects in emotional behavior are far from being completely understood at a mechanistic level, but it has been shown that GCs modulate on the long-term the activity of particular neural pathways. In this perspective it is interesting to refer that early life stress/GC exert a powerful effect in the developing dopaminergic neurons, especially at the mesolimbic level (7). For example, we have shown that animals prenatally exposed to GC present marked hypodopaminergia and D2 epigenetic/expression changes, responsible for their anhedonia and motivational deficits, since administration of a dopamine precursor, L-DOPA, fully reverted the molecular and behavioral impairments (14, 15). Obviously, this dopaminergic de-regulation may also occur indirectly through modulation of upstream neurotransmitter systems. One promising candidate is the ascending cholinergic pathway, comprising projections from acetylcholine (ACh)-rich nuclei within the pons, particularly the laterodorsal (LDT) and pedunculopontine tegmental nuclei (PPT) to the ventral tegmental area (VTA) (16–18). In support of this, it was already shown that stress/GC strongly elicit cholinergic activity of these regions (19), and that they, in turn, critically affect basal and phasic activity of VTA neurons (20–23). Additionally, GC can putatively bind to GC-responsive elements of cholinergic players and control their expression, namely choline acetyltransferase (ChAT) (24) and acetylcholine esterase (AChE) (25), two enzymes of the cholinergic pathway. Also relevant is to note that the ascending cholinergic system is implicated in the control of the stress response by modulating hypothalamic pituitary adrenal (HPA) axis function (26–28) and in mediating the anxiogenic effects of stress (29–31).

Surprisingly, very few studies have focused on the impact of stress/GC exposure in the cholinergic system. Considering the lack of evidence, herein, we focused on the impact of prenatal exposure to GCs in the ascending cholinergic system, and the relevance of such changes in stress-related anxious and fear behaviors.

MATERIALS AND METHODS

ANIMALS AND TREATMENTS

All manipulations were conducted in accordance with local regulations on animal care and experimentation (European Union Directive 2010/63/EU). Pregnant Wistar Han rats were subcutaneously injected with the synthetic GC dexamethasone at 1 mg kg^{-1} (*in utero* glucocorticoid exposure, iuGC animals), or with vehicle (CONT; control animals), on days 18 and 19 of gestation.

Dexamethasone dosage was selected based on our previous studies showing that this regimen effectively impairs HPA axis activity in a long-term basis (12). From a clinical perspective, guidelines on prenatal corticotherapy (32, 33) recommend single course administration (0.3–0.5 mg/kg), however, multiple courses of GCs are often administered (34), despite the lack of evidence of increased therapeutic efficacy. Nevertheless, we must consider the difficulty in the transposition of human doses to rodents due to ADME species specificity.

At weaning day, male offspring were house-paired randomly, according with prenatal treatment, under standard laboratory conditions: artificial 12 h light/dark cycle (lights on from 08:00 a.m. to 08:00 p.m.); room temperature 22°C; food and water were provided *ad libitum*. Animals derived from at least three different litters were used for all the experimental procedures.

BEHAVIORAL TESTS

All tests were performed during the day period, except the confined cage and fear-conditioning protocols that were conducted during the night period (08:30 p.m. to 03:00 a.m.). All behavioral equipment was cleaned between animals (ethanol 10%) in order to remove any olfactory cues.

OPEN FIELD

The open field (OF) test was conducted in an arena (43.2 cm × 43.2 cm) with transparent acrylic walls and white floor (Med Associates Inc., St. Albans, VT, USA). Rats were placed in the center of the arena and movement was monitored over a period of 10 min with the aid of two 16-beam infrared arrays. Total distance traveled was used as an indicator of locomotor activity.

ELEVATED PLUS MAZE

The elevated plus maze (EPM) test was carried out under bright white light. Animals were placed individually for 5 min in the center of a black polypropylene plus-shaped platform elevated 72.4 cm above the floor. The apparatus consisted of two open arms (50.8 cm × 10.2 cm) and two closed arms (50.8 cm × 10.2 cm × 40.6 cm) (MedAssociates Inc., St. Albans, VT, USA). The number of entries into each arm and the time spent therein were recorded.

LIGHT/DARK BOX TEST

The light/dark box (L/D) test was performed inside the OF arena (43.2 cm × 43.2 cm) (MedAssociates Inc., St. Albans, VT, USA). A dark compartment was attached to one side with an opening facing the center of the arena. Animals were individually placed in the center of the illuminated part. The distance traveled and time spent in each compartment was recorded in a single trial of 10 min.

CONFINED CAGE TEST

The confined cage test was performed in a non-restrictive Plexiglas cylinder (inner diameter 8.8 cm, length 22.2 cm), mounted on a Plexiglas platform and placed in a ventilated, sound-attenuated chamber (SR-LAB, San Diego Instruments, San Diego, CA, USA). A stainless steel grid was placed inside the cylinder, through which an electric current could be passed (shock chamber). A microphone and a video camera were placed inside the sound-attenuated chamber. The protocol was performed in two consecutive days, in which the animals were placed inside the shock chamber for 3 min. The ultrasonic vocalizations (USVs) and the percentage of total freezing time were measured.

FEAR-CONDITIONING PARADIGM

The fear-conditioning test was performed in a non-restrictive Plexiglas cylinder (inner diameter 8.8 cm, length 22.2 cm), mounted on a Plexiglas platform and placed in a ventilated, sound-attenuated chamber (SR-LAB, San Diego Instruments, San Diego, CA, USA). The protocol was performed in three consecutive days (35). On the first day (habituation), each animal was placed in the shock chamber for 11 min. On the second day (conditioning), each subject was positioned inside the shock chamber for 3 min (no light, no shock). Afterwards, animals were exposed to six lights/shock pairings (0.4 ± 0.1 mA), with an inter-stimulus-interval (ISI) of 60 s. The shock was given for 500 ms, immediately after the cue light was turned off. On the following day (test day), after an initial phase of 3 min without light, animals were presented with a 20 s cue light for six times, but no shock was given (ISI of 60 s). During all procedures, USVs and freezing behavior were recorded.

USVs ANALYSIS

An ultrasound microphone (CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) sensitive to frequencies of 10–200 kHz, placed 15 cm above the floor, was used in all experiments. The microphone was connected via an Avisoft UltrasoundGate 416H (Avisoft Bioacoustics) to a personal computer; USVs were recorded using the Avisoft-Recorder (version 5.1.04) with the following settings: sampling rate: 250,000; format: 16 bit. For acoustical analysis, recordings were transferred to Avisoft SASLab Pro (version 5.1.22, Avisoft Bioacoustics). This program was used in order to produce spectrograms of USVs by conducting a fast Fourier transformation (256 FFT-length, 100% frame, Hamming window filter, 50% time window overlap). These spectrograms had a frequency resolution ∼1.2 kHz and a temporal resolution ∼0.4 ms.

Twenty-two kilohertz call detection was provided by an automated threshold-based algorithm (threshold: −40 dB) and a hold time mechanism (hold time: 20 ms). A lower-cut-off-frequency of 18 kHz was used to reduce background noise.

Calls were also inspected manually to ensure that, when necessary, USVs not detected automatically could be subsequently included in the automatic parameter analysis.

IMMUNOHISTOCHEMISTRY

For immunohistochemistry (IHC), 11 rats were sacrificed by pentobarbital (Eutasil, Sanofi) anesthesia and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde (pH 7.4 in 0.1 M phosphate buffer). Brains were removed and post-fixed for 48 h in 4% paraformaldehyde and then rinsed and stored in 30% of sucrose until sectioning. Brains were sectioned coronally, at a thickness of 50 μm, on a vibratome (VT1000S, Leica, Germany) and stored in cryoprotectant solution at −20°C until use.

Briefly, free-floating sections were pre-treated with 3% H_2O_2 in PBS, thoroughly rinsed in PBS, blocked with 2.5% fetal bovine serum (FBS) in PBS-Triton 0.3% for 2 h at room temperature, and then incubated overnight at 4°C with primary antibody goat anti-ChAT (Millipore, MA, USA; 1:1000). Afterwards, sections were washed, incubated with the secondary biotinylated anti-goat (Vector Lab., USA; 1:200) for 1 h, and processed with an avidin-biotin complex solution (ABC-Elite Vectastain reagent; Vector Lab, USA). Detection was done using 0.5 mg/ml 3,3′-diaminobenzidine. Sections were washed and mounted on glass slides, air-dried, counterstained with hematoxylin, and cover slipped with Entellan-New (Merck, Darmstadt, Germany).

IMMUNOFLUORESCENCE

Ninety minutes after completion of the fear-conditioning paradigm, 12 animals were deeply anesthetized with pentobarbital (Eutasil, Sanofi) and were transcardially perfused with 0.9% saline followed by 4% paraformaldehyde. Brains were removed and post-fixed in 4% paraformaldehyde. Coronal vibratome sections (50 μm) were first incubated with rabbit anti-c-fos primary antibody (1:1000; Ab-5, Calbiochem, USA), followed by incubation with goat anti-ChAT primary antibody (1:1000; anti-ChAT, Millipore, MA, USA). Secondary fluorescent antibodies were: Dylight 488-conjugated donkey anti-rabbit IgG (1:500, BioLegend), and Alexa Fluor 568-conjugated donkey anti-goat IgG (1:500, Invitrogen), respectively. Finally, all sections were stained with 4′,6-diamidino-2-phenylindole (DAPI; 1 mg/ml). For each animal, c-fos-positive cells within the PPT and LDT were analyzed after double staining with cholinergic (ChAT) marker and cell counts were performed by confocal microscopy (Olympus FluoViewTMFV1000, Hamburg, Germany). Estimation of cell density was obtained by crossing cell number values with the corresponding areas, determined using an Olympus BX51 optical microscope and the StereoInvestigator software (Microbrightfield, VT, USA).

STEREOLOGICAL PROCEDURES

Eleven animals were perfused transcardially with 4% paraformaldehyde, under deep pentobarbital (Eutasil, Sanofi) anesthesia. Brains were included in glycolmethacrylate (Tecnovit 7100, Heraeus Kulzer, Wehrheim, Germany) and sectioned on a microtome as described in detail elsewhere (36). Every other 30 μm thick coronal section was collected on a gelatinized slide, stained with Giemsa, mounted with Entellan-New (Merck, Darmstadt, Germany), and cover slipped. Stereological procedures were performed by a blind observer.

Laterodorsal tegmental and PPT regions were outlined according to the atlas of Paxinos and Watson (37) and based on noticeable cytoarchitectural differences, namely density of cells and size of the perikarya.

Volume and neuronal number estimations were performed using StereoInvestigator software (Microbrightfield, VT, USA) and a camera attached to a motorized microscope (Axioplan 2, Carl Zeiss, Germany). The Cavalieri's principle was used to assess volume. Briefly, every second section was used and the cross-sectional area was estimated by point counting (final magnification 112×). We used a test point system in which the interpoint distance, at the tissue level, was 150 μm for LDT and 200 μm for PPT. The volume of the region of interest was calculated from the number of points within its boundaries and the distance between sampled sections.

Average cell numbers were estimated using the optical fractionator method (38). Briefly, a grid of virtual 3D-boxes (LDT: 30 μm × 30 μm × 20 μm; PPT: 40 μm × 40 μm × 20 μm) equally spaced (using the same grid spacing as for volume estimations) was superimposed on every second section of the lamina of interest and cells within boxes were counted. Coefficients of error (CE) were automatically computed, according to the formulas of Gundersen et al. (39) for cell numbers and Gundersen et al. (40) for volume estimations. Glial cells were not included in the estimations, and the discrimination between neuronal and glial cell body profiles was based on the criteria described by Ling et al. (41) and Peinado et al. (42).

STATISTICAL ANALYSIS

Statistical analysis was performed in GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical analysis between two groups was made using Student's *t*-test or Mann–Whitney tests. Two-way analysis of variance (ANOVA) was used when appropriate. Bonferroni's *post hoc* multiple comparison test was used for group differences determination. Non-parametric analysis (Mann–Whitney test) was used when normality of data was not assumed. Results are presented as mean ± SEM. Statistical significance was accepted for $p \leq 0.05$.

RESULTS

IN UTERO GLUCOCORTICOID EXPOSURE IMPAIRS EMOTIONAL BEHAVIOR

Animals were exposed to a battery of behavioral tests that consisted of paradigms studying spontaneous exploratory behavior (OF), tasks of innate anxiety (EPM, L/D test, confined cage), and reactivity to adverse stimulus (version of fear-conditioning paradigm). Since USVs can give information on the emotional status of the animal, we decided to further complement the behavioral characterization by measuring USVs in these different paradigms.

In the OF, iuGC animals presented a decrease in the number of ambulatory counts (**Figure 1A**, $t = 2.197$, $p = 0.037$) and total distance traveled (**Figure 1B**, $t = 3.002$, $p = 0.006$) when compared with control animals. In addition, iuGC animals exhibited a decrease in the percentage of time spent in the center of the arena (**Figure 1C**, $t = 2.416$, $p = 0.023$).

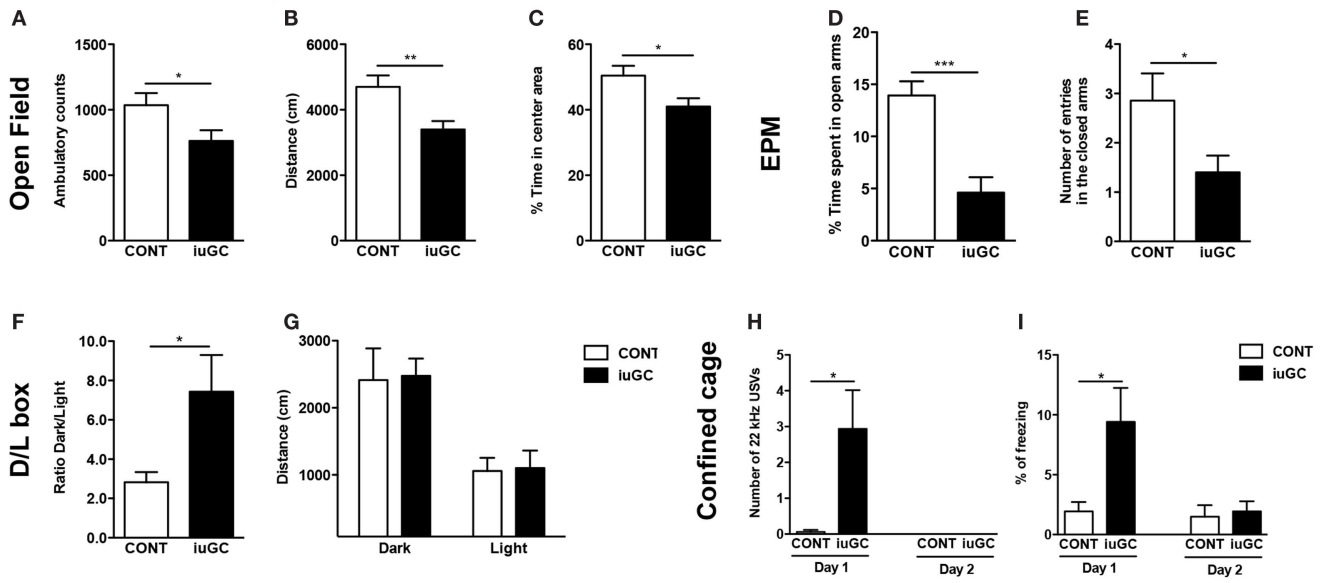


FIGURE 1 | ***In utero*** **glucocorticoid exposure induces an anxious-like behavior**. iuGC group presented a decrease in ambulatory counts **(A)**, total distance traveled **(B)**, and percentage of time in the center of the arena **(C)**. In the EPM, iuGC animals exhibited a decrease in the percentage of time spent **(D)** and number of entries **(E)** in the open arms of the maze, when compared with control group. **(F)** In the L/D test, iuGC presented an increase in the ratio dark/light, with no differences on the distance traveled in both compartments **(G)**. In the confined cage, iuGC group presented an increase in the number of 22 kHz USVs **(H)** and freezing behavior **(I)**. Upon habituation, iuGC animals no longer presented this anxious behavior (day 2). CONT, control animals; iuGC, *in utero* GC exposed animals; USVs, ultrasonic vocalizations. $*p < 0.05$, $**p < 0.001$, and $***p < 0.0001$; **(A–C)** $n = 16$; **(D,E)** $n = 11$–13; **(F,G)** $n = 10$, **(H,I)** $n = 8$–16.

In the EPM, iuGC animals spent significantly less time in the open arms (**Figure 1D**, $t = 2.947$, $p = 0.009$) and presented a reduction in the number of open arms entries (**Figure 1E**, $t = 2.375$, $p = 0.031$), when compared with control animals. No differences were found in the time and entries in the closed arms (data not shown; time: $t = 0.479$, $p = 0.637$; entries: $t = 0.872$, $p = 0.392$).

In the L/D test, iuGC animals presented an increase in the ratio dark/light (**Figure 1F**, $t = 2.765$, $p = 0.014$) and no differences in distance traveled in both compartments (**Figure 1G**, dark: $t = 0.117$, $p = 0.909$; light: $t = 0.137$, $p = 0.893$).

In the confined cage, while control animals rarely emitted aversive 22 kHz USVs, iuGC animals vocalized throughout the exposure (**Figure 1H**; $U = 81$, $p = 0.016$). Similarly, iuGC group presented increased freezing behavior (**Figure 1I**; $t = 2.846$, $p = 0.013$). Upon habituation to the cage (2 days of exposure), iuGC animals no longer presented this anxious-like response.

To investigate reactivity to an adverse stimulus, we performed a variation of the classical fear-conditioning paradigm. Animals were conditioned to a cue (light) predicting painful electric shocks. After cage habituation, animals were given six pairs of cue-shock. No emission of 22 kHz USVs was observed in the baseline phase (0–3 min), but upon light/shock pairings, as expected, both groups significantly emitted more negative USVs. iuGC animals emitted negative USVs to a greater extent than control animals (**Figure 2A**, $t = 1.562$, $p = 0.130$) and also presented an increase in freezing behavior time (**Figure 2B**, $t = 2.355$, $p = 0.034$). After this conditioning session, in the next day, animals were exposed to the cue, but no shock was given. In the initial period, both groups emitted more 22 kHz USVs than the day before ($F_{1,58} = 4.10$, $p = 0.047$); cue exposure elicited more negative USVs in both groups, but again, iuGC animals were over-reactive (**Figure 2C**, $t = 2.804$, $p = 0.011$). Analysis of freezing behavior further confirmed the phenotype of iuGC group (**Figure 2D**, $t = 2.087$, $p = 0.049$). Plotting the number of negative USVs along time further confirms that iuGC animals were over-reactive to the cue predicting the adverse stimulus, since iuGC group emitted more context-induced 22 kHz vocalizations than control group, especially in the first 60 s (**Figure 2E**, $F_{1,131} = 104.42$, $p < 0.0001$). Upon the first cue exposure (ON period), both groups increased the number of USVs with no major differences between them. However, upon second cue exposure, iuGC animals emitted more negative USVs than control group and remained over-reactive throughout. The pattern of 22 kHz USVs emission in both groups is interesting since immediately after the light is turned off, both groups emit more negative vocalizations, indicative of the consolidated association of the cue with the electric shock.

iuGC EXPOSURE INDUCES PROMINENT CHOLINERGIC ALTERATIONS

Pharmacological studies suggest that the ascending cholinergic tegmental system is responsible for the initiation and production of negative vocalizations in rodents (43–45). In addition, cholinergic signaling is highly responsive to stress/GC (46) and is important for the manifestation of aversive behaviors (47). Considering these findings, we decided to further explore the impact of iuGC exposure in the cholinergic circuitry. To do so,

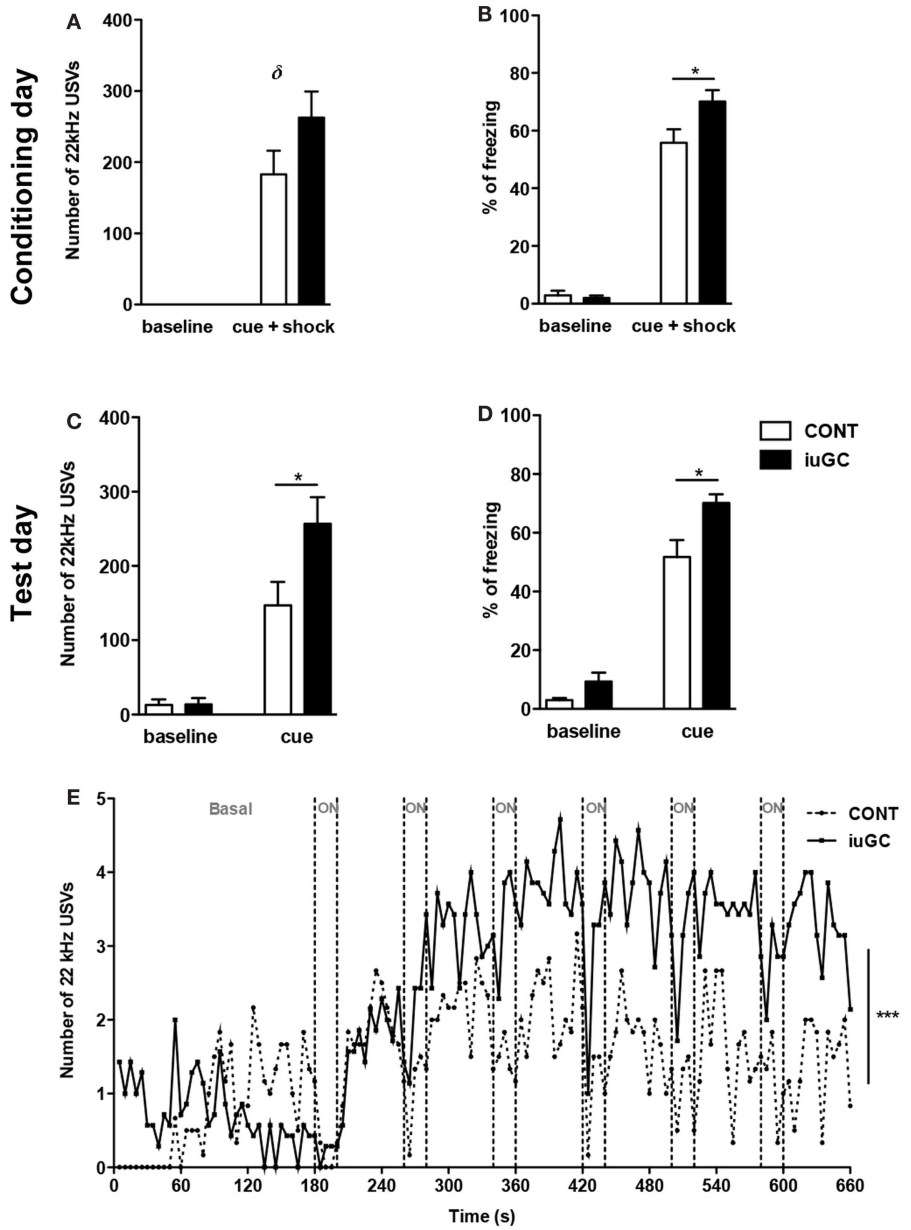


FIGURE 2 | Prenatal exposure to GC leads to amplified response to adverse stimulus. **(A)** Number of 22 kHz USVs on the conditioning day (cue + shock) of the fear-conditioning paradigm. iuGC group emitted more negative calls than control animals in the light/shock period. **(B)** Similarly, iuGC group displayed increased percentage of freezing behavior in comparison with control animals. On the test day, animals were exposed six times to cue but no shock was given. iuGC presented an increase in the number of USVs **(C)** and in freezing time **(D)**. **(E)** iuGC animals are hyper-reactive throughout time, since they emit more context-induced 22 kHz vocalizations than control group, especially in the first 60 s. Upon first cue exposure (ON period), both groups increased the number of USVs with no major differences between them. However, upon second cue exposure, iuGC animals emitted more negative USVs than controls and remained over-reactive during time. CONT, control animals; iuGC, *in utero* GC exposed animals; USVs, ultrasonic vocalizations. $^{*}p < 0.05$, $^{**}p < 0.001$, $^{***}p < 0.0001$, δ: trend, $p = 0.130$; **(A–E)** $n = 8$–16.

we have performed IHC against ChAT, the key enzyme in ACh synthesis. The number of ChAT-positive cells in the LDT was significantly higher in iuGC animals when compared to control group (**Figures 3A,B**; 49% increase; $U = 3$, $p = 0.026$). The PPT, a LDT adjacent region, also presented substantial increase in the number of ChAT-positive cells (**Figures 3C,D**; $U = 2$, $p = 0.024$). On the contrary, the nucleus basalis of Meynert did not show any significant alteration in the number of cholinergic positive cells (**Figures 3E,F**; $U = 8$, $p = 0.315$). Other regions such as the NAc (core and shell), rich in cholinergic interneurons, also did not show any differences in the number of ChAT-positive cells (**Figures 3G,H**; core: $U = 13$, $p = 0.927$; shell: $U = 6$, $p = 0.164$).

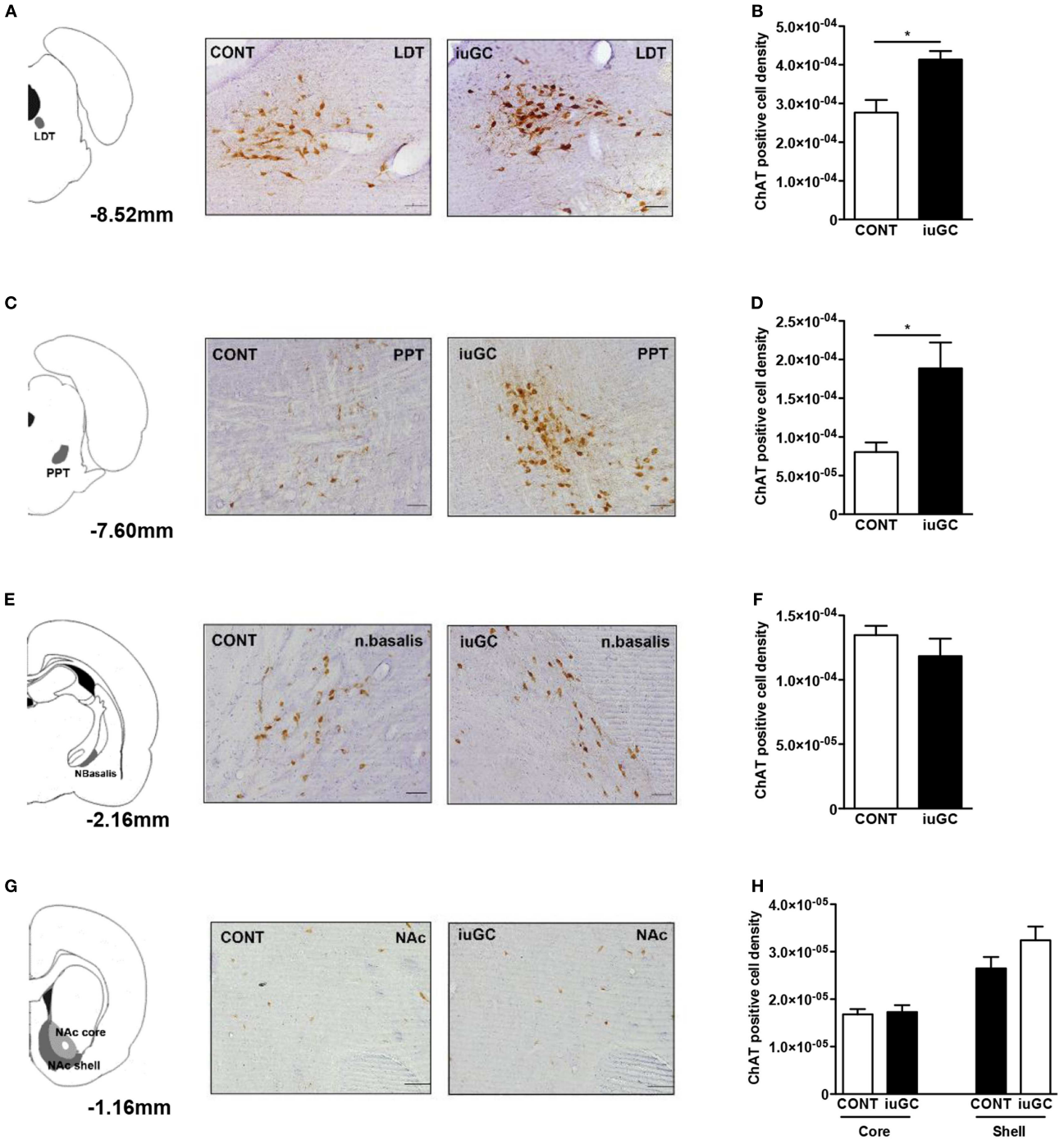


FIGURE 3 | Mesopontine cholinergic de-regulation in iuGC animals. **(A)** LDT ChAT immunohistochemistry revealed that iuGC animals presented increased ChAT expression **(B)**. **(C,D)** Similarly, iuGC animals present increased ChAT staining in the PPT region. Conversely, no differences were found in ChAT expression in the nucleus basalis of Meynert **(E,F)** or in the nucleus accumbens core or shell **(G,H)**. Representative images of coronal brain sections; numbers represent distance in mm posterior to bregma. CONT, control animals; iuGC, *in utero* GC exposed animals; LDT, laterodorsal tegmental nucleus; NAc, nucleus accumbens; PPT, pedunculopontine tegmental nucleus. $*p < 0.05$, $**p < 0.001$, $***p < 0.0001$; **(A)** $n = 4$–7. Scale bars: 50 μm.

Because iuGC can induce relevant structural changes, we measured the volume and number of cells in the LDT and PPT nuclei. No statistical differences regarding the volume of LDT were found between control and iuGC animals (**Figures 4A–C**; volume: $U = 5$, $p = 0.486$; cell numbers: $U = 3$, $p = 0.200$). In the PPT, no significant differences in volume and cell numbers were found

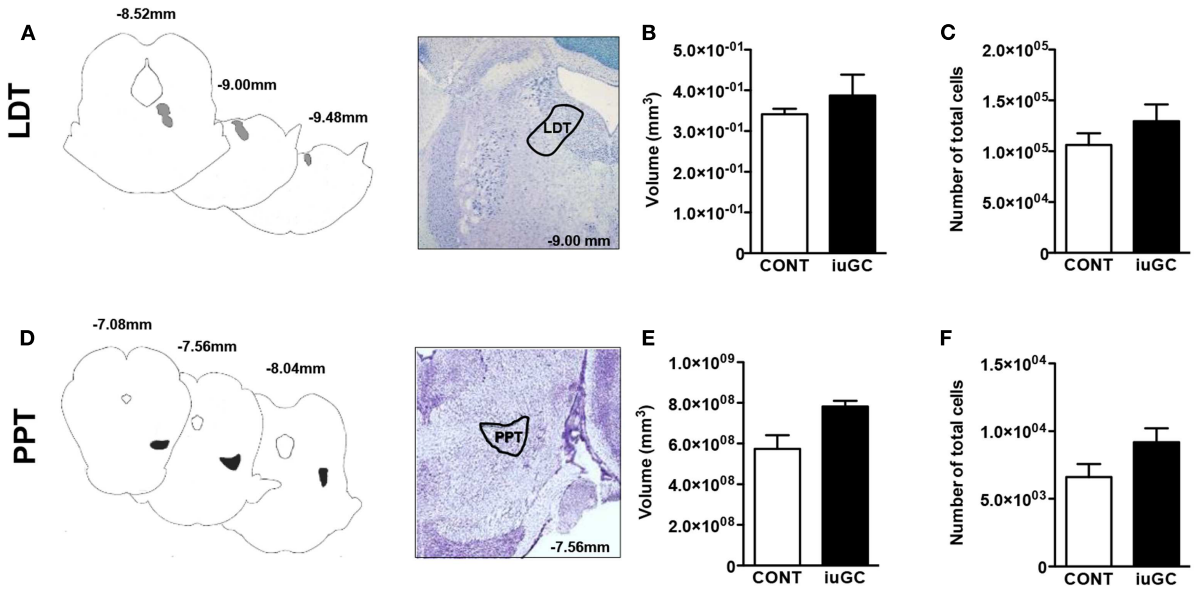


FIGURE 4 | Stereological data of LDT and PPT regions. No major differences were observed in the volumes and cell numbers of LDT **(A–C)** and PPT regions **(D–F)** of iuGC animals when compared to controls. Representative images of coronal brain sections; numbers represent distance in millimeters posterior to bregma. CONT, control animals; iuGC, *in utero* GC exposed animals; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nucleus. **(B,C)** $n = 5$–6; **(E,F)** $n = 4$.

(**Figures 4D–F**; volume: $U = 2$, $p = 0.114$; cell numbers: $U = 2$, $p = 0.229$).

To assess if the observed increase in ChAT-positive cells in iuGC group was translated into augmented ACh release, we measured the levels of ACh in cholinoceptive regions. A trend for increased ACh levels in the hypothalamus and the amygdala was found (data not shown).

CHOLINERGIC NEURONS IN THE LDT AND PPT ARE DIFFERENTIALLY ACTIVATED UPON ADVERSE STIMULUS IN iuGC ANIMALS

To better determine the impact of iuGC in cholinergic neurons, and the relevance of such changes in reaction to adverse stimuli, we evaluated neuronal activation patterns using c-fos labeling in combination with ChAT after the fear-conditioning protocol.

Briefly, animals were subjected to the modified fear-conditioning protocol, and sacrificed 90 min after stimuli on the test day. As depicted in **Figure 5**, after an adverse stimulus, iuGC animals presented a significant increase (85%) in the number of c-fos-positive cells in the LDT (**Figures 5A,B**; $U = 0$, $p = 0.008$). The number of ChAT-positive cells was also substantially augmented (64%) in iuGC animals when compared to control animals (**Figure 5C**; $U = 0$, $p = 0.014$).

Regarding the PPT, after the adverse stimuli, iuGC animals present 2.5 times more c-fos-positive cells than control animals (**Figures 5E,F**; $U = 0$, $p = 0.002$). Similarly, the number of ChAT-positive cells was also increased in iuGC animals (**Figure 5G**; $U = 0$, $p = 0.002$).

To determine if the observed increase in c-fos-positive cells was due to enhanced cholinergic activation, we also quantified the number of c-fos/ChAT-positive cells after fear conditioning. Remarkably, iuGC animals presented a substantial increase (186%) in the number of c-fos/ChAT-positive cells in the LDT in comparison to control animals (**Figure 5D**; $U = 4$, $p = 0.029$). Similarly, in the PPT, we observed that iuGC animals display a four-time increase in the number of double c-fos/ChAT-positive cells (**Figure 5H**; 3.358 vs. 0.801%, $U = 4.5$, $p = 0.030$).

DISCUSSION

Confirming previous findings (12, 14, 48), we observed that iuGC animals present an anxious phenotype. In the EPM, iuGC animals presented a decrease in the time and entries in the open arms when compared with control animals, in accordance with the L/D test, where they spent less time in the anxiogenic compartment. Moreover, iuGC animals emitted more 22 kHz negative calls and presented enhanced freezing behavior in the confined cage paradigm. These differences were eliminated by habituation. This is in accordance with previous work showing that rats that are highly anxious tend to vocalize more often and present augmented freezing time during aversive stimuli than rats that display low anxiety-like trait (35). In the fear-conditioning paradigm, iuGC animals emitted more negative calls and enhanced freezing behavior on the conditioning day. This suggests an emotional over-reactivity of iuGC animals to adverse stimulus. In further support of this idea, iuGC animals emitted more negative calls than control subjects in response to the cue predicting the harmful stimulus and during ISI, suggesting an over-reactive response. Altogether, our data confirms that iuGC exposure leads to anxious behavior and exacerbated response to stressful events in adulthood, a finding also observed in other stress models (49–51). Similarly, a rat line that displays signs of extreme trait anxiety also presents increased stress vulnerability and reactivity (52).

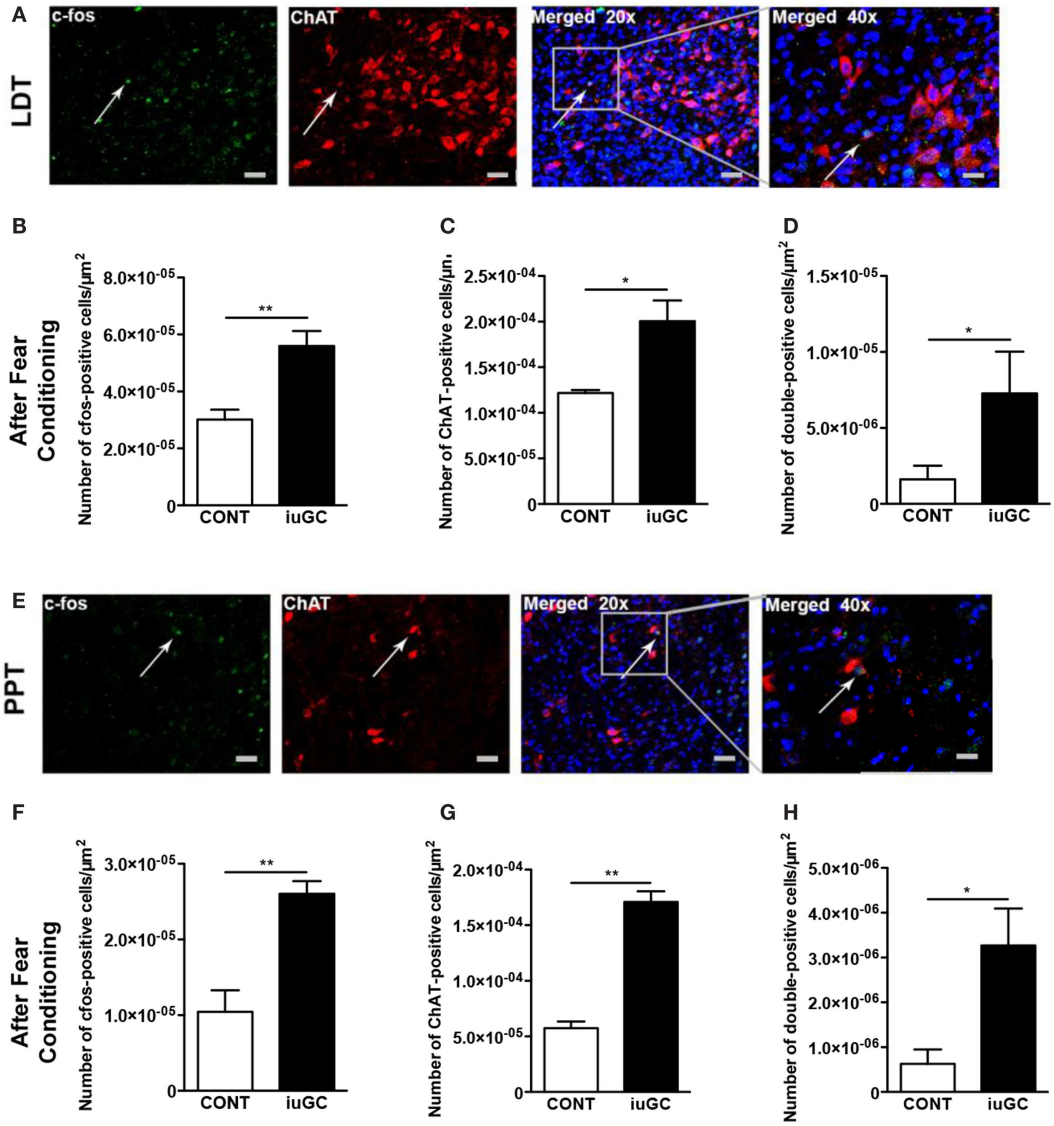


FIGURE 5 | Differential cholinergic activation of the LDT and PPT regions in iuGC animals after an adverse stimulus. **(A)** Representative images of LDT ChAT (red labeling) and c-fos (green labeling) immunostaining in animals exposed to the fear-conditioning test. **(B)** Increased c-fos activation in iuGC animals. **(C)** ChAT expression was increased in iuGC animals in comparison to controls. **(D)** The number of c-fos/ChAT-positive cells was substantially increased in the LDT of iuGC animals. **(E)** Representative images of PPT ChAT and c-fos immunostaining in animals exposed to the fear-conditioning test. **(F)** Increased c-fos staining in iuGC animals. **(G)** ChAT expression was augmented in iuGC animals. **(H)** The number of c-fos/ChAT-positive cells is significantly increased in the PPT of iuGC group. CONT, control animals; iuGC, *in utero* GC exposed animals; LDT, laterodorsal tegmental nucleus; PPT, pedunculopontine tegmental nucleus. $*p < 0.05$, $**p < 0.001$; **(A–H)** $n = 6$. Scale bars: 50 µm.

One remarkable finding was the increased emission of negative calls in iuGC animals, hinting differential activity of the mesopontine cholinergic circuitry, that mainly comprises projections from the LDT region (43, 44, 53, 54). In accordance, we found increased ChAT expression in the LDT of iuGC animals in a basal situation, suggesting overproduction of ACh. Upon exposure to a cue predicting an adverse stimulus, there was a marked increase in the number of c-fos/ChAT-positive cells, potentially explaining iuGC behavioral response. Indeed it was shown that LDT activation induces a complex state of defensiveness, critical for alarm responses to dangerous stimuli (44, 45). Our results are in accordance with previous work, in which they have shown that pharmacological stimulation of the LDT lead to an increase in the emission of 22 kHz USVs (44). In addition, the increased LDT cholinergic signaling could also be implicated in the impaired negative HPA axis feedback of iuGC animals (12) since LDT-arising ACh enhances ACTH and CRF release (28, 55, 56). On the other hand, GC also modulate cholinergic signaling (19), suggesting a reciprocal ACh-GC control that may culminate in a vicious loop.

Alike the LDT, iuGC animals presented increased PPT cholinergic signaling both in a basal situation and after exposure to the cue predicting an adverse stimulus. Apart from the classical role of PPT in sleep and arousal, lesion studies suggest a role in anxiety modulation, although both anxiogenic and anxiolytic effects were found depending on the degree and type of lesion. Excitotoxic and electrolytic PPT lesions induce an anxiogenic-like status (57, 58), contrary to one study that suggests a slight anxiolytic effect (59). On the other hand, ibotenic lesions seem to reduce anxious-like phenotype (60). Apart from distinct technical procedures, it has been suggested that opposing results may arise from damage in different sub-regions within the PPT (pars compacta vs. dissipata) or in surrounding regions, namely the cuneiform nucleus (59, 61).

This programing effect of iuGC exposure is not so surprising considering the importance of GC receptors for the maturation of medial septal and hippocampal cholinergic neurons (62, 63), yet, more studies need to be performed to understand how GC exert long-lasting functional and molecular changes in these neurons.

In utero glucocorticoid exposure-induced alterations in the mesopontine cholinergic pathway may go beyond a direct effect of cholinergic inputs on behavioral output. For instance, LDT-dependent cholinergic activation of VTA evokes dopamine release in the NAc (20, 21, 64) driving motivational and reward behaviors (65, 66). However, these findings are somewhat contradictory to the VTA-accumbal hypodopaminergia and anhedonia observed in iuGC animals (6, 14, 15). One possible explanation is that the sustained augmented LDT-VTA ACh signaling could desensitize or alter the expression/epigenetic status of the nicotinic/muscarinic receptors as a compensatory mechanism. Indeed, GC or acute stress can induce prominent ACh release in specific brain regions (46, 67, 68), and transiently change the expression levels of different cholinergic players through c-fos binding to promoter regions of target genes in order to maintain the homeostasis (19). Additional studies focusing on the regulation of the expression of different cholinergic players by GC will be critical to better comprehend our findings.

In summary, our results show for the first time that prenatal GC exposure programs the mesopontine cholinergic pathway, leading to cholinergic hyperactivation of both the LDT and PPT, which in turn can underlie the anxious behavior and enhanced stress reactivity observed in these animals.

ACKNOWLEDGMENTS

Authors would like to thanks Tircia Santos and Mariana Cerqueira (3B's group) for the technical help with the fluorimeter. This project was supported by a grant of Institute for the Study of Affective Neuroscience (ISAN) and by Janssen Neuroscience Prize. Sónia Borges, Carina Soares-Cunha, Miguel M. Carvalho, Ana P. Ventura-Silva, and Ana João Rodrigues have Fundação para a Ciência e Tecnologia (FCT) fellowships (Sónia Borges: SFRH/BD/89936/2012; Carina Soares-Cunha: SFRH/BD/51992/2012; Miguel M. Carvalho: SRFH/BD/51061/2010, Ana P. Ventura-Silva: SFRH/BD/52078/2013; Ana João Rodrigues: SFRH/BPD/33611/2009).

REFERENCES

1. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* (2001) **49**:1023–39. doi:10.1016/S0006-3223(01)01157-X
2. Heim C, Nemeroff CB. Neurobiology of early life stress: clinical studies. *Semin Clin Neuropsychiatry* (2002) **7**:147–59. doi:10.1053/scnp.2002.33127
3. Seckl JR, Meaney MJ. Glucocorticoid programming. *Ann N Y Acad Sci* (2004) **1032**:63–84. doi:10.1196/annals.1314.006
4. Matthews SG. Antenatal glucocorticoids and programming of the developing CNS. *Pediatr Res* (2000) **47**:291–300. doi:10.1203/00006450-200003000-00003
5. McArthur S, McHale E, Dalley JW, Buckingham JC, Gillies GE. Altered mesencephalic dopaminergic populations in adulthood as a consequence of brief perinatal glucocorticoid exposure. *J Neuroendocrinol* (2005) **17**:475–82. doi:10.1111/j.1365-2826.2005.01331.x
6. Leao P, Sousa JC, Oliveira M, Silva R, Almeida OF, Sousa N. Programming effects of antenatal dexamethasone in the developing mesolimbic pathways. *Synapse* (2007) **61**:40–9. doi:10.1002/syn.20341
7. Rodrigues AJ, Leao P, Carvalho M, Almeida OF, Sousa N. Potential programming of dopaminergic circuits by early life stress. *Psychopharmacology (Berl)* (2011) **214**:107–20. doi:10.1007/s00213-010-2085-3
8. Wyrwoll CS, Holmes MC. Prenatal excess glucocorticoid exposure and adult affective disorders: a role for serotonergic and catecholamine pathways. *Neuroendocrinology* (2012) **95**:47–55. doi:10.1159/000331345
9. Vallee M, Mayo W, Dellu F, Le Moal M, Simon H, MacCari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. *J Neurosci* (1997) **17**:2626–36.
10. Welberg LA, Seckl JR, Holmes MC. Prenatal glucocorticoid programming of brain corticosteroid receptors and corticotrophin-releasing hormone: possible implications for behaviour. *Neuroscience* (2001) **104**:71–9. doi:10.1016/S0306-4522(01)00065-3
11. MacCari S, Darnaudery M, Morley-Fletcher S, Zuena AR, Cinque C, Van Reeth O. Prenatal stress and long-term consequences: implications of glucocorticoid hormones. *Neurosci Biobehav Rev* (2003) **27**:119–27. doi:10.1016/S0149-7634(03)00014-9
12. Oliveira M, Bessa JM, Mesquita A, Tavares H, Carvalho A, Silva R, et al. Induction of a hyperanxious state by antenatal dexamethasone: a case for less detrimental natural corticosteroids. *Biol Psychiatry* (2006) **59**:844–52. doi:10.1016/j.biopsych.2005.08.020
13. Oliveira M, Rodrigues AJ, Leao P, Cardona D, Pego JM, Sousa N. The bed nucleus of stria terminalis and the amygdala as targets of antenatal glucocorticoids: implications for fear and anxiety responses. *Psychopharmacology (Berl)* (2012) **220**:443–53. doi:10.1007/s00213-011-2494-y
14. Rodrigues AJ, Leao P, Pego JM, Cardona D, Carvalho MM, Oliveira M, et al. Mechanisms of initiation and reversal of drug-seeking behavior induced by prenatal exposure to glucocorticoids. *Mol Psychiatry* (2012) **17**:1295–305. doi:10.1038/mp.2011.126
15. Borges S, Coimbra B, Soares-Cunha C, Miguel Pego J, Sousa N, Joao Rodrigues A. Dopaminergic modulation of affective and social deficits induced by prenatal glucocorticoid exposure. *Neuropsychopharmacology* (2013) **38**:2068–79. doi:10.1038/npp.2013.108
16. Henderson Z, Sherriff FE. Distribution of choline acetyltransferase immunoreactive axons and terminals in the rat and ferret brainstem. *J Comp Neurol* (1991) **314**:147–63. doi:10.1002/cne.903140114
17. Oakman SA, Faris PL, Kerr PE, Cozzari C, Hartman BK. Distribution of pontomesencephalic cholinergic neurons projecting to substantia nigra differs significantly from those projecting to ventral tegmental area. *J Neurosci* (1995) **15**:5859–69.
18. Holmstrand EC, Sesack SR. Projections from the rat pedunculopontine and laterodorsal tegmental nuclei to the anterior thalamus and ventral tegmental area arise from largely separate populations of neurons. *Brain Struct Funct* (2011) **216**:331–45. doi:10.1007/s00429-011-0320-2
19. Kaufer D, Friedman A, Seidman S, Soreq H. Acute stress facilitates long-lasting changes in cholinergic gene expression. *Nature* (1998) **393**:373–7. doi:10.1038/30741
20. Blaha CD, Allen LF, Das S, Inglis WL, Latimer MP, Vincent SR, et al. Modulation of dopamine efflux in the nucleus accumbens after cholinergic stimulation of the ventral tegmental area in intact, pedunculopontine tegmental nucleus-lesioned,

and laterodorsal tegmental nucleus-lesioned rats. *J Neurosci* (1996) **16**: 714–22.
21. Forster GL, Blaha CD. Laterodorsal tegmental stimulation elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the ventral tegmental area. *Eur J Neurosci* (2000) **12**:3596–604. doi:10.1046/j.1460-9568.2000.00250.x
22. Floresco SB, West AR, Ash B, Moore H, Grace AA. Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat Neurosci* (2003) **6**:968–73. doi:10.1038/nn1103
23. Lodge DJ, Grace AA. The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. *Proc Natl Acad Sci U S A* (2006) **103**:5167–72. doi:10.1073/pnas.0510715103
24. Berse B, Blusztajn JK. Modulation of cholinergic locus expression by glucocorticoids and retinoic acid is cell-type specific. *FEBS Lett* (1997) **410**:175–9. doi:10.1016/S0014-5793(97)00568-1
25. Meshorer E, Erb C, Gazit R, Pavlovsky L, Kaufer D, Friedman A, et al. Alternative splicing and neuritic mRNA translocation under long-term neuronal hypersensitivity. *Science* (2002) **295**:508–12. doi:10.1126/science.1066752
26. Ohmori N, Itoi K, Tozawa F, Sakai Y, Sakai K, Horiba N, et al. Effect of acetylcholine on corticotropin-releasing factor gene expression in the hypothalamic paraventricular nucleus of conscious rats. *Endocrinology* (1995) **136**:4858–63. doi:10.1210/en.136.11.4858
27. Llorente I, Lizcano F, Alvarez R, Diez N, Sopena M, Gil MJ, et al. Cholinergic modulation of spontaneous hypothalamic-pituitary-adrenal activity and its circadian variation in man. *J Clin Endocrinol Metab* (1996) **81**:2902–7. doi:10.1210/jc.81.8.2902
28. Rhodes ME, Rubin RT. Functional sex differences ('sexual diergism') of central nervous system cholinergic systems, vasopressin, and hypothalamic-pituitary-adrenal axis activity in mammals: a selective review. *Brain Res Brain Res Rev* (1999) **30**:135–52. doi:10.1016/S0165-0173(99)00011-9
29. Ross SA, Wong JY, Clifford JJ, Kinsella A, Massalas JS, Horne MK, et al. Phenotypic characterization of an alpha 4 neuronal nicotinic acetylcholine receptor subunit knock-out mouse. *J Neurosci* (2000) **20**:6431–41.
30. Salas R, Pieri F, Fung B, Dani JA, De Biasi M. Altered anxiety-related responses in mutant mice lacking the beta4 subunit of the nicotinic receptor. *J Neurosci* (2003) **23**:6255–63.
31. Booker TK, Butt CM, Wehner JM, Heinemann SF, Collins AC. Decreased anxiety-like behavior in beta3 nicotinic receptor subunit knockout mice. *Pharmacol Biochem Behav* (2007) **87**:146–57. doi:10.1016/j.pbb.2007.04.011
32. National Institutes of Health. The effect of antenatal steroids for fetal maturation on perinatal outcomes. *NIH Consens Statement* (1994) **12**(2):1–24.
33. Miracle X, Di Renzo GC, Stark A, Fanaroff A, Carbonell-Estrany X, Saling E. Guideline for the use of antenatal corticosteroids for fetal maturation. *J Perinat Med* (2008) **36**:191–6. doi:10.1515/JPM.2008.032
34. Smith LM, Qureshi N, Chao CR. Effects of single and multiple courses of antenatal glucocorticoids in preterm newborns less than 30 weeks' gestation. *J Matern Fetal Med* (2000) **9**:131–5. doi:10.1002/(SICI)1520-6661(200003/04)9:2<131::AID-MFM9>3.3.CO;2-D
35. Borta A, Wohr M, Schwarting RK. Rat ultrasonic vocalization in aversively motivated situations and the role of individual differences in anxiety-related behavior. *Behav Brain Res* (2006) **166**:271–80. doi:10.1016/j.bbr.2005.08.009
36. Keuker JI, Vollmann-Honsdorf GK, Fuchs E. How to use the optical fractionator: an example based on the estimation of neurons in the hippocampal CA1 and CA3 regions of tree shrews. *Brain Res Brain Res Protoc* (2001) **7**:211–21. doi:10.1016/S1385-299X(01)00064-2
37. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. 6th ed. San Diego: Academic Press (2007).
38. West MJ, Slomianka L, Gundersen HJ. Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat Rec* (1991) **231**:482–97. doi:10.1002/ar.1092310411
39. Gundersen HJ, Jensen EB, Kieu K, Nielsen J. The efficiency of systematic sampling in stereology – reconsidered. *J Microsc* (1999) **193**:199–211. doi:10.1046/j.1365-2818.1999.00457.x
40. Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. *J Microsc* (1987) **147**:229–63. doi:10.1111/j.1365-2818.1987.tb02837.x
41. Ling EA, Paterson JA, Privat A, Mori S, Leblond CP. Investigation of glial cells in semithin sections. I. Identification of glial cells in the brain of young rats. *J Comp Neurol* (1973) **149**:43–71. doi:10.1002/cne.901490104
42. Peinado MA, Quesada A, Pedrosa JA, Martinez M, Esteban FJ, Del Moral ML, et al. Light microscopic quantification of morphological changes during aging in neurons and glia of the rat parietal cortex. *Anat Rec* (1997) **247**:420–5. doi:10.1002/(SICI)1097-0185(199703)247:3<420::AID-AR14>3.3.CO;2-O
43. Brudzynski SM, Barnabi F. Contribution of the ascending cholinergic pathways in the production of ultrasonic vocalization in the rat. *Behav Brain Res* (1996) **80**:145–52. doi:10.1016/0166-4328(96)00029-0
44. Brudzynski SM. Pharmacological and behavioral characteristics of 22 kHz alarm calls in rats. *Neurosci Biobehav Rev* (2001) **25**:611–7. doi:10.1016/S0149-7634(01)00058-6
45. Brudzynski SM. Ultrasonic calls of rats as indicator variables of negative or positive states: acetylcholine-dopamine interaction and acoustic coding. *Behav Brain Res* (2007) **182**:261–73. doi:10.1016/j.bbr.2007.03.004
46. Imperato A, Puglisi-Allegra S, Casolini P, Angelucci L. Changes in brain dopamine and acetylcholine release during and following stress are independent of the pituitary-adrenocortical axis. *Brain Res* (1991) **538**:111–7. doi:10.1016/0006-8993(91)90384-8
47. Tinsley MR, Quinn JJ, Fanselow MS. The role of muscarinic and nicotinic cholinergic neurotransmission in aversive conditioning: comparing Pavlovian fear conditioning and inhibitory avoidance. *Learn Mem* (2004) **11**:35–42. doi:10.1101/lm.70204
48. Roque S, Oliveira TG, Nobrega C, Barreira-Silva P, Nunes-Alves C, Sousa N, et al. Interplay between depressive-like behavior and the immune system in an animal model of prenatal dexamethasone administration. *Front Behav Neurosci* (2011) **5**:4. doi:10.3389/fnbeh.2011.00004
49. Romeo RD, Mueller A, Sisti HM, Ogawa S, McEwen BS, Brake WG. Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. *Horm Behav* (2003) **43**:561–7. doi:10.1016/S0018-506X(03)00063-1
50. Tsoory M, Cohen H, Richter-Levin G. Juvenile stress induces a predisposition to either anxiety or depressive-like symptoms following stress in adulthood. *Eur Neuropsychopharmacol* (2007) **17**:245–56. doi:10.1016/j.euroneuro.2006.06.007
51. Uchida S, Hara K, Kobayashi A, Funato H, Hobara T, Otsuki K, et al. Early life stress enhances behavioral vulnerability to stress through the activation of REST4-mediated gene transcription in the medial prefrontal cortex of rodents. *J Neurosci* (2010) **30**:15007–18. doi:10.1523/JNEUROSCI.1436-10.2010
52. Landgraf R, Wigger A. Born to be anxious: neuroendocrine and genetic correlates of trait anxiety in HAB rats. *Stress* (2003) **6**:111–9. doi:10.1080/1025389031000104193
53. Bihari A, Hrycyshyn AW, Brudzynski SM. Role of the mesolimbic cholinergic projection to the septum in the production of 22 kHz alarm calls in rats. *Brain Res Bull* (2003) **60**:263–74. doi:10.1016/S0361-9230(03)00041-8
54. Brudzynski SM. Communication of adult rats by ultrasonic vocalization: biological, sociobiological, and neuroscience approaches. *ILAR J* (2009) **50**:43–50. doi:10.1093/ilar.50.1.43
55. Risch SC, Kalin NH, Janowsky DS. Cholinergic challenges in affective illness: behavioral and neuroendocrine correlates. *J Clin Psychopharmacol* (1981) **1**:186–92. doi:10.1097/00004714-198107000-00003
56. Hasey G, Hanin I. The cholinergic-adrenergic hypothesis of depression reexamined using clonidine, metoprolol, and physostigmine in an animal model. *Biol Psychiatry* (1991) **29**:127–38. doi:10.1016/0006-3223(91)90041-J
57. Podhorna J, Franklin KB. Lesions of the pedunculopontine tegmental nucleus increase anxiety in rats. *Neuroreport* (1998) **9**:1783–6. doi:10.1097/00001756-199806010-00020
58. Leri F, Franklin KBJ. Learning impairments caused by lesions to the pedunculopontine tegmental nucleus: an artifact of anxiety? *Brain Res* (1998) **807**:187–92. doi:10.1016/S0006-8993(98)00762-8
59. Homs-Ormo S, Coll-Andreu M, Satorra-Marin N, Arevalo-Garcia R, Morgado-Bernal I. Effects of pedunculopontine tegmental nucleus lesions on emotional reactivity and locomotion in rats. *Brain Res Bull* (2003) **59**:495–503. doi:10.1016/S0361-9230(02)00966-8
60. Satorra-Marín N, Homs-Ormo S, Arévalo-García R, Morgado-Bernal I, Coll-Andreu M. Effects of pre-training pedunculopontine tegmental nucleus lesions on delayed matching- and non-matching-to-position in a T-maze in rats. *Behav Brain Res* (2005) **160**:115–24. doi:10.1016/j.bbr.2004.11.022
61. Walker SC, Winn P. An assessment of the contributions of the pedunculopontine tegmental and cuneiform nuclei to anxiety and neophobia. *Neuroscience* (2007) **150**:273–90. doi:10.1016/j.neuroscience.2007.09.018

62. Takahashi LK, Goh CS. Glucocorticoid facilitation of cholinergic development in the rat hippocampus. *Neuroscience* (1998) **83**:1145–53. doi:10.1016/S0306-4522(97)00472-7
63. Guijarro C, Rutz S, Rothmaier K, Turiault M, Zhi Q, Naumann T, et al. Maturation and maintenance of cholinergic medial septum neurons require glucocorticoid receptor signaling. *J Neurochem* (2006) **97**:747–58. doi:10.1111/j.1471-4159.2006.03728.x
64. Miller AD, Blaha CD. Midbrain muscarinic receptor mechanisms underlying regulation of mesoaccumbens and nigrostriatal dopaminergic transmission in the rat. *Eur J Neurosci* (2005) **21**:1837–46. doi:10.1111/j.1460-9568.2005.04017.x
65. Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl)* (2007) **191**:391–431. doi:10.1007/s00213-006-0578-x
66. Wise RA. Dopamine and reward: the anhedonia hypothesis 30 years on. *Neurotox Res* (2008) **14**:169–83. doi:10.1007/BF03033808
67. Gilad GM, Mahon BD, Finkelstein Y, Koffler B, Gilad VH. Stress-induced activation of the hippocampal cholinergic system and the pituitary-adrenocortical axis. *Brain Res* (1985) **347**:404–8. doi:10.1016/0006-8993(85)90209-4
68. Mark GP, Rada PV, Shors TJ. Inescapable stress enhances extracellular acetylcholine in the rat hippocampus and prefrontal cortex but not the nucleus accumbens or amygdala. *Neuroscience* (1996) **74**:767–74. doi:10.1016/0306-4522(96)00211-4

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 October 2013; paper pending published: 16 November 2013; accepted: 25 November 2013; published online: 13 December 2013.
*Citation: Borges S, Coimbra B, Soares-Cunha C, Ventura-Silva AP, Pinto L, Carvalho MM, Pêgo J-M, Rodrigues AJ and Sousa N (2013) Glucocorticoid programing of the mesopontine cholinergic system. Front. Endocrinol. **4**:190. doi: 10.3389/fendo.2013.00190*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 09 July 2014
doi: 10.3389/fendo.2014.00100

Context modulates outcome of perinatal glucocorticoid action in the brain

***E. Ronald de Kloet*[1,2]*, Sanne E. F. Claessens*[1] *and Jiska Kentrop*[1]**

[1] *Department of Medical Pharmacology, Leiden University Medical Center, Leiden University, Leiden, Netherlands*
[2] *Department of Endocrinology and Metabolism, Leiden University Medical Center, Leiden, Netherlands*

Edited by:
Nikolaos P. Daskalakis, Icahn School of Medicine at Mount Sinai, USA

Reviewed by:
Gábor B. Makara, Hungarian Academy of Sciences, Hungary
Nuno Sousa, University of Minho, Portugal

****Correspondence:***
E. Ronald de Kloet, Department of Endocrinology and Metabolism, Division of Medical Pharmacology, LACDR, Leiden University Medical Center, Leiden University, PO Box 9503, Leiden 2300 RA, Netherlands
e-mail: e.kloet@lacdr.leidenuniv.nl; erdekloet@gmail.com

Prematurely born infants may be at risk, because of inadequate maturation of tissues. If there are signs of preterm birth, it has become common practice therefore to treat either antenatally the mother or postnatally the infant with glucocorticoids to accelerate tissue development, particularly of the lung. However, this life-saving early glucocorticoid treatment was found to increase the risk of adverse outcome in later life. In one animal study, the authors reported a 25% shorter lifespan of rats treated as newborns with the synthetic glucocorticoid dexamethasone, but so far this finding has not been replicated. After a brief clinical introduction, we discuss studies in rodents designed to examine how perinatal glucocorticoid action affects the developing brain. It appears that the perinatal action of the glucocorticoid depends on the context and the timing as well as the type of administered steroid. The type of steroid is important because the endogenous glucocorticoids cortisol and corticosterone bind to two distinct receptor populations, i.e., mineralocorticoid and glucocorticoid receptors (GR), while synthetic glucocorticoids predominantly bind to the GR. In addition, if given antenatally hydrocortisone is inactivated in the placenta by 11β-HSD type 2, and dexamethasone is not. With respect to timing, the outcome of glucocorticoid effects is different in early vs. late phases of brain development. The context refers to the environmental input that can affect the susceptibility to glucocorticoid action in the newborn rodent brain; early handling of pups and maternal care obliterate effects of post-natal dexamethasone treatment. Context also refers to coping with environmental conditions in later life, for which the individual may have been programed epigenetically by early-life experience. This knowledge of determinants affecting the outcome of perinatal glucocorticoid exposure may have clinical implications for the treatment of prematurely born infants.

Keywords: preterm birth, stress, glucocorticoids, antenatal, post-natal, human, rodent, brain

INTRODUCTION

The endogenous glucocorticoids cortisol and corticosterone promote the development of various tissues and organ systems during development. However, in prematurely born infants this effect of the steroids on maturation is abrogated resulting in a life-threatening situation. Therefore, it has become common practice to administer synthetic glucocorticoids such as dexamethasone to accelerate maturation, particularly of lung tissue (1, 2). If given antenatally to the mother the mortality of the prematurely born infant is decreased. While the infants obviously benefit from perinatal glucocorticoids, the treatment reportedly can have negative implications for later life outcome, particularly with respect to dexamethasone or its analogs (3–5). This health risk at adulthood includes impaired cardiovascular and immune functions as well as deficits in motor and cognitive performance, altered emotional reactivity, and neuroendocrine regulations.

In this contribution, data from animal and human studies with respect to the perinatal (antenatal and post-natal) action of glucocorticoids are presented. The action of the naturally occurring glucocorticoids is mediated by mineralocorticoid receptors (NR3C2, MR) and glucocorticoid receptors (NR3C1, GR) (6, 7). These glucocorticoids, cortisol, and corticosterone (the latter steroid only in rodents), bind to MR and GR with an order of magnitude difference in binding affinity (6). This implies that corticosterone and cortisol bind to the brain MR with high affinity resulting in substantial occupation even during the very low circulating hormone levels that are typically observed during the circadian trough and in rodents during the early post-natal stress-hyporesponsive-period (SHRP). These same steroids bind to GR only in adequate amounts when in adult animals their concentration has increased after stress and at the circadian peak. The membrane variants of MR and GR also display a lower affinity suggesting that their rapid effects preferably occur only at rising and high steroid concentrations during stressful situations and circadian peaks (8). No data exist on the role of these membrane variants during development. The synthetic glucocorticoids preferentially bind to GR, usually with high affinity (9).

We first review the human studies on antenatal activity of the hypothalamic–pituitary–adrenal (HPA)-axis, development of corticosteroid receptors and the effect of antenatal glucocorticoid

treatment. Then, after an overview of antenatal rodent data, we will discuss the action of dexamethasone during the first post-natal days (pnds) of the newborn rodent, since this resembles most the antenatal treatment protocol in the human. Most animal studies have been performed with male animals; if females are used this is indicated. This contribution is concluded with a translational perspective that may be helpful in the clinical management of perinatal glucocorticoid treatment.

HUMAN STUDIES

ANTENATAL HPA-AXIS DEVELOPMENT

During pregnancy, the activity of the maternal HPA-axis gradually increases over time toward a state of hypercortisolism that is caused by the steadily increasing production of corticotropin-releasing hormone (CRH) in the placenta and fetal membranes (10–12)). Fetal levels of CRH, adrenocorticotropic hormone (ACTH), and cortisol also rise, but not as much as the maternal values (13). Most of the cortisol present in amniotic fluid has a maternal origin and has successfully traversed the placenta. However, this accounts for only 10–20% of maternal cortisol because the remaining 80–90% is converted to inactive cortisone by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) to protect the fetus from excess glucocorticoid exposure (14, 15). During the third trimester, fetal 11β-HSD2 levels fall and the fetus is exposed to high levels of CRH and endogenous cortisol (16). In this period, cortisol is able to exert its positive influence on the maturation of several organ systems, in preparation of survival after birth (17, 18).

ANTENATAL MR AND GR EXPRESSION

Very limited information is available on human MR and GR expression patterns during gestation. Condon and colleagues reported minimal expression of MR and GR in peripheral tissues during gestational week 6, which becomes detectable from gestational weeks 8–16, with in general higher GR compared to MR expression (19). Noorlander et al. (20) studied MR and GR mRNA expression in the hippocampus from gestational week 24–34. They reported that both MR and GR mRNA are present during this period, with higher MR compared to GR mRNA levels and little change in expression levels over time (20). However, humans are in part altricial species and antenatal MR and GR expression appear to follow a pattern similar to that in guinea pigs and sheep rather than mice and rats. When comparing rodent brain development to human brain development, the last days of rat gestation (day 22–23) resemble human fetal brain development in week 16–17 regarding relative aspects of neurogenesis, neuron size, and number (21, 22).

ANTENATAL GLUCOCORTICOIDS IN THE CLINIC

In 1972, Liggins and Howie were the first to demonstrate the beneficial effects of antenatal synthetic glucocorticoid treatment in the prevention of respiratory distress syndrome of premature infants (23). It is now known that treatment with the synthetic glucocorticoids dexamethasone and betamethasone accelerates the maturation process of several organ systems (e.g., lungs, heart, and kidneys) and antenatal synthetic glucocorticoid has become the standard treatment for women at risk of preterm delivery (1). In short-term, antenatal synthetic glucocorticoids reduce neonatal death, incidence of respiratory distress syndrome, cerebroventricular hemorrhage, necrotizing enterocolitis, respiratory support, intensive care admissions, and systemic infections in the first 48 h of life (2).

There is not much information on the long-term consequences of antenatal synthetic glucocorticoid treatment in humans. Roberts and Dalziel reported in a 2006 Cochrane systematic review, four follow-up studies into childhood and two into adulthood on the effect of a single course of antenatal glucocorticoid treatment (2). No psychological differences were found between antenatal glucocorticoid treatment and placebo for both children and adults. Another Cochrane review failed to show any long-term (adverse) effects of either single or repeated doses of antenatal betamethasone (24).

Despite this positive news on the clinical outcome of perinatal synthetic glucocorticoid treatment in the short-term, there are concerns about the potential negative long-term effects of antenatal overexposure. This is because of data from animal experiments with these synthetic glucocorticoids or derived from endogenous glucocorticoid action during stress (see below). Epidemiological studies have shown an association between high maternal stress, nutritional factors, or infections during pregnancy, which suggested an association of perinatal overexposure to endogenous glucocorticoid with mental disorders in later life. These include retrospective studies in man, such as those involving children born from Dutch mothers that were pregnant during the Dutch "Hunger winter" of 1944–1945. The adults exposed as fetus to these major stressors showed a higher incidence of schizophrenia (25), affective disorder (26), and addiction (27) as well as metabolic disorders (28). Furthermore, depression was associated with maternal influenza infection (29) and autism with mothers suffering from family problems (30). One review focused on animal models derived from epidemiological studies to examine the antenatal influence of maternal stress and nutritional status (31). In a meta-analysis of 60 human and 43 animal studies Beydoun and Saftlas (32) concluded independent effects of antenatal and postnatal outcomes. A vast literature is emerging on the outcome of early post-natal adversity and abuse (33, 34). Attempts are under way to ameliorate outcome using micronutrient supplements (35).

However, the consequences of treatment with the synthetic glucocorticoids betamethasone and dexamethasone cannot be directly compared to the impact of exposure to the endogenous glucocorticoids cortisol and corticosterone. First, the synthetic glucocorticoids are poor substrates for 11β-HSD2 and can pass the placental barrier more readily than endogenous glucocorticoids (36, 37). Second, the synthetic glucocorticoids bind the GR with higher affinity than endogenous glucocorticoids (9). Third, the synthetic glucocorticoids are GR-specific rather than MR-specific, while cortisol and corticosterone bind with a 10-fold higher affinity to MR compared to GR (6). And fourth, in the guinea pig MR and GR both are involved in the induction of multidrug resistance P-glycoprotein (P-gp) in the fetal blood–brain-barrier in response to endogenous cortisol, aldosterone, and dexamethasone (38).This finding suggests that upon repeated exposure to dexamethasone access of the synthetic steroid itself may be hampered, because the steroid is a substrate for the transporter (39–41). Regardless, the

overall picture clearly indicates that glucocorticoid overexposure has long-term consequences and the question is therefore rather how these effects occur.

ANIMAL STUDIES

ANTENATAL MR AND GR EXPRESSION

The programing effects of glucocorticoids during fetal brain development depend on the expression of MR and GR, which are time, location, and species specific. A distinction can be made between altricial and precocial species. Rats and mice are altricial species, which are relatively underdeveloped when born, with critical brain development occurring ante- and postnatally. In precocial species such as guinea pigs and sheep critical brain development occurs mostly antenatal. Primates including humans are considered a mixture of altricial and precocial: in aspects of body development they may be considered precocial, but behaviorally they are altricial.

In rats, GR mRNA expression was found in e.g., hippocampus, hypothalamus, cerebellum, raphe nuclei, locus coeruleus, and olfactory bulb, in midgestation from gestational day E12.5 onward (16, 42). At the end of the gestational period, when endogenous levels of corticosterone are high and the fetal HPA-axis becomes active, GR mRNA expression increases throughout the brain. MR mRNA expression is more limited in the hippocampus, parts of the hypothalamus and the superior colliculus piriform cortex, lateral septum, brainstem, and pituitary and starts in late gestation, 3 days before birth (16). A similar expression pattern was seen in mice (43–45).

In precocial species such as the guinea pig and sheep, a different and more complex MR and GR expression pattern was found (46–48). In guinea pigs, from gestational day 40 onward both MR and GR mRNA is present in the cortex, hippocampus, and dentate gyrus. In the period between gestational day 40 and 50, which is 60–75% of the total gestation length, GR mRNA levels increase while MR mRNA levels decrease. GR mRNA in the hippocampus increases to peak levels near term, while MR mRNA levels remain consistently low. However, in the paraventricular nucleus (PVN), GR mRNA levels are at peak level around gestational day 40 and 50 and show a large decline afterward (48).

ANIMAL MODELS OF ANTENATAL GLUCOCORTICOID EXPOSURE

Many experimental animal studies have demonstrated that glucocorticoid overexposure *in utero*, both endogenous and exogenous, can have long-term effects on the offspring. Studies in rodents have shown a relationship between antenatal glucocorticoid overexposure and signs of schizophrenia and depression, increased anxiety and impaired learning and memory [see review in Ref. (49)]. Antenatal glucocorticoid exposure in rats, guinea pigs, and non-human primates caused permanently elevated baseline corticosteroid levels and increased corticosteroid levels in response to stress (50–53). Excess antenatal glucocorticoids are also capable of permanently altering MR and GR expression in multiple brain areas (54–56) and can influence entire neurotransmitter systems (49, 57). Experimental animal studies in comparison to studies in man are far better capable of controlling variables, yet there are many differences in methodology that can influence the outcome, such as the type of stressor or synthetic glucocorticoid used and timing and duration of exposure. Additionally, the effects of glucocorticoid overexposure are also age, sex, and species specific, making it very difficult to translate the outcome of animal experiments to the clinic.

POST-NATAL MR AND GR EXPRESSION

The ontogeny of the receptors has been carefully mapped over the years using different techniques such as receptor binding assays, immunocytochemistry, and *in situ* hybridization (58–62). In general, MR is abundantly expressed in limbic-cortical regions, such as hippocampus, septum, amygdala, and fronto-cortical regions that have a function in processing of stressful information. With all detection methods, MR density is found to be high already early postnatally in mice and rats and localized in the nucleus. Pituitary GR expression is also very high in early-life. In the brain, GR binding and mRNA expression gradually increase postnatally and at weaning have reached about 50% of its adult level in the rat, and already adult levels in the mouse.

Using immunocytochemistry, a profound change in translocation of GR was observed. Immediately after birth, high nuclear immunoreactivity (ir) of GR occurs widespread over the rodent brain, particularly in stress regulating centers such as PVN, the ascending biogenic amine neurons, hippocampus, and amygdala as well as fronto-cortical regions. Then, possibly because of the low circulating levels of corticosterone during the SHRP, nuclear GR-ir is very low before reappearing at around pnd 12. Interestingly, the initial high GR-ir in the hippocampal CA3 pyramidal neurons and the suprachiasmatic nucleus does not re-appear after pnd 7, suggesting that the function of the latter nucleus now is entrained by exposure to daylight rather than the circadian variation in glucocorticoids from the mother (63, 64).

MR and GR in hippocampus show a profound downregulation after prolonged maternal absence, while a procedure like handling can induce their expression (65–67). Interestingly, MR and GR density changes in parallel with the amount of maternal care the pup is exposed to, a change in receptor concentration that can be explained by the extent of methylation of the receptor gene promotor region (68, 69). In a classical experiment (70), infant rats were deprived of maternal contact for 24 h on pnd 3–4 and injected with saline or ACTH1-24 at the end of the deprivation period. They were then returned to their dams and weaned on pnd 21. At pnd 48, they were sacrificed (24 h post adrenalectomy) and the hippocampal MR and GR measured using an *in vitro* cytosol binding assay. Using this procedure in the male rats, deprivation and deprivation + ACTH resulted in a reduction of GR. MR was also significantly downregulated in the deprived males. In contrast, in the female rats, saline injections in deprived female rats resulted in increased GR capacity and ACTH injections lead to a further up-regulation of the GR. None of the early manipulations influenced the regulation of the MR in females. These results in adult (7-week-old) rats indicate that the corticosteroid receptor systems in the brain are sensitive to brief manipulations in maternal care as well as corticosterone levels occurring early in development. Moreover, there is a striking effect of sex.

ANIMAL MODELS OF ENDOGENOUS POST-NATAL GLUCOCORTICOID VARIATION

In rodents, the first 2 weeks of life are characterized by a SHRP during which mild psychological stressors or exogenous ACTH,

that trigger a pronounced corticosterone rise in adulthood, produce only a weak adrenocortical response (71, 72). The SHRP lasts from pnd 4 to 14 in rats, and from pnd 2 to 12 in mice (73). During the SHRP, circulating corticosterone levels are low and stable, but the steroid that circulates is free, since corticosteroid binding globulin (CBG) is not detectable during this period. Also metabolism of corticosterone is changed, and hence the pharmacodynamics of the hormone is different from that during the previous perinatal days as well as the postweaning period. Because of the lack of ACTH, the size of the adrenal is small and thus the capacity to secrete steroids is very low, demonstrating that adrenal hyporesponsiveness is actual the most proximal cause of the SHRP (74).

The mechanism underlying this period of adrenal quiescence has been examined thoroughly. It has been proposed that neural connections involved in processing of stressful information are still immature at the time. However, since adrenalectomy (ADX) during the SHRP triggers a large ACTH response, it seems that enhanced corticosterone feedback at the pituitary level is causal for the SHRP. Moreover, Schmidt et al. (75) demonstrated that the GR antagonist RU486, administered during the SHRP, triggered a profound ACTH and corticosterone response. Next, mice carrying a conditional knockout of the GR gene specifically targeted at the pituitary corticotrophs showed excessive amounts of circulating corticosterone during the SHRP (76). This elevated corticosterone was resistant to dexamethasone suppression suggesting that in the pup the pituitary is the primary feedback site of stress-induced HPA-axis activity. Indeed, a single exposure to a psychological stressor is capable to mount a central response in c-fos and CRH, but the adrenal remains quiescent under these conditions (77).

It is important to realize that GR-mediated feedback is in operation to maintain the SHRP and this phenomenon by definition only can be revealed in the context of a stressor. Hence, administration of RU486 in saline did not trigger a stress response and therefore no further disinhibition by the GR antagonist. If RU486 was administered in polyethylene glycol (PEG) as solvent, an immune response caused by inflammation at the injection site occurred that was sufficient to trigger adrenocortical corticosterone secretion revealing the effect of the antagonist (78).

Thus, during the SHRP, corticosterone inhibits the stress-induced HPA-axis activity primarily via a pituitary feedback site. The GR-mediated mechanism maintaining the SHRP can be revealed by pituitary GR knockout or GR antagonist treatment, but only if there is a corticosterone rise after stress. In case of resting conditions when GR blockade cannot operate because corticosterone levels are too low, the quiescence of the HPA-axis is maintained via a centrally driven MR mechanism. These MR's operate brain circuits appraising salient information as they do during adulthood (78).

MATERNAL CARE AND NOT DEXAMETHASONE EXERTS LONG-TERM HPA-AXIS CONTROL

There is evidence that the effects of dexamethasone on the developing brain precede independent of psychosocial and attachment effects. The experiments were inspired by the seminal studies of Levine in the 1950's demonstrating that daily handling of rat pups attenuated HPA-axis activity and emotional reactivity in adulthood, an effect that could be mimicked by enhanced maternal care (71, 72). MR and GR also respond; both handling and maternal care induced MR and GR, probably because of enhanced demethylation of the receptor gene (68, 79, 80). The opposite outcome was reached under conditions of prolonged maternal absence, a procedure that has become known as maternal deprivation.

In response to 24 h of maternal deprivation of rat or mouse pups ACTH and corticosterone initially both rise during the first 8 h of maternal absence. Subsequently, the rise of corticosterone continues and reaches maximal levels after 24 h, while ACTH levels normalize for the remainder of the 24 h period. With the rise of corticosterone, the pituitary pro-opiomelanocortin (POMC) and CRH- and GR mRNA levels decrease in the PVN (66). If these 24-h deprived pups are exposed to an injection stressor a profound response occurs in pituitary ACTH and c-fos mRNA in the PVN. Amazingly, the pituitary ACTH and PVN c-fos response is normalized after stroking the pups for 45 s every 8 h with a warm wet artist brush in the anogenital region, mimicking maternal licking and grooming (81). For normalization of the corticosterone and the PVN GR mRNA response feeding is required in addition to stroking. Interestingly, dexamethasone (100 μg/kg) administration completely inhibits the ACTH and corticosterone response, but does not affect the central responses to maternal deprivation and the stressor (81) (**Figure 1**).

This differentiation between the effects of behavioral manipulations and the action of dexamethasone also persists. In a subsequent experiment, the rats deprived from pnd 11 to 12 for 24 h were pre-treated with dexamethasone to suppress the corticosterone response to maternal deprivation. Another group was only stroked for 45 s every 8 h or stroked + fed at that time, the latter procedure like dexamethasone also suppresses the deprivation-induced HPA-axis activity. At 20 days of age, the previously deprived + dexamethasone-treated animals did not differ in their endocrine and central responses. However, feeding and stroking did normalize the persistent effect of maternal deprivation on ACTH and corticosteroid receptors. Hence this study not only shows a lack of a persistent effect of dexamethasone, but also that the suppression of corticosterone by dexamethasone has no long-term consequences. It is the re-instatement of specific aspects of maternal care that counts in the long-term control of neuroendocrine function (82).

PUPS BECOME USED TO REPEATED MATERNAL SEPARATION

That the central components of the stress response are already in operation during the SHRP can be demonstrated also in another way. If the 24-h maternal deprivation at pnd 3–4 was split into three episodes of each 8 h per day from pnd 3 to 5, we observed during the first separation the predicted rise in ACTH and corticosterone secretion. These neuroendocrine responses disappeared with the next separations as if the pup has learned to anticipate the return of the dam. When the anti-glucocorticoid RU486 was administered the response to the first separation was further enhanced, but the effect of the antagonist declined in the second and was abolished after the third separation on pnd 5. In contrast, a MR antagonist decreased corticosterone levels after the first, but increased corticosterone secretion after the third separation. Surprisingly, while

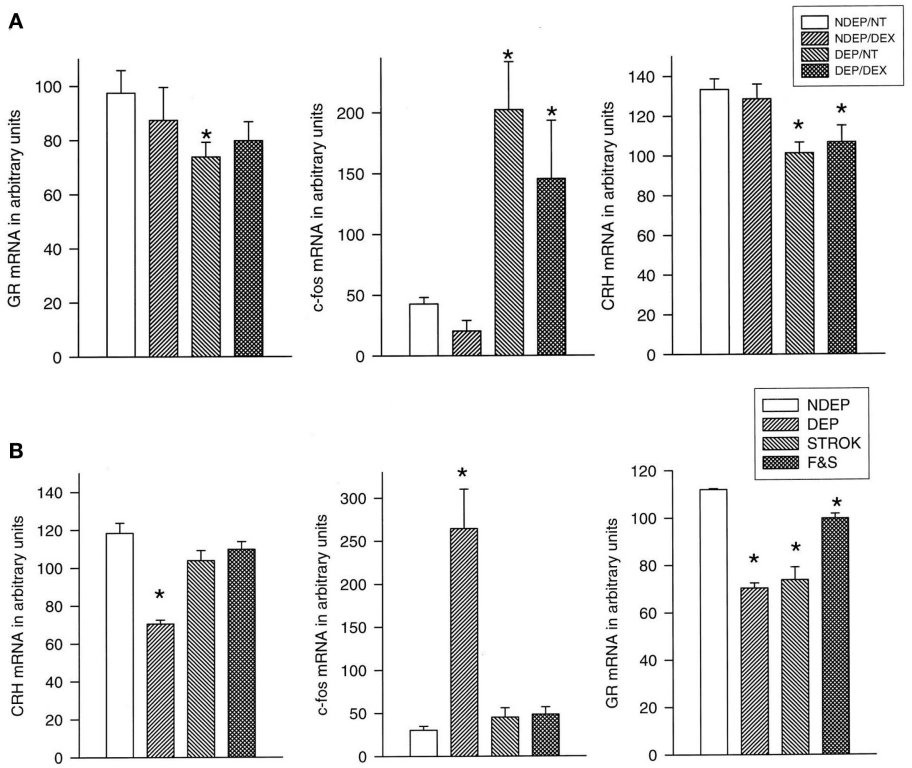


FIGURE 1 | Basal CRH stress-induced (30 min after saline injection) c-fos and basal GR mRNA expression in the PVN of 12-day-old pups. **(A)** Effect of dexamethasone (DEX): the deprived (DEP) group had been deprived 24 h before testing ($n = 10$–12 per group). Non-deprived (NDEP) animals served as controls. The DEX animals had received a dexamethasone injection at the onset of deprivation (or equivalent time for NDEP) of 100 μg/kg BW. NT animals had received a saline injection instead of a DEX injection, $^*p < 0.05$, significant from NDEP counterparts. **(B)** Effect of feeding and stroking: litters were deprived for 24 h on P11, during which time they were either left undisturbed (ISO), stroked (STROK), or stroked and episodically fed (F & S) ($n = 10$–12 per group). NDEP animals served as controls, $^*p < 0.05$, significant from NDEP counterparts. [Reprinted with permission from Ref. (81)].

the newborn becomes used to repeated daily maternal separation as reflected by its normalization of basal HPA-axis activity to SHRP levels, the pups continue to respond to a novelty stressor (78, 83).

The importance of this information cannot be emphasized enough. It first shows that the pups as young as they are already have learned to cope with maternal absence. Their stress system stays on alert, however, and this process seems to involve the functioning of the MR. In the adult, this receptor has been shown to be important in controlling the circuits underlying the appraisal of salient events, vigilance, and emotional reactivity, and apparently this salience system already seems to be in operation in the newborn. In the adult amygdala, corticosterone is capable to impose metaplasticity in the amygdala (8, 84, 85). It appears that also in the pup the cooperative MR- and GR-mediated actions of corticosterone can enhance a lasting activity in the amygdala as can be read from the increased c-fos activity that persists into later life (86, 87). Whether glucocorticoids acting via GR are capable early postnatally to reallocate energy resources to circuits underlying executive function as they do in the adult, remains to be demonstrated (7, 88–90). That at this early age likely the salient rather than the fronto-cortical network operates also has become apparent from studies demonstrating the efficacy of the MR-dependent network in response to corticosterone during the SHRP (91).

In these experiments not only the duration of the separation leaves its marks, also the timing is relevant. If 24 h of maternal separation occurs at pnd 3–4, an enhanced stress-induced ACTH and hypothalamic c-fos response is observed at pnd 20. Deprivation for 24 h at pnd 11–12, however, results in the opposite, an attenuated ACTH and c-fos response to stress 8 days later at pnd 20 (92). The corticosterone response to stress in the pnd 20 pups deprived at either pnd 3 or 11 was not different from the non-deprived controls, however. Accordingly, the persistent alterations in central and ACTH responses as a function of pnd of deprivation are not reflected in corticosterone levels.

In conclusion, the findings reported in the previous paragraphs demonstrate that post-natal dexamethasone effects are overridden by centrally regulated processes that underlie coping of the pup with changing conditions. The newborn is aware of these changing conditions and the corticosterone receptor system seems already to cooperate with the sympathetic nervous system in organizing the salience neuronal network response expressing signs of appraisal, attention, vigilance, and emotional reactivity. Why this network does not seem to be affected by dexamethasone may be because the synthetic steroid does not bind to the MR, which is one of the initial drivers of the salience network (**Figure 2**).

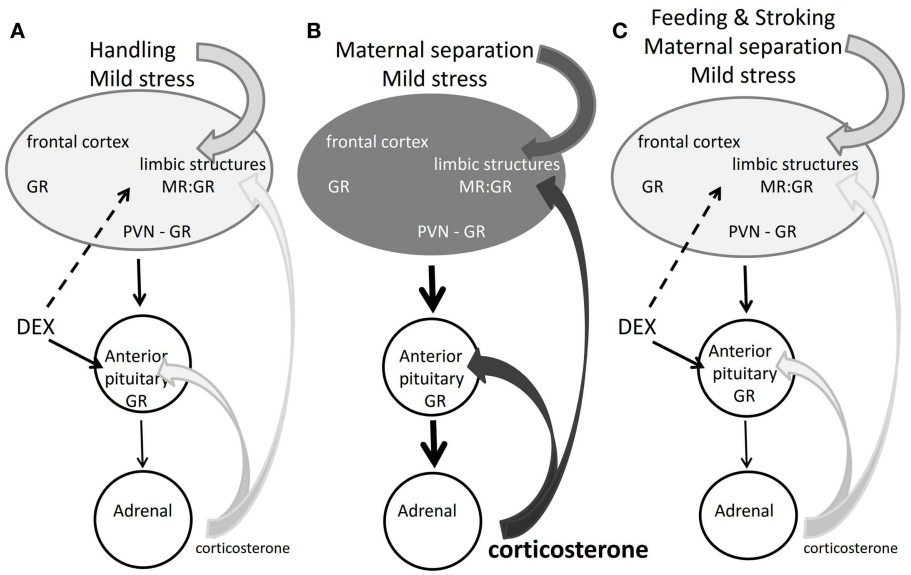


FIGURE 2 | Corticosterone, dexamethasone, and the rat SHRP. **(A)** Mild stress + handling: corticosterone maintains quiescence in the HPA-axis, but the brain responds to the mild stressor. Dexamethasone acts on brain and pituitary via GR. Handling obliterates long-term effects of dexamethasone (93). **(B)** Maternal separation and mild stress: stressful information from the brain overrides the pituitary feedback action of corticosterone. The elevated corticosterone acts via MR and GR to modulate programing of limbic brain pathways (87). The darker gray tone indicates enhanced limbic activity by increased sensory and corticosterone input. **(C)** Feeding and stroking the maternally separated rat inhibits the brain, pituitary, and adrenal response to the stressor. Dexamethasone also blocks the pituitary–adrenal axis but does not give the same outcome as feeding and stroking (81).

ANIMAL MODELS OF POST-NATAL GLUCOCORTICOID EXPOSURE

Animal models of post-natal treatment with dexamethasone have demonstrated profound effects on later life outcome. Among the most serious outcomes is the finding that the lifespan of early dexamethasone-treated rats was reduced with 25% (94). The cause of death was cardiac and kidney failure, while also auto-immunity was noted. Impaired cognitive functions were also reported. The dexamethasone-treated animals had deficits in spatial learning and showed disturbances in the neuroendocrine response pattern to stress (95, 96).

We started a research project with the aim to ameliorate the putative negative prognosis of early dexamethasone treatment. In the first series of experiments, we have used an intracerebroventricular (ICV) administration of the anti-glucocorticoid RU486 to protect the brain against early dexamethasone treatment (97). We adopted the treatment regimen of tapering daily doses of dexamethasone-21-phosphate during the first 3 pnds, because it is thought to resemble the clinical administration of the drug: 0.5 μg/g pup on pnd 1 followed by 0.3 and 0.1 μg on pnd 2 and 3. The treatments were preceded by an ICV administration of 0.1 ng RU486. First, we examined with immunostaining for the Ki-67 marker the neuronal proliferation in the hippocampal dentate gyrus and found a profound reduction the day after dexamethasone treatment, which however was not inhibited by GR blockade. These effects were normalized by pnd 10. We also analyzed astrogliosis on the basis of glial fibrillary acidic protein (GFAP) immunocytochemistry and found a significant suppression in the corpus callosum 1 week after dexamethasone administration, that was blocked by pre-treatment with RU486 (**Figure 3**).

These data are consistent with previous studies showing that dexamethasone can transiently suppress neuronal proliferation and astrogliosis and that the effects are reversible possibly because of rebound effect later during development (98). The data also show that some of these effects can be prevented by local GR blockade in the hippocampus.

LATER LIFE OUTCOME

We were unable to reproduce the striking negative outcome of early post-natal dexamethasone treatment and discovered several factors that may account for this discrepancy with the published studies. First, in our studies we have used Long Evans rats, while most other studies were performed with Wistars or Sprague Dawley rats. More importantly, however, might have been differences in the design of the studies. While for instance Kamphuis et al. (96) used a whole litter design, we have consistently used a split-litter design with both dexamethasone and saline treated animals within every litter, to assure similar treatment conditions for each individual animal. Moreover, in the course of our experiments we noted that the handling procedure, required to inject and mark the pups, actually triggered bouts of enhanced maternal care that are known since long to reduce emotional reactivity and stress responsivity of the pups in later life. Indeed in a comparative study we found that a non-handled group of rats was more impaired in cognitive function than the handled dexamethasone and saline groups that each did not differ significantly from each other (93, 99).

Then in a controlled study, we examined whether daily handling actually could prevent adverse effects of post-natal dexamethasone treatment (93). Thus four groups were compared: first, a

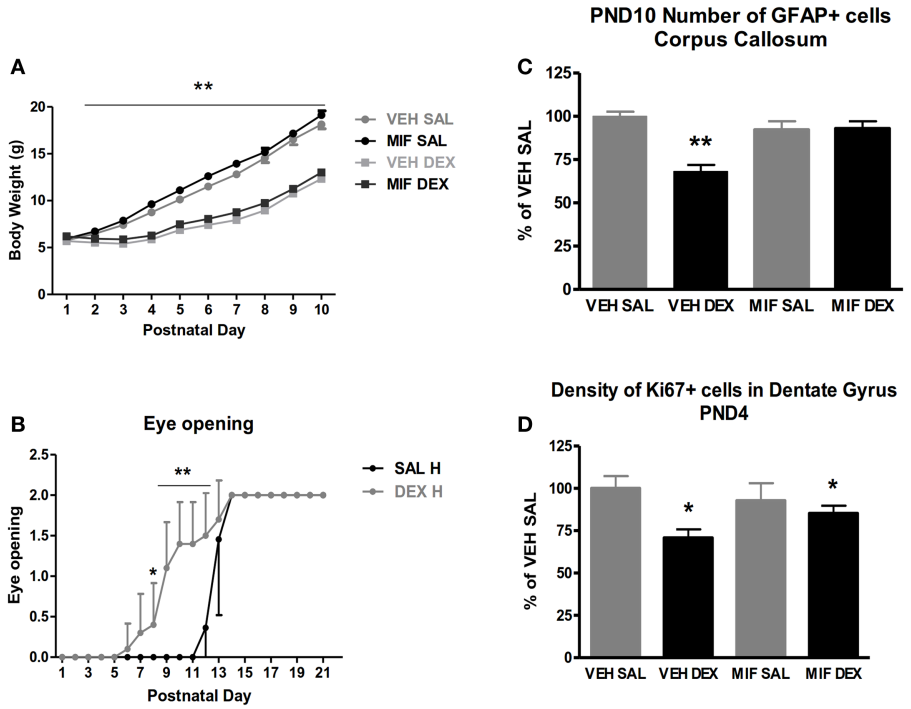


FIGURE 3 | Short-term effect of post-natal dexamethasone. **(A)** Body weight on pnd 1–10 of saline (SAL) and dexamethasone (DEX) treated animals with intracerebroventricular (ICV) treatment of the glucocorticoid antagonist RU486 (mifepristone, MIF). DEX treatment significantly reduced body weight; this effect is not prevented by central mifepristone pre-treatment. **(B)** DEX treatment resulted in accelerated eye opening; on pnd 8–12 DEX treated animals show enhanced level of eye opening compared to SAL treated animals. Data represent mean ± SEM, $*p < 0.05$, $**p < 0.01$. **(C)** Number of glial fibrillary acidic protein (GFAP)-positive cells in the corpus callosum 7 days post treatment in SAL and DEX treated animals with ICV MIF pre-treatment. **Interaction between subcutaneous (SC) and ICV treatment $p < 0.01$. #Interaction between SC and ICV treatment $p < 0.10$. **(D)** Density of Ki-67 positive cells in the dentate gyrus of the hippocampus 24 h post treatment in SAL and DEX treated animals with ICV MIF pre-treatment. **Main effect of DEX treatment $p < 0.01$; *main effect of DEX treatment $p < 0.05$. [Reprinted with permission from Ref. (97)].

saline and a dexamethasone group that was only handled briefly for injections on the first 3 days of life and afterward was left undisturbed until weaning. Second, a saline and a dexamethasone group that was handled daily until weaning for 15 min. Eye opening was accelerated in dexamethasone-treated animals from day 12 to 8; a phenomenon that could not be evaluated in the non-handled groups. Dexamethasone treatment reduced body weight gain by 20% during the first 3 weeks of life. These differences in body weight lasted up to 10 months of age. Maternal care was enhanced the first week of life in the handled groups. This effect was only observed post-reunion when licking and grooming was increased from about 8 to 12% of the time. These differences in maternal care are known to have profound effects on later life outcome (100).

A number of behavioral observations were made over the next 10 months. Handling improved impaired spatial learning of the dexamethasone-treated non-handled animals the T-maze (**Figure 4**). In another hippocampal-associated spatial learning test, the water maze, handling reduced the susceptibility to the impact of dexamethasone treatment. Furthermore, in a fear conditioning paradigm the acquisition of fear as expressed by a freezing response was reduced in both handling groups both immediately after the shock as well as upon re-exposure to the context after 24 h, without any effect of dexamethasone treatment, which is in agreement with Kamphuis et al. (95). Handling had a beneficial effect on pre-pulse inhibition at the 2 dB level in saline treated animals, without affecting dexamethasone-treated animals. Finally, both dexamethasone treatment and handling resulted in enhanced negative feedback of the stress-induced corticosterone response and reduced startle reactivity (**Figure 4**). Thus, we find that the handling procedure, which enhances maternal care received by the newborn, was able to attenuate specific dexamethasone-induced impairments in the behavioral phenotype of the adult animals (93).

DEVELOPMENTAL ORIGIN OF DISEASE

One may wonder how the outcome of early dexamethasone exposure may fit into the theory on the developmental origin of health and disease (DOHaD). This theory has its basis in the Barker hypothesis on the relationship between low birth weight and health outcome in later life and refers to the efficiency of food and energy transfer of the mother to the fetus (101). There can be many reasons for this deficit including the stressful events occurring antenatally and even the pre-implantation hormonal conditions (102). Ovarian hyperstimulation is a stressful condition that compromises vascularization of the blastocyst implantation site and hence caused decreased supply of nutrients. Ovarian hyperstimulation is commonly used in the generation of transgenic mice and might therefore have introduced a bias in all studies involving mutant

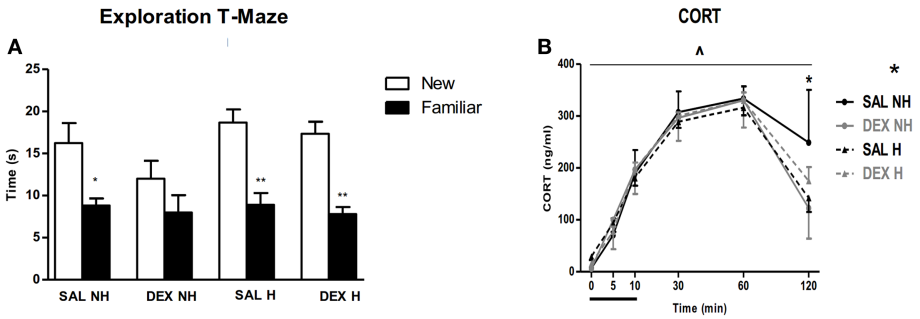


FIGURE 4 | Long-term outcome of post-natal dexamethasone. **(A)** All groups except dexamethasone non-handled (DEX NH) spent significantly more time in the new compared to the familiar arm during re-exposure to T-maze. Data represent mean ± SEM, $*p < 0.05$, $**p < 0.01$. **(B)** Cortcosterone (CORT) levels before, during, and following exposure to 10 min restraint stress. Both handling and DEX treatment result in enhanced negative feedback of the HPA-axis at $t = 120$ min. Data represent mean ± SEM. Time × drug treatment × handling interaction $p = 0.01$, $*p < 0.05$ (from Ref. (93)).

mice (103, 104), particularly if urinary rather than recombinant gonadotropins have been used.

The DOHaD theory describes how experiences during early-life from blastocyst implantation, to fetal and post-natal life into puberty may induce developmental changes that affect the susceptibility to often comorbid cardiovascular and metabolic diseases as well as mental disorders in later life. It is thought that stressful experiences during early-life can modulate the genetic programing of specific brain circuits underlying emotional and cognitive aspects of behavioral adaptation. Although such a scenario implies multiple hits we have used for practical reasons to restrict the hits to three main categories: hit 1: genetic pre-disposition, hit 2: early-life experiences and hit 3: experiences during puberty (87, 105, 106).

CUMULATIVE STRESS EXPOSURE: THE MATCH/MISMATCH THEORY

It was found that either each subsequent hit accumulates damage and predisposes for disease or that gene X environment in early-life (hit 1 × hit 2 × hit 3) prepares for coping with challenges in later life. The latter situation predicts that experiencing adversity in early-life actually promotes coping with similar adverse conditions in later life. These findings led to the formulation of the predictive adaptive capacity or match/mismatch hypothesis (107). The latter hypothesis predicts increased vulnerability in case of a mismatch between early-life experiences that have re-programed the behavioral repertoire to cope with the actual circumstances the individual is facing in later life (108).

In our research, we observed that male rats that had experienced as pups abundant maternal care were well prepared at adulthood to execute cognitive tasks under relatively mild stressful conditions. Their hippocampus showed signs of extensive dendritic arborizations richly endowed by synaptic boutons and *in vitro* an efficient long-term potential (LTP) response could be evoked from these hippocampal circuits. However, under more stressful conditions this well-groomed offspring performed poorly, a finding that gives support to the match/mismatch hypothesis (100, 109–113).

What determines whether early-life adversity actually prepares for life ahead or for cumulative damage and enhanced vulnerability is not known. One line of research suggests that the genotype is critical. When newborn rats of a line that was genetically selected for enhanced responsiveness of the dopamine system by apomorphine-induced gnawing were deprived repeatedly from maternal care for a prolonged period, but only if the pups had also experienced a stressor (87). Subsequent exposure to isolation rearing during puberty promoted at adulthood signs of schizophrenia such as an impaired PPI response.

How early-life dexamethasone treatment fits into these hypothesis is not known. It is conceivable that the reduced body weight after pre- and post-natal dexamethasone exposure bears some relationship with the phenotype that led to the Barker and the DOHaD theories, but this needs to be investigated. At the same time, however, we also presented evidence that with respect to the brain the dexamethasone effects are overridden by maternal factors. This was demonstrated in two ways. First, in the deprived rat the normalization of HPA-axis activity persisted by mimicking maternal care through feeding and stroking the pups, but not if dexamethasone was given that also causes like maternal care an immediate suppression of the HPA axis. Second, adverse effects of dexamethasone on higher brain functions were ameliorated by neonatal handling causing enhanced maternal care.

MECHANISM

How do these maternal factors override the lasting effects of dexamethasone? Research in the past decade has clearly established that early-life experience may cause stable changes in histone acetylation and DNA methylation. These findings have led to the recognition of the epigenome as the molecular basis for altered plasticity in organ systems including the brain (114). These epigenomic signatures are a determinant of plasticity in neuronal networks underlying later emotional expression and cognitive performance, and seem a significant factor in the precipitation of stress-related mental disorders such as depression and addiction (115).

In recent years, DNA methylation of HPA-axis genes, among others the promotor regions of the GR (80, 116), the vasopressin (VP) gene (117), and 11βHSD (118) was observed. In particular, the epigenetic change in GR was found associated with a

behavioral response pattern and HPA-axis setpoint in later life, and therefore is considered to represent some sort of "molecular memory" for salient events (68). Hence, decreased expression of GR in the hippocampus was found associated with emotional neglect in early-life (80, 119). Suderman et al. (116) recently stated that a global epigenetic signature of early-life experience becomes apparent that is maintained across species and centered around GR gene regulation, while more detailed analysis of the methylation profile may reveal more subtle intrinsic differences between species. Indeed, altered responsiveness in gene expression is maintained until adulthood and reflected in transcriptome analysis of subregions of the hippocampus (120). In this type of analysis it appeared already in the adult animals that the history of chronic stress experience causes a profound change in the transcriptional response to an acute corticosterone or stress stimulus with an overrepresentation of genes involved in chromatin remodeling, epigenetic processes, and cell adhesion (121–123).

The mechanism by which perinatal dexamethasone exposure causes long-term effects is not completely understood. Recent studies suggest that these long-term effects are possibly mediated through GR-activated epigenetic modulations such as DNA methylation and histone acetylation. Matthews and colleagues have extensively studied the epigenetic modifications associated with antenatal synthetic glucocorticoid treatment in the guinea pig. They observed that during the endogenous glucocorticoid surge in late gestation considerable changes in global DNA methylation in several organ systems take place that affect the expression of genes involved in the methylation process. Betamethasone administered before the natural glucocorticoid surge also affected global methylation and expression of methylation-related genes, though slightly different compared to the endogenous surge, and in addition affected histone-3 lysine 9 acetylation (124, 125). The effects of betamethasone treatment not only persisted into adulthood but also into the next generation (124).

In the male hippocampus it was also shown that concurrent with changes in DNA methylation, GR DNA binding was altered during both the endogenous glucocorticoid surge as well as synthetic glucocorticoid treatment (126). Though, this study clearly shows a relationship between altered GR DNA binding and epigenetic changes in gene expression, further research is necessary to provide conclusive evidence for a GR-mediated mechanism of epigenetic modification. This type of ChIP-seq analysis of GR binding to the hippocampal genome of ADX rats recently identified in the adult rat hippocampus 2460 significant binding sites of which 40% were associated with GRE's in promoter regions (127).

CONCLUDING REMARKS

This contribution was focused on the mode and mechanism of action in the brain of perinatal dexamethasone administration using pre- and post-natal animal models. This topic is of relevance because glucocorticoids are critical for survival of prematurely born infants. The glucocorticoid is required to ensure adequate maturation of in particular lung tissue. Regarding the mode of action the timing of glucocorticoids is important. If given preterm to the mother the synthetic glucocorticoids are used in a restricted number of courses [see Cochrane data base, (128)]. Hydrocortisone is ineffective preterm since the hormone is largely inactivated by 11β-HSD2 and does only poorly pass the placenta barrier. Postnatally hydrocortisone is used and reportedly there is so far no noticeable adverse outcome in later life (129). The underlying mechanism of the steroid effects depends on epigenetic modulation of the stress response system and possibly the changes in plasticity of limbic-forebrain glucocorticoid target regions.

The rodent is frequently used to examine adversity of dexamethasone treatment, because it is an altricial species and pnd 1 in the rat resembles the developmental stage at the beginning of the third trimester of human pregnancy. Alternatively, guinea pigs are used to mimic antenatal administration of dexamethasone to pregnant females. Here, we have discussed *in extenso* the role of glucocorticoids in development of the brain in relation to the HPA-axis. Some authors reported alarming effects of perinatal dexamethasone on health and brain development even causing a life shortening of 25% (94). However, we and others (130) were unable to find these detrimental effects, probably because our design included enhanced levels of maternal care. Using two different approaches, we demonstrated that such maternal care effects override dexamethasone effects in the brain and the HPA-axis. Hence, other approaches based on environmental input may be beneficial. For instance, in rats environmental enrichment during puberty appeared to neutralize the adverse outcome in the offspring of antenatally stressed pregnant dams (131).

Hence context is capable to modulate the outcome of perinatal glucocorticoid action. The context becomes manifest in two ways. First, maternal care and probably other contextual factors such as e.g., environmental enrichment are capable to modulate the outcome of glucocorticoid action, possibly by an epigenetic machinery. Second, context is equally important in later life where it either does or does not match the phenotype shaped by early experiences in sensitive windows of brain development during early post-natal life and puberty. Probably depending on genotype and epigenomic signature susceptibility to stress-related disorders may develop. Because of this interaction between dexamethasone-induced effects and experience-related factors, behavioral interventions appear to be crucial in the clinical management of preterm babies (132–134).

ACKNOWLEDGMENTS

The support by the Royal Netherlands Academy of Arts and Sciences and Eu-Lifespan is gratefully acknowledged.

REFERENCES

1. Padbury JF, Ervin MG, Polk DH. Extrapulmonary effects of antenatally administered steroids. *J Pediatr* (1996) **128**(2):167–72. doi:10.1016/S0022-3476(96)70384-0
2. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev* (2006) **3**:CD004454. doi:10.1002/14651858.CD004454.pub2
3. Barrington KJ. The adverse neuro-developmental effects of postnatal steroids in the preterm infant: a systematic review of RCTs. *BMC Pediatr* (2001) **1**:1. doi:10.1186/1471-2431-1-1
4. Barrington KJ. Postnatal steroids and neurodevelopmental outcomes: a problem in the making. *Pediatrics* (2001) **107**(6):1425–6. doi:10.1542/peds.107.6.1425
5. Yeh TF, Lin YJ, Lin HC, Huang CC, Hsieh WS, Lin CH, et al. Outcomes at school age after postnatal dexamethasone therapy for lung disease of prematurity. *N Engl J Med* (2004) **350**:1304–13. doi:10.1056/NEJMoa032089

6. Reul JM, de Kloet ER. Two receptor systems for corticosterone in rat brain: microdistribution and differential occupation. *Endocrinology* (1985) **117**(6):2505–11. doi:10.1210/endo-117-6-2505
7. de Kloet ER, Joëls M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* (2005) **6**(6):463–75. doi:10.1038/nrn1683
8. Karst H, Berger S, Turiault M, Tronche F, Schütz G, Joëls M. Mineralocorticoid receptors are indispensable for non-genomic modulation of hippocampal glutamate transmission by corticosterone. *Proc Natl Acad Sci U S A* (2005) **102**(52):19204–7. doi:10.1073/pnas.0507572102
9. Grossmann C, Scholz T, Rochel M, Bumke-Vogt C, Oelkers W, Pfeiffer AF, et al. Transactivation via the human glucocorticoid and mineralocorticoid receptor by therapeutically used steroids in CV-1 cells: a comparison of their glucocorticoid and mineralocorticoid properties. *Eur J Endocrinol* (2004) **151**(3):397–406. doi:10.1530/eje.0.1510397
10. Rosenthal HE, Slaunwhite WR Jr, Sandberg AA. Transcortin: a corticosteroid-binding protein of plasma. X. Cortisol and progesterone interplay and unbound levels of these steroids in pregnancy. *J Clin Endocrinol Metab* (1969) **29**(3):352–67. doi:10.1210/jcem-29-3-352
11. Mastorakos G, Illias I. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. *Ann N Y Acad Sci* (2003) **997**:136–49. doi:10.1196/annals.1290.016
12. Riley SC, Challis JR. Corticotrophin-releasing hormone production by the placenta and fetal membranes. *Placenta* (1991) **12**(2):105–19. doi:10.1016/0143-4004(91)90015-8
13. Davis EP, Sandman CA. The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Dev* (2010) **81**(1):131–48. doi:10.1111/j.1467-8624.2009.01385.x
14. Edwards CR, Benediktsson R, Lindsay RS, Seckl JR. Hydroxysteroid dehydrogenase: key enzymes in determining tissue-specific glucocorticoid effects. *Steroids* (1996) **61**(4):263–9. doi:10.1016/0039-128X(96)00033-5
15. Welberg LA, Seckl JR, Holmes MC. Inhibition of 11beta-hydroxysteroid dehydrogenase, the foeto-placental barrier to maternal glucocorticoids, permanently programs amygdala GR mRNA expression and anxiety-like behaviour in the offspring. *Eur J Neurosci* (2000) **12**(3):1047–54. doi:10.1046/j.1460-9568.2000.00958.x
16. Diaz R, Brown RW, Seckl JR. Distinct ontogeny of glucocorticoid and mineralocorticoid receptor and 11β-hydroxysteroid dehydrogenase types I and II mRNAs in the fetal rat brain suggest a complex control of glucocorticoid actions. *J Neurosci* (1998) **18**(7):2570–80.
17. Ohkawa T, Takeshita S, Murase T, Kambegawa A, Okinaga S, Arai K. Ontogeny of the response of the hypothalamic-pituitary-adrenal axis to maternal immobilization stress in rats. *Endocrinol Jpn* (1991) **38**(2):187–94. doi:10.1507/endocrj1954.38.187
18. Reichardt HM, Schütz G. Feedback control of glucocorticoid production is established during fetal development. *Mol Med* (1996) **2**(6):735–44.
19. Condon J, Gosden C, Gardener D, Nickson P, Hewison M, Howie AJ, et al. Expression of type 2 11beta-hydroxysteroid dehydrogenase and corticosteroid hormone receptors in early human fetal life. *J Clin Endocrinol Metab* (1998) **83**(12):4490–7. doi:10.1210/jcem.83.12.5302
20. Noorlander CW, de Graan PN, Middeldorp J, van Beers JJ, Visser GH. Ontogeny of hippocampal corticosteroid receptors: effects of antenatal glucocorticoids in human and mouse. *J Comp Neurol* (2006) **499**(6):924–32. doi:10.1002/cne.21162
21. Bayer SA, Altmann J, Russo RJ, Zhang X. Timetables of neurogenesis in the human brain based on experimentally determined patterns in the rat. *Neurotoxicology* (1993) **14**(1):83–144.
22. Dobbing J, Sands J. Comparative aspects of the brain growth spurt. *Early Hum Dev* (1979) **3**(1):79–83. doi:10.1016/0378-3782(79)90022-7
23. Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. *Pediatrics* (1972) **50**(4):515–25.
24. Crowther CA, Harding JE. Repeat doses of prenatal corticosteroids for women at risk of preterm birth for preventing neonatal respiratory disease. *Cochrane Database Syst Rev* (2007) **3**:CD003935. doi:10.1002/14651858.CD003935.pub2
25. Susser ES, Lin SP. Schizophrenia after prenatal exposure to Dutch hunger winter of 1944-1945. *Arch Gen Psychiatry* (1996) **49**(12):983–8. doi:10.1001/archpsyc.1992.01820120071010
26. Brown AS, Susser ES, Lin SP, Neugebauer R, Gorman JM. Increased risk of affective disorder in males after second trimester prenatal exposure to the Dutch hunger winter of 1944-1945. *Br J Psychiatry* (1995) **166**:601–60. doi:10.1192/bjp.166.5.601
27. Franzek EJ, Sprangers N, Janssens AC, van Duijn CM, van de Wetering BJ. Prenatal exposure to the 1944-45 Dutch 'hunger winter' and addiction in later life. *Addiction* (2008) **103**(3):433–8. doi:10.1111/j.1360-0443.2007.02084.x
28. Roseboom TJ, Painter RC, van Abeelen AF, Veenendaal MV, de Rooij SR. Hungry in the womb: what are the consequences? Lessons from the Dutch famine. *Maturitas* (2011) **70**:141–5. doi:10.1016/j.maturitas.2011.06.017
29. Watson JB, Mednick SA, Huttenen M, Wang X. Prenatal teratogens and the development of adult mental illness. *Dev Psychopathol* (1999) **11**(3):457–66. doi:10.1017/S0954579499002151
30. Ward AJ. A comparison and analysis of the presence of family problems during pregnancy of mothers of "autistic" children and mothers of normal children. *Child Psychiatry Hum Dev* (1990) **20**(4):279–88. doi:10.1007/BF00706020
31. Meyer U, Feldon J. Epidemiology-driven neurodevelopmental animal models of schizophrenia. *Prog Neurobiol* (2010) **90**:285–326. doi:10.1016/j.pneurobio.2009.10.018
32. Beydoun H, Saftlas AF. Physical and mental health outcomes of prenatal maternal stress in human and animal studies: a review of recent evidence. *Paediatr Perinat Epidemiol* (2008) **22**:438–66. doi:10.1111/j.1365-3016.2008.00951
33. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* (2001) **49**:1023–39. doi:10.1016/S0006-3223(01)01157-X
34. Hornung OP, Heim CM. Gene-environment interactions and intermediate phenotypes: early trauma and depression. *Front Endocrinol (Lausanne)* (2014) **5**:14. doi:10.3389/fendo.2014.00014
35. Marques AH, O'Connor TG, Roth C, Susser E, Bjørke-Monsen AL. The influence of maternal prenatal and early childhood nutrition and maternal prenatal stress on offspring immune system development and neurodevelopmental disorders. *Front Neurosci* (2013) **31**(7):120. doi:10.3389/fnins.2013.00120
36. Blanford AT, Murphy BE. In vitro metabolism of prednisolone, dexamethasone, betamethasone, and cortisol by the human placenta. *Am J Obstet Gynecol* (1977) **127**(3):264–7.
37. Murphy VE, Fittock RJ, Zarzycki PK, Delahunty MM, Smith R, Clifton VL. Metabolism of synthetic steroids by the human placenta. *Placenta* (2007) **28**(1):39–46. doi:10.1016/j.placenta.2005.12.010
38. Iqbal M, Gibb W, Matthews SG. Corticosteroid regulation of P-glycoprotein in the developing blood-brain barrier. *Endocrinology* (2011) **152**(3):1067–79. doi:10.1210/en.2010-1227
39. Meijer OC, de Lange EC, Breimer DD, de Boer AG, Workel JO, de Kloet ER. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. *Endocrinology* (1998) **139**(4):1789–93. doi:10.1210/endo.139.4.5917
40. Karssen AM, Meijer OC, van der Sandt IC, Lucassen PJ, de Lange EC, de Boer AG, et al. Multidrug resistance P-glycoprotein hampers the access of cortisol but not of corticosterone to mouse and human brain. *Endocrinology* (2001) **142**:2686–94. doi:10.1210/endo.142.6.8213
41. Karssen AM, Meijer OC, Berry A, Sanjuan Piñol R, de Kloet ER. Low doses of dexamethasone can produce a hypocorticosteroid state in the brain. *Endocrinology* (2005) **146**(12):5587–95. doi:10.1210/en.2005-0501
42. Cintra A, Solfrini V, Bunneman B, Okret S, Bortolotti F, Gustafsson JA, et al. Prenatal development of glucocorticoid receptor gene expression and immunoreactivity in the rat brain and pituitary gland: a combined in situ hybridization and immunocytochemistry analysis. *Neuroendocrinology* (1993) **57**(6):1133–47. doi:10.1159/000126480
43. Brown RW, Diaz R, Robson AC, Kotelevtsev YV, Mullins JJ, Kaufman MH, et al. The ontogeny of 11β-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. *Endocrinology* (1996) **137**(2):794–7. doi:10.1210/endo.137.2.8593833
44. Speirs HJ, Seckl JR, Brown JR. Ontogeny of glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase type-1 gene expression identifies potential critical periods of glucocorticoid susceptibility during development. *J Endocrinol* (2004) **181**(1):105–16. doi:10.1677/joe.0.1810105

45. Thompson A, Han VK, Yang K. Differential expression of 11β-hydroxysteroid dehydrogenase types 1 and 2 mRNA and glucocorticoid receptor protein during mouse embryonic development. *J Steroid Biochem Mol Biol* (2004) **88**(4–5):367–75. doi:10.1016/j.jsbmb.2003.12.014
46. Yang K, Haynes NB, Lamming GE. Changes in glucocorticoid receptor number in the hypothalamus and pituitary of the sheep fetus with gestational age and after adrenocorticotropin treatment. *Endocrinology* (1990) **126**(1):11–7. doi:10.1210/endo-126-1-11
47. Andrews MH, Matthews SG. Regulation of glucocorticoid receptor mRNA and heat shock protein 70 mRNA in the developing sheep brain. *Brain Res* (2000) **878**(1–2):174–82. doi:10.1016/S0006-8993(00)02735-9
48. Matthews SG. Dynamic changes in glucocorticoid and mineralocorticoid receptor mRNA in the developing guinea pig brain. *Brain Res Dev Brain Res* (1998) **107**(1):123–32. doi:10.1016/S0165-3806(98)00008-X
49. Huizink AC, Mulder EJ, Buitelaar JK. Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? *Psychol Bull* (2004) **130**(1):115–42. doi:10.1037/0033-2909.130.1.115
50. Levitt NS, Lindsay RS, Holmes MC, Seckl JR. Dexamethasone in the last week of pregnancy attenuates hippocampal glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. *Neuroendocrinology* (1996) **64**(6):412–8. doi:10.1159/000127146
51. Owen D, Matthews SG. Prenatal glucocorticoid exposure alters hypothalamic-pituitary-adrenal function in juvenile guinea pigs. *J Neuroendocrinol* (2007) **19**(3):172–80. doi:10.1111/j.1365-2826.2006.01517.x
52. Uno H, Eisele S, Sakai A, Shelton S, Baker E, DeJesus O, et al. Neurotoxicity of glucocorticoid in the primate brain. *Horm Behav* (1994) **28**(4):336–48. doi:10.1006/hbeh.1994.1030
53. Muneoka K, Mikuni M, Ogawa T, Kitera K, Kamei K, Takigawa M, et al. Prenatal dexamethasone exposure alters brain monoamine metabolism and adrenocortical response in rat offspring. *Am J Physiol* (1997) **273**(5):R1669–75.
54. Henry C, Kabbaj M, Simon H, Le Moal M, Maccari S. Prenatal stress increases the hypothalamo-pituitary-adrenal axis response in young and adult rats. *J Neuroendocrinol* (1994) **6**(3):341–5. doi:10.1111/j.1365-2826.1994.tb00591.x
55. Barbazanges A, Piazza PV, Le Moal M, Maccari S. Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. *J Neurosci* (1996) **16**(12):3943–9.
56. Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, et al. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nat Genet* (1999) **23**(1):99–103. doi:10.1038/12703
57. Virdee K, McArthur S, Brischoux F, Caprioli D, Ungless MA, Robbins TW, et al. Antenatal glucocorticoid treatment induces adaptations in adult midbrain dopamine neurons, which underpin sexually dimorphic behavioral resilience. *Neuropsychopharmacology* (2013) **39**:1–12. doi:10.1038/npp.2013.196
58. Rosenfeld P, Sutanto W, Levine S, de Kloet ER. Ontogeny of type I and type II corticosteroid receptors in the rat hippocampus. *Brain Res* (1988) **42**(1):113–8. doi:10.1016/0165-3806(88)90206-4
59. Rosenfeld P, van Eekelen JA, Levine S, de Kloet ER. Ontogeny of the type 2 glucocorticoid receptor in discrete rat brain regions: an immunocytochemical study. *Brain Res* (1988) **42**(1):119–27. doi:10.1016/0165-3806(88)90207-6
60. Rosenfeld P, Sutanto W, Levine S, de Kloet ER. Ontogeny of mineralocorticoid (type 1) receptors in brain and pituitary: an in vivo autoradiographical study. *Brain Res Dev Brain Res* (1990) **52**(1–2):57–62. doi:10.1016/0165-3806(90)90221-J
61. Rosenfeld P, van Eekelen JA, Levine S, de Kloet ER. Ontogeny of corticosteroid receptors in the brain. *Cell Mol Neurobiol* (1993) **13**(4):295–319. doi:10.1007/BF00711575
62. van Eekelen JA, Bohn MC, de Kloet ER. Postnatal ontogeny of mineralocorticoid and glucocorticoid receptor gene expression in regions of the rat tel- and diencephalon. *Brain Res Dev Brain Res* (1991) **61**(1):33–43. doi:10.1016/0165-3806(91)90111-U
63. van Eekelen JAM, Rosenfeld P, Levine S, Westphal HM, de Kloet ER. Postnatal disappearance of glucocorticoid receptor immunoreactivity. *Neurosci Res Commun* (1987) **1**:129–33.
64. de Kloet ER, Han F, Meijer OC. From the stalk to down-under: about glucocorticoid receptors, stress and development. *Neurochem Res* (2008) **33**(4):637–42. doi:10.1007/s11064-007-9520-9
65. Vázquez DM, Van Oers HJ, Levine S, Akil H. Regulation of glucocorticoid and mineralocorticoid receptor mRNAs in the hippocampus of the maternally deprived infant rat. *Brain Res* (1996) **731**(1–2):79–90. doi:10.1016/S0006-8993(96)00465-9
66. Schmidt MV, Oitzl MS, Levine S, de Kloet ER. The HPA system during the postnatal development of CD1 mice and the effects of maternal deprivation. *Brain Res Dev Brain Res* (2002) **139**(1):39–49. doi:10.1016/S0165-3806(02)00519-9
67. 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):1659–62. doi:10.1126/science.277.5332.1659
68. Meaney MJ, Szyf M, Seckl JR. Epigenetic mechanisms of perinatal programming of hypothalamic-pituitary-adrenal function and health. *Trends Mol Med* (2007) **13**(7):269–77. doi:10.1016/j.molmed.2007.05.003
69. Oberlander TF, Weinberg J, Papsdorf M, Grunau R, Misri S, Devlin AM. Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. *Epigenetics* (2008) **3**(2):97–106. doi:10.4161/epi.3.2.6034
70. Sutanto W, Rosenfeld P, de Kloet ER, Levine S. Long-term effects of neonatal maternal deprivation and ACTH on hippocampal mineralocorticoid and glucocorticoid receptors. *Brain Res Dev Brain Res* (1996) **92**(2):156–63. doi:10.1016/0165-3806(95)00213-8
71. Levine S. Infantile experience and resistance to physiological stress. *Science* (1957) **126**(3270):405. doi:10.1126/science.126.3270.405
72. Levine S. Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology* (2005) **30**(1):939–46. doi:10.1016/j.psyneuen.2005.03.013
73. Schmidt MV, Enthoven L, van der Mark M, Levine S, de Kloet ER, Oitzl MS. The postnatal development of the hypothalamic-pituitary-adrenal axis in the mouse. *Int J Dev Neurosci* (2003) **21**(3):125–32. doi:10.1016/S0736-5748(03)00030-3
74. Rosenfeld P, Suchecki D, Levine S. Multifactorial regulation of the hypothalamic-pituitary-adrenal axis during development. *Neurosci Biobehav Rev* (1992) **16**(4):553–68. doi:10.1016/S0149-7634(05)80196-4
75. Schmidt MV, Levine S, Oitzl MS, van der Mark M, Müller MB, Holsboer F, et al. Glucocorticoid receptor blockade disinhibits pituitary-adrenal activity during the stress hyporesponsive period of the mouse. *Endocrinology* (2005) **146**(3):1458–64. doi:10.1210/en.2004-1042
76. Schmidt MV, Sterlemann V, Wagner K, Niederleitner B, Ganea K, Liebl C, et al. Postnatal glucocorticoid excess due to pituitary glucocorticoid receptor deficiency: differential short- and long-term consequences. *Endocrinology* (2009) **150**(6):2709–16. doi:10.1210/en.2008-1211
77. Smith MA, Kim SY, van Oers HJ, Levine S. Maternal deprivation and stress induce immediate early genes in the infant rat brain. *Endocrinology* (1997) **138**(11):4622–8. doi:10.1210/endo.138.11.5529
78. Enthoven L, Oitzl MS, Koning N, van der Mark M, de Kloet ER. Hypothalamic-pituitary-adrenal axis activity of newborn mice rapidly desensitizes to repeated maternal absence but becomes highly responsive to novelty. *Endocrinology* (2008) **149**(12):6366–77. doi:10.1210/en.2008-0238
79. Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science* (1999) **286**(5442):1155–8. doi:10.1126/science.286.5442.1155
80. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. *Nat Neurosci* (2004) **7**(8):847–54. doi:10.1038/nn1276
81. 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):10171–9.
82. van Oers HJ, de Kloet ER, Levine S. Persistent effects of maternal deprivation on HPA regulation can be reversed by feeding and stroking, but not by dexamethasone. *J Neuroendocrinol* (1999) **11**(8):581–8. doi:10.1046/j.1365-2826.1999.00329.x
83. Daskalakis NP, Claessens SE, Laboyrie JJ, Enthoven L, Oitzl MS, Champagne DL, et al. The newborn rat's stress system readily habituates to repeated and prolonged maternal separation, while continuing to respond to stressors in context dependent fashion. *Horm Behav* (2011) **60**(2):165–76. doi:10.1016/j.yhbeh.2011.04.003

84. Karst H, Berger S, Erdmann G, Schütz G, Joëls M. Metaplasticity of amygdalar responses to the stress hormone corticosterone. *Proc Natl Acad Sci U S A* (2010) **107**(32):14449–54. doi:10.1073/pnas.0914381107
85. Joëls M, Sarabdjitsingh RA, Karst H. Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. *Pharmacol Rev* (2012) **64**(4):901–38. doi:10.1124/pr.112.005892
86. Moriceau S, Wilson DA, Levine S, Sullivan RM. Dual circuitry for odor-shock conditioning during infancy: corticosterone switches between fear and attraction via amygdala. *J Neurosci* (2006) **26**(25):6737–48. doi:10.1523/JNEUROSCI.0499-06.2006
87. Daskalakis NP, Diamantopoulou A, Claessens SE, Remmers E, Tjälve M, Oitzl MS, et al. Early experience of a novel-environment in isolation primes a fearful phenotype characterized by persistent amygdala activation. *Psychoneuroendocrinology* (2014) **39**:39–57. doi:10.1016/j.psyneuen.2013.09.021
88. Myers B, McKlveen JM, Herman JP. Neural regulation of the stress response: the many faces of feedback. *Cell Mol Neurobiol* (2012) **32**(5):683–94. doi:10.1007/s10571-012-9801-y
89. Hermans EJ, Henckens M, Joëls M, Fernandez G. Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends Neurosci* (2014) **37**:304–14. doi:10.1016/j.tins.2014.03.006
90. de Kloet ER. From receptor balance to rational glucocorticoid therapy. *Endocrinology* (2014). doi:10.1210/en.2014-1048
91. van Oers HJ, de Kloet ER, Li C, Levine S. The ontogeny of glucocorticoid negative feedback: influence of maternal deprivation. *Endocrinology* (1998) **139**(6):2838–46. doi:10.1210/endo.139.6.6037
92. van Oers HJ, de Kloet ER, Levine S. Early vs. late maternal deprivation differentially alters the endocrine and hypothalamic responses to stress. *Brain Res Dev Brain Res* (1998) **111**(2):245–52. doi:10.1016/S0165-3806(98)00143-6
93. Claessens SE, Daskalakis NP, Oitzl MS, de Kloet ER. Early handling modulates outcome of neonatal dexamethasone exposure. *Horm Behav* (2012) **62**(4):433–41. doi:10.1016/j.yhbeh.2012.07.011
94. Kamphuis PJ, de Vries WB, Bakker JM, Kavelaars A, van Dijk JE, Schipper ME, et al. Reduced life expectancy in rats after neonatal dexamethasone treatment. *Pediatr Res* (2007) **61**(1):72–6. doi:10.1203/01.pdr.0000249980.95264.dd
95. Kamphuis PJ, Bakker JM, Broekhoven MH, Kunne C, Croiset G, Lentjes EG, et al. Enhanced glucocorticoid feedback inhibition of hypothalamo-pituitary-adrenal responses to stress in adult rats neonatally treated with dexamethasone. *Neuroendocrinology* (2002) **76**(3):158–69. doi:10.1159/000064526
96. Kamphuis PJ, Gardoni F, Kamal A, Croiset G, Bakker JM, Cattabeni F, et al. Long-lasting effects of neonatal dexamethasone treatment on spatial learning and hippocampal synaptic plasticity: involvement of the NMDA receptor complex. *FASEB J* (2003) **17**(8):911–3. doi:10.1096/fj.02-0333fje
97. Claessens SE, Belanoff JK, Kanatsou S, Lucassen PJ, Champagne DL, de Kloet ER. Acute effects of neonatal dexamethasone treatment on proliferation and astrocyte immunoreactivity in hippocampus and corpus callosum: towards a rescue strategy. *Brain Res* (2012) **1482**:1–12. doi:10.1016/j.brainres.2012.08.017
98. Bohn MC, Lauder JM. Cerebellar granule cell genesis in the hydrocortisone-treated rats. *Dev Neurosci* (1980) **3**(2):81–9. doi:10.1159/000112380
99. Claessens SEF. *Programming the Brain: Towards intervention strategies*. Ph.D. thesis, Leiden University, Leiden (2012).
100. 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):6037–45. doi:10.1523/JNEUROSCI.0526-08.2008
101. Barker DJ. Fetal growth and adult disease. *Br J Obstet Gynaecol* (1992) **99**(4):275–6. doi:10.1111/j.1471-0528.1992.tb13719.x
102. de Kloet ER, Sibug RM, Helmerhorst FM, Schmidt MV. Stress, genes and the mechanism of programming the brain for later life. *Neurosci Biobehav Rev* (2005) **29**(2):271–81. doi:10.1016/j.neubiorev.2004.10.008
103. Sibug RM, de Koning J, Tijssen AM, de Ruiter MC, de Kloet ER, Helmerhorst FM. Urinary gonadotrophins but not recombinant gonadotrophins reduce expression of VEGF120 and its receptors flt-1 and flk-1 in the mouse uterus during the peri-implantation period. *Hum Reprod* (2005) **20**(3):649–56. doi:10.1093/humrep/deh648
104. Sibug RM, Datson N, Tijssen AM, Morsink M, de Koning J, de Kloet ER, et al. Effects of urinary and recombinant gonadotrophins on gene expression profiles during the murine peri-implantation period. *Hum Reprod* (2007) **22**(1):75–82. doi:10.1093/humrep/del363
105. Daskalakis NP, Oitzl MS, Schächinger H, Champagne DL, de Kloet ER. Testing the cumulative stress and mismatch hypotheses of psychopathology in a rat model of early-life adversity. *Physiol Behav* (2012) **106**(5):707–21. doi:10.1016/j.physbeh.2012.01.015
106. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER. The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* (2013) **38**(9):1858–73. doi:10.1016/j.psyneuen.2013.06.008
107. Gluckman PD, Hanson MA. Developmental plasticity and human disease: research directions. *J Intern Med* (2007) **261**(5):461–71. doi:10.1111/j.1365-2796.2007.01802.x
108. Nederhof E, Schmidt MV. Mismatch or cumulative stress: toward an integrated hypothesis of programming effects. *Physiol Behav* (2012) **106**:691–700. doi:10.1016/j.physbeh.2011.12.008
109. Champagne DL, de Kloet ER, Joëls M. Fundamental aspects of the impact of glucocorticoids on the (immature) brain. *Semin Fetal Neonatal Med* (2009) **14**(3):136–42. doi:10.1016/j.siny.2008.11.006
110. Oitzl MS, Champagne DL, van der Veen R, de Kloet ER. Brain development under stress: hypotheses of glucocorticoid actions revisited. *Neurosci Biobehav Rev* (2010) **34**(6):853–66. doi:10.1016/j.neubiorev.2009.07.006
111. Claessens SE, Daskalakis NP, van der Veen R, Oitzl MS, de Kloet ER, Champagne DL. Development of individual differences in stress responsiveness: an overview of factors mediating the outcome of early life experiences. *Psychopharmacology (Berl)* (2011) **214**:141–54. doi:10.1007/s00213-010-2118-y
112. Bagot RC, van Hasselt FN, Champagne DL, Meaney MJ, Krugers HJ, Joëls M. Maternal care determines rapid effects of stress mediators on synaptic plasticity in adult rat hippocampal dentate gyrus. *Neurobiol Learn Mem* (2009) **92**(3):292–300. doi:10.1016/j.nlm.2009.03.004
113. Bagot RC, Tse YC, Nguyen HB, Wong AS, Meaney MJ, Wong TP. Maternal care influences hippocampal N-methyl-D-aspartate receptor function and dynamic regulation by corticosterone in adulthood. *Biol Psychiatry* (2012) **72**(6):491–8. doi:10.1016/j.biopsych.2012.03.016
114. Hoffmann A, Spengler D. DNA memories of early social life. *Neuroscience* (2014) **264**:64–75. doi:10.1016/j.neuroscience.2012.04.003
115. Peña CJ, Bagot RC, Labonté B, Nestler EJ. Epigenetic signaling in psychiatric disorders. *J Mol Biol* (2014). doi:10.1016/j.jmb.2014.03.016
116. Suderman M, McGowan PO, Sasaki A, Huang TC, Hallett MT, Meaney MJ, et al. Conserved epigenetic sensitivity to early life experience in the rat and human hippocampus. *Proc Natl Acad Sci U S A* (2012) **109**(2):17266–72. doi:10.1073/pnas.1121260109
117. Murgatroyd C, Patchev AV, Wu Y, Micale V, Bockmühl Y, Fischer D, et al. Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat Neurosci* (2009) **12**(12):1559–66. doi:10.1038/nn.2436
118. Wyrwoll CS, Holmes MC. Prenatal excess glucocorticoid exposure and adult affective disorder: a role of serotonergic and catecholamine pathways. *Neuroendocrinology* (2012) **95**:47–55. doi:10.1159/000331345
119. McGowan PO, Sasaki A, D'Alessio AC, Dymov S, Labonté B, Szyf M, et al. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat Neurosci* (2009) **12**(3):342–8. doi:10.1038/nn.2270
120. Weaver IC, Meaney MJ, Szyf M. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc Natl Acad Sci U S A* (2006) **103**(9):3480–5. doi:10.1073/pnas.0507526103
121. Polman JA, Hunter RG, Speksnijder N, van den Oever JME, Korobko OB, McEwen BS, et al. Glucocorticoids modulate the mTOR pathway in the hippocampus: differential effects depending on stress history. *Endocrinology* (2012) **153**(3):4317–27. doi:10.1210/en.2012-1255
122. Datson NA, van den Oever JM, Korobko OB, Magarinos AM, de Kloet ER, McEwen BS. Previous history of chronic stress changes the transcriptional response to glucocorticoid challenge in the dentate gyrus region of the male rat hippocampus. *Endocrinology* (2013) **154**(9):3261–72. doi:10.1210/en.2012-2233
123. Gray JD, Rubin TG, Hunter RG, McEwen BS. Hippocampal gene expression changes underlying stress sensitization and recovery. *Mol Psychiatry* (2013). doi:10.1038/mp.2013.175

124. Crudo A, Petropoulos S, Moisiadis VG, Iqbal M, Kostaki A, Machnes Z, et al. Prenatal synthetic glucocorticoid treatment changes DNA methylation states in male organ systems: multigenerational effects. *Endocrinology* (2012) **153**(7):3269–83. doi:10.1210/en.2011-2160
125. Crudo A, Suderman M, Moisiadis VG, Petropolous S, Kostaki A, Hallett M, et al. Glucocorticoid programming of the fetal male hippocampal epigenome. *Endocrinology* (2013) **154**(3):1168–80. doi:10.1210/en.2012-1980
126. Crudo A, Petropoulos S, Suderman M, Moisiadis VG, Kostaki A, Hallett M, et al. Effects of antenatal synthetic glucocorticoid on glucocorticoid receptor binding, DNA methylation, and genome-wide mRNA levels in the fetal male hippocampus. *Endocrinology* (2013) **154**(11):4170–81. doi:10.1210/en.2013-1484
127. Polman JA, de Kloet ER, Datson NA. Two populations of glucocorticoid receptor-binding sites in the male rat hippocampal genome. *Endocrinology* (2013) **154**(5):1832–44. doi:10.1210/en.2012-2187
128. Brownfoot FC, Gagliardi DI, Bain E, Middleton P, Crowther CA. Different corticosteroids and regimens for accelerating fetal lung maturation for women at risk of preterm birth. *Cochrane Database Syst Rev* (2013) **8**:CD006764. doi:10.1002/14651858.CD006764.pub3
129. Kersbergen KJ, de Vries LS, van Kooij BJ, Išgum I, Rademaker KJ, van Bel F, et al. Hydrocortisone treatment for bronchopulmonary dysplasia and brain volumes in preterm infants. *J Pediatr* (2013) **163**(3):666.e–71.e. doi:10.1016/j.jpeds.2013.04.001
130. Hauser J, Feldon J, Pryce CR. Direct and dam-mediated effects of prenatal dexamethasone on emotionality, cognition and HPA axis in adult Wistar rats. *Horm Behav* (2009) **56**(4):364–75. doi:10.1016/j.yhbeh.2009.07.003
131. Morley-Fletcher S, Rea M, Maccari S, Laviola G. Environmental enrichment during adolescence reverses the effects of prenatal stress on play behaviour and HPA axis reactivity in rats. *Eur J Neurosci* (2003) **18**:3367–74. doi:10.1111/j.1460-9568.2003.03070.x
132. Field T, Diego M, Hernandez-Reif M. Preterm infant massage therapy research: a review. *Infant Behav Dev* (2010) **33**(2):115–24. doi:10.1016/j.infbeh.2009.12.004
133. Middlemiss W, Granger DA, Goldberg WA, Nathans L. Asynchrony of mother-infant hypothalamic-pituitary-adrenal axis activity following extinction of infant crying responses induced during the transition to sleep. *Early Hum Dev* (2012) **88**(4):227–32. doi:10.1016/j.earlhumdev.2011.08.010
134. Lewis AJ, Galbally M, Gannon T, Symeonides C. Early life programming as a target for prevention of child and adolescent mental disorders. *BMC Med* (2014) **12**:33. doi:10.1186/1741-7015-12-33

Conflict of Interest Statement: E. Ronald de Kloet is on the Scientific Advisory Board and owns stock of Corcept Therapeutics, and also served on the Advisory Boards of Dynacorts Therapeutics and Pharmaseed Ltd. The other co-authors report no conflicts of interest.

Received: 25 April 2014; accepted: 11 June 2014; published online: 09 July 2014.
*Citation: de Kloet ER, Claessens SEF and Kentrop J (2014) Context modulates outcome of perinatal glucocorticoid action in the brain. Front. Endocrinol. **5**:100. doi: 10.3389/fendo.2014.00100*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
ENDOCRINOLOGY

ORIGINAL RESEARCH ARTICLE
published: 05 November 2014
doi: 10.3389/fendo.2014.00190

Immediate effects of maternal deprivation on the (re)activity of the HPA-axis differ in CD1 and C57Bl/6J mouse pups

***Nikolaos P. Daskalakis*[1,2,3,4,5]*, Leo Enthoven[1], Edwige Schoonheere[1], Edo Ronald de Kloet[1,2] and Melly S. Oitzl[1,6]**

[1] *Division of Medical Pharmacology, Leiden Academic Center for Drug Research, Leiden University, Leiden, Netherlands*
[2] *Department of Endocrinology and Metabolism, Leiden University Medical Center, Leiden University, Leiden, Netherlands*
[3] *Traumatic Stress Studies Division, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
[4] *Laboratory of Molecular Neuropsychiatry, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA*
[5] *PTSD Research Program, Mental Health Patient Care Center, James J. Peters Veterans Affairs Medical Center, Bronx, NY, USA*
[6] *Center for Neuroscience, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, Netherlands*

Edited by:
Mitsuhiro Kawata, Kyoto Prefectural University of Medicine, Japan

Reviewed by:
Aldo Lucion, Universidade Federal do Rio Grande do Sul, Brazil
Gábor B. Makara, Hungarian Academy of Sciences, Hungary

****Correspondence:***
Nikolaos P. Daskalakis, Laboratory of Molecular Neuropsychiatry and Traumatic Stress Studies Division, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1668, New York, NY 10029-6574, USA
e-mail: nikolaos.daskalakis@mssm.edu

The postnatal development of the mouse is characterized by a period of hypo-responsiveness of the hypothalamic–pituitary–adrenal (HPA) axis to mild stressors. Maternal deprivation (MD) during this period can disrupt the quiescence of the HPA-axis. The present study examined the influence of strain (outbred CD1 vs. inbred C57BL/6J mice) on some central and peripheral components of the HPA-axis in neonatal mice (5-day-old) in the presence of their mother or after 24 h MD (on postnatal day 4) under basal or mild stressful conditions. In the presence of the dam, adrenal corticosterone (CORT) secretion was low in both mouse strains. Compared to CD1 mice, C57BL/6J had lower CORT levels associated with higher ACTH levels and ACTH/CORT ratio (i.e., lower adrenal sensitivity to ACTH), and higher glucocorticoid receptor (GR) mRNA expression in the paraventricular nucleus. Although MD disinhibited the HPA-axis in both strains as reflected by increased basal CORT and ACTH, we found a strain-dependent pattern. MD increased CORT more in C57BL/6J compared to CD1 mice together with a lower ACTH/CORT ratio (i.e., higher adrenal sensitivity to ACTH), while GR mRNA was no longer different in the two strains. However, this increased adrenal sensitivity in maternally deprived C57BL/6J mice was not reflected in their CORT response to a subsequent novelty stressor, possibly due to an MD-induced ceiling effect in their steroidogenic capacity. In conclusion, the immediate outcome of MD depends on the genetic background of the mother–infant dyad, suggesting that maybe also the outcome in later-life cannot be generalized.

Keywords: hypothalamic–pituitary–adrenal axis, corticosterone, ACTH, CRH, GR, neonate, maternal deprivation, genetic

INTRODUCTION

Maternal stimuli play a central role in the postnatal development of the hypothalamic–pituitary–adrenal axis (HPA-axis) in rodents (1, 2) especially during the stress-hyporesponsive period (SHRP). The SHRP lasts from postnatal day (pnd) 1–12 in mice, (pnd 3–14 in rats) and is characterized by low basal levels of corticosterone (CORT) and an inability to elicit a CORT response to mild stress (3, 4). Rodent dams do not leave often their nest for longer than 15–20 min during the SHRP (5). Removal of the mother for prolonged time periods (>3–8 to 24 h) has been shown to activate the HPA-axis, while the axis also becomes responsive to mild stressors, which may modulate ongoing developmental programs (6, 7).

Large individual variations in the long-term biobehavioral outcome of early-life traumatic experiences have been reported in humans (8) and rodents (9, 10). This raised the idea that early-life trauma might shape pre-existing genetic vulnerability to certain stressful conditions in later life (11). Maternal deprivation (MD) is a commonly used animal paradigm to study the consequences of early-life trauma on adult stress–responses and related behaviors (12). The MD paradigm has been applied in various designs ranging from single 24 h deprivations to repeated daily separations in time periods ranging between 3 and 8 h (9, 11).

Most of our knowledge on the effects of MD on HPA-axis and stress-related behaviors is based on research in outbred rodent strains. Although it is known that genetically selected lines of rats display differential sensitivity to the long-term effects of MD (13–15), the aspect of genetic predisposition has been given little attention. In recent experiments, we showed that responsiveness to mild stressors following prolonged maternal absence is strain-dependent (16). We actually observed that while maternally separated pups (i.e., repeatedly for 8 h) habituate toward the maternal absence *per se* by displaying low basal CORT levels (16–18), their CORT response toward a subsequent heterotypic stressor sensitizes in a strain-dependent fashion: deprived Long Evans pups were more re-active to the subsequent stressor than similarly deprived Wistar rats (16).

The inbred C57BL/6J mouse strain is most widely used as genetic background strain for engineering genetic mouse models for human diseases. A few studies compared C57BL/6J mice with common outbred mice strains (e.g., CD1 mice) on stress-related physiology and behavior. C57BL/6J and CD1 mice have differences in their circadian pattern of the stress–response (19). C57BL/6J mice have lower stress responsiveness in a light/dark exploration test for anxiety (20) and display a reduced exploration in a novel environment (21). Furthermore, CD1 mice showed better avoidance learning in a Y-maze task (22). Interestingly, C57BL/6J and CD1 mice seem to display differences on the long-term outcome of maternal separation on the stress–response, cognitive performance, anxiety/depression-like, or schizophrenia-like behaviors (23–33). Generally, the reported effects indicate more often significant effects in C57BL/6J than in CD1 mice.

Studying the immediate effects of MD on the development of the stress system responsiveness might give insights on the salient factors that influence the long-term outcome. This is an approach proven to be successful using a variety of early-life stress paradigms (18, 34). In the current study, we compared C57BL/6J with CD1 mouse pups with regard to the immediate effects of pnd 4 MD on HPA-axis stress reactivity.

MATERIALS AND METHODS

ANIMALS

Offspring of CD1 and C57BL/6J mice were used in this study. All mice were housed under a 12:12 h light/dark cycle (lights on at 07:00 hours) and constant temperature (23 ± 2°C) and humidity (55 ± 5%) conditions. Food (SRM-A; Hope Farms, Woerden, The Netherlands) and water (172 ml HCl/200l H_2O) was provided *ad libitum*. Three females were mated with one male in polycarbonate boxes (820 cm^3) containing sawdust bedding. Pregnant females were transferred to clean polycarbonate cages containing sawdust and two sheets of paper towels for nesting material. Pregnant females were checked for litters daily between 09:00 and 10:00 hours. If litters were found, the day of birth was defined as day 0 for that litter. On the day after parturition, day 1, each litter was culled to eight healthy pups (four males and four females) for the CD1 strain and to six healthy pups (three males and three females) for the C57BL/6J strain and then remained undisturbed until used in the experiment. A total of four CD1 litters and six C57BL/6J litters were used in the study. Animal experiments were approved by the Local Committee for Animal Health, Ethics, and Research of Leiden University and carried out in accordance with European Communities Council Directive 86/609/EEC.

EXPERIMENTAL DESIGN

At postnatal day 4, mothers from nests randomly selected for MD (two CD1 and three C57BL/6J nests) were removed from the home cage. The home cage containing the pups was transferred to an adjacent room with similar light and temperature conditions and placed on a heating pad set at 30–33°C. Neither food nor water was available during MD. At pnd 5, half of the non-deprived (NON-DEP) and half of the deprived (DEP) pups were decapitated immediately to provide a basal sample for measurements in blood and brain. The remaining NON-DEP and DEP pups were placed individually in novel cages containing clean sawdust bedding on heating pads set at 30–33°C and decapitated after 30 min to provide a novelty stress sample.

BLOOD PROCESSING AND HORMONE DETERMINATION

Trunk blood from all decapitated pups was collected in EDTA-coated microcentrifuge tubes; plasma was extracted and stored frozen at −20°C until hormone determination. ACTH was measured by radioimmunoassay (RIA; MP Biomedicals, LLC, NY, USA; sensitivity 10 pg/ml, intra-assay variation 4.1%, inter-assay variation 4.4%) as described before (16). CORT was measured by RIA (MP Biomedicals, LLC, NY, USA; sensitivity 1.25 ng/ml, intra-assay variation, 4.4%, interassay variation 6.5%) as described before (16). We calculated the ratio of ACTH to CORT as an indirect measure of adrenal sensitivity to ACTH (18).

IN SITU HYBRIDIZATION

Frozen brains and pituitaries were sectioned at −20°C in a cryostat microtome at 16 μm in the coronal plane. Sections were thaw-mounted on poly-L-lysine coated slides, air-dried, and kept at −80°C. *In situ* hybridization using 35S-UTP labeled ribonucleotide probes [CRH and glucocorticoid receptor (GR)] was performed as described previously (17, 18). The slides were opposed to Kodak Biomax MR film (Eastman Kodak Co., Rochester, NY, USA) and developed. Autoradiographs were digitized, and relative expression of CRH and GR mRNA was determined by computer-assisted optical densitometry (analysis 3.1, Soft Imaging System GmbH, Münster, Germany). The mean of four to six measurements was calculated for each mouse.

STATISTICS

Data were analyzed by analysis of variance (ANOVA) using strain (CD1 or C57BL/6J), treatment (NON-DEP or DEP), and time (basal or novelty) as fixed factors. When appropriate, *post hoc* Tukey test was performed. The initial analysis included sex as a factor; once it was determined that sex was not a significant factor, the data were collapsed across this variable. The level of significance was set at $P < 0.05$. Data are presented as mean ± SEM.

RESULTS

WEIGHT

Two-way ANOVA revealed main effects of strain ($F_{1,64} = 141.34$; $p < 0.001$) and treatment ($F_{1,64} = 141.34$; $p < 0.001$). C57BL/6J were lighter than CD1 mice ($p < 0.001$) in both treatment conditions (**Table 1**). DEP pups were lighter than NON-DEP pups ($p < 0.001$ for both strains).

Table 1 | Body weight (grams) of non-deprived (NON-DEP) and 24 h deprived (DEP) pups in CD1 and C57BL/6J mice at postnatal day 5.

Strain	NON-DEP			DEP			% Change
	Mean	*N*	SEM	Mean	*N*	SEM	
CD1	3.15	16	0.06	2.51[#]	16	0.08	↓20
C57BL/6J	2.51[$]	16	0.10	1.78[#$]	17	0.11	↓29

Data represent mean ± SEM. # vs. NON-DEP, $ vs. CD1. Significance level was set at p = 0.05.

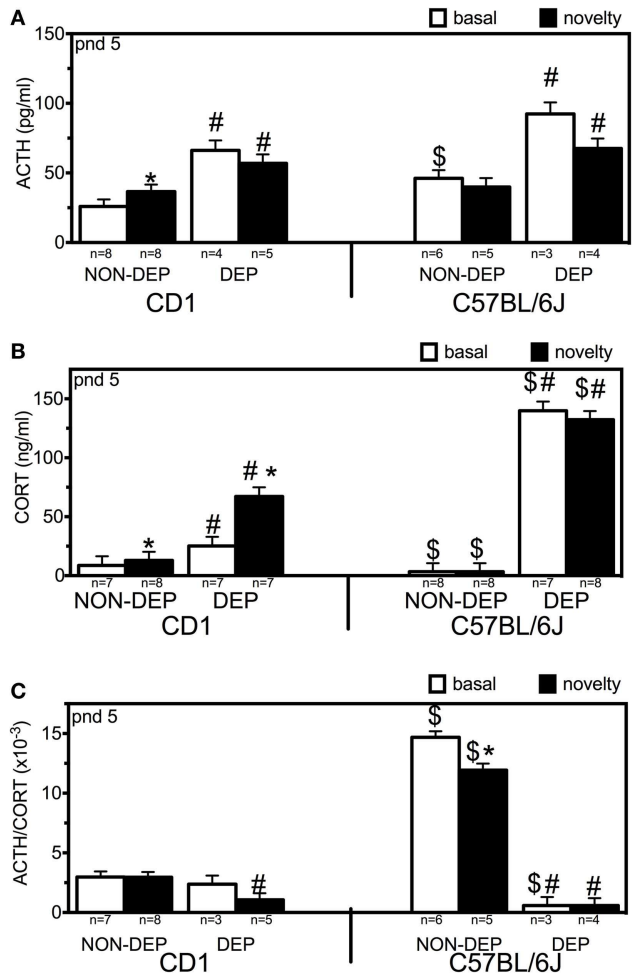


FIGURE 1 | ACTH [(A); picogram/milliliter], corticosterone [(B); CORT in nanogram/milliliter] blood plasma levels, and their ratio [(C); ACTH/CORT] of non-deprived (NON-DEP) and 24 h deprived (DEP) pups measured at basal conditions (basal; white bars) or after 30 min of novelty exposure (novelty; black bars) at postnatal day (pnd) 5. Data represent mean ± SEM. * vs. basal, # vs. NON-DEP, $ vs. CD1. Significance level was set at $p = 0.05$.

ACTH

Three-way ANOVA revealed main effects of strain ($F_{1,42} = 10.79$; $p < 0.001$), treatment ($F_{1,42} = 53.65$; $p < 0.001$), and interaction of treatment and time ($F_{1,42} = 4.41$; $p = 0.043$) (**Figure 1A**). Strain differences were found at NON-DEP basal levels ($p = 0.005$). Novelty exposure increased ACTH levels in CD1 (↑41%, $p = 0.025$) but not in C57BL/6J mice. After 24 h MD, ACTH basal levels were elevated (↑156%, $p = 0.001$ for CD1; ↑100%, $p = 0.006$ for C57BL/6J). Subsequent novelty exposure did not produce further increase in ACTH in either CD1 or C57BL/6J mice, while in both strains ACTH levels were higher than the respective NON-DEP levels ($p = 0.010$ for CD1, $p = 0.040$ for C57BL/6J).

CORTICOSTERONE

Three-way ANOVA revealed main effects of strain ($F_{1,59} = 59.86$; $p < 0.001$), treatment ($F_{1,59} = 248.76$; $p < 0.001$), interaction strain and treatment ($F_{1,59} = 83.52$; $p < 0.001$), interaction strain and time ($F_{1,59} = 6.38$; $p = 0.015$), and interaction of strain, treatment, and time ($F_{1,59} = 4.50$; $p = 0.039$) (**Figure 1B**). Novelty exposure increased CORT levels in CD1 (↑50%, $p = 0.002$) but not in C57BL/6J mice. After 24 h MD, CORT basal levels were elevated in both strains (↑191%, $p < 0.001$ for CD1; ↑4099%, $p < 0.001$ for C57BL/6J). Subsequent novelty exposure further increased CORT only in CD1 mice (additional ↑167%, $p < 0.001$), while in both strains CORT levels were higher than the respective NON-DEP levels ($p < 0.001$). Strain differences were found at all four conditions: C57BL/6J CORT levels being lower than in CD1 at NON-DEP conditions ($p < 0.001$ for both basal and novelty), and higher at DEP conditions (for basal: $p < 0.001$, for novelty: $p = 0.003$).

ACTH/CORT RATIO

Three-way ANOVA revealed main effects of strain ($F_{1,40} = 126.05$; $p < 0.001$), treatment ($F_{1,40} = 290.46$; $p < 0.001$), time ($F_{1,40} = 6.24$; $p = 0.018$), interaction strain and treatment ($F_{1,40} = 196.03$; $p < 0.001$), and interaction of strain, treatment, and time ($F_{1,40} = 6.12$; $p = 0.019$) (**Figure 1C**). At NON-DEP basal conditions, C57BL/6J displayed much higher ACTH/CORT than CD1 mice (↑393%, $p < 0.001$). Novelty exposure decreased the ratio in C57BL/6J (↓19%, $p = 0.048$) but not in CD1 mice. After 24 h MD, ACTH/CORT ratio decreased in C57BL/6J (↓96%, $p < 0.001$) but not in CD1 mice in such an extent that the C57BL/6J displayed even less ratio than CD1 mice ($p = 0.029$). For both strains, ACTH/CORT ratios after subsequent novelty exposure were lower than the respective NON-DEP levels (CD1: $p = 0.004$, C57BL/6J: $p < 0.001$).

CRH mRNA EXPRESSION IN THE PVN

Two-way ANOVA revealed main effects of treatment ($F_{1,30} = 5.41$; $p = 0.028$) (**Figure 2A**). Twenty-four hours of MD downregulated CRH mRNA ($p = 0.036$) in CD1 mice but not in C57BL/6J.

GR mRNA EXPRESSION IN THE PVN

Two-way ANOVA revealed main effects strain ($F_{1,27} = 10.77$; $p = 0.003$), treatment ($F_{1,27} = 17.97$; $p < 0.001$), and interaction of strain and treatment ($F_{1,27} = 5.02$; $p = 0.035$) (**Figure 2B**). At basal conditions, C57BL/6J displayed higher levels of GR mRNA than CD1 mice ($p = 0.002$). Twenty-four hours of MD downregulated GR mRNA in C57BL/6J ($p < 0.001$).

GR mRNA EXPRESSION IN PITUITARY (DATA NOT SHOWN)

There were no main effects of strain or treatment on GR mRNA in pituitary.

DISCUSSION

Our data show that the two mouse strains, CD1 and C57BL/6J mice, differ in the neonatal HPA-axis activity at basal conditions as well as after a 24 h MD period.

Regarding basal HPA-axis activity, C57BL/6J displayed higher ACTH and lower CORT than CD1 mice, indicating lower basal adrenal sensitivity to ACTH as reflected by a higher ACTH/CORT ratio. Additionally, basal GR mRNA expression in the PVN is higher than in CD1 mice. We propose that this increased GR

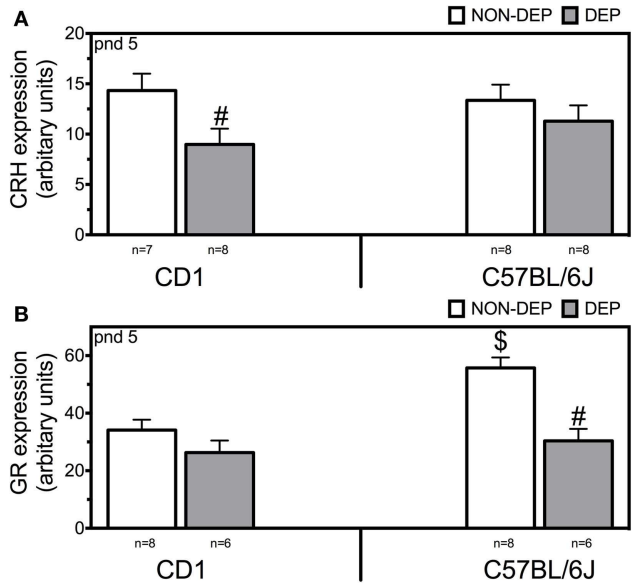


FIGURE 2 | CRH (A) and GR (B) mRNA expression in the paraventricular nucleus of the hypothalamus measured in non-deprived (NON-DEP) and deprived (DEP) mice at postnatal day (pnd) 5. Data represent mean ± SEM. # vs. NON-DEP, $ vs. CD1. Significance level was set at $p = 0.05$.

mRNA expression might be a result of the lower CORT production. The higher GR mRNA is not likely to be an indication of stronger negative feedback capacity because there was no strain difference in basal CRH mRNA expression in the PVN or GR mRNA expression in the pituitary. Exposure of NON-DEP pups to novelty resulted in a subtle statistically significant rise in both ACTH and CORT in CD1 mice only. This finding underlines strain-dependent effects and confirms that the SHRP is a period of stress-hypo-responsiveness (3).

Maternal deprivation elicited in both strains the expected increase of ACTH (35) and CORT (35, 36). ACTH rose at a similar extent in both strains. CORT levels were dramatically increased in C57BL/6J compared to a more moderate increase in CD1 pups. Previous findings in rats showed that during the time-course of 24 h maternal separation, adrenal sensitivity to stress increased (37) through increases in melanocortin type 2 receptors for ACTH (16) or other mechanisms (38) in a strain-dependent manner (16). The decrease in ACTH/CORT ratio in C57BL/6J compared to CD1 pups (from higher ACTH/lower CORT to comparable ACTH/higher CORT) indicates that, C57BL/6J after MD are no longer less sensitive to ACTH than CD1 mice at the adrenal level, but actually they display increased adrenal sensitivity compared to CD1 mice. In that, CORT secretion may be influenced also by factors other than ACTH, direct measures of neonatal adrenal sensitivity to ACTH need to be undertaken in future experiments. Only CD1 mice displayed a CORT response to novelty stress after MD. The absence of an additional novelty-induced CORT increase in C57BL/6J might be related to a ceiling effect in their steroidogenic capacity.

It is interesting that C57BL/6J do not show the expected reduction in CRH mRNA expression following MD (35, 39) that was seen in the CD1 pups. This might be associated with the reduction in GR mRNA expression in the PVN and, thus, with potentially less efficient negative feedback actions of CORT at the cells that produce and release CRH. This might be an indication that, in C57BL/6J, MD causes a greater disruption of SHRP, which is characterized by enhanced negative feedback (40).

Another contributing factor to the strain differences here might be the transcortin levels and ultimately the free (biologically active) CORT, which is the HPA-axis feedback signal. RIA does not distinguish between free and transcortin-bound cortisol. Transcortin levels are low during SHRP (41) and strain differences are possible. Peripheral and central metabolic factors (e.g., blood glucose, arcuate nucleus NPY) can also mediate the activation of the HPA-axis induced by maternal separation (42, 43). Indeed, in terms of body weight changes, MD caused the greatest metabolic challenge in C57BL/6J pups, which also displayed the highest activation of the HPA-axis expressed by CORT. Other factors not related to feeding might be also involved. Actually feeding is more related with the adrenal CORT secretion and tactile stimulation more related to pituitary ACTH release (1).

We have to acknowledge some limitations of the study. Preweaning pups from small litters (<5 pups) have higher body weight and higher basal CORT levels than pups from large litters (>15 pups) (44). The C57BL/6J litters are naturally smaller in size than the CD1 litters. This has created an unavoidable, without cross-fostering, confound that might have interfered with the strain differences reported. We opted for an equal sex-ratio (1:1) that removed the sex-ratio bias. Nevertheless, the litter-size difference between the strains was small (two pups) and did not seem to have a noticeable effect; in this experiment, the pups of the C57BL/6J strain (with the smaller litter size of six pups) displayed lower body weight and lower basal CORT than the pups of the CD1 strain (with the larger litter size of eight pups). Future studies could illuminate the role of litter-size, but also of the basal mother–pup interactions and other related epigenetically mediated mechanisms (45) on the neonatal basal and post-MD HPA-axis activity.

Specific genetic contributions could be clarified in the future with the use of transgenic mice, but the strain differences in immediate effects of MD observed, here, in mice and, previously, in rats (16) emphasize the importance of genetic background on the effects of early maternal environment on the development of the stress system. Late-life consequences may also depend on genetic background, but this remains to be tested.

ACKNOWLEDGMENTS

This work was supported by the Top Institute of Pharmaceutical Sciences T5#209 (Edo Ronald de Kloet, Nikolaos P. Daskalakis), NWO-NDRF/STIGON (Leo Enthoven), Royal Netherlands Academy of Arts and Sciences (Edo Ronald de Kloet), NWO-Aspasia (Melly S. Oitzl), NWO-IRTG 95-420 (Melly S. Oitzl).

REFERENCES

1. 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):10171–9.

2. Rosenfeld P, Gutierrez YA, Martin AM, Mallett HA, Alleva E, Levine S. Maternal regulation of the adrenocortical response in preweanling rats. *Physiol Behav* (1991) **50**(4):661–71. doi:10.1016/0031-9384(91)90001-5
3. 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. doi:10.1016/0165-0173(86)90010-X
4. Schmidt MV, Enthoven L, van der Mark M, Levine S, de Kloet ER, Oitzl MS. The postnatal development of the hypothalamic-pituitary-adrenal axis in the mouse. *Int J Dev Neurosci* (2003) **21**(3):125–32. doi:10.1016/S0736-5748(03)00030-3
5. Ader R, Grota LJ. Rhythmicity in the maternal behaviour of *Rattus norvegicus*. *Anim Behav* (1970) **18**(1):144–50. doi:10.1016/0003-3472(70)90083-7
6. Schmidt MV, Oitzl MS, Levine S, de Kloet ER. The HPA system during the postnatal development of CD1 mice and the effects of maternal deprivation. *Brain Res Dev Brain Res* (2002) **139**(1):39–49. doi:10.1016/S0165-3806(02)00519-9
7. Stanton ME, Gutierrez YR, Levine S. Maternal deprivation potentiates pituitary-adrenal stress responses in infant rats. *Behav Neurosci* (1988) **102**(5):692–700. doi:10.1037/0735-7044.102.5.692
8. Nederhof E, Schmidt MV. Mismatch or cumulative stress: toward an integrated hypothesis of programming effects. *Physiol Behav* (2012) **106**(5):691–700. doi:10.1016/j.physbeh.2011.12.008
9. Lehmann J, Feldon J. Long-term biobehavioral effects of maternal separation in the rat: consistent or confusing? *Rev Neurosci* (2000) **11**(4):383–408. doi:10.1515/REVNEURO.2000.11.4.383
10. Claessens SE, Daskalakis NP, van der Veen R, Oitzl MS, de Kloet ER, Champagne DL. Development of individual differences in stress responsiveness: an overview of factors mediating the outcome of early life experiences. *Psychopharmacology (Berl)* (2011) **214**(1):141–54. doi:10.1007/s00213-010-2118-y
11. Daskalakis NP, Bagot RC, Parker KJ, Vinkers CH, de Kloet ER. The three-hit concept of vulnerability and resilience: toward understanding adaptation to early-life adversity outcome. *Psychoneuroendocrinology* (2013) **38**(9):1858–73. doi:10.1016/j.psyneuen.2013.06.008
12. Kosten TA, Kim JJ, Lee HJ. Early life manipulations alter learning and memory in rats. *Neurosci Biobehav Rev* (2012) **36**(9):1985–2006. doi:10.1016/j.neubiorev.2012.07.003
13. Carboni L, Becchi S, Piubelli C, Mallei A, Giambelli R, Razzoli M, et al. Early-life stress and antidepressants modulate peripheral biomarkers in a gene-environment rat model of depression. *Prog Neuropsychopharmacol Biol Psychiatry* (2010) **34**(6):1037–48. doi:10.1016/j.pnpbp.2010.05.019
14. Neumann ID, Wigger A, Kromer S, Frank E, Landgraf R, Bosch OJ. Differential effects of periodic maternal separation on adult stress coping in a rat model of extremes in trait anxiety. *Neuroscience* (2005) **132**(3):867–77. doi:10.1016/j.neuroscience.2005.01.034
15. Ellenbroek BA, Sluyter F, Cools AR. The role of genetic and early environmental factors in determining apomorphine susceptibility. *Psychopharmacology (Berl)* (2000) **148**(2):124–31. doi:10.1007/s002130050033
16. Daskalakis NP, Claessens SE, Laboyrie JJ, Enthoven L, Oitzl MS, Champagne DL, et al. The newborn rat's stress system readily habituates to repeated and prolonged maternal separation, while continuing to respond to stressors in context dependent fashion. *Horm Behav* (2011) **60**(2):165–76. doi:10.1016/j.yhbeh.2011.04.003
17. Enthoven L, Oitzl MS, Koning N, van der Mark M, de Kloet ER. Hypothalamic-pituitary-adrenal axis activity of newborn mice rapidly desensitizes to repeated maternal absence but becomes highly responsive to novelty. *Endocrinology* (2008) **149**(12):6366–77. doi:10.1210/en.2008-0238
18. Daskalakis NP, Diamantopoulou A, Claessens SE, Remmers E, Tjalve M, Oitzl MS, et al. Early experience of a novel-environment in isolation primes a fearful phenotype characterized by persistent amygdala activation. *Psychoneuroendocrinology* (2014) **39**:39–57. doi:10.1016/j.psyneuen.2013.09.021
19. Hotchkiss AK, Pyter LM, Neigh GN, Nelson RJ. Nyctohemeral differences in response to restraint stress in CD-1 and C57BL/6 mice. *Physiol Behav* (2004) **80**(4):441–7. doi:10.1016/j.physbeh.2003.09.010
20. Bouwknecht JA, Paylor R. Behavioral and physiological mouse assays for anxiety: a survey in nine mouse strains. *Behav Brain Res* (2002) **136**(2):489–501. doi:10.1016/S0166-4328(02)00200-0
21. Dellu F, Contarino A, Simon H, Koob GF, Gold LH. Genetic differences in response to novelty and spatial memory using a two-trial recognition task in mice. *Neurobiol Learn Mem* (2000) **73**(1):31–48. doi:10.1006/nlme.1999.3919
22. Heyser CJ, McDonald JS, Polis IY, Gold LH. Strain distribution of mice in discriminated Y-maze avoidance learning: genetic and procedural differences. *Behav Neurosci* (1999) **113**(1):91–102. doi:10.1037/0735-7044.113.1.91
23. Millstein RA, Ralph RJ, Yang RJ, Holmes A. Effects of repeated maternal separation on prepulse inhibition of startle across inbred mouse strains. *Genes Brain Behav* (2006) **5**(4):346–54. doi:10.1111/j.1601-183X.2005.00172.x
24. Millstein RA, Holmes A. Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. *Neurosci Biobehav Rev* (2007) **31**(1):3–17. doi:10.1016/j.neubiorev.2006.05.003
25. Parfitt DB, Levin JK, Saltstein KP, Klayman AS, Greer LM, Helmreich DL. Differential early rearing environments can accentuate or attenuate the responses to stress in male C57BL/6 mice. *Brain Res* (2004) **1016**(1):111–8. doi:10.1016/j.brainres.2004.04.077
26. Parfitt DB, Walton JR, Corriveau EA, Helmreich DL. Early life stress effects on adult stress-induced corticosterone secretion and anxiety-like behavior in the C57BL/6 mouse are not as robust as initially thought. *Horm Behav* (2007) **52**(4):417–26. doi:10.1016/j.yhbeh.2007.06.002
27. Romeo RD, Mueller A, Sisti HM, Ogawa S, McEwen BS, Brake WG. Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. *Horm Behav* (2003) **43**(5):561–7. doi:10.1016/S0018-506X(03)00063-1
28. Martini M, Valverde O. A single episode of maternal deprivation impairs the motivation for cocaine in adolescent mice. *Psychopharmacology (Berl)* (2012) **219**(1):149–58. doi:10.1007/s00213-011-2385-2
29. MacQueen GM, Ramakrishnan K, Ratnasingan R, Chen B, Young LT. Desipramine treatment reduces the long-term behavioural and neurochemical sequelae of early-life maternal separation. *Int J Neuropsychopharmacol* (2003) **6**(4):391–6. doi:10.1017/S1461145703003729
30. Enthoven L, de Kloet ER, Oitzl MS. Effects of maternal deprivation of CD1 mice on performance in the water maze and swim stress. *Behav Brain Res* (2008) **187**(1):195–9. doi:10.1016/j.bbr.2007.08.037
31. Fabricius K, Wortwein G, Pakkenberg B. The impact of maternal separation on adult mouse behaviour and on the total neuron number in the mouse hippocampus. *Brain Struct Funct* (2008) **212**(5):403–16. doi:10.1007/s00429-007-0169-6
32. van Heerden JH, Russell V, Korff A, Stein DJ, Illing N. Evaluating the behavioural consequences of early maternal separation in adult C57BL/6 mice; the importance of time. *Behav Brain Res* (2010) **207**(2):332–42. doi:10.1016/j.bbr.2009.10.015
33. Boasen JF, McPherson RJ, Hays SL, Juul SE, Gleason CA. Neonatal stress or morphine treatment alters adult mouse conditioned place preference. *Neonatology* (2009) **95**(3):230–9. doi:10.1159/000165379
34. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. *Nat Neurosci* (2004) **7**(8):847–54. doi:10.1038/nn1276
35. Schmidt M, Enthoven L, van Woezik JH, Levine S, de Kloet ER, Oitzl MS. The dynamics of the hypothalamic-pituitary-adrenal axis during maternal deprivation. *J Neuroendocrinol* (2004) **16**(1):52–7. doi:10.1111/j.1365-2826.2004.01123.x
36. Levine S, Huchton DM, Wiener SG, Rosenfeld P. Time course of the effect of maternal deprivation on the hypothalamic-pituitary-adrenal axis in the infant rat. *Dev Psychobiol* (1991) **24**(8):547–58. doi:10.1002/dev.420240803
37. Okimoto DK, Blaus A, Schmidt MV, Gordon MK, Dent GW, Levine S. Differential expression of c-fos and tyrosine hydroxylase mRNA in the adrenal gland of the infant rat: evidence for an adrenal hyporesponsive period. *Endocrinology* (2002) **143**(5):1717–25. doi:10.1210/endo.143.5.8819
38. Walker CD. Chemical sympathectomy and maternal separation affect neonatal stress responses and adrenal sensitivity to ACTH. *Am J Physiol* (1995) **268**(5 Pt 2):R1281–8.
39. Enthoven L, Schmidt MV, Cheung YH, van der Mark MH, de Kloet ER, Oitzl MS. Ontogeny of the HPA axis of the CD1 mouse following 24 h maternal deprivation at pnd 3. *Int J Dev Neurosci* (2010) **28**(2):217–24. doi:10.1016/j.ijdevneu.2009.10.006
40. Schmidt MV, Levine S, Oitzl MS, van der Mark M, Muller MB, Holsboer F, et al. Glucocorticoid receptor blockade disinhibits pituitary-adrenal activity during the stress hyporesponsive period of the mouse. *Endocrinology* (2005) **146**(3):1458–64. doi:10.1210/en.2004-1042

41. Viau V, Sharma S, Meaney MJ. Changes in plasma adrenocorticotropin, corticosterone, corticosteroid-binding globulin, and hippocampal glucocorticoid receptor occupancy/translocation in rat pups in response to stress. *J Neuroendocrinol* (1996) **8**(1):1–8. doi:10.1111/j.1365-2826.1996.tb00680.x
42. Schmidt MV, Liebl C, Sterlemann V, Ganea K, Hartmann J, Harbich D, et al. Neuropeptide Y mediates the initial hypothalamic-pituitary-adrenal response to maternal separation in the neonatal mouse. *J Endocrinol* (2008) **197**(2):421–7. doi:10.1677/JOE-07-0634
43. 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):865–74. doi:10.1111/j.1365-2826.2006.01482.x
44. Rodel HG, Meyer S, Prager G, Stefanski V, Hudson R. Litter size is negatively correlated with corticosterone levels in weanling and juvenile laboratory rats. *Physiol Behav* (2010) **99**(5):644–50. doi:10.1016/j.physbeh.2010.01.032
45. Caldji C, Hellstrom IC, Zhang TY, Diorio J, Meaney MJ. Environmental regulation of the neural epigenome. *FEBS Lett* (2011) **585**(13):2049–58. doi:10.1016/j.febslet.2011.03.032

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 28 May 2014; accepted: 18 October 2014; published online: 05 November 2014.
*Citation: Daskalakis NP, Enthoven L, Schoonheere E, de Kloet ER and Oitzl MS (2014) Immediate effects of maternal deprivation on the (re)activity of the HPA-axis differ in CD1 and C57Bl/6J mouse pups. Front. Endocrinol. **5**:190. doi: 10.3389/fendo.2014.00190*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 20 February 2014
doi: 10.3389/fendo.2014.00013

Age- and sex-dependent effects of early life stress on hippocampal neurogenesis

***Manila Loi*[1]*, *Sylwia Koricka*[1], *Paul J. Lucassen*[2] *and Marian Joëls*[1]**

[1] *Department of Translational Neuroscience, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, Netherlands*
[2] *Swammerdam Institute for Life Sciences – Center for Neuroscience, University of Amsterdam, Amsterdam, Netherlands*

Edited by:
Nikolaos P. Daskalakis, Icahn School of Medicine at Mount Sinai, USA

Reviewed by:
Thomas Dickmeis, Karlsruhe Institute of Technology, Germany
Gábor B. Makara, Hungarian Academy of Sciences, Hungary

***Correspondence:**
Manila Loi, Department of Translational Neuroscience, Brain Center Rudolf Magnus, University Medical Center Utrecht, Universiteitsweg 100, 3584 CG Utrecht, Netherlands
e-mail: m.loi@umcutrecht.nl

Early life stress is a well-documented risk factor for the development of psychopathology in genetically predisposed individuals. As it is hard to study how early life stress impacts human brain structure and function, various animal models have been developed to address this issue. The models discussed here reveal that perinatal stress in rodents exerts lasting effects on the stress system as well as on the structure and function of the brain. One of the structural parameters strongly affected by perinatal stress is adult hippocampal neurogenesis. Based on compiled literature data, we report that postnatal stress slightly enhances neurogenesis until the onset of puberty in male rats; when animals reach adulthood, neurogenesis is reduced as a consequence of perinatal stress. By contrast, female rats show a prominent reduction in neurogenesis prior to the onset of puberty, but this effect subsides when animals reach young adulthood. We further present preliminary data that transient treatment with a glucocorticoid receptor antagonist can normalize cell proliferation in maternally deprived female rats, while the compound had no effect in non-deprived rats. Taken together, the data show that neurogenesis is affected by early life stress in an age- and sex-dependent manner and that normalization may be possible during critical stages of brain development.

Keywords: maternal deprivation, maternal separation, stress, rat, dentate gyrus, adult neurogenesis, proliferation, hippocampus

EARLY LIFE STRESS AND BRAIN DEVELOPMENT

Early life represents a critical phase in brain development as many regions are not fully formed at birth or undergo extended postnatal maturation. The dentate gyrus (DG), part of the hippocampal formation, is an extreme case where the majority of neurons are generated after birth (1). The continued formation of new neurons after birth, known as adult neurogenesis, is restricted to a limited number of brain areas: in addition to the DG, neurogenesis occurs in the subventricular zone (SVZ) and in the olfactory bulb (2). Even in other parts of the brain, growth is not completed at birth. For instance, the prefrontal cortex (PFC) continues to develop well into adulthood (3). Cortical thickness in humans reaches a maximum around age 35 (4). In addition to the progressive growth until adulthood, new connections continue to be formed, too. The intricate formation and pruning of essential contacts eventually lead to an effective connectome and functional network (5).

It is therefore not surprising that potential or actual perturbations in the individual's environment and "homeostasis" – subjectively experienced as "stress" – particularly when these take place during the critical phase of early development, can have important lasting consequences for brain structure and function later in life (**Figure 1**). In interaction with the genetic profile, early environmental influences "shape" brain maturation as well as the way in which an individual deals with environmental challenges throughout the rest of life. In humans, brain structure and function as well as the ability to cope with stress together determine the vulnerability to psychopathology. Retrospective case–control studies for various psychiatric illnesses, including post-traumatic stress disorder (PTSD) (6), depression (7), schizophrenia (8), and also borderline syndrome (9), have consistently shown that early life adversity is a significant risk factor. The risk increases when early life adversity is severe, prolonged, repeated, and/or characterized by a lack of control over the situation (10). Prospective studies, though more rare, confirm this view [see e.g., Ref. (11–13)].

The sequential steps through which early life adversity changes brain structure and function in a lasting manner, and hence the later risk for psychopathology, is hard to investigate in human subjects, given the long duration of brain maturation, the restrictions in obtaining detailed information about signal transduction in the brain and the lack of control over both genetic and environmental factors. Therefore, research has resorted to animal models, which have fewer of these drawbacks.

In rodents, many models for early life adversity have been developed (14, 15). Some intervene with the prenatal environment, e.g., by stressing the pregnant female (16–19) or by exposing her to compounds acting on stress hormone receptors, e.g., dexamethasone (20). The majority of models, though, focus on the postnatal environment. Since the care provided by the dam represents a strong environmental influence during the first postnatal weeks, many models have specifically concentrated on (disturbed) mother–pup interactions. One model, developed largely by Michael Meaney and coworkers, makes use of natural variations in maternal care provided by the dams (21). Their licking and grooming behavior shows a classic normal distribution,

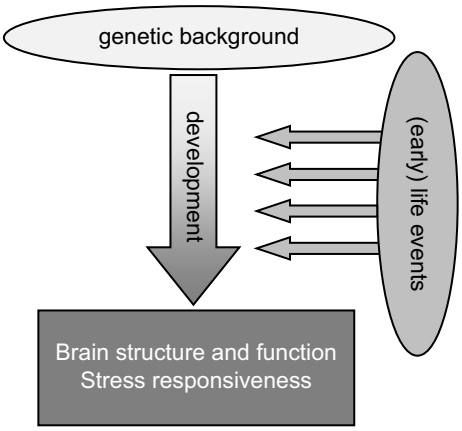


FIGURE 1 | General scheme highlighting that life events, especially when experienced during the early developmental stage, may strongly impact the development of the brain, especially in genetically susceptible individuals. These gene–environment interactions during development will strongly contribute to the overall brain structure and function as well as stress responsiveness in adulthood.

with the majority of the mothers providing moderate amounts of care (22). However, some mothers show extremely high or low amounts of licking–grooming behavior (>1 standard deviation above or below the mean respectively). Their offspring can be examined in adulthood and even into the next generation to study consequences of maternal care (22). Notably, through cross-fostering, the influence of maternal care can be dissociated from the contributions of the genetic background (23).

Many other models actively intervene with the mother–pup interaction, e.g., by limiting the bedding and nesting material in the cage, which induces fragmented care in the dam (24). This, in turn, has lasting consequences for brain development and behavior in the offspring. Separation of the pups from the mother has also been applied in various models. This can range from brief, daily separations to more extreme conditions where the dam and her offspring are separated for up to 24 h. Brief separations, e.g., 15 min handling of the pups during the first postnatal weeks, actually results in an overall enhancement of maternal care, because the mother bestows more care on her pups upon their return to the cage; this has been used as a model for environmental enrichment (25). More prolonged separations, e.g., for 3 h daily between postnatal days (PND) 2 and 14 or deprivation of the mother for 24 h at PND 3 or 9, can be considered models for impoverished and poor care or even neglect (26).

Studies using these models have shown that many aspects of brain structure and function are strongly affected by early life adversity, some of which can be normalized by environmental enrichment (27, 28). One set of data pertains to the development of the stress response itself. Stress rapidly activates the autonomic nervous system, eventually causing the release of (nor)adrenaline from the adrenal medulla. Slightly later, the hypothalamic–pituitary–adrenal (HPA) axis is activated, which leads to release of corticosteroid hormones (corticosterone in rodents and cortisol in humans) from the adrenal cortex into the circulation; this response is terminated 1–2 h later by negative feedback actions of corticosteroids at the level of the pituitary gland, the hypothalamus, and extra-hypothalamic regions (29, 30). Thus, after stress, the brain is exposed to successive waves of noradrenaline and (slightly later) corticosteroid hormones, which have their own and combined roles in mediating effects of stress on the brain, that normalize some hours later (31). In the brain, corticosteroids bind to discretely localized intracellular receptors, most notably the glucocorticoid receptor (GR) that is enriched in hippocampal CA1 and dentate granule cells (29). Corticosteroids also bind to another receptor, the mineralocorticoid receptor, but the affinity for this receptor is very high, so that it is substantially bound to corticosteroids already under conditions of rest (29, 32). Early life adversity was found to reduce the number of hippocampal GRs and impair the negative feedback, causing prolonged exposure of the brain to corticosteroids in the aftermath of stress (33).

In addition to effects on stress responsiveness, also brain structure and function are affected by the early life environment (26). Many studies have shown that, e.g., the complexity of dendritic trees, the number of synaptic contacts and growth factor levels depend on early life history, although the direction of these effects can be region dependent (34). Similarly, the extent of neurogenesis in adolescence and adulthood is affected by circumstances experienced earlier in life (7). In this paper, we will highlight the effects of the early life environment on hippocampal neurogenesis (see below), focusing on stress experienced just prior to, or during, the first 2 postnatal weeks.

Not only structural plasticity but also functional plasticity has been the subject of study. For instance, hippocampal long-term potentiation, i.e., the prolonged strengthening of synaptic contacts, which is thought to underlie memory formation (35), is generally impaired in adult rats that experienced fragmented or low levels of maternal care, or were separated from the mother during the first postnatal weeks (36, 37). These structural and functional changes contribute to behavioral changes. For instance, contextual hippocampus-dependent memory is impaired in adult rats that experienced reduced amounts of maternal care, be it due to natural variations (27, 37, 38) or imposed by separation (39, 40). However, other cognitive domains – e.g., decision making or reward processes that depend on an optimal function of the PFC – are also disturbed in adult rodents with a history of early life adversity (41, 42).

The overall adaptive value of these long-term changes in stress responsiveness, brain structure and function after early life adversity can be best appreciated when studying individuals under various circumstances later in life. For instance, long-term potentiation and hippocampus-dependent learning are impaired under non-stressful experimental conditions in adult offspring from low licking–grooming mothers, or in animals with a history of 24 h of MD at PND 3 (37, 38, 40, 43). In contrast, when these animals are tested under conditions of elevated corticosterone levels, long-term potentiation and hippocampus-dependent learning are actually improved (37, 38, 40). This suggests that the early life environment may affect brain development in such a way that the network can optimally perform under comparable, i.e., matching, conditions later in life. Inadequate responses may arise when there is a mismatch between early life, and the predictions, or "settings"

based on that environment on the one hand, and the actual, later life conditions the individual experiences at an adult age on the other hand (44–46).

NEUROGENESIS

Adult neurogenesis refers to the formation of new, functional neurons that originate from stem cells present in the adult brain. This form of structural plasticity occurs in at least two brain regions; the SVZ of the lateral ventricles, from where cells migrate through the rostro-migratory stream toward the olfactory bulb, and in the subgranular zone (SGZ) of the hippocampal DG. Adult neurogenesis is strongly affected by the early life environment (7, 47, 48). Whereas in the SVZ, the newborn cells participate in olfactory learning, and newborn cells in the DG have been implicated in specific aspects of spatial memory formation and cognition such as pattern separation (49).

During the dynamic process of neurogenesis, stem cells go through several, distinct stages of development (50). Following an initial phase of proliferation during which the initial stem cell pool mainly undergoes expansion, a selection process occurs after approximately 1 week during which around 50% of the newly generated cells die through apoptosis. The surviving cells use radial glia cells as a scaffold to migrate into the granule cell layer, where they eventually differentiate into a mature neuronal phenotype. The proliferation phase is often studied using immunocytochemical markers like Ki-67 or proliferating cell nuclear antigen, while the differentiation phase is usually investigated with doublecortin (DCX), a microtubule-associated protein expressed in young migratory neurons (51). The spatio-temporal expression pattern of DCX largely coincides with the process of adult neurogenesis in the rat hippocampus. Cell survival and cell fate can be studied several weeks after (intra-peritoneal) pulse labeling with bromo-deoxy-uridine (BrdU), a compound that is incorporated into the DNA of dividing cells. The fate and progeny of BrdU-incorporating cells can then later be monitored using double-immunofluorescent labeling with markers for mature neuronal or glial cells. With viral vectors, which label only dividing cells, it has been shown that most adult-born cells, 3–4 weeks after their birth, express adult neuronal markers and are functionally incorporated within the existing DG network (52).

The process of neurogenesis is regulated by several environmental factors including enriched environmental housing or physical exercise, both stimulating the survival of the newborn neurons (53). By contrast, aging and exposure to acute or chronic stress strongly suppress one or more phases of the neurogenic process (54).

Elevated stress hormone levels or an activated HPA-axis is commonly observed in depressed patients. Recent studies have further suggested that also impairments in structural plasticity, including neurogenesis, may be involved in the pathophysiology of depression and in the hippocampal volume reduction in this disorder (55). This "neurogenic theory" of depression postulates that a suppressed rate of cyto- or neurogenesis contributes to the (vulnerability for) depression (56–58), and is supported by the findings that: (1) stress inhibits neurogenesis in animals and is a risk factor for depression; (2) depressed patients often display hippocampal volume reductions parallel to cognitive deficits and HPA activation; (3) most antidepressant drugs do not exert their therapeutic effect until after 3–4 weeks of administration, a time-to-effect that parallels the maturation period of adult newborn neurons; (4) many antidepressants increase or normalize reductions in neurogenesis, particularly in young animals; and (5) disruption of neurogenesis blocks the behavioral response to antidepressant drugs (59, 60). However, this theory is not always supported and still under debate (58, 61, 62).

While stress-induced reductions of neurogenesis occurring during adulthood are generally reversible, e.g., after appropriate recovery periods or antidepressant drug treatment, the changes induced by *early life* stress are generally longer lasting and the consequences often persist throughout life (see further below). One reason for this difference could be that stress occurring during early life interferes with the development of the DG, which is largely postnatal in rodents. However, it remains poorly understood why such deficits are so long-lasting and whether they can be prevented or reversed at all.

AGE- AND SEX-DEPENDENT EFFECTS OF PERINATAL STRESS

Adult neurogenesis is sensitive to the early life environment. As summarized in **Table 1**, perinatal stress in male rats was generally found to suppress neurogenesis (17, 63). The effects appear to be *region-specific*: for instance, prenatal stress impaired neurogenesis in the DG but not in the olfactory bulb (64).

The overall effect of stress on neurogenesis also depends on the *developmental stage* during which the organism experiences stress. Thus, *in utero* exposure to stress or to a variety of pharmacological agents almost invariably reduces neurogenesis in adulthood (**Table 1**). Postnatal exposure to stress yields more variable results, though suppression of neurogenesis prevails here also.

More importantly, the consequences of early life environment depend on the *moment at which neurogenesis is determined*. When tested in adulthood or middle-age, cell proliferation and neurogenesis were usually found to be decreased (**Table 1**, **Figure 2**). Yet, at earlier stages, e.g., at PND 21 (70, 75), neurogenesis in males was actually found to be *enhanced* by early life stress (**Figure 3**), as was BDNF expression and performance in a stressful version of the Morris water maze (75). Apparently, early life adversity can transiently improve dentate functionality, possibly to allow the organism to survive in the adverse conditions. However in the long run, early life adversity seems to program structural plasticity such that it may become a disadvantage, most notably under low to moderately stressful conditions. Overall, this gives rise to a significant negative correlation between the number of proliferating (Ki-67 or BrdU-positive cells; $r^2 = -0.464$, $p = 0.05$; Pearson test) or DCX-positive ($r^2 = -0.623$, $p = 0.017$) neurons and age in male rodents.

Strikingly different effects of early life stress on neurogenesis are seen in female rats (**Figure 2**). Whereas neurogenesis is enhanced at PND 21 in male rats exposed to 24 h of maternal deprivation at PND 3, a strong suppression was reported in females (**Figure 3**). However, in females the consequence of early life adversity for the number of DCX-positive cells subsides with age, resulting in an overall positive correlation between the number of DCX-positive cells and age ($r^2 = 0.737$, $p = 0.037$). By PND 29, the effects of maternal deprivation on neurogenesis are far less

Table 1 | Overview of the effects of perinatal stress on cell proliferation and neurogenesis in rodents, as measured at various times in life and as a function of sex.

Type of early life intervention	Sex	Age during stress	Age when neurogenesis is studied	Marker	Effect on neurogenesis	Reference
Prenatal stress: restraint stress	Males	Gestational day: 14–21	PND 28 3 months 10 months 22 months	BrdU, used as cell proliferation marker	Reduction	Koehl et al. (65)
Prenatal stress: restraint stress	Males	Gestational day: 15–delivery	4 months	DCX, Ki-67, BrdU injected 1 day before the animals were sacrificed	Reduction	Lemaire et al. (18)
		Gestational day: 15–delivery	6 months	BrdU injected 3 weeks before the animals were sacrificed		
	Males	Gestational day: 15–delivery	26 months	DCX, Ki-67, BrdU injected 1 day before the animals were sacrificed		
Prenatal stress: restraint stress or randomized stressors	Males vs. Females	Gestational day: 14–21	5–6 months	DCX	Reduction in females (controls and stressed group)	Mandyam et al. (66)
	Males	Gestational day: 14–21	5–6 months	Ki-67	Reduction	
	Females	Gestational day: 14–21	5–6 months	Ki-67	Reduction	
Prenatal stress: interaction with resident + restraint stress	Males (from selective line LAB and HAB)	Gestational day: 5–20	PND 43	DCX	Reduction in HAB No effect in LAB	Lucassen et al. (63)
				BrdU injected at PND 11	Reduction in HAB No effect in LAB	
Prenatal stress: restraint stress (animals were tested in behavioral task before to assess neurogenesis)	Males	Gestational day: 15–21	PND 42	DCX Ki-67	Reduction	Rayen et al. (67)
	Females	Gestational day: 15–21	PND 42	DCX Ki-67		
Prenatal stress: restraint stress	Males and females (combined together)	Gestational day: 1–10	PND 40	DCX	Reduction	Madhyastha et al. (68)
	Males and females (combined together)	Gestational day: 11-delivery	PND 40	DCX		
Prenatal nicotine treatment + Maternal separation	Males and females	Gestational day:7–21 Maternal separation: PND 2–21	PND 14	DCX	Reduction	Wang et al. (69)

(Continued)

Table 1 | Continued

Type of early life intervention	Sex	Age during stress	Age when neurogenesis is studied	Marker	Effect on neurogenesis	Reference
Maternal deprivation (MD)	Males	PND 3 (24 h)	PND 4	Ki-67	No effect	Oomen et al. (70)
	Females	PND 3 (24 h)	PND 4	Ki-67		
	Males	PND 3 (24 h)	PND 21	DCX	Increase	
				Ki-67, BrdU, injected at PND 3	No effect	
	Females	PND 3 (24 h)	PND 21	DCX	Reduction	
				Ki-67, BrdU, injected at PND 3	No effect	
Maternal deprivation (MD)	Males	PND 3 (24 h)	10 weeks	DCX, BrdU, injected at PND 51	Reduction in caudal part of DG but not rostral	Oomen et al. (40)
				Ki-67	Reduction	
Maternal deprivation (MD)	Females	PND 3 (24 h)	10 weeks	DCX, Ki-67, BrdU, injected at PND 51	No effect	Oomen et al. (71)
Maternal separation	Males	PND 1–14: Handling + 180 min. MS	PND 60–70:	BrdU injection: 2 h	Reduction vs. AFR and EH	Mirescu et al. (72)
				BrdU injection: 1 week	Reduction vs. AFR and EH	
				BrdU injection: 3 week	No effect	
Maternal separation	Males	PND 2–14: Handling + 180 min MS	11 weeks	BrdU, injected at 8 weeks for 7 days	No effect compared to EH	Kumar et al. (73)
	Females	PND 2–14: Handling + 180 min MS	11 weeks	BrdU, injected at 8 weeks for 7 days	No effect compared to EH	
Maternal separation (animals were tested in behavioral task before assessing neurogenesis)	Males	PND 2–15 (3 h per day)	± PND 80	DCX, BrdU injection ± PND 65	No effect	Hulshof et al. (74)
				Ki-67	Reduction in ventral but not dorsal hippocampus	
Maternal separation	Males	PND 2–14 (3 h per day)	PND 21	DCX, BrdU, injected 2 h before sacrifice	Increase	Suri et al. (75)
			2 months		No effect	
			15 months		Reduction	
Low licking/grooming	Males	Behavior checked first week of life	PND 8	BrdU injected at PND 7	No effect	Bredy et al. (27)
			PND 21		Reduction	
			PND 90			
Social defeat stress	Males	PND 35–41	PND 42	DCX	Reduction	Buwalda et al. (76)
				BrdU, injected 2 h before sacrifice		

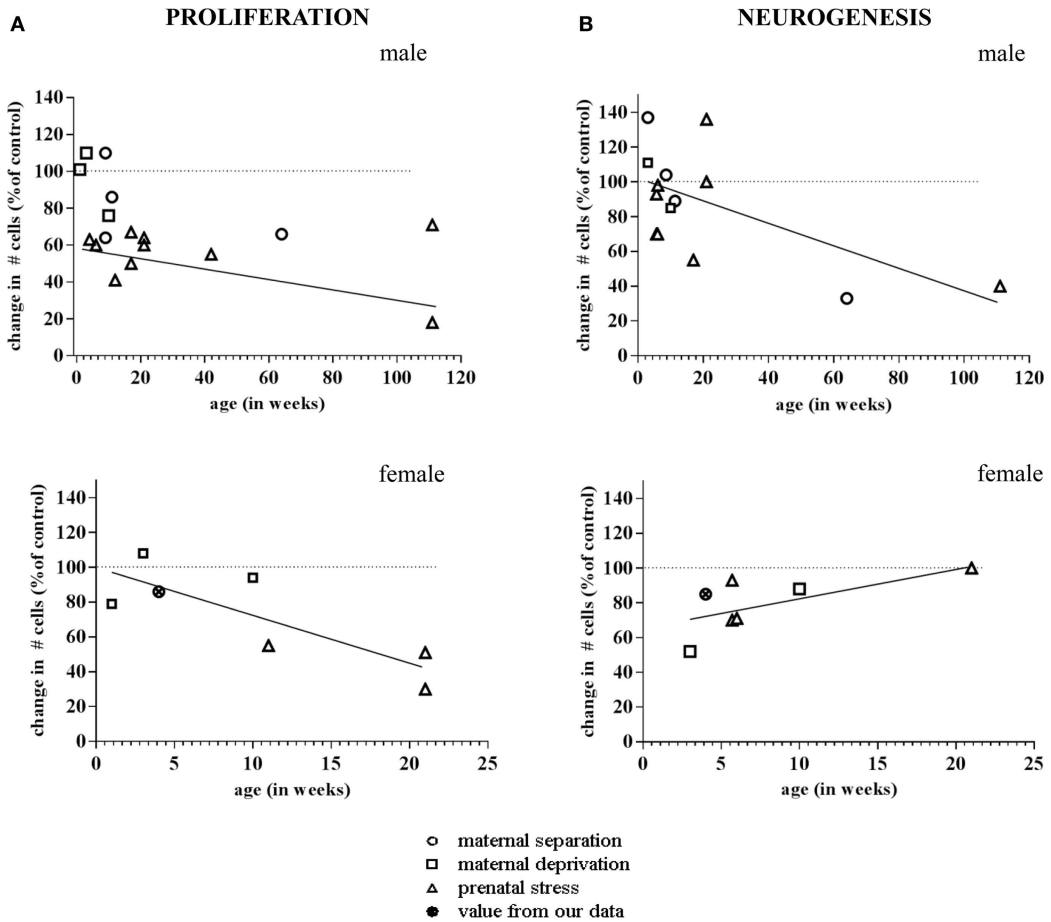


FIGURE 2 | Meta-analysis of age- and sex-dependent effects of early life adversity on neurogenesis. The graphs show the percentage change in number of Ki-67 and Doublecortin (DCX)-positive cells after perinatal stress (*y*-axis; 100% is control), as a function of the age at which the change in neurogenesis was determined (*x*-axis, in weeks). **(A)** Both in males (top) and females (bottom), the change in number of Ki-67 positive cells due to prenatal (triangle), maternal separation (diamond) or maternal deprivation (square) was negatively correlated to the age at which the effect was determined. **(B)** In male rodents (top), the number of DCX-positive cells was found to be enhanced by perinatal stress when examined at a very young age. When studied at time-points >2 months of age, generally a decrease in the number of DCX-positive cells was observed. Overall, this resulted in a negative correlation between the effect of early life stress on neurogenesis and the age at which these effects were apparent. In female rats (bottom), we observed a correlation in the opposite direction. The data points are based on the references summarized in **Table 1**. The one data-point depicted by the filled symbol in the graphs of the females represents the percentage change found in the pilot study described in **Figure 4** (not incorporated in **Table 1**). The striped horizontal line in the graphs indicates the control condition (i.e., the non-stressed groups mentioned in the same publications) against which the number of cells in the stress groups was expressed. The drawn line indicates the best fit for the linear correlation.

prominent than seen at PND 21 [**Figure 2** (filled symbol) and **Figure 4**]. However, the correlation between the change in number of proliferating neurons due to early life adversity and the age at which the effects were determined was comparable between males and females ($r^2 = -0.816$, $p = 0.025$). This could suggest that in female proliferation of non-neuronal (e.g., glial) cells in adulthood is very sensitive to early life adversity or, *vice versa*, that proliferation of non-neuronal cells is stimulated around weaning, compensating for the loss in young neurons due to perinatal stress.

ESSENTIAL MEDIATORS

The molecular pathways through which early life stress can lastingly change stress responsiveness, brain structure and functional performance are only starting to be explored. There is now evidence that epigenetic programing is involved (48, 78), possibly targeting diverse mediators such as NFκB (79), SGK1 (80) or critical steps in the glutamate signaling pathway (81). The GR seems to be a particularly critical element in the cascade leading to lasting changes in brain structure and function. For instance, exon I-7 of the GR promoter is transiently methylated during early development, and again subject to demethylation after PND 6 (82). This demethylation did not occur in the offspring from low licking and grooming mothers. Temporary treatment with a histone deacetylase inhibitor, a compound that prevents the removal of acetyl groups from histones, thus enabling transcription, could fully prevent the development of the phenotype – characterized by reduced hippocampal GR

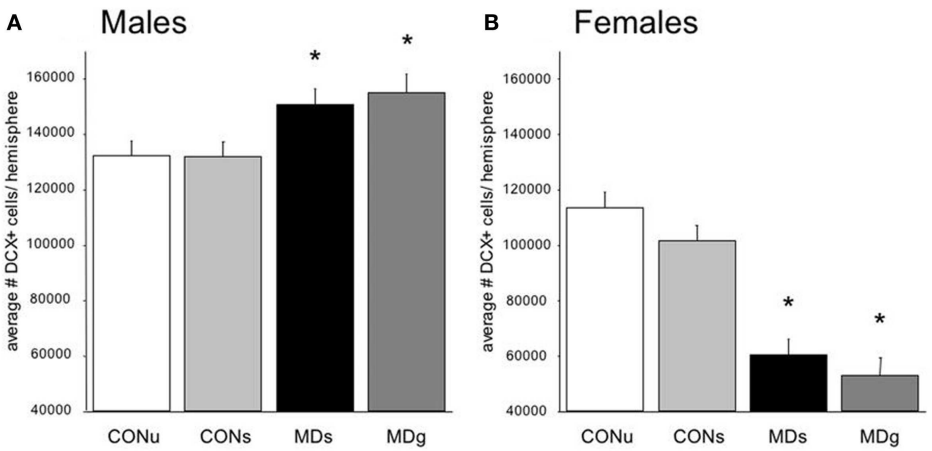


FIGURE 3 | Sex-dependent effects of maternal deprivation on neurogenesis. **(A)** The number of Doublecortin (DCX)-positive neurons in the entire dentate gyrus from 21-days-old male rats, which underwent MD for 24 h at postnatal day (PND) 3, was significantly (*$p < 0.05$; $n = 7$ animals) enhanced compared to the non-deprived controls (CON). Half of the animals received glucose (g) on day 3, to compensate for the loss in nutrients, while the remaining animals received saline (s). There was no effect of sucrose compared to saline treatment. **(B)** The opposite effect was observed in the female littermates: i.e., the number of DCX-positive neurons on PND 21 was significantly reduced in maternally deprived compared to non-deprived rats, regardless of sucrose/saline treatment ($n = 7$). From Ref. (70).

expression and an impaired negative feedback of the stress induced stress response – in adult offspring of low licking–grooming mothers (82, 83). This suggests that the reduction of GR expression in the offspring of low licking–grooming mothers – and hence corticosteroid over-exposure, particularly after stress – may at least partly be responsible for the structural and functional alterations reported along the lifespan and are likely mediated by epigenetic changes.

If corticosteroid over-exposure is indeed an essential step in the cascade, one would expect to see beneficial effects of treatment with pharmacological agents that block the GR, i.e., the receptor most prominently activated after stress. To test this, we performed a pilot study in which female rats, exposed to maternal deprivation at PND 3, were treated during a critical developmental window with the GR antagonist mifepristone. We selected the period of PND 26–28 for treatment with mifepristone, as earlier studies have shown that interventions at this stage of development have significant consequences for the development of the brain and the response to stress later in life (84). Moreover, we had demonstrated before that even a brief treatment with mifepristone is very powerful in normalizing the effects of chronic stress in adult rats (77).

As shown in **Figure 4**, the number of Ki67-positive cells was significantly higher in the hilus (but not in the DG as a whole, data not shown) in MD rats treated with mifepristone compared to those treated with vehicle, whereas the drug did not affect the number of Ki67-positive cells in non-deprived rats. Similarly, mifepristone treatment tended to cause higher levels of DCX-positive cells in the dentate supra-pyramidal blade of MD rats compared to vehicle treated MD controls, although this did not reach significance ($p = 0.08$); mifepristone did not affect the number of DCX-positive cells in non-deprived rats. It should be noted that the effects were modest, possibly due to the age (PND 29) at which the effects of maternal deprivation were determined. More definite conclusions about the potential of mifepristone to reverse effects of maternal deprivation on cell proliferation and neurogenesis require extension of the current pilot experiment to analysis at an earlier time-point – when effects on neurogenesis are more clearly discernable in females, e.g., at PND 21, combined with mifepristone treatment at an earlier time-point, too. Nevertheless, the results are generally in line with earlier findings that brief treatment with the GR-antagonist mifepristone can quickly normalize effects of stress on neurogenesis (77).

CONCLUDING REMARKS

Rodent studies over the past decades have shown that neurogenesis appears to be very sensitive to stress, particularly when stress occurs during the perinatal period. As has become evident from the current overview, these effects of perinatal stress are clearly age-dependent: the consequences seem to change in nature depending on the interval between early life adversity and the time of analysis of the effects on neurogenesis. Interestingly, the effects of perinatal stress on neurogenesis are also sex-dependent. Male rats show a brief period in adolescence during which neurogenesis, BDNF expression, and spatial learning are actually improved, possibly allowing the individual to temporarily compensate for the effects of early life adversity. Female rats do not show such a period of improved performance but rather show a very strong suppression of neurogenesis during the pre-pubertal period, which then subsides with age. The consequences of this period of suppressed neurogenesis in females, though, may be long-lasting. For instance, female rats exposed to 24 h of maternal deprivation at PND 3 exhibited a lower total number of mature granule cells in adulthood (71), potentially limiting the number of synaptic contacts that can be established in this region. Preliminary studies indicate that intervention at the pre-pubertal stage is possible, e.g., by

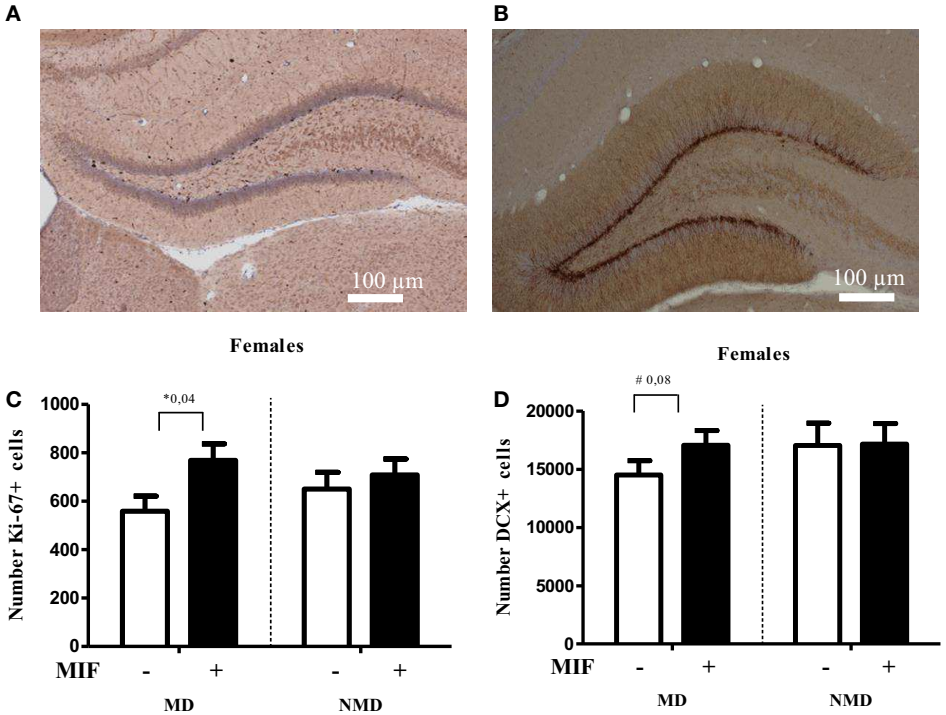


FIGURE 4 | Brief treatment with the GR antagonist mifepristone protects female rats against the effects of early life stress. Rats were deprived from their mother for 24 h at PND 3, following the procedure as described in Oomen et al. (70). After weaning at PND 21, they were group-housed with same-sex same-littermates. On PND 26–28 each rat received mifepristone twice daily (5 mg of RU-38486 (Sigma) per 100 g of body weight, dissolved first in 15 µl ethanol and then in 1.5 ml coffee cream (Campina, Woerden, The Netherlands) and administered by oral gavage (77). One day later, at PND 29, female rats were transcardially perfused with saline, followed by 4% paraformaldehyde in phosphate buffer (0.1M; pH 7.4). Tissue handling and staining for DCX and the proliferation marker Ki67 was conducted as described in Oomen et al. (70). **(A)** Typical example of Ki-67 staining in the DG of a control female rat. **(B)** Typical example of DCX staining in the DG of a control female rat. **(C)** Cell proliferation at PND 29, as determined with Ki-67 staining, was significantly increased in the hilus of MD female rats treated with mifepristone (MIF) on PND 26–28, compared to those treated with vehicle. MIF had no effect in non-deprived (NMD) rats. No significant differences between the experimental groups were seen in the dentate as a whole (data not shown). For each animal, we counted the number of Ki67-positive cells in every 10th section and from this the total number of Ki67-positive cells per hemisphere was inferred. All bars represent the mean + SEM per group ($n = 7$–9 animals per group). **(D)** In the supra-pyramidal blade, a trend ($p = 0.08$) toward a significant increase in the number of DCX-positive cells in MD female rats treated with MIF vs. those treated with vehicle was observed. Mifepristone did not alter neurogenesis at all in NMD rats. Though the percentage change in the infrapyramidal blade and the dentate gyrus as a whole showed a comparable pattern, these differences were not significant ($p > 0.1$, data not shown). For each animal, we counted every 10th section sampled in an unbiased stereological manner, yielding up to a total of nine sections per animal. We then expressed the average number of DCX-positive cells per section per animal. All bars represent the mean + SEM per group ($n = 11$–12 animals per group).

blocking GRs for a limited number of days. Clearly, these studies on successful intervention strategies require more extensive follow-up, to precisely determine the effectiveness of various treatment regimes.

One can speculate about the implications of findings in animal models of perinatal stress for human brain development and the vulnerability to psychopathology. In general, the study of actual neurogenesis in the human brain is difficult and, although its existence has been convincingly demonstrated, it has largely relied on immunocytochemical approaches using proliferation markers in postmortem tissues. Although neurogenesis in adult and aged individuals is generally rare, much larger levels are present at earlier ages (85, 86). It is currently thought that antidepressant treatment may target the neurogenetic process and in fact requires the newborn cells for their antidepressant action to be exerted (87), although this is mostly based on studies in young animal models (60). The finding that especially female rats showed suppressed neurogenesis during a critical developmental stage in response to early life adversity is of interest, given the higher prevalence of many psychiatric illnesses in the female population. In humans, careful monitoring of genetically predisposed females with a history of early life adversity during their development, including possibilities for early intervention, may therefore be one approach to mitigate the development of psychopathology.

ACKNOWLEDGMENTS

Manila Loi was financially supported by Corcept Therapeutics. Paul J. Lucassen is supported by ISAO, Alzheimer Nederland and the HersenStichting Nederland. We thank Mr. G. Meerhoff (SILS, UvA) for histotechnical assistance and Hazel Hunt and Joe Belanoff for helpful comments.

REFERENCES

1. Kohman RA, Rhodes JS. Neurogenesis, inflammation and behavior. *Brain Behav Immun* (2013) **27**:22–32. doi:10.1016/j.bbi.2012.09.003
2. Kempermann G. New neurons for 'survival of the fittest'. *Nat Rev Neurosci* (2012) **13**:727–36. doi:10.1038/nrn3319
3. Kolb B, Mychasiuk R, Muhammad A, Li Y, Frost DO, Gibb R. Experience and the developing prefrontal cortex. *Proc Natl Acad Sci U S A* (2012) **109**(Suppl 2):17186–93. doi:10.1073/pnas.1121251109
4. Brans RG, Kahn RS, Schnack HG, van Baal GC, Posthuma D, van Haren NE, et al. Brain plasticity and intellectual ability are influenced by shared genes. *J Neurosci* (2010) **30**:5519–24. doi:10.1523/JNEUROSCI.5841-09.2010
5. Sporns O. The human connectome: a complex network. *Ann N Y Acad Sci* (2011) **1224**:109–25. doi:10.1111/j.1749-6632.2010.05888.x
6. Raabe FJ, Spengler D. Epigenetic risk factors in PTSD and depression. *Front Psychiatry* (2013) **4**:80. doi:10.3389/fpsyt.2013.00080
7. Korosi A, Naninck EF, Oomen CA, Schouten M, Krugers H, Fitzsimons C, et al. Early-life stress mediated modulation of adult neurogenesis and behavior. *Behav Brain Res* (2012) **227**:400–9. doi:10.1016/j.bbr.2011.07.037
8. Jiang Z, Cowell RM, Nakazawa K. Convergence of genetic and environmental factors on parvalbumin-positive interneurons in schizophrenia. *Front Behav Neurosci* (2013) **7**:116. doi:10.3389/fnbeh.2013.00116
9. Schmahl CG, Vermetten E, Elzinga BM, Douglas BJ. Magnetic resonance imaging of hippocampal and amygdala volume in women with childhood abuse and borderline personality disorder. *Psychiatry Res* (2003) **122**:193–8. doi:10.1016/S0925-4927(03)00023-4
10. 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**:1509–22. doi:10.1016/S0006-3223(99)00224-3
11. Zimmermann P, Brückl T, Nocon A, Pfister H, Binder EB, Uhr M, et al. Interaction of FKBP5 gene variants and adverse life events in predicting depression onset: results from a 10-year prospective community study. *Am J Psychiatry* (2011) **168**:1107–16. doi:10.1176/appi.ajp.2011.10111577
12. Belsky DW, Caspi A, Arseneault L, Bleidorn W, Fonagy P, Goodman M, et al. Etiological features of borderline personality related characteristics in a birth cohort of 12-year-old children. *Dev Psychopathol* (2012) **24**:251–65. doi:10.1017/S0954579411000812
13. Breslau N, Koenen KC, Luo Z, Agnew-Blais J, Swanson S, Houts RM, et al. Childhood maltreatment, juvenile disorders and adult post-traumatic stress disorder: a prospective investigation. *Psychol Med* (2013) **29**:1–9. doi:10.1017/S0033291713002651
14. Pryce CR, Ruedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, et al. Long-term effects of early-life environmental manipulations in rodents and primates: potential animal models in depression research. *Neurosci Biobehav Rev* (2005) **29**:649–74. doi:10.1016/j.neubiorev.2005.03.011
15. Schmidt MV, Wang XD, Meijer OC. Early life stress paradigms in rodents: potential animal models of depression? *Psychopharmacology (Berl)* (2011) **214**:131–40. doi:10.1007/s00213-010-2096-0
16. Koenig JI, Walker CD, Romeo RD, Lupien SJ. Effects of stress across the lifespan. *Stress* (2011) **14**:475–80. doi:10.3109/10253890.2011.604879
17. Lemaire V, Koehl M, Le MM, Abrous DN. Prenatal stress produces learning deficits associated with an inhibition of neurogenesis in the hippocampus. *Proc Natl Acad Sci U S A* (2000) **97**:11032–7. doi:10.1073/pnas.97.20.11032
18. Lemaire V, Lamarque S, Le MM, Piazza PV, Abrous DN. Postnatal stimulation of the pups counteracts prenatal stress-induced deficits in hippocampal neurogenesis. *Biol Psychiatry* (2006) **59**:786–92. doi:10.1016/j.biopsych.2005.11.009
19. van den Hove DL, Leibold NK, Strackx E, Martinez-Claros M, Lesch KP, Steinbusch HW, et al. Prenatal stress and subsequent exposure to chronic mild stress in rats; interdependent effects on emotional behavior and the serotonergic system. *Eur Neuropsychopharmacol* (2013) (in press). doi:10.1016/j.euroneuro.2013.09.006
20. Welberg LA, Seckl JR. Prenatal stress, glucocorticoids and the programming of the brain. *J Neuroendocrinol* (2001) **13**:113–28. doi:10.1111/j.1365-2826.2001.00601.x
21. Zhang TY, Labonte B, Wen XL, Turecki G, Meaney MJ. Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. *Neuropsychopharmacology* (2013) **38**:111–23. doi:10.1038/npp.2012.149
22. Champagne FA. Maternal imprints and the origins of variation. *Horm Behav* (2011) **60**:4–11. doi:10.1016/j.yhbeh.2011.02.016
23. 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**:1335–46. doi:10.1016/j.neubiorev.2005.04.012
24. Baram TZ, Davis EP, Obenaus A, Sandman CA, Small SL, Solodkin A, et al. Fragmentation and unpredictability of early-life experience in mental disorders. *Am J Psychiatry* (2012) **169**:907–15. doi:10.1176/appi.ajp.2012.11091347
25. Francis DD, Diorio J, Plotsky PM, Meaney MJ. Environmental enrichment reverses the effects of maternal separation on stress reactivity. *J Neurosci* (2002) **22**:7840–3.
26. de Kloet ER, Sibug RM, Helmerhorst FM, Schmidt MV. Stress, genes and the mechanism of programming the brain for later life. *Neurosci Biobehav Rev* (2005) **29**:271–81. doi:10.1016/j.neubiorev.2004.10.008
27. Bredy TW, Grant RJ, Champagne DL, Meaney MJ. Maternal care influences neuronal survival in the hippocampus of the rat. *Eur J Neurosci* (2003) **18**:2903–9. doi:10.1111/j.1460-9568.2003.02965.x
28. Cirulli F, Berry A, Bonsignore LT, Capone F, D'Andrea I, Aloe L, et al. Early life influences on emotional reactivity: evidence that social enrichment has greater effects than handling on anxiety-like behaviors, neuroendocrine responses to stress and central BDNF levels. *Neurosci Biobehav Rev* (2010) **34**:808–20. doi:10.1016/j.neubiorev.2010.02.008
29. de Kloet ER, Joëls M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* (2005) **6**:463–75. doi:10.1038/nrn1683
30. Handa RJ, Weiser MJ. Gonadal steroid hormones and the hypothalamo-pituitary-adrenal axis. *Front Neuroendocrinol* (2013) (in press). doi:10.1016/j.yfrne.2013.11.001
31. Joels M, Sarabdjitsingh RA, Karst H. Unraveling the time domains of corticosteroid hormone influences on brain activity: rapid, slow, and chronic modes. *Pharmacol Rev* (2012) **64**:901–38. doi:10.1124/pr.112.005892
32. Oitzl MS, Champagne DL, van der Veen R, de Kloet ER. Brain development under stress: hypotheses of glucocorticoid actions revisited. *Neurosci Biobehav Rev* (2010) **34**:853–66. doi:10.1016/j.neubiorev.2009.07.006
33. Champagne FA. Early environments, glucocorticoid receptors, and behavioral epigenetics. *Behav Neurosci* (2013) **127**:628–36. doi:10.1037/a0034186
34. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* (2009) **10**:434–45. doi:10.1038/nrn2639
35. Kim JJ, Song EY, Kosten TA. Stress effects in the hippocampus: synaptic plasticity and memory. *Stress* (2006) **9**:1–11. doi:10.1080/10253890600678004
36. Fenoglio KA, Brunson KL, Baram TZ. Hippocampal neuroplasticity induced by early-life stress: functional and molecular aspects. *Front Neuroendocrinol* (2006) **27**:180–92. doi:10.1016/j.yfrne.2006.02.001
37. 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**:6037–45. doi:10.1523/JNEUROSCI.0526-08.2008
38. 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) **92**:292–300. doi:10.1016/j.nlm.2009.03.004
39. Barha CK, Pawluski JL, Galea LA. Maternal care affects male and female offspring working memory and stress reactivity. *Physiol Behav* (2007) **92**:939–50. doi:10.1016/j.physbeh.2007.06.022
40. Oomen CA, Soeters H, Audureau N, Vermunt L, van Hasselt FN, Manders EM, et al. Severe early life stress hampers spatial learning and neurogenesis, but improves hippocampal synaptic plasticity and emotional learning under high-stress conditions in adulthood. *J Neurosci* (2010) **30**:6635–45. doi:10.1523/JNEUROSCI.0247-10.2010
41. Baudin A, Blot K, Verney C, Estevez L, Santamaria J, Gressens P, et al. Maternal deprivation induces deficits in temporal memory and cognitive flexibility and exaggerates synaptic plasticity in the rat medial prefrontal cortex. *Neurobiol Learn Mem* (2012) **98**:207–14. doi:10.1016/j.nlm.2012.08.004
42. van Hasselt FN, de Visser L, Tieskens JM, Cornelisse S, Baars AM, Lavrijsen M, et al. Individual variations in maternal care early in life correlate with later life decision-making and c-fos expression in prefrontal subregions of rats. *PLoS One* (2012) **7**:e37820. doi:10.1371/journal.pone.0037820

43. Cao X, Huang S, Cao J, Chen T, Zhu P, Zhu R, et al. The timing of maternal separation affects morris water maze performance and long-term potentiation in male rats. *Dev Psychobiol* (2013) (in press). doi:10.1002/dev.21130
44. Champagne FA, Curley JP. Epigenetic mechanisms mediating the long-term effects of maternal care on development. *Neurosci Biobehav Rev* (2009) **33**:593–600. doi:10.1016/j.neubiorev.2007.10.009
45. Claessens SE, Daskalakis NP, van der Veen R, Oitzl MS, de Kloet ER, Champagne DL. Development of individual differences in stress responsiveness: an overview of factors mediating the outcome of early life experiences. *Psychopharmacology (Berl)* (2011) **214**:141–54. doi:10.1007/s00213-010-2118-y
46. Nederhof E, Schmidt MV. Mismatch or cumulative stress: toward an integrated hypothesis of programming effects. *Physiol Behav* (2012) **106**:691–700. doi:10.1016/j.physbeh.2011.12.008
47. Karten YJ, Olariu A, Cameron HA. Stress in early life inhibits neurogenesis in adulthood. *Trends Neurosci* (2005) **28**:171–2. doi:10.1016/j.tins.2005.01.009
48. Lucassen PJ, Naninck EF, van Goudoever JB, Fitzsimons C, Joels M, Korosi A. Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics. *Trends Neurosci* (2013) **36**:621–31. doi:10.1016/j.tins.2013.08.002 [Epub ahead of print].
49. Sawada M, Sawamoto K. Mechanisms of neurogenesis in the normal and injured adult brain. *Keio J Med* (2013) **62**:13–28. doi:10.2302/kjm.2012-0005-RE
50. Kempermann G, Jessberger S, Steiner B, Kronenberg G. Milestones of neuronal development in the adult hippocampus. *Trends Neurosci* (2004) **27**:447–52. doi:10.1016/j.tins.2004.05.013
51. von Bohlen und Halbach O. Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus. *Cell Tissue Res* (2011) **345**:1–19. doi:10.1007/s00441-011-1196-4
52. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. *Nature* (2002) **415**:1030–4. doi:10.1038/4151030a
53. Brown J, Cooper-Kuhn CM, Kempermann G, Van Praag H, Winkler J, Gage FH, et al. Enriched environment and physical activity stimulate hippocampal but not olfactory bulb neurogenesis. *Eur J Neurosci* (2003) **17**:2042–6. doi:10.1046/j.1460-9568.2003.02647.x
54. Joels M, Karst H, Krugers HJ, Lucassen PJ. Chronic stress: implications for neuronal morphology, function and neurogenesis. *Front Neuroendocrinol* (2007) **28**:72–96. doi:10.1016/j.yfrne.2007.04.001
55. Czeh B, Lucassen PJ. What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated? *Eur Arch Psychiatry Clin Neurosci* (2007) **257**:250–60. doi:10.1007/s00406-007-0728-0
56. Kempermann G, Krebs J, Fabel K. The contribution of failing adult hippocampal neurogenesis to psychiatric disorders. *Curr Opin Psychiatry* (2008) **21**:290–5. doi:10.1097/YCO.0b013e3282fad375
57. Sahay A, Hen R. Adult hippocampal neurogenesis in depression. *Nat Neurosci* (2007) **10**:1110–5. doi:10.1038/nn1969
58. Lucassen PJ, Fitzsimons CP, Korosi A, Joels M, Belzung C, Abrous DN. Stressing new neurons into depression? *Mol Psychiatry* (2013) **18**:396–7. doi:10.1038/mp.2012.39
59. Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S, et al. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. *Mol Psychiatry* (2011) **16**:738–50. doi:10.1038/mp.2011.26
60. Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, et al. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* (2003) **301**:805–9. doi:10.1126/science.1083328
61. Henn FA, Vollmayr B. Neurogenesis and depression: etiology or epiphenomenon? *Biol Psychiatry* (2004) **56**:146–50. doi:10.1016/j.biopsych.2004.04.011
62. Lucassen PJ, Stumpel MW, Wang Q, Aronica E. Decreased numbers of progenitor cells but no response to antidepressant drugs in the hippocampus of elderly depressed patients. *Neuropharmacology* (2010) **58**:940–9. doi:10.1016/j.neuropharm.2010.01.012
63. Lucassen PJ, Bosch OJ, Jousma E, Kromer SA, Andrew R, Seckl JR, et al. Prenatal stress reduces postnatal neurogenesis in rats selectively bred for high, but not low, anxiety: possible key role of placental 11beta-hydroxysteroid dehydrogenase type 2. *Eur J Neurosci* (2009) **29**:97–103. doi:10.1111/j.1460-9568.2008.06543.x
64. Belnoue L, Grosjean N, Ladeveze E, Abrous DN, Koehl M. Prenatal stress inhibits hippocampal neurogenesis but spares olfactory bulb neurogenesis. *PLoS One* (2013) **8**:e72972. doi:10.1371/journal.pone.0072972
65. Koehl M, Lemaire V, Mayo W, Abrous DN, Maccari S, Piazza PV, et al. Individual vulnerability to substance abuse and affective disorders: role of early environmental influences. *Neurotox Res* (2002) **4**:281–96. doi:10.1080/1029842021000010866
66. Mandyam CD, Crawford EF, Eisch AJ, Rivier CL, Richardson HN. Stress experienced in utero reduces sexual dichotomies in neurogenesis, microenvironment, and cell death in the adult rat hippocampus. *Dev Neurobiol* (2008) **68**:575–89. doi:10.1002/dneu.20600
67. Rayen I, van den Hove DL, Prickaerts J, Steinbusch HW, Pawluski JL. Fluoxetine during development reverses the effects of prenatal stress on depressive-like behavior and hippocampal neurogenesis in adolescence. *PLoS One* (2011) **6**:e24003. doi:10.1371/journal.pone.0024003
68. Madhyastha S, Sekhar S, Rao G. Resveratrol improves postnatal hippocampal neurogenesis and brain derived neurotrophic factor in prenatally stressed rats. *Int J Dev Neurosci* (2013) **31**:580–5. doi:10.1016/j.ijdevneu.2013.06.010
69. Wang H, Gondre-Lewis MC. Prenatal nicotine and maternal deprivation stress de-regulate the development of CA1, CA3, and dentate gyrus neurons in hippocampus of infant rats. *PLoS One* (2013) **8**:e65517. doi:10.1371/journal.pone.0065517
70. Oomen CA, Girardi CE, Cahyadi R, Verbeek EC, Krugers H, Joëls M, et al. Opposite effects of early maternal deprivation on neurogenesis in male versus female rats. *PLoS One* (2009) **4**:e3675. doi:10.1371/journal.pone.0003675
71. Oomen CA, Soeters H, Audureau N, Vermunt L, van Hasselt FN, Manders EM, et al. Early maternal deprivation affects dentate gyrus structure and emotional learning in adult female rats. *Psychopharmacology (Berl)* (2011) **214**:249–60. doi:10.1007/s00213-010-1922-8
72. Mirescu C, Peters JD, Gould E. Early life experience alters response of adult neurogenesis to stress. *Nat Neurosci* (2004) **7**:841–6. doi:10.1038/nn1290
73. Kumar G, Jones NC, Morris MJ, Rees S, O'Brien TJ, Salzberg MR. Early life stress enhancement of limbic epileptogenesis in adult rats: mechanistic insights. *PLoS One* (2011) **6**:e24033. doi:10.1371/journal.pone.0024033
74. Hulshof HJ, Novati A, Sgoifo A, Luiten PG, den Boer JA, Meerlo P. Maternal separation decreases adult hippocampal cell proliferation and impairs cognitive performance but has little effect on stress sensitivity and anxiety in adult Wistar rats. *Behav Brain Res* (2011) **216**:552–60. doi:10.1016/j.bbr.2010.08.038
75. Suri D, Veenit V, Sarkar A, Thiagarajan D, Kumar A, Nestler EJ, et al. Early stress evokes age-dependent biphasic changes in hippocampal neurogenesis, BDNF expression, and cognition. *Biol Psychiatry* (2013) **73**:658–66. doi:10.1016/j.biopsych.2012.10.023
76. Buwalda B, Stubbendorff C, Zickert N, Koolhaas JM. Adolescent social stress does not necessarily lead to a compromised adaptive capacity during adulthood: a study on the consequences of social stress in rats. *Neuroscience* (2013) **249**:258–70. doi:10.1016/j.neuroscience.2012.12.050
77. Hu P, Oomen C, van Dam AM, Wester J, Zhou JN, Joels M, et al. A single-day treatment with mifepristone is sufficient to normalize chronic glucocorticoid induced suppression of hippocampal cell proliferation. *PLoS One* (2012) **7**:e46224. doi:10.1371/journal.pone.0046224
78. McClelland S, Korosi A, Cope J, Ivy A, Baram TZ. Emerging roles of epigenetic mechanisms in the enduring effects of early-life stress and experience on learning and memory. *Neurobiol Learn Mem* (2011) **96**:79–88. doi:10.1016/j.nlm.2011.02.008
79. Koo JW, Russo SJ, Ferguson D, Nestler EJ, Duman RS. Nuclear factor-kappaB is a critical mediator of stress-impaired neurogenesis and depressive behavior. *Proc Natl Acad Sci U S A* (2010) **107**:2669–74. doi:10.1073/pnas.0910658107
80. Anacker C, Cattaneo A, Musaelyan K, Zunszain PA, Horowitz M, Molteni R, et al. Role for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis. *Proc Natl Acad Sci U S A* (2013) **110**:8708–13. doi:10.1073/pnas.1300886110
81. Bagot RC, Tse YC, Nguyen HB, Wong AS, Meaney MJ, Wong TP. Maternal care influences hippocampal N-methyl-D-aspartate receptor function and dynamic regulation by corticosterone in adulthood. *Biol Psychiatry* (2012) **72**:491–8. doi:10.1016/j.biopsych.2012.03.016

82. Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, et al. Epigenetic programming by maternal behavior. *Nat Neurosci* (2004) **7**:847–54. doi:10.1038/nn1276
83. Weaver IC. Epigenetic effects of glucocorticoids. *Semin Fetal Neonatal Med* (2009) **14**:143–50. doi:10.1016/j.siny.2008.12.002
84. Jacobson-Pick S, Richter-Levin G. Differential impact of juvenile stress and corticosterone in juvenility and in adulthood, in male and female rats. *Behav Brain Res* (2010) **214**:268–76. doi:10.1016/j.bbr.2010.05.036
85. Spalding KL, Bergmann O, Alkass K, Bernard S, Salehpour M, Huttner HB, et al. Dynamics of hippocampal neurogenesis in adult humans. *Cell* (2013) **153**:1219–27. doi:10.1016/j.cell.2013.05.002
86. Knoth R, Singec I, Ditter M, Pantazis G, Capetian P, Meyer RP, et al. Murine features of neurogenesis in the human hippocampus across the lifespan from 0 to 100 years. *PLoS One* (2010) **5**:e8809. doi:10.1371/journal.pone.0008809
87. Boldrini M, Hen R, Underwood MD, Rosoklija GB, Dwork AJ, Mann JJ, et al. Hippocampal angiogenesis and progenitor cell proliferation are increased with antidepressant use in major depression. *Biol Psychiatry* (2012) **72**:562–71. doi:10.1016/j.biopsych.2012.04.024

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 25 December 2013; paper pending published: 14 January 2014; accepted: 31 January 2014; published online: 20 February 2014.
*Citation: Loi M, Koricka S, Lucassen PJ and Joëls M (2014) Age- and sex-dependent effects of early life stress on hippocampal neurogenesis. Front. Endocrinol. **5**:13. doi: 10.3389/fendo.2014.00013*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
NEUROSCIENCE

ORIGINAL RESEARCH ARTICLE
published: 27 March 2014
doi: 10.3389/fnins.2014.00045

Social isolation disrupts hippocampal neurogenesis in young non-human primates

Simone M. Cinini[1†], Gabriela F. Barnabe[1†], Nicole Galvão-Coelho[2], Magda A. de Medeiros[3], Patrícia Perez-Mendes[1], Maria B. C. Sousa[2], Luciene Covolan[1] and Luiz E. Mello[1]*

[1] *Departamento de Fisiologia, Universidade Federal de São Paulo, São Paulo, Brazil*
[2] *Departamento de Fisiologia, Universidade Federal do Rio Grande do Norte, Natal, Brazil*
[3] *Departamento de Ciências Fisiológicas, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro Brazil*

Edited by:
Nikolaos P. Daskalakis, Icahn School of Medicine at Mount Sinai, USA

Reviewed by:
Lan Ma, Fudan University, China
Christopher Robert Pryce, University of Zurich, Switzerland

Correspondence:
Luiz E. Mello, Departamento de Fisiologia, Universidade Federal de São Paulo, Rua Botucatu, 862, 5º andar, 04023-062 São Paulo, Brazil
e-mail: lemello@unifesp.br

† *These authors have contributed equally to this work.*

Social relationships are crucial for the development and maintenance of normal behavior in non-human primates. Animals that are raised in isolation develop abnormal patterns of behavior that persist even when they are later reunited with their parents. In rodents, social isolation is a stressful event and is associated with a decrease in hippocampal neurogenesis but considerably less is known about the effects of social isolation in non-human primates during the transition from adolescence to adulthood. To investigate how social isolation affects young marmosets, these were isolated from other members of the colony for 1 or 3 weeks and evaluated for alterations in their behavior and hippocampal cell proliferation. We found that anxiety-related behaviors like scent-marking and locomotor activity increased after social isolation when compared to baseline levels. In agreement, grooming—an indicative of attenuation of tension—was reduced among isolated marmosets. These results were consistent with increased cortisol levels after 1 and 3 weeks of isolation. After social isolation (1 or 3 weeks), reduced proliferation of neural cells in the subgranular zone of dentate granule cell layer was identified and a smaller proportion of BrdU-positive cells underwent neuronal fate (doublecortin labeling). Our data is consistent with the notion that social deprivation during the transition from adolescence to adulthood leads to stress and produces anxiety-like behaviors that in turn might affect neurogenesis and contribute to the deleterious consequences of prolonged stressful conditions.

Keywords: social isolation, young marmosets, hippocampal neurogenesis, anxiety, isolation stress

INTRODUCTION

In the adult hippocampus, progenitor cells in the subgranular zone of the dentate gyrus give rise to new neurons that migrate into the granule cell layer, differentiate into granular neurons, and are capable of functional integration into the hippocampal circuitry (Gould and Gross, 2002; Van Praag et al., 2002; Kee et al., 2007). The functional role of hippocampal neurogenesis has not been fully understood until now, but despite the divergent results from different laboratories and models, most data points toward its involvement with specific aspects of learning, conditioning, and spatial information (for review see Balu and Lucki, 2009).

Reduction in hippocampal neurogenesis is associated with stress (Gould et al., 1998) mainly by means of increased excitatory transmission (Gould et al., 1997; Abraham et al., 1998), pro-inflammatory cytokines (Koo and Duman, 2008), diminished neurotrophins expression (Duman and Monteggia, 2006; Jacobsen and Mork, 2006), and glucocorticoid signaling (Wong and Herbert, 2005, 2006). Social isolation is a form of stress, which affects some hippocampal-related functions such as learning and memory and may lead to affective disorders. In marmosets there is a strong exponential negative correlation between the number of dentate proliferating cells and aging where 2–3 years-old animals are considered young adults, from 4 to 7 years they are middle-aged and above 8 years old they are considered old (Bunk et al., 2011). In the present study we used social isolation of young animals as the stressful event (Laudenslager et al., 1995; Stranahan et al., 2006) in order to characterize behavioral consequences of social isolation during the transition phase from adolescence to adulthood, when the animals are at the peak of dentate neurogenesis, so any disturbance might bear a greater relevance in the onset of future mood disorders.

The long-term effects of social isolation among rodent pups include decreased hippocampal neurogenesis, which can culminate in a reduced ability to cope with stressful events in adulthood (Laudenslager et al., 1995; Mirescu et al., 2004; Karten et al., 2005; Stranahan et al., 2006; Rizzi et al., 2007). As compared to rodents, social interactions in primates are considerably more important for the appropriate neuropsychological development (Rosenblum and Andrews, 1994). Marmosets partially deprived of parental care during infancy develop abnormal patterns of behavior that persist even when they are later reunited with their parents (Dettling et al., 2002a,b). In spite of the well-characterized

Abbreviations: 1W, socially isolated for one week; 3W, socially isolated for three weeks; BrdU, 5-bromo-2′-deoxyuridine; PBS, phosphate buffered saline; DAB, 3,3′-diaminobenzidine-tetrahydrochloride; DCX, doublecortin.

behavioral consequences of social isolation during infancy in these animals, little is known about the neurobiological effects of social isolation during its transition to adulthood. In the present study we investigate the consequences of social isolation in the behavior and hippocampal neurogenesis in these non-human primates.

METHODS

ANIMAL CARE

All experimental procedures were approved by the Research Ethics Board of the Federal University of São Paulo and by the Ethics Committee of the Department of Physiology of Federal University of Rio Grande do Norte. The collected behavioral data was obtained in the animal facilities from both Universities, while the data for brain analysis were obtained from animals raised in Federal University of São Paulo animal facility. The cortisol and behavioral analysis were held at Federal University of Rio Grande do Norte. We are fully aware that under ideal experimental conditions all animals should have been subjected to exactly the same conditions. Here, in order to maximize the availability of animals, to expand the multiple uses of animals and to minimize the unnecessary deaths of non-human primates we opted to use two separate groups of animals under similar laboratory conditions. Indeed, the similarity in the behavioral repertoire of both groups before and after isolation clearly indicates a similar underlying physiological response to social isolation.

Marmosets (*Callithrix sp)* bred in both animal facilities ($n = 16$) were housed in cages, on a standard light/dark cycle (12/12 h). The animals were fed twice a day, around 8:00 am with a protein mixture (powdered milk, corn syrup, eggs, bread, soy bean protein, and bone flour supplemented with vitamins A, D, and E) and around 2:00 pm with a portion of regional tropical fruits. Water was provided *ad libitum*. At baseline, animals were kept with other members of their families (mother, father, and more than one sibling).

In the São Paulo group, 8–10 months old marmosets ($n = 9$) had regular weight (3 males, 198 ± 38 g; 6 females, 223 ± 27 g) in the beginning of the experiments. Animals were subdivided in 3 different groups, each containing 2 females and 1 male. Two groups were socially isolated for 1 or 3 weeks, respectively named here as 1W and 3W groups. Isolated marmosets were kept on individual metallic cages (0.75 × 0.75 × 0.8 m) and had no physical or visual contact with other members of the colony. The third group of animals (control, CTR) remained with their families in metallic cages (1.5 × 1.5 × 0.8 m) for 3 weeks. At the end of the third experimental week all these animals were perfused for immunolabeling of hippocampal proliferating cells.

The Rio Grande do Norte group consisted of 7 animals, with same age and similar weight as the Sao Paulo group (4 males, 200 ± 35 g; 3 females, 220 ± 18 g). Similarly to the São Paulo group, the baseline behavioral repertoire of these sub-adults marmosets was taken for each animal while they were with their families, housed in brick wall cages (2 × 2 × 2 m). Thereafter, animals were subdivided into the social isolated groups 1W and 3W. During isolation marmosets were kept on individual cages (2 × 2 × 1 m) without physical or visual contact with other members of the colony. Finally, after a period of social isolation, marmosets were returned to their family cages and their behavioral repertoire was also assessed to evaluate their familiar reunion.

BEHAVIORAL OBSERVATION AND CORTISOL MEASUREMENT

The behavioral repertoire assessment and the feces collection for cortisol levels measurements were made once a day, 2 days prior to the social isolation in order to establish its baseline levels. Feces samples were taken in the first 2 days of social isolation (early phase) and again in the last 2 days (late phase) totalizing four samples per subject during the isolation period. Behavioral observations were also made during the first and last 2 days of the isolation period. The animals were then moved back to their original family cage and again, feces collection and behavioral observations were made during the two following days, considered the reunion phase. The choice of assessment of cortisol levels from collected feces enabled us to avoid the manipulation of animals and the stress related to daily blood sample collection, which would in turn have influenced the animal's behavior.

The fecal collection was performed between 06:30 and 09:00 a.m. to avoid circadian influence on cortisol (Raminelli et al., 2003). All animals were observed until the defecation occurred. After that, a collector entered the cage and collected the feces from the cage bedding on the floor. Only completely identified feces were collected. Material of defecation was collected in small identified glass tubes with snap caps. After collection, the samples were frozen immediately.

Fecal samples were allowed to reach room temperature for at least 10 min before homogenization. Separation of the steroids by extraction was performed on 0.1 g of well-mixed feces by the addition of 2.5 mL ethanol and 2.5 mL distilled water. The samples were vortexed for 5 min and centrifuged for 10 min at 3000 rpm, and then the aqueous phase was decanted into glass tubes and then frozen at −20°C until solvolysis procedure.

Solvolysis was performed as previously described (Ziegler et al., 1995, 1996). To separate the steroid conjugates, frozen samples were left at room temperature for 10 min before the 500 samples were vortexed for 10 s and were added 100 mL solution of sodium chloride (NaCl) saturated, 50 mL sulfuric acid (H_2SO_4), and 5 mL of organic solvent ethyl acetate ($C_4H_8O_2$). The solutions were vortexed for 1 min and kept under agitation in a water bath (40°C) overnight. Next morning, 4 mL of ethyl acetate was added to the mixture; this was followed by 5 min vortex and centrifuged for 3 min (1000 rpm). After supernatant extraction, 2.5 mL of distilled water was added and the samples were vortexed 5 min, followed by 3 min centrifugation at 1000 rpm. The supernatants were allowed to dry on a water bath at 40°C until its complete evaporation. Finally, the fractions of free steroids were ressuspended in 500 μL of ethanol and assayed for cortisol using a modification of the enzyme immunoassay method developed by (Munro and Stabenfeldt, 1984; Ziegler et al., 1995, 1996) with the standards used for this assay prepared in ethanol. Parallelism using serial dilution of marmoset fecal pool and standards did not differ ($P > 0.05$), and the accuracy was 109.6 ± 4.4%. For this, 50 μL of each sample was placed on a water bath 40°C until its complete evaporation. Immediately following was added 300 μL of solution containing the enzyme conjugate (cortisol:HRP, diluted

at 1: 75,000) to the respective hormone (dilution 1:16,000 in monophosphate buffer:sodium diphosphate). The solution was kept under agitation for 5 min. Then, 100 μL of each sample was placed in multiwell plates prepared with the cortisol antibody (R4866, 1:12,000), which was developed and characterized by Munro and Stabenfeldt (1984). The samples were incubated for 2 h in a humid chamber. After that, 100 μL/well a solution containing 25 mL of 10% citrate buffer, 250 μL of ABTS substrate [2,2′-Azino-bis (3- ethylbenzothiazoline-6-sulfonic acid) diammonium salt, Sigma] and 80 μL of H_2O_2 was added to each well. Thus, the multiwell plate was incubated for 1 h in humidity chamber. A solution containing 5.048 mL of hydrofluoric acid; 1.2 mL of sodium hydroxide (5N) diluted in 500 mL of distilled water was prepared. From this solution, 100 μL of was added to each well to stop the reaction. The optical density reading of the plaque was assessed with a spectrophotometer (Asys/Hitech Expert plus, Eugendorf, Austria) using 410 nm filter.

The whole behavioral repertoire analysis was performed during 2 h daily using the focal continuous observation. This method has been previously demonstrated to be effective to monitor the bimodal pattern in captive common marmosets (Barbosa and Mota, 2009). For statistical analysis we selected anxiety-related behaviors (Barros and Tomaz, 2002; Dettling et al., 2002a), like scent-marking (frequency of rubbing the anogenital on a surface of cage), scratching, piloerection, and locomotor activity (the cage was divided into 60 quadrants of 38 cm each and the frequency of movement between two quadrants was recorded as one movement), as well as behaviors that are indicative of attenuation of tension like auto-grooming: time spent licking, picking at or parting his or her own fur with the fingers (Devries et al., 2007; Wittig et al., 2008), attempts to establish contact, and social-grooming.

BrdU AND DOUBLECORTIN STAINING

On the last day at end of 1 (1W group) or 3 weeks (3W group) of social isolation or 3 weeks after the beginning of the experiments (CTR group), animals ($n = 3$ per group) were given a single injection of 5-bromo-2′-deoxyuridine (BrdU; 75 mg/Kg i.p. dissolved in a solution containing 0.9% NaCl and 0.007 M NaOH). Twenty-four hours later with the animals still in isolation, they were deeply anesthetized with thiopental (50 mg/Kg, i.p) and transcardially perfused with 0.9% saline, followed by a 4% paraformaldehyde fixative solution. The brains were then removed from the skull and post-fixed overnight in the same fixative solution before cryoprotection in 30% sucrose solution. Thirty-two μm thick coronal sections throughout the entire antero-posterior axis of the hippocampus were cut on a cryostat. Sections from each animal were sequentially collected in 24-well plates filled with anti-freezing solution and stored at −20°C.

The immunohistochemistry procedure was a priori defined and it was performed on every 12th section of the entire hippocampus (the distance between each two sections was about 360 μm). With this sampling, we were able to run countings in 1 out of 12 representative sections along the rostro-caudal extension of the dentate gyrus. The tissue was first incubated with 3% H_2O_2 in PB (10 min), washed in PB, followed by 2M HCl at 40°C (30 min), and washed in PBS (3 × 10 min). Sections were then incubated for 1 h in a blocking buffer solution containing 2% normal goat serum (Sigma-Aldrich) and 0.1% TritonX-100 in PBS. Thereafter, they were incubated with monoclonal primary antibody to BrdU (rat anti-BrdU, 1:200, Axyll/Accurate Chemical) in the blocking solution overnightat 4°C. In the next morning, sections were washed in PBS and incubated with biotinylated secondary antibody (goat anti-rat IgG, 1:200, Vector) during 2 h in PB. After 3 washes in PBS (3 × 10 min) were incubated for 1 h in avidin-biotin-peroxidase complex (Vectastain Elite ABC,Vector) and the reaction product was developed using 0.05% 3,3′-diaminobenzidine-tetrahydrochloride(DAB, Sigma-Aldrich). Stained sections were mounted onto gelatin-coated glass slides, air dried, dehydrated, and coverslipped with Entellan (Merk).

Co-localization of BrdU and doublecortin (DCX) was examined in the sections adjacent to those used for BrdU labeling. Sections were incubated in HCl 2M at 40°C for 30 min, followed by incubation with a mixture of the monoclonal primary antibodies to BrdU (rat,1:200; Axyll/Accurate Chemical) and DCX (guinea pig, 1:100; Chemicon) diluted in blocking buffer overnight, at room temperature. After several rinses in PBS, sections were incubated with Alexa Fluor® 488 goat anti-rat IgG (1:300, Molecular Probes) and Alexa Fluor® 546 donkey anti-guinea pig IgG (1:300, Molecular Probes) for 1 h at room temperature. Rinsed tissue sections were then mounted onto gelatin-subbed slides and coverslipped with Fluoromount G (Electron Microscopy Sciences).

CELL COUNTS

Cell counts were conducted on a blinded fashion at 3 different rostro-caudal levels throughout the hippocampal extent of 9 animals. These corresponded to plates A 6.5–A 5.5 (rostral level), A 5.0–A 4.0 (intermediate level) and A3.5–A 1.5 (caudal level) of the marmoset brain atlas by Stephan et al., 1980. Both right and the left hippocampi were assessed, rendering a total of 55, 58, and 30 coronal sections counted in the groups of animals isolated for 1 week, 3 weeks and controls, respectively. Since the number of proliferating cells labeled with BrdU or BrdU/DCX in the dentate gyrus was very low and the spatial distribution was inhomogeneous, the total number of labeled cells was counted in the selected sections, as above described, for each animal. For this, we took care to assure that each analyzed section was similar among all groups.

To quantify BrdU-positive cells, we have used a light microscope (400 × magnification; Olympus® BX 50 microscope, Japan) and a digital image analysis system (Neurozoom software, NY, USA). Small BrdU-labeled nuclei in the hilar border of the dentate gyrus (presumed to be glial precursors) and fusiform immunostained cells (endothelial-like) were excluded from the analysis. In order to normalize data, the number of BrdU-positive cells in the three different groups of animals is expressed as the mean number of labeled cells per section.

The number of cells co-localizing BrdU and DCX was determined with a Zeiss (Oberkochen) LSM 510 confocal laser microscope. Sections were scanned using a 40 × oil-immersion objective and dual-channel excitation with argon (488 nm) and helium-neon (543 nm). Four coronal sections per subject

corresponded to the ones described above were randomly assessed for counting. Co-localization analysis included visual inspection of size and shape of cell throughout a z-stack and orthogonal planes. The percentage of co-localization was achieved by dividing the number of double-labeled cells (DCX/BrdU-positive) by the total number of BrdU-positive cells in each hippocampal section.

STATISTICAL ANALYSIS

The comparison of cortisol levels between baseline, early social isolation, late social isolation, and after family's reunion was assessed by means of repeated measure analysis of variance followed by *post-hoc* Fisher PLSD. The variation of behaviors between phases was assessed with non-parametric tests (Friedman, Wilcoxon, Mann-Whitney). Pearson's correlation coefficient (r) was obtained to investigate the relationship between anxiety-like behaviors with the cortisol levels along different phases of social isolation. Differences in the total number of BrdU-positive cells between groups, across hippocampal plates or the full structure, were assessed with Two-Way and One-Way ANOVA (applying Newman-Keuls *post-hoc* test), respectively. Percentages of BrdU and DCX co-localization were analyzed by Kruskal-Wallis and Mann-Whitney tests. After the percentage was determined for the total BrdU counting, we performed One-Way analysis of variance followed by Newman-Keuls *post-hoc* test. Statistical significance was set at $P \leq 0.05$. Results in the text are expressed as mean ± standard error (SE).

RESULTS

DIFFERENT DURATIONS OF SOCIAL ISOLATION RESULTED IN BEHAVIORAL CHANGES AMONG YOUNG MARMOSETS

Animals that have been isolated for 1 week decreased autogrooming at both early and late phases of isolation, when compared to baseline values ($X^2 = 9.52$, $P = 0.02$; Basal × Early phase: $z = 2.02$; $P = 0.04$; Basal × Late phase: $z = 2.02$; $P = 0.04$) (**Figure 1A**). The 1W group also had higher frequency of scent-marking during both phases of isolation than in the reunion phase ($X^2 = 7.8, P = 0.05$; Reunion × Early phase: $z = 2.02; p = 0.04$; Reunion × Late phase: $z = 2.02$; $P = 0.04$) (**Figure 1C**).

Animals isolated for 3 weeks had higher frequencies of locomotor activity at later phase of isolation than during other phases ($X^2 = 13.8$, $P = 0.03$; Basal × Late phase: $z = 2.52$; $P = 0.01$; Early × Late phase: $z = 2.36$; $P = 0.01$; Reunion × Late phase: $z = 2.36$; $P = 0.01$) (**Figure 1B**) and higher frequencies of scent-marking at late phase of isolation than after reunion ($X^2 = 8.19, P = 0.04$; Reunion × Late phase: $z = 2.02; P = 0.04$) (**Figure 1C**). Altogether these data indicate that marmosets had a higher expression of anxiety-related behaviors and a reduction of those related to attenuation of tension during social isolation, but we were not able to distinguish the effects between 1 and 3 week groups. We did not observe any other behavioral alteration regarding the other analyzed parameters, such as piloerection, attempts to contact and social-grooming. It is important to emphasize that all anxiety-related behaviors returned to their basal levels after family reunion, as well as the social grooming, which is an indicative of good adaptation. This result is confirmed by the cortisol levels measurement in the reunion phase, as described below.

SOCIAL ISOLATION INDUCED STRESS IN MARMOSETS

In order to infer about stress levels during social isolation, each session of behavioral monitoring was followed by cortisol levels measurement, for all animals under study (**Figure 1D**). The early phase of social deprivation caused significant increase in cortisol in both 1W ($P = 0.03$) and 3W groups ($P < 0.01$) when compared to their respective baseline levels. However, thereafter cortisol levels gradually decreased, reaching values between baseline and early isolation phases ($P = 0.057$). After family reunion, the cortisol levels of previously isolated marmosets became similar to their respective baseline values ($P > 0.05$).

Positive correlations were observed between anxiety-related behaviors and cortisol levels indicating that animals had indeed experienced stress. The cortisol level was significantly correlated with the frequencies of scent-marking during the early phase of social deprivation ($r = 0.53$, $P < 0.05$) and with increased locomotion ($r = 0.10$, $P < 0.05$) in the later phase of social deprivation.

HIPPOCAMPAL CELL PROLIFERATION AND NEUROGENESIS ARE IMPAIRED IN ISOLATED MARMOSETS

Cell counts of the entire hippocampus showed that the number of dentate gyrus progenitor cells incorporating BrdU was significantly decreased in animals that were socially isolated for 1 (3.71 ± 0.65 cells/section) or 3 (3.20 ± 0.87 cells/section) weeks, as compared to controls (6.04 ± 0.04 cells/section; $P = 0.04$, $F = 5.82$) (**Figure 2A**). In order to verify if this reduction was homogeneous throughout the rostro-caudal extent of the hippocampus, we further evaluated the distribution of BrdU-labeled cells in rostral, intermediate and caudal levels of hippocampus, as described in Methods. A One-Way ANOVA indicated that major BrdU-labeled cell reduction occurred in rostral levels, where 1W and 3W groups were statistically different from non-isolated animals ($P < 0.01, F = 14.98$). It also revealed that cell proliferation in the intermediate or caudal portions of dentate gyrus was similar between 1W, 3W, or CTR marmosets (**Figure 2B**). No interaction was found between groups and hippocampal levels (Two-Way ANOVA; $P = 0.26$, $F = 1.44$).

To assess whether newborn cells underwent neuronal fate, sections were double-stained for BrdU and DCX (a marker of immature neurons) (**Figure 2D**). Among the BrdU-labeled cells 43.7% co-localized to DCX in non-isolated controls, 35.5% in 1W group, and 19.2% in 3W group. These proportions pointed toward a decrease in the neurogenesis rate after 3 weeks isolation ($P = 0.05$, compared to Control and 1W groups). Taken together, these results suggest that marmosets that were socially isolated not only had a reduced number of dentate gyrus proliferating cells, but also fewer of these cells underwent a neuronal phenotype. Thus, once the number of newborn neurons can be estimated by applying the proportion of double-labeled cells over the total BrdU counting (**Figure 2C**), the neurogenesis impairment among the isolated primates can be clearly appreciated ($P = 0.0004$, $F = 39.17$). The late effects of neurogenesis impairment after 3 weeks of social isolation resulted in an overall reduced DCX immunoreactivity in the dentate gyrus, as illustrated in **Figure 2E**, which includes a representative image for each subject in all three groups.

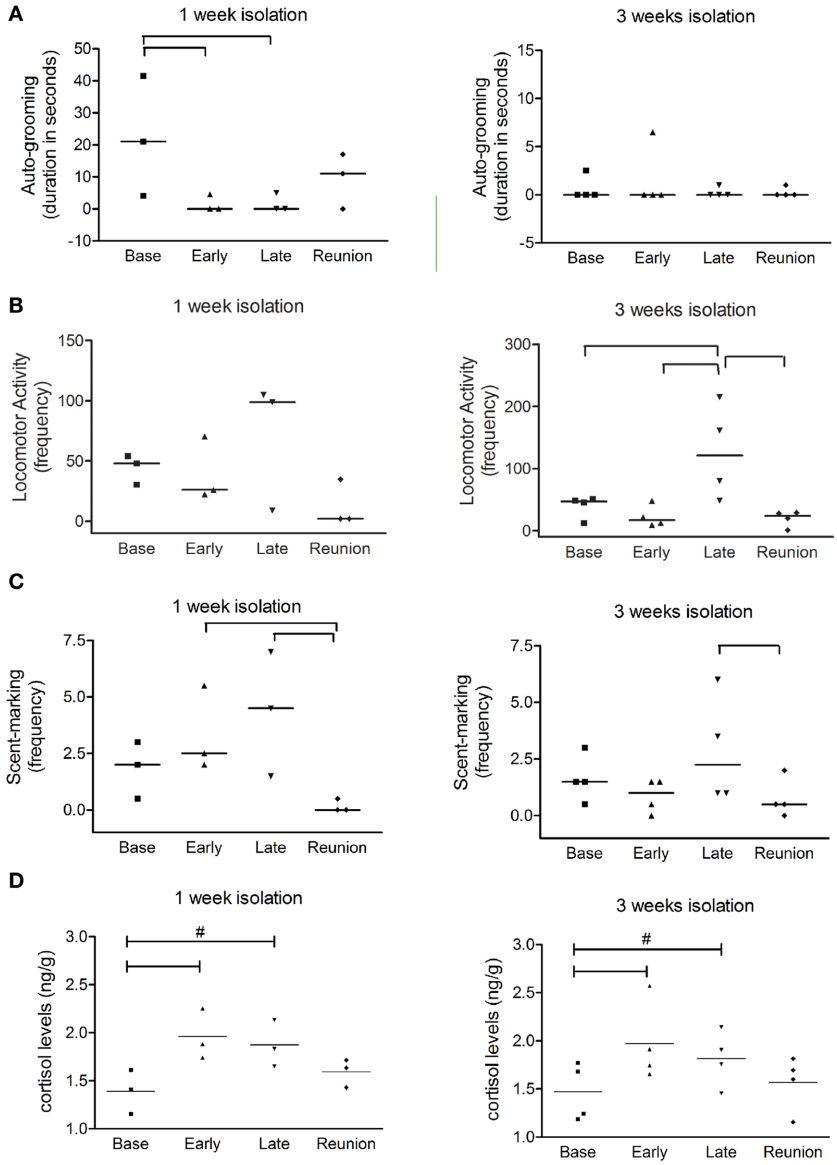


FIGURE 1 | Behavioral changes produced by isolation and cortisol measurement from fecal samples. Scatter plot graphs from behavioral observation data show **(A)** duration of auto-grooming (expressed in seconds); **(B)** frequencies of locomotor activity; and **(C)** frequencies of scent-marking during baseline phase (Base), two initial days of isolation (Early), two finals days of isolation (Late) and reunion for both 1 week and 3 weeks social isolation. In **(D)**, scatter plots represent normalized cortisol levels for marmosets isolated during one and 3 weeks; Horizontal lines indicate median values; brackets designate significant alterations between phases ($P < 0.05$). # Indicate tendency of variation between respective phase and baseline phase ($0.05 < P < 0.06$).

DISCUSSION

In summary, our results led us toward a possible cascade of events from social isolation to hippocampal substrates of anxiety-related behaviors during early adulthood of non-human primates. Here we found that socially isolated young marmosets had an increase in cortisol levels during the first days of isolation, concomitant with changes in affective states and subsequently a steady decrease in hippocampal neurogenesis.

It is well accepted that social isolation among rodent pups affects brain function, leading the organism to adapt to challenges in the environment. However, the long-term resulting stress can produce maladaptive behaviors and ultimately disease (Marsden et al., 2011; Pereda-Perez et al., 2013). Compared to rodents, social interactions in primates are even more complex. Some years ago it has been proposed that callitrichids when exposed to disturbing conditions do not overreact to it. This response was considered to be due to their familiar organization, which is very complex and stable over time, therefore such familiar relationship could have a buffering effect when these animals are exposed to stressful situations (Rylands, 1993). This however, is in opposition to recent findings (Barbosa and Mota, 2009; Yamaguchi et al., 2010). In spite of their complex organization

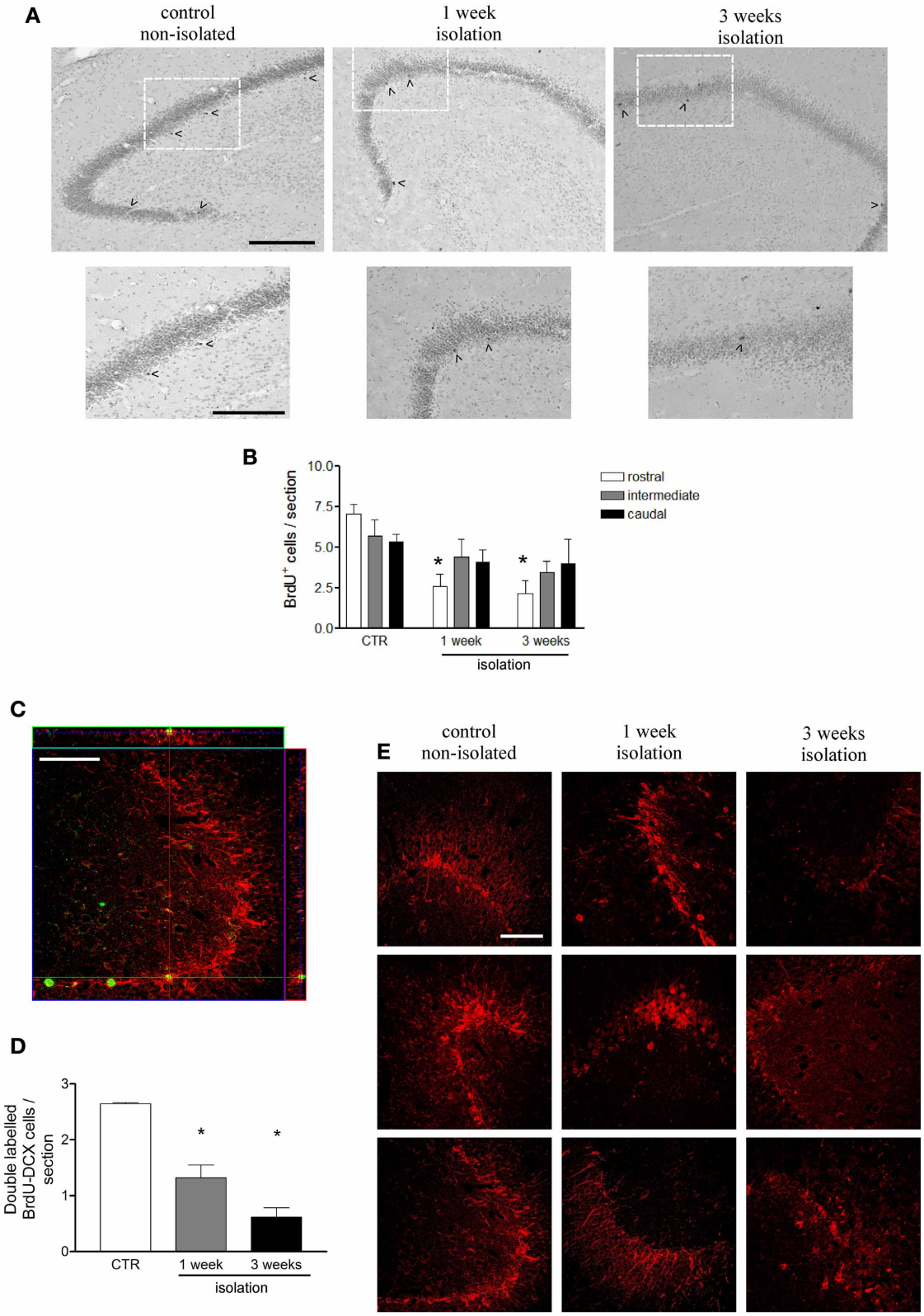


FIGURE 2 | Effects of social isolation on hippocampal cell proliferation. **(A)** Representative images of BrdU-positive cell labeling in dentate gyrus subgranular area in rostral region for each group in a wider view (upper panel) and in detail of the indicated area as showed in the lower panel; arrow heads indicate cells included in the counting; scale bar for upper panel = 200 μm; scale bar for lower panel = 150 μm. **(B)** graphical representation of the total number of BrdU-positive cells per section at three different rostral-caudal

(Continued)

FIGURE 2 | Continued
levels of the hippocampus for animals that were socially isolated for 1 week, 3 weeks, and non-isolated age matched controls (CTR). **(C)** Orthogonal Z-section of confocal microscope image of BrdU-DCX double-labeled cell in hippocampal dentate gyrus used to estimate the number of new cells undergoing a neuronal fate per section, showed in graph **(D)**. Figure **(E)** represents DCX-immunorreactivity density in dentate gyrus for each marmoset under control or 1 weeks or 3 weeks isolation periods. BrdU labels in green and DCX labels in red; scale bar: 50 μm. Data represented as mean ± standard error. * Indicates statistical significance between CTR and the respective group.

and stability, in captive conditions families of callitrichids are easily stressed even by their caregivers, showing higher stress levels during week daily care manipulations than during weekends (Barbosa and Mota, 2009). Thus, we believe that individual isolation of juvenile marmosets for 1 or 3 weeks could adequately model anxiety in non-human primates. Age is critical factor as it has been demonstrated that adult marmosets are insensitive to psychosocial stress paradigms that include a recovery period, as measured by neurogenesis in the dentate gyrus (Marlatt et al., 2011).

The potential impact of the reduced neurogenesis reported here is that it is occurring at a critical period, which is essential to make predictions regarding possible future mental disturbances. The transition between childhood and adulthood is characterized by high impulsivity, high plasticity and the development of complex behavioral repertoires, ultimately leading to stable behavioral patterns that guarantee successful survival (Spear, 2000).The transition from adolescence to adulthood varies in different primate species, yet it is well defined and displays many of the characteristic traits described in humans (Pereira and Altmann, 1985; Steinberg et al., 1989). Recent data based on computational estimates suggested that adult-generated neurons could be additionally incorporated to the population of existing (mature) granule cells leading to gradual increases in the total number of neurons (Aimone et al., 2009). Thus, stressing factors, applied during this critical period, as used in our current study may not only affect neuron birth but also the structural organization of the granule cell layer. We do not have data to test this hypothesis thus future studies should address these specific questions using hippocampal-dependent tasks. Our current results demonstrate that rostral hippocampus levels are the most affected in terms of cell proliferation. Similarly it has recently been reported that depressed adult female cynomolgus monkeys also have hippocampal atrophy in rostral but not caudal portions (Willard et al., 2009). It is noteworthy that previous studies consider that posterior hippocampus in humans (or primates) activation may reflect contextual fear encoding, whereas the engagement of rostral regions during later phases of acquisition may reflect the emotional expression of that fear (Bannerman et al., 2003; Fanselow and Dong, 2010).

The greater manifestation of anxiety-related behaviors right after social deprivation was concomitant with increased cortisol levels, as previously demonstrated by others (Smith et al., 1998; Marlatt et al., 2011). Similarly to our results, these authors reported increases in cortisol levels 48 h after isolation with recovery to basal levels when animals had returned to their family. In the current study, past some days of isolation, cortisol slightly decreased until remaining constant between 1 and 3 weeks after familial separation. On the other hand, unlike cortisol levels, anxiety-related behaviors continuously augmented during late phase of isolation. At first, animals that were socially isolated for 1 week showed signs of increased distress with high number of scent marking and high levels of cortisol, similarly to previous findings (Laudenslager et al., 1990, 1995; Norcross and Newman, 1999; Rukstalis and French, 2005). As the social isolation persists, some authors have reported a drop in physiological indicators of stress, indicative of habituation (Honess and Marin, 2006). Our current results on the other hand indicate that even when individual isolation took longer, animals still had a high number of scent marking and locomotor activities, having these anxiety-related behaviors returned to basal levels only after familiar reunion. At the final days in the 1 week isolation group, cortisol level became stable, but not anxiety-like behaviors, indicating here dissociation between hormones and behaviors. Similar findings were described in others studies with different species (Norcross and Newman, 1999; Hennessy et al., 2008) that in turn show the importance of measuring both indicators for more accurate results.

Direct modulation of dentate gyrus cell population by corticoids has been considered an important mechanism through which stressors reduce hippocampal neurogenesis (Mirescu and Gould, 2006). Indeed, already in the early phase of isolation animals had significantly increased cortisol levels, a hormone involved in mediating the effects of stress on neurogenesis. The hippocampus contains glucocorticoid and mineralocorticoid receptors, besides being an important structure in the modulation of stressful responses (for a review see McEwen, 1999). Accordingly, a recent study has demonstrated that early life stress in primate infants leads in adolescence to mild reductions in the expression of mineralocorticoid and glucocorticoid receptor genes in the hippocampus (Arabadzisz et al., 2010). As concluded by these last authors, it is unlikely that these reductions are only acute mediators of the long-term effects of early life stress. Indeed, increases in the corticosterone levels during social isolation in marmosets did alter the number of proliferating cells in the dentate gyrus (Marlatt et al., 2011).

Decreased hippocampal neurogenesis plays a role in depression and anxiety-related behaviors (Wong and Licinio, 2004; Cryan and Holmes, 2005). Nevertheless, it does not establish a direct link between both since inhibition of neurogenesis in mice did not seem to trigger a depressive or anxious behavioral phenotype, but the full capacity of giving rise to new neurons may be crucial for antidepressants to take effect (Santarelli et al., 2003; Balu and Lucki, 2009). Accordingly, as shown here, inhibition of neurogenesis was exacerbated as a time-dependent outcome of social isolation-induced stress, even when cortisol levels were steady (from 1 until 3 weeks of separation). Some reports have indicated that decreased neurogenesis can keep on even after re-establishment of standard cortisol levels (Malberg and Duman, 2003; Mirescu et al., 2004). Thus, the hypothesis that high cortisol

levels were not necessary to maintain neurogenesis suppressed is supported by the present results. From this work we can suggest that separation-induced stress affects proliferation of progenitor cells, as well as neuronal fate of newborn cells in the dentate gyrus.

Our current interpretations are limited by the number of animals and current experimental conditions. In rodent brain, profound differences have been reported between males and females in response to early life stress, particularly on neurogenesis (Oomen et al., 2009; Negrigo et al., 2011; Korosi et al., 2012; Lima et al., 2014). In the current study, we showed that gender does not interfere in the dentate gyrus neurogenesis rate (Marlatt et al., 2011), and thus we have chosen to pool male and female animals. Unfortunately, here we could not provide a direct correlation between the cortisol measurements and the dentate proliferating rate given these results were obtained from different sets of animals. Yet, behavioral results did not differ between the two sets of animals and due to the current experimental settings we were able to avoid the death of seven animals.

CONCLUSION

In non-human primates, social relationships comprise an important aspect of the juvenile's environment and are crucial for the development of normal behavior. Though these behavioral abnormalities have been somewhat characterized, the mechanisms underlying these events are still elusive. Our data is consistent with the notion that social deprivation leads to stress (indicated by higher cortisol levels), producing anxiety-like behaviors. We showed that some of the consequences of the stressful condition, such as reduction of neurogenesis, have a slower response profile. Our data might be relevant for the understanding of the pathophysiological conditions that ensue after episodes of social separation in humans.

AUTHORS' CONTRIBUTIONS

Simone M. Cinini was responsible for animal care at UNIFESP facility, participated in the study design, performed immunohistochemistry, BrdU cell counts, and drafted the manuscript. Gabriela F. Barnabe helped in study design, performed immunofluorescence, carried out confocal microscopy, statistical analysis, and drafted the manuscript. Nicole Galvão-Coelho conducted all behavioral and cortisol measurement experiments and its analysis. Magda A. de Medeiros conceived of the study and helped with animal care. Patrícia Perez-Mendes helped with animal care and participated in the study design. Maria B. C. Sousa designed the behavioral and cortisol experiments, also contributed with data interpretation. Luciene Covolan participated in experimental design, conducted cell count acquisition and interpretation, drafted the manuscript. Luiz E. Mello conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

FAPESP, CNPq and Instituto do Milênio de Bioengenharia Tecidual; Simone M. Cinini was a CAPES fellow, Gabriela F. Barnabe was a FAPESP fellow. We also thank Ivone de Paulo for technical support and Beatriz Longo for providing reagents, both from Department of Physiology, Universidade Federal de São Paulo, São Paulo, Brazil. We are grateful to Carolina and Elizabeth for operating the confocal microscope.

REFERENCES

Abraham, I., Juhasz, G., Kekesi, K. A., and Kovacs, K. J. (1998). Corticosterone peak is responsible for stress-induced elevation of glutamate in the hippocampus. *Stress* 2, 171–181. doi: 10.3109/10253899809167281

Aimone, J. B., Wiles, J., and Gage, F. H. (2009). Computational influence of adult neurogenesis on memory encoding. *Neuron* 61, 187–202. doi: 10.1016/j.neuron.2008.11.026

Arabadzisz, D., Diaz-Heijtz, R., Knuesel, I., Weber, E., Pilloud, S., Dettling, A. C., et al. (2010). Primate early life stress leads to long-term mild hippocampal decreases in corticosteroid receptor expression. *Biol. Psychiatry* 67, 1106–1109. doi: 10.1016/j.biopsych.2009.12.016

Balu, D. T., and Lucki, I. (2009). Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. *Neurosci. Biobehav. Rev.* 33, 232–252. doi: 10.1016/j.neubiorev.2008.08.007

Bannerman, D. M., Grubb, M., Deacon, R. M., Yee, B. K., Feldon, J., and Rawlins, J. N. (2003). Ventral hippocampal lesions affect anxiety but not spatial learning. *Behav. Brain Res.* 139, 197–213. doi: 10.1016/S0166-4328(02)00268-1

Barbosa, M. N., and Mota, M. T. (2009). Behavioral and hormonal response of common marmosets, Callithrix jacchus, to two environmental conditions. *Primates* 50, 253–260. doi: 10.1007/s10329-009-0137-2

Barros, M., and Tomaz, C. (2002). Non-human primate models for investigating fear and anxiety. *Neurosci. Biobehav. Rev.* 26, 187–201. doi: 10.1016/S0149-7634(01)00064-1

Bunk, E. C., Stelzer, S., Hermann, S., Schafers, M., Schlatt, S., and Schwamborn, J. C. (2011). Cellular organization of adult neurogenesis in the Common Marmoset. *Aging Cell* 10, 28–38. doi: 10.1111/j.1474-9726.2010.00639.x

Cryan, J. F., and Holmes, A. (2005). The ascent of mouse: advances in modelling human depression and anxiety. *Nat. Rev. Drug Discov.* 4, 775–790. doi: 10.1038/nrd1825

Dettling, A. C., Feldon, J., and Pryce, C. R. (2002a). Early deprivation and behavioral and physiological responses to social separation/novelty in the marmoset. *Pharmacol. Biochem. Behav.* 73, 259–269. doi: 10.1016/S0091-3057(02)00785-2

Dettling, A. C., Feldon, J., and Pryce, C. R. (2002b). Repeated parental deprivation in the infant common marmoset (Callithrix jacchus, primates) and analysis of its effects on early development. *Biol. Psychiatry* 52, 1037–1046. doi: 10.1016/S0006-3223(02)01460-9

Devries, A. C., Craft, T. K., Glasper, E. R., Neigh, G. N., and Alexander, J. K. (2007). 2006 Curt P. Richter award winner: social influences on stress responses and health. *Psychoneuroendocrinology* 32, 587–603. doi: 10.1016/j.psyneuen.2007.04.007

Duman, R. S., and Monteggia, L. M. (2006). A neurotrophic model for stress-related mood disorders. *Biol. Psychiatry* 59, 1116–1127. doi: 10.1016/j.biopsych.2006.02.013

Fanselow, M. S., and Dong, H. W. (2010). Are the dorsal and ventral hippocampus functionally distinct structures? *Neuron* 65, 7–19. doi: 10.1016/j.neuron.2009.11.031

Gould, E., and Gross, C. G. (2002). Neurogenesis in adult mammals: some progress and problems. *J. Neurosci.* 22, 619–623.

Gould, E., McEwen, B. S., Tanapat, P., Galea, L. A., and Fuchs, E. (1997). Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J. Neurosci.* 17, 2492–2498.

Gould, E., Tanapat, P., McEwen, B. S., Flugge, G., and Fuchs, E. (1998). Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. *Proc. Natl. Acad. Sci. U.S.A.* 95, 3168–3171. doi: 10.1073/pnas.95.6.3168

Hennessy, M. B., Zate, R., and Maken, D. S. (2008). Social buffering of the cortisol response of adult female guinea pigs. *Physiol. Behav.* 93, 883–888. doi: 10.1016/j.physbeh.2007.12.005

Honess, P. E., and Marin, C. M. (2006). Behavioural and physiological aspects of stress and aggression in nonhuman primates. *Neurosci. Biobehav. Rev.* 30, 390–412. doi: 10.1016/j.neubiorev.2005.04.003

Jacobsen, J. P., and Mork, A. (2006). Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. *Brain Res.* 1110, 221–225. doi: 10.1016/j.brainres.2006.06.077

Karten, Y. J., Olariu, A., and Cameron, H. A. (2005). Stress in early life inhibits neurogenesis in adulthood. *Trends Neurosci.* 28, 171–172. doi: 10.1016/j.tins.2005.01.009

Kee, N., Teixeira, C. M., Wang, A. H., and Frankland, P. W. (2007). Preferential incorporation of adult-generated granule cells into spatial memory networks in the dentate gyrus. *Nat. Neurosci.* 10, 355–362. doi: 10.1038/nn1847

Koo, J. W., and Duman, R. S. (2008). IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc. Natl. Acad. Sci. U.S.A.* 105, 751–756. doi: 10.1073/pnas.0708092105

Korosi, A., Naninck, E. F., Oomen, C. A., Schouten, M., Krugers, H., Fitzsimons, C., et al. (2012). Early-life stress mediated modulation of adult neurogenesis and behavior. *Behav. Brain Res.* 227, 400–409. doi: 10.1016/j.bbr.2011.07.037

Laudenslager, M. L., Boccia, M. L., Berger, C. L., Gennaro-Ruggles, M. M., McFerran, B., and Reite, M. L. (1995). Total cortisol, free cortisol, and growth hormone associated with brief social separation experiences in young macaques. *Dev. Psychobiol.* 28, 199–211. doi: 10.1002/dev.420280402

Laudenslager, M. L., Held, P. E., Boccia, M. L., Reite, M. L., and Cohen, J. J. (1990). Behavioral and immunological consequences of brief mother-infant separation: a species comparison. *Dev. Psychobiol.* 23, 247–264. doi: 10.1002/dev.420230305

Lima, M., Malheiros, J., Negrigo, A., Tescarollo, F., Medeiros, M., Suchecki, D., et al. (2014). Sex-related long-term behavioral and hippocampal cellular alterations after nociceptive stimulation throughout postnatal development in rats. *Neuropharmacology* 77, 268–276. doi: 10.1016/j.neuropharm.2013.10.007

Malberg, J. E., and Duman, R. S. (2003). Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology* 28, 1562–1571. doi: 10.1038/sj.npp.1300234

Marlatt, M. W., Philippens, I., Manders, E., Czeh, B., Joels, M., Krugers, H., et al. (2011). Distinct structural plasticity in the hippocampus and amygdala of the middle-aged common marmoset (Callithrix jacchus). *Exp. Neurol.* 230, 291–301. doi: 10.1016/j.expneurol.2011.05.008

Marsden, C. A., King, M. V., and Fone, K. C. (2011). Influence of social isolation in the rat on serotonergic function and memory–relevance to models of schizophrenia and the role of 5-HT(6) receptors. *Neuropharmacology* 61, 400–407. doi: 10.1016/j.neuropharm.2011.03.003

McEwen, B. S. (1999). Stress and hippocampal plasticity. *Annu. Rev. Neurosci.* 22, 105–122. doi: 10.1146/annurev.neuro.22.1.105

Mirescu, C., and Gould, E. (2006). Stress and adult neurogenesis. *Hippocampus* 16, 233–238. doi: 10.1002/hipo.20155

Mirescu, C., Peters, J. D., and Gould, E. (2004). Early life experience alters response of adult neurogenesis to stress. *Nat. Neurosci.* 7, 841–846. doi: 10.1038/nn1290

Munro, C., and Stabenfeldt, G. (1984). Development of a microtitre plate enzyme immunoassay for the determination of progesterone. *J. Endocrinol.* 101, 41–49. doi: 10.1677/joe.0.1010041

Negrigo, A., Medeiros, M., Guinsburg, R., and Covolan, L. (2011). Long-term gender behavioral vulnerability after nociceptive neonatal formalin stimulation in rats. *Neurosci. Lett.* 490, 196–199. doi: 10.1016/j.neulet.2010.12.050

Norcross, J. L., and Newman, J. D. (1999). Effects of separation and novelty on distress vocalizations and cortisol in the common marmoset (Callithrix jacchus). *Am. J. Primatol.* 47, 209–222. doi: 10.1002/(SICI)1098-2345(1999)47:3<209::AID-AJP3>3.0.CO;2-0

Oomen, C. A., Girardi, C. E., Cahyadi, R., Verbeek, E. C., Krugers, H., Joels, M., et al. (2009). Opposite effects of early maternal deprivation on neurogenesis in male versus female rats. *PLoS ONE* 4:e3675. doi: 10.1371/journal.pone.0003675

Pereda-Perez, I., Popovic, N., Otalora, B. B., Popovic, M., Madrid, J. A., Rol, M. A., et al. (2013). Long-term social isolation in the adulthood results in CA1 shrinkage and cognitive impairment. *Neurobiol. Learn. Mem.* 106, 31–39. doi: 10.1016/j.nlm.2013.07.004

Pereira, M., Altmann, J. (1985). *Nonhuman Primate Models for Human Growth and Development.* New York, NY: Blank Liss.

Raminelli, J., Sousa, M. B. C., Cunha, M. S., and Barbosa, M. F. V. (2003). Morning and afternoon patterns of fecal cortisol excretion among reproductive and non-reproductive male and female common marmosets, callithrix jacchus. *Biol. Rhythm Res.* 32, 159–167. doi: 10.1076/brhm.32.2.159.1357

Rizzi, S., Bianchi, P., Guidi, S., Ciani, E., and Bartesaghi, R. (2007). Neonatal isolation impairs neurogenesis in the dentate gyrus of the guinea pig. *Hippocampus* 17, 78–91. doi: 10.1002/hipo.20247

Rosenblum, L. A., and Andrews, M. W. (1994). Influences of environmental demand on maternal behavior and infant development. *Acta Paediatr. Suppl.* 397, 57–63. doi: 10.1111/j.1651-2227.1994.tb13266.x

Rukstalis, M., and French, J. A. (2005). Vocal buffering of the stress response: exposure to conspecific vocalizations moderates urinary cortisol excretion in isolated marmosets. *Horm. Behav.* 47, 1–7. doi: 10.1016/j.yhbeh.2004.09.004

Rylands, A. (1993). *Marmosets and Tamarins: Systematics, Behaviour, and Ecology*. New York, NY: Oxford University Press.

Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., et al. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301, 805–809. doi: 10.1126/science.1083328

Smith, T. E., McGreer-Whitworth, B., and French, J. A. (1998). Close proximity of the heterosexual partner reduces the physiological and behavioral consequences of novel-cage housing in black tufted-ear marmosets (Callithrix kuhli). *Horm. Behav.* 34, 211–222. doi: 10.1006/hbeh.1998.1469

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. *Neurosci. Biobehav. Rev.* 24, 417–463. doi: 10.1016/S0149-7634(00)00014-2

Steinberg, L., Adams, G., Montemayor R., and Gullotta T. (1989). *Biology of Adolescent Behavior and Development.* Thousand Oaks: Sage Publications.

Stephan, H., Baron, G., and Schwerdtfeger, W. K. (1980). *The Brain of the Common Marmoset (Callithrix Jacchus) A Stereotaxic Atlas.* Berlin, NY: Springer-Verlag. doi: 10.1007/978-3-642-67491-4

Stranahan, A. M., Khalil, D., and Gould, E. (2006). Social isolation delays the positive effects of running on adult neurogenesis. *Nat. Neurosci.* 9, 526–533. doi: 10.1038/nn1668

Van Praag, H., Schinder, A. F., Christie, B. R., Toni, N., Palmer, T. D., and Gage, F. H. (2002). Functional neurogenesis in the adult hippocampus. *Nature* 415, 1030–1034. doi: 10.1038/4151030a

Willard, S. L., Friedman, D. P., Henkel, C. K., and Shively, C. A. (2009). Anterior hippocampal volume is reduced in behaviorally depressed female cynomolgus macaques. *Psychoneuroendocrinology* 34, 1469–1475. doi: 10.1016/j.psyneuen.2009.04.022

Wittig, R. M., Crockford, C., Lehmann, J., Whitten, P. L., Seyfarth, R. M., and Cheney, D. L. (2008). Focused grooming networks and stress alleviation in wild female baboons. *Horm. Behav.* 54, 170–177. doi: 10.1016/j.yhbeh.2008.02.009

Wong, E. Y., and Herbert, J. (2005). Roles of mineralocorticoid and glucocorticoid receptors in the regulation of progenitor proliferation in the adult hippocampus. *Eur. J. Neurosci.* 22, 785–792. doi: 10.1111/j.1460-9568.2005.04277.x

Wong, E. Y., and Herbert, J. (2006). Raised circulating corticosterone inhibits neuronal differentiation of progenitor cells in the adult hippocampus. *Neuroscience* 137, 83–92. doi: 10.1016/j.neuroscience.2005.08.073

Wong, M. L., and Licinio, J. (2004). From monoamines to genomic targets: a paradigm shift for drug discovery in depression. *Nat. Rev. Drug Discov.* 3, 136–151. doi: 10.1038/nrd1303

Yamaguchi, C., Izumi, A., and Nakamura, K. (2010). Time course of vocal modulation during isolation in common marmosets (Callithrix jacchus). *Am. J. Primatol.* 72, 681–688. doi: 10.1002/ajp.20824

Ziegler, T. E., Scheffler, G., and Snowdon, C. T. (1995). The relationship of cortisol levels to social environment and reproductive functioning in female cotton-top tamarins, Saguinus oedipus. *Horm. Behav.* 29, 407–424. doi: 10.1006/hbeh.1995.1028

Ziegler, T. E., Scheffler, G., Wittwer, D. J., Schultz-Darken, N., Snowdon, C. T., and Abbott, D. H. (1996). Metabolism of reproductive steroids during the ovarian cycle in two species of callitrichids, Saguinus oedipus and Callithrix jacchus, and estimation of the ovulatory period from fecal steroids. *Biol. Reprod.* 54, 91–99. doi: 10.1095/biolreprod54.1.91

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 01 December 2013; paper pending published: 05 January 2014; accepted: 19 February 2014; published online: 27 March 2014.

Citation: Cinini SM, Barnabe GF, Galvão-Coelho N, de Medeiros MA, Perez-Mendes P, Sousa MBC, Covolan L and Mello LE (2014) Social isolation disrupts hippocampal neurogenesis in young non-human primates. Front. Neurosci. ***8****:45. doi: 10.3389/fnins.2014.00045*

This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Neuroscience.

frontiers in
ENDOCRINOLOGY

MINI REVIEW ARTICLE
published: 26 February 2014
doi: 10.3389/fendo.2014.00017

Mother–pup interactions: rodents and humans

Aldo B. Lucion[1]* and Maria Cátira Bortolini[2]

[1] *Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[2] *Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*

Edited by:
Nikolaos P. Daskalakis, Icahn School of Medicine at Mount Sinai, USA

Reviewed by:
Regina M. Sullivan, New York University School of Medicine, USA
Antonios Stamatakis, National and Kapodistrian University of Athens, Greece

****Correspondence:***
Aldo B. Lucion, Departamento de Fisiologia, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite 500, Porto Alegre, Rio Grande do Sul 90050-170, Brazil
e-mail: alucion@ufrgs.br

In order to survive after birth, mammalian infants need a caretaker, usually the mother. Several behavioral strategies have evolved to guarantee the transition from a period of intense caregiving to offspring independence. Here, we examine a selection of literature on the genetic, epigenetic, physiological, and behavioral factors relating to development and mother–infant interactions. We intend to show the utility of comparisons between rodent and human models for deepening knowledge regarding this key relationship. Particular attention is paid to the following factors: the distinct developmental stages of the mother–pup relationship as relating to behavior; examples of key genetic components of mammalian mother–infant interactions, specifically those coding for the hormones oxytocin and vasopressin; and the possible functions of gene imprinting in mediating interactions between genetics and environment in the mother–infant relationship. As early mother–infant attachment seems to establish the basic parameters for later social interactions, ongoing investigations in this area are essential. We propose the importance of interdisciplinary collaboration in order to better understand the network of genes, gene regulation, neuropeptide action, physiological processes, and feedback loops essential to understand the complex behaviors of mother–infant interaction.

Keywords: mother–infant attachment, oxytocin receptor gene, innate behavior, social behaviors, behavior synchrony

INTRODUCTION

A social interaction is a dynamic process, composed not of a single set of behaviors at a given moment, but rather of the relationship of a series of behaviors over time. The exchange of information between subjects progressively modifies them. Due to its fundamental role in development, the effects of mother–infant interaction last throughout life.

In this review, we examine research on behavioral strategies that have evolved to guarantee the transition from intense caregiving to offspring independence. Infants display preference for social stimuli, suggesting evolutionary pressure in favor of these stimuli. In humans, the mechanisms involved in mother–infant attachment are still insufficiently understood. Nevertheless, a few areas of research are offering promising insight into the determination of this complex interaction. First, we examine studies that demonstrate olfactory stimuli from the mother to be crucial for infant latching and suckling, a determining factor in mother–infant attachment, feeding, and infant survival (1). Next we offer case studies on the genetic basis of two neurotransmitters, oxytocin (OXT) and vasopressin, which facilitate social recognition by acting on several brain regions, such as the olfactory bulb. In placental mammalians, both OXT and vasopressin are highly involved in key social interactions, including social recognition, pair bonding, and parental behavior. Finally, we consider the possible role of imprinting of candidate genes in determining features of the mother–infant interaction. These fundamental early-life physiological, genetic, and epigenetic processes suggest why, in many ways, mother–infant attachment establishes basic parameters for later social interactions.

THE ACTORS: MOTHER AND INFANT

In rodents and humans, mother–pup interaction involves two essential types of actors: the mother and the infant, or several infants at the same time. Many other elements may be involved and/or affect this interaction: the father, the environment, siblings, and predators (2). However, here we focus on mother and infant. This particular relationship involves two organisms in different developmental stages. In this sense, it is an asymmetric relationship.

Moreover, this interaction occurs over time and is constantly adjusted, especially due to the fact that one organism – the infant – changes dramatically over the course of the relationship. Motherhood also involves behavioral changes, such as long-term memory of mothering mediated by OXT (3) and selectively reduced stress responses (4, 5) and anxiety (6).

Animal models have been developed to analyze the effects of maternal deprivation on the development of offspring (1, 7). Due to the complexity of the neuroendocrine and behavioral peculiarities of this relationship (8), the alteration of one element can induce changes of several orders, many of them not directly related to the original disturbance. Two changing organisms interact in a complex and balanced dynamic (9). Attempts to replace a missing mother or to repair an affection poor childhood (10) are rather difficult. This difficulty probably depends on the fact that there are at least two organisms that are necessarily changed by the interaction.

A mother–infant interaction is unique and depends on many factors (11). For instance, even in large litters, as in rats, mothers show different maternal behaviors toward male and female offspring (12). There are several elements that should be taken

into consideration: the genetic background of the mother and the infant; the past experiences of the mother and the prenatal environment of the infant; the neuroendocrine profile of mother and infant; the stress copying style of the mother; and the environment (social and resource availability), among others. To consider such a wide variety of factors is a challenging task. As such, it is essential for the neuroscientist to collaborate with geneticists and physiologists in order to understand the origins of relevant psychopathologies (13).

TIME: LIFECYCLES AND THE DEVELOPMENT OF THE MOTHER–INFANT INTERACTION

The mother–infant interaction depends upon several elements to establish attachment: the product of genes such as hormones and/or neurotransmitters (e.g., OXT, vasopressin, serotonin, noradrenaline), cytokines (14), opioids (15, 16), and dopamine (17), as well as epigenetic (18) and environmental factors (19). In mammals, the uterus is the first site of mother–infant interaction (20, 21). In both rodents and humans, pregnant females undergo physiological and behavioral changes to support gestation (22); and several studies have shown that the infant responds to the mother from within the uterus (23, 24). During the neonatal period, mother–infant attachment develops and filial bonds form (25, 26). This period seems crucial for behavioral development and the establishment of the infant's stress coping style (27). The mother's behavior conveys important information about the environment in which the newborn will live (28), through sensory stimulation of pups by the mother (29). Additionally, the mother–infant interaction involves the behavior of the mother in synchrony with that of the infant (30). Predictability of mother's behavior and thus a secure attachment is crucial for the neurocognitive development of offspring (31).

Consistent demonstrations in rats have shown that mother–infant interaction is based on infant learning processes, expressed by increased CREB phosphorylation (19) and the development of a memory of the mother (4). This is a simple mechanism involving the noradrenergic activity of the locus coeruleus on the olfactory bulb, where the memory of the mother begins (32). Two aspects seem important: the physiology of the locus coeruleus, whose activity is less inhibited by its noradrenergic inhibitory autoreceptors during infancy than later in life; and plastic changes induced by the learning process. Environmental stimuli (odor and varying forms of body contact with the mother) act on the neural system of the infant, which is particularly receptive and prepared to react to those stimuli; the system has a very low threshold during this specific period. All of these interactions exert long-lasting impact on the development of the infant.

ORIGINS OF THE MOTHER–INFANT INTERACTION: NATURE VERSUS NURTURE

The innateness of behaviors is controversial. Some authors suggest that behaviors presented by an individual early in life are essentially based on the genome and are innate (2, 33, 34). The behaviors would be already "wired" and triggered by specific stimuli, during sensitive period (35). However, consistent studies have shown that mother–infant interaction involves learning mechanisms (4, 11, 36). Physical contact is crucial to establish bonding. In rats, the mother's licking induces olfactory memory that would be the basic mechanism of the mother–infant interaction. This interaction is by its nature dynamic and induces permanent reorganizations of the neuroendocrine systems and behaviors of both mother and offspring. Moreover, the system is open to the environment and can be affected by environmental stimuli in several ways, inducing short-term and/or long-term changes (37).

For the mother–infant interaction, it seems plasticity is not exclusively related to specific learning periods, as in other functions. For comparison, we might look at the classic neurophysiological example of ocular dominance columns in the visual system (38). These columns exist in rudimentary form at birth, but need to be stimulated by specific stimuli during a set period to fully develop their function. In the case of the mother–infant interaction, we would predict that plasticity is not time-limited as in the development of ocular dominance columns, but rather is cumulative. The constant learning processes that occur over time in the mother–infant relationship provoke related plastic changes. Thus the development of this interaction and the consequent attachment would not be an all-or-nothing phenomenon. Nevertheless, there are periods of greater impact, such as birth and the initial contact directly following birth.

In order to conceptualize the origins of the mother–infant interaction, let us consider an analogy between that interaction and the rhythmic activity of locomotion. Although these two behaviors are very different in complexity, it is interesting to imagine a similar basic working model. In this model, for locomotion, the output of a hierarchical neural system is the result of the activity of several circuits working in coordination. This system could be triggered by specific environmental stimuli. The generation and fine control of locomotion involves a basic central pattern generator in the spinal cord modulated by several other hierarchical disposed structures (39). In the case of the mother–infant interaction, this model would imply that several "layers" of interconnected neuroendocrine structures are the basis for behavior. The central pattern generator would first need to be properly triggered and then modulated by the interaction that it generates. Feedback is essential in social behaviors; the interaction generated by a behavior can be modulated by the response of the partner. However, in its very early stage, a supposed innate "central pattern generator" would require feed-forward action. Based on this model of the development of the mother–infant interaction, the functional characteristics of the infant locus coeruleus during the period after birth would be an innate system, prepared to be stimulated by the mother.

Animals are born with the capacity to develop complex behaviors (40), but not with behaviors *per se*: an infant must build them. The constructive nature of this process is analogous to those of other systems; for instance, in cognitive functions, an animal builds internally a unique sensation of a particular stimulus.

ROLE OF GENES

It is well known that behavioral traits such as parent–infant bonding are the product of a sophisticated combination of genetic, environmental, and epigenetic factors. The adequate interplay of these elements, both over the course of the evolutionary trajectory of a species, and during the development and life of an individual,

ensures a behavioral repertoire sufficient for survival and reproductive success. Identifying the contribution of genetic factors is the starting point for understanding the formation and expression of a particular phenotype. Although there is still much to learn regarding the genes implicated in specific mammalian behavioral phenotypes, a number of them have been characterized. This is the case of the genes for the OXT and arginine vasopressin (AVP) nonapeptides, which act as hormones and neurotransmitters.

It has been proposed that *OXT* and *AVP*-like genes originated due to a tandem duplication of an ancestral gene ~500 million years. This event would explain the presence of at least one homolog in jawed vertebrates (41), although many aspects of the origin and evolution of these genes remain unknown (42). In humans, *OXT* and *AVP* are located on the same chromosome, at 20p13, but with opposite orientation (43).

In placental mammalians, OXT and AVP nonapeptides differ by two amino acids at positions 3 and 8 (OXT: Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–Gly, and AVP: Cys–Tyr–Phe–Gln–Asn–Cys–Pro–Arg–Gly) and play an important role during lactation, uterine contractions, offspring care, stress-response, and other reproductive and social behavioral traits (41, 44, 45).

Although they boast a wide spectrum of activity, OXT and AVP are best known for their contribution to the regulation of social behaviors. Pedersen and Prange (46) were pioneers in showing that artificial administration of OXT promoted maternal nurturing in virgin and steroid-primed female rats, indicating that this hormone plays a specific role in the mother–pup interaction. Other authors replicated and extended this initial study in several other mammalian species, including humans [(45) and references therein]. For example, Bosch (47) showed that the OXT and AVP systems are key factors regulating maternal aggression and anxiety in rodents, while Apter-Levi et al. (48) found that both OXT and AVP are associated with human maternal and paternal behaviors in distinct, sometimes overlapping, ways.

Both nonapeptides are highly conserved in placental mammalians, with a few rare exceptions. For example, Lee et al. (49) showed that five New World monkey species had fixed a T > C mutation changing leucine to proline at position 8 of OXT. Other authors described an *AVP* mutation leading to a substitution of arginine for lysine at position 8 in opossums and pigs, but the phenotypic consequences of these changes remain unknown (42, 50). It is noteworthy that changes at amino acid chain position 8 characterize some non-placental AVP/OXT-like nonapeptides such as mesotocin in marsupials (51, 52).

The oxytocin receptor (OXTR) and the three AVP receptors (A2, V1a, and V1b) are encoded by the *OXTR, AVPA2*, *AVPR1a*, and *AVPR1b* genes located on human chromosomes 3, X, 12, and 1, respectively. These receptors, which use G-proteins as transducer signals across the cell membranes, have seven transmembrane domains, four extracellular, and four intracellular domains. Both OXT and AVP can bind to each one of the four above-indicated receptors, but not with the same affinity, since the most important binding sites differ slightly among the four receptors (53, 54).

Oxytocin and AVP are produced in greatest quantity in the hypothalamus, but their activity outside of the brain depends on their interaction with the receptors produced in various organs and tissues. For instance, the presence of OXTR in the uterus and mammary glands guarantee uterine contraction and milk ejection (42, 55). Hammock and Levitt (56), on the other hand, showed that OXTR is also found in several tissues of the mouse embryo, such as the adrenal glands, brown adipose tissue, and the oronasal cavity.

In contrast to the observed *OXT* and *AVP* gene conservation, hundreds of mutations in the *OXTR*, *AVPR2*, *AVPR1a*, and *AVPR1b* genes have been reported in placental mammalians. For instance, in pigs, *AVPR1a* and *AVPR1b* mutations have been associated with stress and aggressive behavior through their connection with ACTH, an important component of the hypothalamic–pituitary–adrenal (HPA) axis (57, 58). In humans, polymorphisms in these receptor genes have also been linked to attachment, generosity, and pair bonding behaviors (59).

These examples illustrate that variations in candidate genes can be related to some intra and inter-specific mammalian behaviors. As seen above, however, other non-genetic factors have important roles in determining behavioral phenotype; this may be the case for the differential care of offspring between father and mother. Although males and females can have the same alleles in genes related to pup-care behaviors, only ~10% of mammalian species show significant male parental care, the majority of which are primates, carnivores, and rodents (60). This suggests that some social behavior candidate genes, located on autosomal chromosomes, can be subject to the imprinting process, an epigenetic phenomenon where changes in gene function can be heritable despite the fact that no alteration in the DNA sequence is detected. If genetic variation in key peptides and their receptors is understood as a first step, the imprinting of genes can be understood as a second step in determining the features of the mother–infant interaction. Below, we will see some examples of imprinted genes.

IMPRINTED GENES

The term "imprinted genes" refers to genes in which either the maternal or paternal copy is exclusively expressed. Previous study (61) presented evidence that the imprinted gene *PEG3* is involved in sexual behavior and it would also be related to maternal care. More recently, Garfield et al. (62) showed that the imprinted gene *GRB10* influences, in mice, distinct physiological processes, fetal growth, and adult social behavior, due to actions of the two parental alleles in different tissues. An evolutionary explanation for this parent-of-origin-dependent gene expression is the "conflict hypothesis." A good example of evidence for this hypothesis, which will also help illustrate the meaning of imprinted genes, is the case of insulin-like growth factor (IGF2) and its receptor (IGF2R). As it is an imprinted gene, organisms that predominantly express the paternal allele of *IGF2* show high levels of offspring resource extraction from the mother. However, if selection favors high expression of the maternally inherited allele of *IGF2R*, the offspring would show low levels of resource extraction from the mother (63). Thus, the balance of these imprinted genes would define a very initial and essential relationship pattern between mother and offspring interaction regarding nurturing.

Nevertheless, the likelihood of epigenetic influence on imprinted genes raises the question of the importance of environmental intervention in determining behavior. In rats and humans, several studies have shown that changes in the expression of

candidate genes due to epigenetic mechanisms are connected with early parental care. Furthermore, McGowan et al. (64) suggested that the epigenetic response to maternal care in rats does not involve a single candidate gene, but rather includes changes in the expression of hundreds of additional genes. It is possible to speculate that the same happens with other placental mammalians, including humans.

In conclusion, early-life interactions clearly mold the neural basis for social behaviors (65), and sociability is perhaps the most important natural evolutionary force for mammalian species (66). In this context, it can be assumed that interactions with the mother are essential, not only in quantity, but perhaps most importantly in quality. A crucial question that emerges, among many, is whether it is possible to predict the consequences of the absence of a mother for her offspring, since several studies have indicated a genetic basis for animal behavior.

REFERENCES

1. Raineki C, Moriceau S, Sullivan RM. Developing a neurobehavioral animal model of infant attachment to an abusive caregiver. *Biol Psychiatry* (2010) **67**(12):1137–45. doi:10.1016/j.biopsych.2009.12.019
2. Mogi K, Nagasawa M, Kikusui T. Developmental consequences and biological significance of mother–infant bonding. *Prog Neuropsychopharmacol Biol Psychiatry* (2011) **35**:1232–41. doi:10.1016/j.pnpbp.2010.08.024
3. Lopatina O, Inzhutova A, Pichugina YA, Okamoto H, Salmina AB, Higashida H. Reproductive experience affects parental retrieval behaviour associated with increased plasma oxytocin levels in wild-type and CD38-knockout mice. *J Neuroendocrinol* (2011) **23**:1125–33. doi:10.1111/j.1365-2826.2011.02136.x
4. Sullivan RM, Holman PJ. Transitions in sensitive period attachment learning in infancy: the role of corticosterone. *Neurosci Biobehav Rev* (2010) **34**(6):835–44. doi:10.1016/j.neubiorev.2009.11.010
5. Windle RJ, Wood SA, Kershaw YM, Lightman SL, Ingram CD. Adaptive changes in basal and stress-induced HPA activity in lactating and post-lactating female rats. *Endocrinology* (2013) **154**(2):749–61. doi:10.1210/en.2012-1779
6. Lonstein JL. Reduced anxiety in postpartum rats requires recent physical interactions with pups, but is independent of suckling and peripheral sources of hormones. *Horm Behav* (2005) **47**:241–255. doi:10.1016/j.yhbeh.2004.11.001
7. Carlyle BC, Duque A, Kitchen RR, Bordner KA, Coman D, Doolittle E, et al. Maternal separation with early weaning: a rodent model providing novel insights into neglect associated developmental deficits. *Dev Psychopathol* (2012) **24**(4):1401–16. doi:10.1017/S095457941200079X
8. Moriceau S, Raineki C, Holman JD, Holman JG, Sullivan RM. Enduring neurobehavioral effects of early life trauma mediated through learning and corticosterone suppression. *Front Behav Neurosci* (2009) **3**:22. doi:10.3389/neuro.08.022.2009
9. Walker CD, Deschamps S, Proulx K, Tu M, Salzman C, Woodside B, et al. Mother to infant or infant to mother? Reciprocal regulation of responsiveness to stress in rodents and the implications for humans. *J Psychiatry Neurosci* (2004) **29**(5):364–82.
10. Loman MM, Gunnar MR. Early experience and the development of stress reactivity and regulation in children. *Neurosci Biobehav Rev* (2010) **34**(6):867–76. doi:10.1016/j.neubiorev.2009.05.007
11. Barrett J, Fleming AS. All mothers are not created equal: neural and psychobiological perspectives on mothering and the importance of individual differences. *J Child Psychol Psychiatry* (2011) **52**(4):368–97. doi:10.1111/j.1469-7610.2010.02306.x
12. Oomen CA, Girardi CE, Cahyadi R, Verbeek EC, Krugers H, Joels M, et al. Opposite effects of early maternal deprivation on neurogenesis in male versus female rats. *PLoS One* (2009) **4**(1):e3675. doi:10.1371/journal.pone.0003675
13. Jaffee SR, Price TS. The implications of genotype-environment correlation for establishing causal processes in psychopathology. *Dev Psychopathol* (2012) **24**(4):1253–1264. doi:10.1017/S0954579412000685
14. Bilbo SD, Schwarz JM. The immune system and developmental programming of brain and behavior. *Front Neuroendocrinol* (2012) **33**:267–86. doi:10.1016/j.yfrne.2012.08.006
15. Resendez SL, Kuhnmuench M, Krzywosinski T, Aragona BJ. Kappa-opioid receptors within the nucleus accumbens shell mediate pair bond maintenance. *J Neurosci* (2012) **32**(20):6771–84. doi:10.1523/JNEUROSCI.5779-11.2012
16. Lutz PE, Kieffer BL. Opioid receptors: distinct roles in mood disorders. *Trends Neurosci* (2013) **36**(3):195–206. doi:10.1016/j.tins.2012.11.002
17. Stolzenberg DS, Numan M. Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. *Front Neuroendocrinol* (2009) **30**:46–64. doi:10.1016/j.yfrne.2008.10.002
18. Zhang TY, Labonté B, Wen XL, Turecki G, Meaney MJ. Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. *Neuropsychopharmacology* (2013) **38**:111–23. doi:10.1038/npp.2012.149
19. Raineki C, De Souza MA, Szawka RE, Lutz ML, De Vasconcellos LFT, Sanvitto GL, et al. Neonatal handling and the maternal odor preference in rat pups: involvement of monoamines and cyclic amp response element-binding protein pathway in the olfactory bulb. *Neuroscience* (2009) **159**:31–8. doi:10.1016/j.neuroscience.2008.12.012
20. Mueller BR, Bale TL. Sex-specific programming of offspring emotionality following stress early in pregnancy. *J Neurosci* (2008) **28**(36):9055–65. doi:10.1523/JNEUROSCI.1424-08.2008
21. Pankevich DE, Mueller BR, Brockel B, Bale TL. Prenatal stress programming of offspring feeding behavior and energy balance begins early in pregnancy. *Physiol Behav* (2009) **98**(1-2):94–102. doi:10.1016/j.physbeh.2009.04.015
22. Brunton PJ, Russell JA. Endocrine induced changes in brain function during pregnancy. *Brain Res* (2010) **1364**:198–215. doi:10.1016/j.brainres.2010.09.062
23. Feldman R, Weller A, Zagoory-Sharon O, Levine A. Evidence for a neuroendocrinological foundation of human affiliation: plasma oxytocin levels across pregnancy and the postpartum period predict mother-infant bonding. *Psychol Sci* (2007) **18**(11):965–70. doi:10.1111/j.1467-9280.2007.02010.x
24. Howerton CL, Morgan CP, Fischer DB, Bale TL. O-GlcNAc transferase (OGT) as a placental biomarker of maternal stress and reprogramming of CNS gene transcription in development. *Proc Natl Acad Sci U S A* (2013) **110**(13):5169–74. doi:10.1073/pnas.1300065110
25. Botbol M. Towards an integrative neuroscientific and psychodynamic approach to the transmission of attachment. *J Physiol Paris* (2010) **104**:263–71. doi:10.1016/j.jphysparis.2010.08.005
26. Stoesz BM, Hare JF, Snow WM. Neurophysiological mechanisms underlying affiliative social behavior: insights from comparative research. *Neurosci Biobehav Rev* (2013) **37**:123–32. doi:10.1016/j.neubiorev.2012.11.007
27. Lucassen PJ, Naninck EFG, van Goudoever JB, Fitzsimons C, Joels M, Korosi A. Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics. *Trends Neurosci* (2013) **36**(11):621–31. doi:10.1016/j.tins.2013.08.002
28. Sheriff MJ, Love OP. Determining the adaptive potential of maternal stress. *Ecol Lett* (2013) **16**:271–80. doi:10.1111/ele.12042
29. Hofer MA. On the nature and consequences of early loss. *Psychosom Med* (1996) **58**:570–81.
30. Feldman R. Parent–infant synchrony and the construction of shared timing; physiological precursors, developmental outcomes, and risk conditions. *J Child Psychol Psychiatry* (2007) **48**(3/4):329–54. doi:10.1111/j.1469-7610.2006.01701.x
31. Baram TZ, Davis EP, Obenaus A, Sandman CA, Small SL, Solodkin A, et al. Fragmentation and unpredictability of early-life experience in mental disorders. *Am J Psychiatry* (2012) **169**:907–15. doi:10.1176/appi.ajp.2012.11091347
32. Moriceau S, Shionoya K, Jakubs K, Sullivan RM. Early-life stress disrupts attachment learning: the role of amygdala corticosterone, locus coeruleus, corticotropin releasing hormone, and olfactory bulb norepinephrine. *J Neurosci* (2009) **29**(50):15745–55. doi:10.1523/JNEUROSCI.4106-09.2009
33. Kim EJ, Horovitz O, Pellman BA, Tan LM, Li Q, Richter-Levin G, et al. Dorsal periaqueductal gray-amygdala pathway conveys both innate and learned fear responses in rats. *Proc Natl Acad Sci U S A* (2013) **110**(36):14795–800. doi:10.1073/pnas.1310845110
34. Xu X. Modular genetic control of innate behaviors. *Bioessays* (2013) **35**:421–4. doi:10.1002/bies.201200167
35. Bateson P. How do sensitive periods arise and what are they for? *Anim Behav* (1979) **27**:470–86. doi:10.1016/0003-3472(79)90184-2
36. Robinson GE. Beyond nature and nurture. *Science* (2004) **304**:397–9. doi:10.1126/science.1095766

37. Keverne EB, Curley JP. Epigenetics, brain evolution and behavior. *Front Neuroendocrinol* (2008) **29**:398–412. doi:10.1016/j.yfrne.2008.03.001
38. Kandel ER, Jessell T. Early experience and the fine tuning of synaptic connections. In: Kandel ER, Schwartz JH, Jessell TM editors. *Principles of Neural Science.* Amsterdam: Elsevier Science Publishing (1991). p. 945–58.
39. Guertin PA. Central pattern generator for locomotion: anatomical, physiological, and pathophysiological considerations. *Front Neurol* (2013) **3**:183. doi:10.3389/fneur.2012.00183
40. Marler P. Innateness and the instinct to learn. *An Acad Bras Cienc* (2004) **76**(2):189–200. doi:10.1590/S0001-37652004000200002
41. Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. *Physiol Rev* (2001) **81**:629–83.
42. Yamashita K, Kitano T. Molecular evolution of the oxytocin-oxytocin receptor system in eutherians. *Mol Phylogenet Evol* (2013) **67**:520–8. doi:10.1016/j.ympev.2013.02.017
43. Rao VV, Löffler C, Battey J, Hansmann I. The human gene for oxytocin-neurophysin I (OXT) is physically mapped to chromosome 20p13 by in situ hybridization. *Cytogenet Cell Genet* (1992) **61**:271–3. doi:10.1159/000133420
44. McCall C, Singer T. The animal and human neuroendocrinology of social cognition, motivation and behavior. *Nat Neurosci* (2012) **15**:681–8. doi:10.1038/nn.3084
45. Young LJ, Flanagan-Cato LM. Editorial comment: oxytocin, vasopressin and social behavior. *Horm Behav* (2012) **61**:227–9. doi:10.1016/j.yhbeh.2012.02.019
46. Pedersen CA, Prange AJ Jr. Induction of maternal behavior in virgin rats after intracerebroventricular administration of oxytocin. *Proc Natl Acad Sci U S A* (1979) **76**:6661–5. doi:10.1073/pnas.76.12.6661
47. Bosch OJ. Maternal aggression in rodents: brain oxytocin and vasopressin mediate pup defence. *Philos Trans R Soc Lond B Biol Sci* (2013) **368**:20130085. doi:10.1098/rstb.2013.0085
48. Apter-Levi Y, Zagoory-Sharon O, Feldman R. Oxytocin and vasopressin support distinct configuration of social synchrony. *Brain Res* (2013). doi:10.1016/j.brainres.2013.10.052
49. Lee AG, Cool DR, Grunwald WCJr, Neal DE, Buckmaster CL, Cheng MY, et al. A novel form of oxytocin in New World monkeys. *Biol Lett* (2011) **7**:584–7. doi:10.1098/rsbl.2011.0107
50. Ferguson DR. Genetic distribution of vasopressins in the peccary (*Tayassu angulatus*) and warthog (*Phacochoerus aethiopicus*). *Gen Comp Endocrinol* (1969) **12**:609–13. doi:10.1016/0016-6480(69)90181-6
51. Wallis M. Molecular evolution of the neurohypophysial hormone precursors in mammals: comparative genomics reveals novel mammalian oxytocin and vasopressin analogues. *Gen Comp Endocrinol* (2012) **179**:313–8. doi:10.1016/j.ygcen.2012.07.030
52. Koehbach J, Stockner T, Bergmayr C, Muttenthaler M, Gruber CW. Insights into the molecular evolution of oxytocin receptor ligand binding. *Biochem Soc Trans* (2013) **41**:197–204. doi:10.1042/BST20120256
53. Zingg HH, Laporte SA. The oxytocin receptor. *Trends Endocrinol Metab* (2003) **14**:222–7. doi:10.1016/S1043-2760(03)00080-8
54. Koshimizu TA, Nakamura K, Egashira N, Hiroyama M, Nonoguchi H, Tanoue A. Vasopressin V1a and V1b receptors: from molecules to physiological systems. *Physiol Rev* (2012) **92**:1813–64. doi:10.1152/physrev.00035.2011
55. Kimura T, Tanizawa O, Mori K, Brownstein MJ, Okayama H. Structure and expression of a human oxytocin receptor. *Nature* (1992) **356**:526–9. doi:10.1038/356526a0
56. Hammock EAD, Levitt P. Oxytocin receptor ligand binding in embryonic tissue and postnatal brain development of the C57BL/6J mouse. *Front Behav Neurosci* (2013) **7**:195. doi:10.3389/fnbeh.2013.00195
57. Muráni E, Ponsuksili S, D'Eath RB, Turner SP, Kurt E, Evans G, et al. Association of HPA axis-related genetic variation with stress reactivity and aggressive behavior in pigs. *BMC Genet* (2010) **11**:e74. doi:10.1186/1471-2156-11-74
58. Terenina E, Babigumira BM, Le MignonG, Bazovkina D, Rousseau S, Salin F, et al. Association study of molecular polymorphisms in candidate genes related to stress responses with production and meat quality traits in pigs. *Domest Anim Endocrinol* (2013) **44**:81–97. doi:10.1016/j.domaniend.2012.09.004
59. Ebstein RP, Knafo A, Mankuta D, Chew SH, Lai PS. The contributions of oxytocin and vasopressin pathway genes to human behavior. *Horm Behav* (2012) **61**:359–79. doi:10.1016/j.yhbeh.2011.12.014
60. Schradin C, Reeder DM, Mendoza SP, Anzenberger G. Prolactin and paternal care: comparison of three species of monogamous new world monkeys (*Callicebus cupreus, Callithrix jacchus, and Callimico goeldii*). *J Comp Psychol* (2003) **117**:166–75. doi:10.1037/0735-7036.117.2.166
61. Swaney WT, Curley JP, Champagne FA, Keverne EB. Genomic imprinting mediates sexual experience-dependent olfactory learning in male mice. *Proc Natl Acad Sci U S A* (2007) **104**(14):6084–9. doi:10.1073/pnas.0609471104
62. Garfield AS, Cowley M, Smith FM, Moorwood K, Stewart-Cox JE, Gilroy K, et al. Distinct physiological and behavioural functions for parental alleles of imprinted *Grb10*. *Nature* (2011) **469**(7331):534–8. doi:10.1038/nature09651
63. Day T, Bonduriansky R. Intralocus sexual conflict can drive the evolution of genomic imprinting. *Genetics* (2004) **167**:1537–46. doi:10.1534/genetics.103.026211
64. McGowan PO, Suderman M, Sasaki A, Huang TCT, Hallett M, Meaney MJ, et al. Broad epigenetic signature of maternal care in the brain of adult rats. *PLoS One* (2011) **6**(2):e14739. doi:10.1371/journal.pone.0014739
65. Wersinger SR. The neuroscience of social behavior. *ILAR J* (2009) **50**(1):1–4. doi:10.1093/ilar.50.1.1
66. Dunbar RI, Shultz S. Evolution in the social brain. *Science* (2007) **317**(5843):1344–7. doi:10.1126/science.1145463

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 22 December 2013; paper pending published: 11 January 2014; accepted: 08 February 2014; published online: 26 February 2014.
*Citation: Lucion AB and Bortolini MC (2014) Mother–pup interactions: rodents and humans. Front. Endocrinol. **5**:17. doi: 10.3389/fendo.2014.00017*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
ENDOCRINOLOGY

REVIEW ARTICLE
published: 21 March 2014
doi: 10.3389/fendo.2014.00033

Early life trauma and attachment: immediate and enduring effects on neurobehavioral and stress axis development

***Millie Rincón-Cortés*[1,2,3]* and *Regina M. Sullivan*[1,2,3]**

[1] *Department of Neuroscience and Physiology, Sackler Institute for Graduate Biomedical Sciences, New York University School of Medicine, New York, NY, USA*
[2] *Emotional Brain Institute, Nathan Kline Institute for Psychiatric Research, New York, NY, USA*
[3] *New York University Child Study Center, Department of Child and Adolescent Psychiatry, New York University School of Medicine, New York, NY, USA*

Edited by:
Nikolaos P. Daskalakis, Icahn School of Medicine at Mount Sinai, USA

Reviewed by:
Aniko Korosi, University of Amsterdam, Netherlands
James A. Carr, Texas Tech University, USA

***Correspondence:**
Millie Rincón-Cortés, Sullivan Laboratory, New York University Child Study Center, Department of Child and Adolescent Psychiatry, New York University School of Medicine, 1 Park Avenue, New York, NY 10016, USA
e-mail: millie.rinconcortes@med.nyu.edu

Over half a century of converging clinical and animal research indicates that early life experiences induce enduring neuroplasticity of the HPA-axis and the developing brain. This experience-induced neuroplasticity is due to alterations in the frequency and intensity of stimulation of pups' sensory systems (i.e., olfactory, somatosensory, gustatory) embedded in mother–infant interactions. This stimulation provides "hidden regulators" of pups' behavioral, physiological, and neural responses that have both immediate and enduring consequences, including those involving the stress response. While variation in stimulation can produce individual differences and adaptive behaviors, pathological early life experiences can induce maladaptive behaviors, initiate a pathway to pathology, and increase risk for later-life psychopathologies, such as mood and affective disorders, suggesting that infant-attachment relationships program later-life neurobehavioral function. Recent evidence suggests that the effects of maternal presence or absence during this sensory stimulation provide a major modulatory role in neural and endocrine system responses, which have minimal impact on pups' immediate neurobehavior but a robust impact on neurobehavioral development. This concept is reviewed here using two complementary rodent models of infant trauma within attachment: infant paired-odor-shock conditioning (mimicking maternal odor attachment learning) and rearing with an abusive mother that converge in producing a similar behavioral phenotype in later-life including depressive-like behavior as well as disrupted HPA-axis and amygdala function. The importance of maternal social presence on pups' immediate and enduring brain and behavior suggests unique processing of sensory stimuli in early life that could provide insight into the development of novel strategies for prevention and therapeutic interventions for trauma experienced with the abusive caregiver.

Keywords: infant-attachment, maternal programming, development, amygdala, social behavior, rodent models, stress

INTRODUCTION

Both animal and human research demonstrate that early life experiences interact with genetics to program the central nervous and endocrine systems, including the hypothalamus–pituitary–adrenal (HPA)-axis (1–5). Infant experiences typically occur within the context of the mother and the quality of caregiving by the mother, determined by the patterning and intensity of maternal stimulation of pups' sensory systems, is a key regulator of HPA-axis neuroplasticity in the neonatal period (6–10). Dissecting the mother–infant dyad has characterized maternal control over infant brain and behavior through "hidden regulators" present during mother–infant interactions (11, 12). Lack or loss of typical parental stimulation is a potent stressor during early life (13, 14), and removal of these hidden regulators through maternal deprivation, modulation of maternal behavior, and/or traumatic interactions with the mother, produce immediate changes in pups and result in wide-spread dysregulation of physiological and behavioral responses during development (15–26). Within the range of typical parenting, normal variations in maternal care during infancy program individual differences in behavioral and endocrine responses to stress in rodents and humans; although pathological experiences, including abuse and neglect, produce vulnerability to later-life psychiatric disorders (7, 27–37).

Here, we focus on infant experiences and the effects of early life stress and HPA-axis activation as experienced within the mother–infant dyad, as well as the pups' attachment to the caregiver and learning about the caregiver. We review two complementary rodent models of infant trauma within attachment: infant paired-odor-shock conditioning and rearing with an abusive mother, which converge in producing a similar neurobehavioral phenotype in later-life consisting of depressive-like behavior as well as disrupted HPA-axis and amygdala function, thus enabling us to explore both the immediate and enduring effects of abusive attachment as well as role of the HPA-axis and the stress hormone corticosterone (CORT). Although infant trauma resulting from abusive attachment affects neural substrates of stress vulnerability and resilience, these can be engaged by sensory cues learned

during infancy (i.e., artificial or natural maternal odor), which have the ability to normalize adult neurobehavioral dysregulation stemming from early life trauma.

ATTACHMENT

Attachment is a psychosocial process referring to the deep and enduring emotional bond that connects two individuals across space and time, with an individual deriving security from physical and psychological contact with the attachment figure (38–40). Attachment requires experience-dependent learning of the sensory stimuli associated with infant–caregiver interactions, and a strong attachment to the caregiver is crucial for survival in altricial species, including humans (41–48). In children, attachment is characterized by specific behaviors such as seeking proximity to the caregiver, whom provides a sense of safety and security for the infant (49–51). Like humans, infants from altricial species also exhibit attachment related behaviors to their caregiver shortly after birth that elicit nurturing and attachment from the caregiver, which entails responding appropriately to the infant's needs by providing nourishment, protection, and warmth necessary for survival (51–53). Thus, infant-attachment is an adaptive and reciprocal process consisting of a dynamic and complex exchange of mother–infant behavioral interactions that enhance the infant's chance of survival by maintaining contact with the caregiver.

The mother–infant attachment bond is among the strongest social attachments formed by most mammals (54). As such, human infants seek proximity to and maintain contact with the caregiver despite the quality of care they receive (55), including attachment to an abusive caregiver. This paradoxical phenomenon also occurs in dogs, chicks, and non-human primates, suggesting a phylogenetically preserved system (32, 41, 43, 56–63). From an evolutionary perspective, attachment to an abusive caregiver is thought to be adaptive because it provides immediate benefits, as the infant still has access to some care (48, 64). Albeit infant organisms are biologically predisposed to attach to their caregiver and possess behavioral systems that allow them to rely on these bonds for survival (38), clinical and preclinical studies suggest that adverse parental care compromises brain development and has longstanding effects in stress-responsive neurobiological systems, including the HPA-axis, neurotransmitter systems, as well as cortical and limbic structures such as the prefrontal cortex, amygdala, and hippocampus (65–75). Moreover, traumatic early life experiences involving the caregiver increase the risk for a wide-range of deleterious mental health and behavioral outcomes, including developmental psychopathology, affective, and mood disorders (37, 72, 76–86). Therefore, perturbations in infant-attachment appear to induce immediate neurobiological changes that shape subsequent development and lead to neurobehavioral dysregulation associated with compromised emotionality and increased vulnerability to psychopathology during later-life, suggesting that the quality of an infant's first social relationships programs the infant's emotional and cognitive capabilities to adapt to later-life environments.

Despite the fact that childhood abuse remains a major public health concern (87–91), the mechanisms by which infant trauma initiates the pathway to psychopathology are poorly understood, although the stress axis is evidently implicated. However, animal models have provided some insight into the mechanisms by which disruptions in parental care alter the development of stress response systems (92, 93), which may contribute to our understanding of resilience following infant trauma (62, 94–98). For example, research using animal models of maternal deprivation in rodents and non-human primates parallel human imaging studies suggesting that disruptions in infant-attachment also produce long-term alterations in the limbic system and the stress axis that may compromise the development of emotion- and attention-regulatory systems, which has been used to explain the heightened risk of behavioral and affective disorders in human children experiencing adverse parental care (13, 31, 32, 75, 93, 99–108). Overall, these studies demonstrate that parental care affects the maturation of these brain areas and offers potential sites to understand the damaging effects of early life abuse on subsequent neurobehavioral development (31, 70, 71, 74, 84, 94, 109–115). For these reasons, we employ rodent models of abusive attachment and study the infant's immediate response to trauma as well as the neurobiological sequelae leading to later-life neurobehavioral dysregulation to better understand the infant mechanisms that initiate the pathway to later-life psychopathologies.

THE STRESS-HYPORESPONSIVE PERIOD AND MATERNAL REGULATION OF THE HPA-AXIS

In rats, infant-attachment occurs within a unique developmental context – the stress-hyporesponsive period (SHRP) – during which neonates show low basal plasma concentrations of CORT and reduced stress-reactivity, as indexed by limited adrenocorticotropic hormone (ACTH) and CORT responses to stressful stimuli compared to older animals, as well as low levels of corticosteroid binding globulin (CBG), which regulates glucocorticoid (GC) access into the brain (92, 116–123). Thus, the neuroendocrine stress response of the neonatal rat is characterized by attenuated hormonal responses and altered gene regulation in response to stress compared to adults due to hyporesponsiveness at all levels of the HPA-axis, namely: (1) a blunted pituitary ACTH secretion, resulting from a combination of immaturity of neural inputs to the corticotropin releasing hormone (CRH) neurons, (2) decreased pituitary peptide content or decreased sensitivity to CRH stimulus; and (3) an adrenal gland hyporesponsive to circulating ACTH levels (18, 119, 121, 124–130). Accumulating evidence suggests that human infants exhibit a period of dampened cortisol reactivity analogous to the rodent SHRP, which develops gradually over the course of the first year of life (~6–12 months), although it remains unclear how long it extends (131–135). In both humans and rodents, the SHRP is thought to protect the developing brain from the detrimental effects of elevated HPA-axis activity and excess GC exposure, and the sensitivity and responsiveness from the caregiver appears critical in maintaining low cortisol activity and controlling the offspring's physiological and behavioral responses to stressors during this period (3, 30, 32, 68, 100, 122, 127, 129, 136–140).

However, the SHRP during development appears to be stressor specific, since the HPA-axis is fully capable of responding to stimuli that may be considered stressful to a neonatal rat such as cold or saline injection (141–145). Indeed, the HPA-axis and CORT receptors are functional at birth, but are modulated by

the sensory stimulation provided by the mother (100, 119, 126, 146–152). Moreover, the mother is able to directly regulate the pups' CORT levels through hidden regulators embedded in typical mother–infant interactions, such as the sensory, motor, nutrient, and thermal events associated with caregiving, which exert regulatory influence over the infant's immediate and long-term behavioral and physiological responses by affecting sleep-wake states, cardiac rates, and HPA-axis function (6, 10–12, 17, 129, 153, 154). Removal of maternal sensory stimulation during the SHRP, such as that occurring when the pups are separated from the mother for a prolonged period of time (i.e., maternal deprivation paradigm), increases CORT secretion (16), elevates CORT levels in pups (11, 12, 129), and enables higher CORT/ACTH responses to acute stress (15, 19, 100, 145, 155). Importantly, these changes are similar to those induced by normal variations in maternal care (i.e., maternal high/low licking paradigm) (7, 27, 29) as well as atypical or abusive maternal care (20, 144, 156), suggesting that the hypothalamic mechanisms controlling physiological stress responses in the pup are regulated by elements of maternal care. Taken together, these findings suggest that maternal deprivation, variations in maternal care, and abusive maternal care influence the development and function of the HPA-axis (8, 9, 30, 112, 114, 157–160). In summary, maternal stimulation modulates the infant's HPA-axis and maintains the SHRP, although potent stressors involving disruptions in maternal stimulation (i.e., cold, maternal deprivation, atypical maternal care) can activate the HPA-axis and override maternal control of the SHRP.

ATTACHMENT LEARNING DURING A SENSITIVE-PERIOD IN RAT PUPS

Infants possess a predisposition to approach the mother as well as specific sensory cues associated with her care, such as her odor and vocalizations (161, 162). Within an evolutionary context, the infant-attachment system serves to establish a preference for the mother regardless of whether or not she is associated with pain or pleasure (48, 64). This type of survival-dependent learning is known as imprinting, has wide phylogenetic representation, and is temporally confined to a sensitive-period in development (50, 161, 163) typically involving a hypofunctioning HPA-axis – the principal pathway of the mammalian stress response that regulates the production of GCs (cortisol in humans, CORT in rodents) (40, 164). In rats, we refer to this period of enhanced attachment/preference learning as the "sensitive-period," or postnatal (PN) days 1–9 (see **Figure 1**). As we will discuss below, sensitive-period learning is due to the pup's unique learning circuit, presumably one sculpted through evolution to provide infants with the neural circuitry required to survive and maximize attachment to a caregiver (48).

Intriguingly, the sensitive-period for attachment learning in rat pups overlaps with the SHRP, suggesting that low levels of CORT and reduced HPA-axis responsiveness may contribute to the neonate's unique neural circuitry for attachment learning. However, in order for infant-attachment to occur, the rat pup must first learn to identify the caregiver and exhibit the social behaviors necessary for survival such as orienting to and approaching the caregiver, grasping the nipple and nursing (50, 168, 169). Infant-attachment learning in rodents revolves around the pup's ability to learn and develop a preference for the mother's odor, which is diet dependent and can change postnatally (47, 170–174). Since rat pups are born deaf and blind, they must rapidly learn their mother's odor, which conveys distal and proximal information about the mother's location, and helps the pups orient to the mother, approach her and elicit care (169, 175). The maternal odor is critical in guiding infant-attachment; without it, pups show reduced contact with the mother, are unable to nipple attach and exhibit low survival rates (25, 176). Moreover, any neutral odor can acquire properties of the natural maternal odor and act as a new maternal odor by simply being placed on the mother, in a cage during mother-infant interactions (177–182) or learned in classical conditioning paradigms (i.e., odor-stroke, odor-shock) performed outside the nest in the absence of the mother (111, 165, 171, 183–188).

Our lab uses infant olfactory classical conditioning in which an artificial odor (i.e., peppermint) is paired with a 0.5 mA shock as a rodent model of abusive attachment. While the adult rat responds to shock with a robust CORT response, the neonatal rat does not (9, 100, 189). Unlike older animals, which readily learn odor aversions to painful stimuli paired with an odor, rat pups actually exhibit an odor preference and approach the odor (111, 165, 190–193). This odor preference, however, is not due to the inability of pups to feel pain, since the pain threshold varies little during the neonatal period and pups emit vocalizations to the shock, suggesting that they are experiencing distress (165, 190, 194–197). Instead, infant paired-odor-shock conditioning produces a new artificial maternal odor that acquires the ability to regulate pup behaviors typically controlled by the maternal odor; it induces proximity-seeking/approach responses in pups (distal cues), guides mother–infant interactions by facilitating contact with the mother and nipple attachment (proximal cues), and activates the same neural circuitry as the natural maternal odor (25, 111, 165, 169), suggesting that this odor has comparable qualities to the natural maternal odor. Importantly, infant odor-shock conditioning is a useful experimental paradigm for understanding how early life trauma (i.e., pain-shock) can support and maintain attachment and provide insights into the particular ways the infant brain processes painful stimuli and its relationship to the enduring effects of this experience due to the well documented neural circuitry underlying this type of learning (198–202). Three brain structures have been shown to play a role in the neonatal rat's sensitive-period for enhanced odor learning: the olfactory bulb (OB), the noradrenergic locus coeruleus (LC), and the amygdala (50, 203).

NEUROBIOLOGY OF INFANT-ATTACHMENT AND THE ROLE OF THE HPA-AXIS IN TERMINATING ATTACHMENT LEARNING

Neonatal odor learning produces changes in the OB, which can be induced both naturally in the nest and experimentally in controlled learning experiments outside the nest (182, 186, 204–210). For example, both natural and learned odors produce a similar enhancement of OB responding during the sensitive-period, which has been assessed through a variety of techniques including 2-deoxy-glucose (2-DG) uptake, c-Fos immunoreactivity (ir), CREB phosphorylation, electrophysiology, and optical imaging (205–208, 211–214). Thus, olfactory-based attachment learning

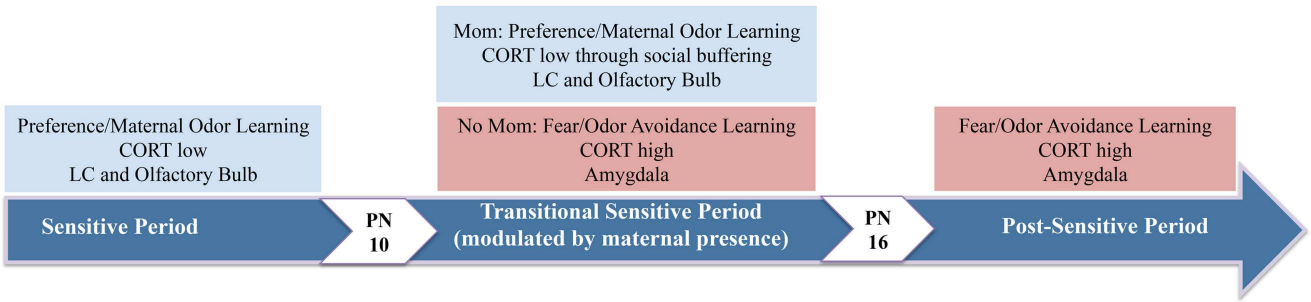


FIGURE 1 | The neural circuitry underlying pup attachment learning changes over development. During the earliest days of life, pups have a sensitive-period in which odor-shock conditioning produces an odor preference. At 10 days of age, pups begin the transitional sensitive-period, when pups endogenous CORT levels have increased sufficiently to enable amygdala-dependent fear/avoidance learning. However, with the mother present at this age, pups will revert back to preference learning and the neural circuitry of the sensitive-period. Thus, the mother's presence socially buffers pups (i.e., attenuates pups shock-induced CORT release) and pups learn a preference. As pups mature and enter the post-sensitive-period, odor-shock conditioning induces amygdala-dependent fear and odor avoidance learning (25, 165–167).

in neonatal rats is associated with the acquisition of odor-specific neural changes in the OB, which can only be acquired during the sensitive-period, and are retained throughout development (111, 201, 215–218).

Infant rats (PN1–9) readily learn an odor preference to neutral odors paired with pleasant (i.e., milk, stroking) (47, 171, 179, 183–185, 219) or painful stimuli, such as 0.5 mA shock or tail pinch (111, 165, 190, 191, 201), which is partly due to a uniquely large noradrenergic input to the OB from the LC, the sole source of norepinephrine (NE) for the OB (220, 221), which prompts abundant release of NE into the OB (203, 222). Furthermore, the neonatal LC shows prolonged stimulus-evoked excitation and greater NE release to odors during the sensitive-period compared to later-life due to the immaturity of the LC alpha-2 inhibitory autoreceptors, which functionally emerge around PN10 and cause a shift from prolonged excitatory alpha-1 mediated responses to inhibitory alpha-2 mediated responses, resulting in brief excitation due to inhibited LC firing and decreased NE output (203, 222–227). Importantly, NE release from the LC is both necessary and sufficient for odor preference learning during the sensitive-period (228–232).

Experimental evidence indicates a lack of amygdala participation in the neural circuitry underlying infant paired-odor-shock conditioning during the sensitive-period, as suggested by amygdala lesions, 2-DG, and c-Fos-ir (111, 201, 203, 216, 232), although the amygdala is strongly implicated in adult classical conditioning (198–200, 202, 233). These data suggest that the infant amygdala is not part of the sensitive-period learning circuit during which aversions are difficult to learn because of its failure to exhibit the plasticity required for this type of learning (234–236), although the amygdala is responsive to odors and other environmental stimuli by PN10 (165, 201, 237). Like the infant amygdala, the infant HPA-axis is limited in function, resulting in reduced shock-induced CORT release during the neonatal sensitive-period (189), which limits pups' ability to acquire learned odor aversions (201, 238). Endogenous CORT levels increase gradually and reach a critical level by PN10 (92, 136, 239, 240), at which time stressful or painful stimuli are able to elicit a sufficient CORT response that permits infant amygdala plasticity and avoidance learning (**Figure 1**) (201, 218, 241).

Indeed, the natural increase of stress-induced CORT release marks the end of sensitive-period learning (165, 201, 203, 238), which has been demonstrated experimentally by increasing CORT systemically (3 mg/kg, i.p.) or through intra-amygdala CORT infusions (50–100 ng) prior to odor-shock conditioning, which enables sensitive-period pups to learn an odor aversion and exhibit learning-evoked neural activity (i.e., enhanced 2-DG uptake) in the amygdala, while preventing the acquisition of learning-induced changes in the OB (201, 238, 241, 242). In contrast, CORT depletion (via adrenalectomy or social buffering, discussed below) in PN12 pups results in shock-induced odor preference learning and acquisition of OB neural changes. Thus, within the context of paired-odor-shock conditioning, CORT appears to play a modulatory role on infant learning by switching whether the amygdala learns attraction or avoidance: if CORT is low, pups learn a preference to an odor paired with shock due to a lack of amygdala involvement; if CORT is high, the amygdala is activated by odor-shock conditioning and pups learn an avoidance. Recently, we have identified a role for amygdala dopamine (DA) in mediating these infant learning transitions, as conditions that block aversion/fear learning are associated with downregulated DA function (243). Altogether, these findings suggest that neonatal rat pups have unique learning capabilities that aid olfactory-based attachment to the mother, which are dependent on low levels of CORT.

In summary, the infant learning circuit is characterized by an enhanced ability to learn odor preferences to aversive stimuli, due to a hyper-functioning LC, as well as a decreased ability to learn odor aversions that may interfere with proximity-seeking during the sensitive-period due to a hypo-functional amygdala, suggesting that the infant brain is specialized for maximizing attachment to a caregiver (**Figure 1**) (41, 165, 186, 221, 225, 229, 234, 235, 244–247). As the sensitive-period ends, owing to the natural emergence of CORT, odor aversions can be learned because of changes in the infant learning circuit, including maturation of LC autoinhibition, which reduces NE release and greatly attenuates rapid odor preference learning, but also due to the functional emergence of the

amygdala, all of which enable the plasticity required for aversion learning (50, 165, 223, 225, 229, 232, 241).

MATERNAL MODULATION OF HPA-AXIS FUNCTION AND SENSITIVE-PERIOD DURATION

Empirical evidence suggests that social support is a powerful modulator of individual differences in response to potentially stressful events in both humans and animals (248–253). In rodents, maternal presence is known to blunt CORT release to stressful and painful stimuli in older pups (>PN12) through olfactory and somatosensory cues (9, 148, 152, 166, 167, 254, 255). The process by which the presence of a social companion and/or social sensory cues can dampen HPA responses to stressors (i.e., decrease CORT levels) is termed "social buffering" and has been reported in humans and other species (139, 249, 250, 253, 256–259). Our lab has identified a transitional sensitive-period in pups from PN10–15, during which odor-shock conditioning produces either olfactory preference or aversion in infant rats depending on social context (166, 260). In the absence of the mother, paired-odor-shock conditioning yields a learned odor avoidance that is accompanied by amygdala activation. However, maternal presence is able to suppress amygdala activity and block aversion learning induced by odor-shock conditioning, indicating that maternal presence reengages the sensitive-period attachment circuitry to reinstate odor preference learning through modulation of CORT (see **Figure 1**), and therefore CORT regulation of amygdala activity. Importantly, these animal data are consistent with the principles of attachment theory (38), in which access to a secure base provided by the attachment figure reduces the probability of HPA/CRF stress reactions that could have unfavorable long-term consequences on brain development (9, 137, 261).

Yet, human parental care is disturbed under conditions of chronic stress (262), which can be modeled in rodents by creating an abnormal rearing environment that alters maternal behavior (20, 23, 111) and mimics the effects of a stressful environment as a risk factor for potentiating infant abuse, including humans (62, 77, 263, 264). Because bedding type and volume are important components of the dam's nesting environment, limiting the amount of bedding available constitutes a continuous stressor for the dam and her pups, disrupts mother–pup interactions, and alters the development of the pup's HPA-axis by reducing the frequency of positive maternal behaviors (i.e., licking, grooming, nursing) and increasing the frequency of negative maternal behaviors that are painful to the pup and elicit vocalizations, such as stepping, dragging, and rough handling of the pups (20, 25, 111, 156, 188, 265). Thus, one could conceptualize a stressed dam as a poor regulator, which is supported by findings showing that ICV infusion of corticotropin releasing factor (CRF) reduces maternal responsivity (266).

Furthermore, because maternal stimulation of pups modulates pups' endogenous CORT, maternal care quality alters sensitive-period duration. Pups reared with a stressed mother (i.e., poor regulator) exhibit a precocious emergence of CORT, which is delivered through the mother's milk (267), that facilitates aversion learning and engages the amygdala, as indexed by increased odor-shock-induced amygdala neural activity (188), suggesting that experience with a stressed mother prematurely ends the SHRP and the sensitive-period for attachment learning. In addition, this procedure results in striking changes in the expression and activity patterns of key regulatory elements of the neuroendocrine stress response, which result in persistent alterations of HPA-axis function such as elevated basal GC concentrations, impaired GC feedback, and modifications in CRF-receptor regulation (20, 25, 114, 156, 174). Since the mother serves as a primary link between the environment and the infant, environmentally driven alterations in maternal care could transduce an environmental signal to the pups, alter the development of central CRF systems activating behavioral, endocrine and autonomic responses to stress, as well as systems regulating CRF and HPA-axis activity, which may serve to increase or decrease stress-reactivity in the offspring, so that it mirrors that of the mother.

IMMEDIATE AND ENDURING EFFECTS OF EARLY LIFE STRESS

Responses to stressors, or conditions that threaten or are perceived to threaten physiological equilibrium, are mediated by the activation of stress-responsive neurobiological systems that help preserve allostasis, or stability through change, thereby making the stress response an essential endocrine mechanism for survival (268–270). Stressors, which can include psychological and physical challenges, increase the amount of hypothalamic CRF that is released into the anterior pituitary gland, stimulating ACTH secretion in the anterior pituitary and resulting in GC production in the adrenal gland (268, 271, 272). GCs facilitate the mobilization of substrates for energy sources, potentiate the release of catecholamines, and enhance cardiovascular tone while suppressing "non-essential systems" for immediate survival, such as immunity, growth, and reproduction (273–276). Stress-induced HPA-axis activation is associated with acute release of stress-related neuropeptides, hormones, and neurotransmitters, including NE, serotonin (5-HT), and DA, in cortical and limbic structures (21, 27, 277–289). Although acutely elevated GCs help orchestrate physiological and behavioral responses that promote allostasis, chronic activation of the HPA-axis, and prolonged elevations of GCs and CRF increase the risk of stress-related disorders and psychological illnesses during later-life (269, 290–292).

The effects of HPA-axis activation depend on multiple factors, including the developmental stage in which the insult occurs, number of exposures, and type of adversity (71, 293–297). Numerous behavioral, endocrine, and clinical studies have shown that various early life stressors cause a premature increase in CORT levels (129) that produces profound alterations in growth and development and negatively affects mental health (40, 72, 135, 298, 299). Moreover, repeated exposure to early life stressors, both physical and psychological, induce changes in endocrine (HPA-axis), neurotransmitter (DA, 5-HT), and brain memory systems, including the hippocampus, amygdala, and PFC that persist throughout the life-span (8, 67, 101, 300, 301). Furthermore, the HPA-axis is modulated by limbic and cortical regions such as the amygdala, hippocampus, and the PFC (269, 302), which enable the activation of stress responses by psychosocial stressors (303–307). Importantly, the timing of early life stress may affect brain regions undergoing specific growth spurts during that time (308, 309), so that brain regions rich in GC receptors and characterized by extended PN development, such as the amygdala, hippocampus, and PFC, are

particularly susceptible to the long-term effects of stress (71, 92), which affects later-life memory, cognitive, executive, and affective function as well as stress-reactivity in humans (296, 297). Alterations in stress-sensitive neurobiological systems, including regulation of GCs and CRF, have been posited as mechanisms through which early life stress, including inadequate/disorganized parental care, increases the likelihood of psychopathology by influencing HPA hyperreactivity to stressors and promoting the development of stress-induced illnesses throughout life (31, 40, 290, 310–312).

Early life adversity may lead to a maladaptive outcome to a given later environmental context. Depression is a common outcome of childhood abuse, and children with a comorbid history of depression and abuse have elevated CRF levels in the cerebrospinal fluid (313) as well as an increased ACTH response to a CRF challenge compared to children with depression without abuse, suggesting excessive CRF release (3, 314, 315). Additional clinical evidence indicates that severe early life stressors in childhood are associated with the long-term HPA-axis disturbances in depressed patients (316–319), which is supported by preclinical studies of non-human primates showing that poor rearing conditions and conditions that disrupt responsive maternal care have a long-term impact on the neurobiology of stress and negative emotionality (21, 31, 32, 109, 158). For example, variable foraging paradigms that result in neglectful maternal care produce adult offspring that are more fearful, low in dominance, have elevated levels of CRF in the CSF and high in brain levels of CRH, exhibit persistent alterations in metabolites of 5-HT, DA, and NE, as well as changes in noradrenergic and serotonergic responses to stress (99, 320–324). Given the importance of noradrenergic and serotonergic systems in mood disorders, these findings postulate a mechanism by which early life stress may predispose an individual to later-life depression (32, 300, 325–327).

CONVERGENCE OF BOTH ABUSIVE ATTACHMENT MODELS IN PRODUCING A DEPRESSIVE-LIKE BEHAVIORAL PHENOTYPE DURING LATER-LIFE

Recently, our lab has demonstrated that both rodent models of abusive attachment (paired-odor-shock, abusive mother) during infancy result in later-life depressive-like behavior in the Forced Swim Test (FST), a measure of behavioral despair in rodents (328, 329), that is accompanied by changes in amygdala function and preceded by disruptions in social behavior (26). When employed from PN8–12, these two complementary rodent models of early life abuse produced a reduction in sociability, as indexed by spending significantly less time in a social chamber compared to control animals reared with a normal mother – a behavioral pattern that was observable prior to weaning (PN23) and maintained in adolescence (PN45). However, animals experiencing early life abuse only showed depressive-like behavior in the FST during adolescence (PN45), as indicated by immobility – the passive state in which the animal makes only those movements necessary to keep its head above water (328, 330). In addition, depressive-like behavior in the FST in animals experiencing early life abuse was associated with increased c-Fos-ir in the basal, lateral, and central amygdala nuclei, suggesting that increased neural activity in these structures may contribute to the expression of depressive-like behavior in the FST (26). A causal relationship between amygdala function and depressive-like behavior in the FST was suggested through temporary inactivation (i.e., muscimol) of amygdala function during the FST, which normalized these behaviors to a level comparable to controls (26). Collectively, these findings suggest that the expression of depressive-like behavior in the FST following early life abuse is characterized by a hyper-functioning amygdala. Thus, abusive attachment appears to disrupt the developmental trajectory of the amygdala and modify the way that it responds to future stressors, which is supported by our work using rodent models of early life abuse.

Our findings are in accordance with clinical and animal literature indicating that early life adversity constitutes a prime risk factor for the development of psychopathologies characterized by dysregulated HPA-axis function (1, 5, 24, 32, 133, 319, 331–334), such as mood and affective disorders (37, 93, 335, 336), which also exhibit a developmental delay (309, 337, 338) Thus, these rodent models of early life abuse allow us to explore the ontogeny of depressive-like behavior and amygdala dysregulation, which is of clinical relevance because abnormal amygdala function and social behavior deficits as well as their relationship to later-life depressive-like behaviors have been documented in individuals with a history of early life abuse (71, 310, 331, 336).

MODULATION OF ADULT NEUROBEHAVIORAL FUNCTION BY INFANT-ATTACHMENT RELATED CUES

An ample body of evidence suggests that the quality of infant-attachment relationships results in long-term adaptations that have the ability to program subsequent behavioral, endocrine, and neural function (28, 109, 261, 310, 336). Results from our laboratory have shown that infant paired-odor-shock conditioning results in reduced fear learning and attenuated related amygdala function, dysregulation in neural networks underlying olfactory learning, and depressive-like behavior during adulthood (339–342). Importantly, attachment related sensory cues learned during infancy can play a critical role in modulating neurobehavioral responses during later-life. In humans, for example, cues associated with early life abuse elicit strong attraction and feelings of comfort (343). In rodents, presentation of an artificial maternal odor, resulting from infant paired-odor-shock conditioning, is able to reverse the behavioral effects of abusive attachment in rodent measures of depressive-like behavior, such as the sucrose consumption test and the FST (342). Specifically, the odor increased the latency to immobility and reduced the time spent immobile in the FST, but also increased the percentage of sucrose consumed during a sucrose preference test to levels comparable to controls. Furthermore, these restorative effects of a learned infant maternal odor on adult function were also observable at electrophysiological level, as odor presentation also normalized paired-pulse inhibition deficits in the amygdala. Collectively, these data suggest that early life experiences are able to shape adult neural circuits underlying behavior and that adult behaviors can be modified under environmental conditions in which learned infant cues are present. The discovery that infant cues can retain their value throughout the life-span and regulate later-life behaviors controlled by circuits implicated in emotion, learning, and social behavior is of great interest because it provides an opportunity for intervention and possibly correction of maladaptive outcomes related to psychopathology induced by adverse early life experiences within attachment. Thus, it appears that the enduring neurobehavioral

dysregulation stemming from early life abuse can be positively modulated by learned sensory cues related to infant-attachment.

CONCLUSION

In species requiring parental care, evolution has ensured that infants quickly learn and express robust preferences to the caregiver, regardless of the quality of care (48, 50). However, trauma within attachment leaves the infant particularly vulnerable to adult psychiatric disorders, behavioral changes in fear and anxiety, and alterations in neural circuits, particularly those regulating stress and emotion (71, 133, 334, 344, 345). In addition, early life stress can have negative effects on the neurobiology of the developing brain that are comparable to those induced by disruptions in infant-caregiver interactions (25, 346) Thus, early life experiences have enduring effects on the neuroplasticity of the HPA-axis, suggesting the HPA-axis is programmable via multiple environmental sources across development. In early development, stressors and maternal care jointly program HPA-axis responses and later-life function. The HPA-axis, however, remains modifiable during later stages of development during which infant-attachment related cues can exert a positive modulatory effect on later-life HPA-axis function as well as behavioral and endocrine responses to stress.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation Graduate Research Fellowship (DGE-1137475) to Millie Rincón-Cortés and NIH-MH091451, NIH-DC009910 to Regina M. Sullivan.

REFERENCES

1. Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early-life events permanently alter behavioral and hormonal responses to stressors? *Int J Dev Neurosci* (1998) **16**:149–64. doi:10.1016/S0736-5748(98)00025-2
2. Meaney MJ, Szyf M. Environmental programming of stress responses through DNA methylation: life at the interface between a dynamic environment and a fixed genome. *Dialogues Clin Neurosci* (2005) **7**:103–23.
3. Romeo RD, Tang AC, Sullivan RM. Early life experiences: enduring behavioral, neurological and endocrinological consequences. In: Anne DWP, Etgen M, editors. *Molecular Mechanisms of Hormone Actions on Behavior*. San Diego, CA: Academic Press (2009). p. 543–72.
4. Bale TL, Baram TZ, Brown AS, Goldstein JM, Insel TR, McCarthy MM, et al. Early life programming and neurodevelopmental disorders. *Biol Psychiatry* (2010) **68**:314–9. doi:10.1016/jbiopsych.2010.05.028
5. Grace CE, Kim SJ, Rogers JM. Maternal influences on epigenetic programming of the developing hypothalamic-pituitary-adrenal axis. *Birth Defects Res A Clin Mol Teratol* (2011) **91**:797–805. doi:10.1002/bdra.20824
6. Levine S. Maternal and environmental influences on the adrenocortical response to stress in weanling rats. *Science* (1967) **156**:258–60. doi:10.1126/science.156.3772.258
7. 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**:1659–62. doi:10.1126/science.277.5332.1659
8. Francis DD, Meaney MJ. Maternal care and the development of stress responses. *Curr Opin Neurobiol* (1999) **9**:128–34. doi:10.1016/S0959-4388(99)80016-6
9. Levine S. Primary social relationships influence the development of the hypothalamic – pituitary – adrenal axis in the rat. *Physiol Behav* (2001) **73**:255–60. doi:10.1016/S0031-9384(01)00496-6
10. Walker CD. Maternal touch and feed as critical regulators of behavioral and stress responses in the offspring. *Dev Psychobiol* (2010) **52**:638–50. doi:10.1002/dev.20492
11. Hofer MA. Hidden regulatory processes in early social relationships. In: Klopfer PH, editor. *Perspectives in Ethology: Social Behavior*. New York, NY: Plenum Press (1978). p. 135–66.
12. Hofer MA. Hidden regulators in attachment, separation, and loss. *Monogr Soc Res Child Dev* (1994) **59**:192–207. doi:10.2307/1166146
13. Dettling AC, Feldon J, Pryce CR. Repeated parental deprivation in the infant common marmoset (*Callithrix jacchus*, primates) and analysis of its effects on early development. *Biol Psychiatry* (2002) **52**:1037–46. doi:10.1016/S0006-3223(02)01460-9
14. Levine S. Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology* (2005) **30**:939–46. doi:10.1016/j.psyneuen.2005.03.013
15. Stanton ME, Gutierrez YR, Levine S. Maternal deprivation potentiates pituitary-adrenal stress responses in infant rats. *Behav Neurosci* (1988) **102**:692–700. doi:10.1037/0735-7044.102.5.692
16. Kuhn CM, Pauk J, Schanberg SM. Endocrine responses to mother-infant separation in developing rats. *Dev Psychobiol* (1990) **23**:395–410. doi:10.1002/dev.420230503
17. Levine S, Huchton DM, Wiener SG, Rosenfeld P. Time course of the effect of maternal deprivation on the hypothalamic-pituitary-adrenal axis in the infant rat. *Dev Psychobiol* (1991) **24**:547–58. doi:10.1002/dev.420240803
18. Rosenfeld P, Suchecki D, Levine S. Multifactorial regulation of the hypothalamic-pituitary-adrenal axis during development. *Neurosci Biobehav Rev* (1992) **16**:553–68. doi:10.1016/S0149-7634(05)80196-4
19. Avishai-Eliner S, Yi SJ, Newth CJ, Baram TZ. Effects of maternal and sibling deprivation on basal and stress induced hypothalamic-pituitary-adrenal components in the infant rat. *Neurosci Lett* (1995) **192**:49–52. doi:10.1016/0304-3940(95)11606-W
20. Gilles EE, Schultz L, Baram TZ. Abnormal corticosterone regulation in an immature rat model of continuous chronic stress. *Pediatr Neurol* (1996) **15**:114–9. doi:10.1016/0887-8994(96)00153-1
21. Ladd CO, Owens MJ, Nemeroff CB. Persistent changes in corticotropin-releasing factor neuronal systems induced by maternal deprivation. *Endocrinology* (1996) **137**:1212–8. doi:10.1210/endo.137.4.8625891
22. Cirulli F, Berry A, Alleva E. Early disruption of the mother-infant relationship: effects on brain plasticity and implications for psychopathology. *Neurosci Biobehav Rev* (2003) **27**:73–82. doi:10.1016/S0149-7634(03)00010-1
23. Ivy AS, Brunson KL, Sandman C, Baram TZ. Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. *Neuroscience* (2008) **154**:1132–42. doi:10.1016/j.neuroscience.2008.04.019
24. McEwen BS. Understanding the potency of stressful early life experiences on brain and body function. *Metabolism* (2008) **57**(Suppl 2):S11–5. doi:10.1016/j.metabol.2008.07.006
25. Raineki C, Moriceau S, Sullivan RM. Developing a neurobehavioral animal model of infant attachment to an abusive caregiver. *Biol Psychiatry* (2010) **67**:1137–45. doi:10.1016/j.biopsych.2009.12.019
26. Raineki C, Cortes MR, Belnoue L, Sullivan RM. Effects of early-life abuse differ across development: infant social behavior deficits are followed by adolescent depressive-like behaviors mediated by the amygdala. *J Neurosci* (2012) **32**:7758–65. doi:10.1523/JNEUROSCI.5843-11.2012
27. Plotsky PM, Meaney MJ. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Brain Res Mol Brain Res* (1993) **18**:195–200. doi:10.1016/0169-328X(93)90189-V
28. Canetti L, Bachar E, Galili-Weisstub E, De-Nour AK, Shalev AY. Parental bonding and mental health in adolescence. *Adolescence* (1997) **32**:381–94.
29. Caldji C, Tannenbaum B, Sharma S, Francis D, Plotsky PM, Meaney MJ. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc Natl Acad Sci U S A* (1998) **95**:5335–40. doi:10.1073/pnas.95.9.5335
30. Caldji C, Diorio J, Meaney MJ. Variations in maternal care in infancy regulate the development of stress reactivity. *Biol Psychiatry* (2000) **48**:1164–74. doi:10.1016/S0006-3223(00)01084-2
31. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* (2001) **49**:1023–39. doi:10.1016/S0006-3223(01)01157-X

32. 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**:419–49. doi:10.1017/S0954579401003029
33. De Bellis MD. The psychobiology of neglect. *Child Maltreat* (2005) **10**:150–72. doi:10.1177/1077559505275116
34. Hane AA, Fox NA. Ordinary variations in maternal caregiving influence human infants' stress reactivity. *Psychol Sci* (2006) **17**:550–6. doi:10.1111/j.1467-9280.2006.01742.x
35. Zhang TY, Bagot R, Parent C, Nesbitt C, Bredy TW, Caldji C, et al. Maternal programming of defensive responses through sustained effects on gene expression. *Biol Psychol* (2006) **73**:72–89. doi:10.1016/j.biopsycho.2006.01.009
36. Neigh GN, Gillespie CF, Nemeroff CB. The neurobiological toll of child abuse and neglect. *Trauma Violence Abuse* (2009) **10**:389–410. doi:10.1177/1524838009339758
37. Gershon A, Sudheimer K, Tirouvanziam R, Williams LM, O'Hara R. The long-term impact of early adversity on late-life psychiatric disorders. *Curr Psychiatry Rep* (2013) **15**:352. doi:10.1007/s11920-013-0352-9
38. Bowlby J. *Attachment. Attachment and Loss: Vol. I. Loss.* New York, NY: Basic Books (1969).
39. Ainsworth MDS. The development of infant-mother attachment. In: Ricciuti BCH, editor. *Review of Child Developmental Research.* Chicago: University of Chicago Press (1973). p. 1–94.
40. Gunnar M, Quevedo K. The neurobiology of stress and development. *Annu Rev Psychol* (2007) **58**:145–73. doi:10.1146/annurev.psych.58.110405.085605
41. Hess E. Ethology: an approach to the complete analysis of behavior. In: Brown EGR, Hess E, Mendler G, editors. *New Directions in Psychology.* New York, NY: Holt, Rineheart and Winston (1962). p. 159–99.
42. Ainsworth MDS. Object relations, dependency, and attachment: a theoretical review of the infant-mother relationship. *Child Dev* (1969) **40**:969–1025. doi:10.2307/1127008
43. Rajecki DWL, Michael E, Obmascher P. Toward a general theory of infantile attachment: a comparative review of aspects of the social bond. *Behav Brain Sci* (1978) **1**:417–64. doi:10.1017/S0140525X00075816
44. DeCasper AJ, Fifer WP. Of human bonding: newborns prefer their mothers' voices. *Science* (1980) **208**:1174–6. doi:10.1126/science.7375928
45. Hennessy MB, Li J, Levine S. Infant responsiveness to maternal cues in mice of 2 inbred lines. *Dev Psychobiol* (1980) **13**:77–84. doi:10.1002/dev.420130111
46. Gewirtz SBP. The attachment learning process and its relation to cultural and biological evolution: proximate and ultimate considerations. In: Field MRAT, editor. *The Psychobiology of Attachment and Separation.* Orlando, FL: Academic Press Inc (1985). p. 259–86.
47. Polan HJ, Hofer MA. Olfactory preference for mother over home nest shavings by newborn rats. *Dev Psychobiol* (1998) **33**:5–20. doi:10.1002/(SICI)1098-2302(199807)33:1<5::AID-DEV2>3.0.CO;2-P
48. Hofer MA, Sullivan RM. Towards a neurobiology of attachment. In: Nelson CA, editor. *Developmental Cognitive Neuroscience.* Cambridge: MIT Press (2001). p. 599–616.
49. Bowlby J. The nature of the child's tie to his mother. *Int J Psychoanal* (1958) **39**:350–73.
50. Sullivan RM. Developing a sense of safety: the neurobiology of neonatal attachment. *Ann N Y Acad Sci* (2003) **1008**:122–31. doi:10.1196/annals.1301.013
51. Sullivan R, Perry R, Sloan A, Kleinhaus K, Burtchen N. Infant bonding and attachment to the caregiver: insights from basic and clinical science. *Clin Perinatol* (2011) **38**:643–55. doi:10.1016/j.clp.2011.08.011
52. Ainsworth MS. Infant – mother attachment. *Am Psychol* (1979) **34**:932–7. doi:10.1037/0003-066X.34.10.932
53. Ainsworth MDS. Patterns of infant-mother attachments: antecedents and effects on development. *Bull N Y Acad Med* (1985) **61**:771–91.
54. Bowlby J. *Attachment: Attachment and Loss.* New York, NY: Basic Books (1982).
55. Bowlby J. *Attachment.* New York, NY: Basic Books (1965).
56. Fisher AE. *The Effects of Differential Early Treatment on the Social and Exploratory Behavior in Puppies.* Ph.D. thesis. University Park (PA): Pennsylvania State University (1955).
57. Stanley W. Differential human handling as reinforcing events and as treatments influencing later social behavior in basenji puppies. *Psychol Rep* (1962) **10**:775–88. doi:10.2466/pr0.1962.10.3.775
58. Harlow HF, Harlow MK. The affectional systems. In: Schrier HF, Stollnitz F, editors. *Behavior of Nonhuman Primates.* New York, NY: Academic Press (1965). p. 287–344.
59. Salzen EA. Imprinting and environmental learning. In: Aronson LR, Lehrman DS, Rosenblatt J, editors. *Development and Evolution of Behavior.* San Francisco, CA: W.H. Freeman (1970). p. 158–78.
60. Helfer ME, Kempe RS, Krugman RD. *The Battered Child.* Chicago, IL: University Press (1997).
61. Carroll KA, Maestripieri D. Infant abuse and neglect in monkeys – a discussion of definitions, epidemiology, etiology, and implications for child maltreatment: reply to Cicchetti (1998) and Mason (1998). *Psychol Bull* (1998) **123**:234–7. doi:10.1037/0033-2909.123.3.234
62. Maestripieri D, Carroll KA. Child abuse and neglect: usefulness of the animal data. *Psychol Bull* (1998) **123**:211–23. doi:10.1037/0033-2909.123.3.211
63. Hofer MA, Sullivan RM. Toward a neurobiology of attachment. In: Nelson CA, Luciana M, editors. *Handbook of Developmental Cognitive Neuroscience.* Cambridge: MIT Press (2008). p. 787–806.
64. Sapolsky R. Any kind of mother in a storm. *Nat Neurosci* (2009) **12**:1355–6. doi:10.1038/nn1109-1355
65. Joseph R. Environmental influences on neural plasticity, the limbic system, emotional development and attachment: a review. *Child Psychiatry Hum Dev* (1999) **29**:189–208. doi:10.1023/A:1022660923605
66. Glaser D. Child abuse and neglect and the brain – a review. *J Child Psychol Psychiatry* (2000) **41**:97–116. doi:10.1017/S0021963099004990
67. Bremner JD, Vermetten E. Stress and development: behavioral and biological consequences. *Dev Psychopathol* (2001) **13**:473–89. doi:10.1017/S0954579401003042
68. Dent GW, Smith MA, Levine S. Stress-induced alterations in locus coeruleus gene expression during ontogeny. *Brain Res Dev Brain Res* (2001) **127**:23–30. doi:10.1016/S0165-3806(01)00108-0
69. Vythilingam M, Heim C, Newport J, Miller AH, Anderson E, Bronen R, et al. Childhood trauma associated with smaller hippocampal volume in women with major depression. *Am J Psychiatry* (2002) **159**:2072–80. doi:10.1176/appi.ajp.159.12.2072
70. Bremner JD. Long-term effects of childhood abuse on brain and neurobiology. *Child Adolesc Psychiatr Clin N Am* (2003) **12**:271–92. doi:10.1016/S1056-4993(02)00098-6
71. Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP, Kim DM. The neurobiological consequences of early stress and childhood maltreatment. *Neurosci Biobehav Rev* (2003) **27**:33–44. doi:10.1016/S0149-7634(03)00007-1
72. Cirulli F, Francia N, Berry A, Aloe L, Alleva E, Suomi SJ. Early life stress as a risk factor for mental health: role of neurotrophins from rodents to non-human primates. *Neurosci Biobehav Rev* (2009) **33**:573–85. doi:10.1016/j.neubiorev.2008.09.001
73. Braun K, Bock J. The experience-dependent maturation of prefronto-limbic circuits and the origin of developmental psychopathology: implications for the pathogenesis and therapy of behavioural disorders. *Dev Med Child Neurol* (2011) **53**(Suppl 4):14–8. doi:10.1111/j.1469-8749.2011.04056.x
74. Blaze J, Scheuing L, Roth TL. Differential methylation of genes in the medial prefrontal cortex of developing and adult rats following exposure to maltreatment or nurturing care during infancy. *Dev Neurosci* (2013) **35**:306–16. doi:10.1016/j.ijdevneu.2013.10.001
75. Gee DG, Gabard-Durnam LJ, Flannery J, Goff B, Humphreys KL, Telzer EH, et al. Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation. *Proc Natl Acad Sci U S A* (2013) **110**:15638–43. doi:10.1073/pnas.1307893110
76. Rutter M. The long-term effects of early experience. *Dev Med Child Neurol* (1980) **22**:800–15. doi:10.1111/j.1469-8749.1980.tb03751.x
77. Cicchetti D. How research on child maltreatment has informed the study of child development: perspectives from development psychopathology. In: Cicchetti D, Carlson V, editors. *Child Maltreatment: Theory and Research on the Causes and Consequences of Child Abuse and Neglect.* New York, NY: Cambridge University Press (1990). p. 377–431.
78. Shields AM, Cicchetti D, Ryan RM. The development of emotional and behavioral self-regulation and social competence among maltreated school-age children. *Dev Psychopathol* (1994) **6**:57–75. doi:10.1017/S0954579400005885
79. Cicchetti D, Toth SL. A developmental psychopathology perspective on child abuse and neglect. *J Am Acad Child Adolesc Psychiatry* (1995) **34**:541–65. doi:10.1097/00004583-199505000-00008

80. Rutter M. Developmental catch-up, and deficit, following adoption after severe global early privation. English and Romanian Adoptees (ERA) Study Team. *J Child Psychol Psychiatry* (1998) **39**:465–76. doi:10.1017/S0021963098002236
81. O'Connor TG, Rutter M. Attachment disorder behavior following early severe deprivation: extension and longitudinal follow-up. English and Romanian Adoptees Study Team. *J Am Acad Child Adolesc Psychiatry* (2000) **39**:703–12. doi:10.1097/00004583-200006000-00008
82. De Bellis MD. Developmental traumatology: the psychobiological development of maltreated children and its implications for research, treatment, and policy. *Dev Psychopathol* (2001) **13**:539–64. doi:10.1017/S0954579401003078
83. Schore AN. Dysregulation of the right brain: a fundamental mechanism of traumatic attachment and the psychopathogenesis of posttraumatic stress disorder. *Aust N Z J Psychiatry* (2002) **36**:9–30. doi:10.1046/j.1440-1614.2002.00996.x
84. Nemeroff CB. Neurobiological consequences of childhood trauma. *J Clin Psychiatry* (2004) **65**(Suppl 1):18–28.
85. Rogosch FA, Cicchetti D. Child maltreatment, attention networks, and potential precursors to borderline personality disorder. *Dev Psychopathol* (2005) **17**:1071–89. doi:10.1017/S0954579405050509
86. Anda RF, Felitti VJ, Bremner JD, Walker JD, Whitfield C, Perry BD, et al. The enduring effects of abuse and related adverse experiences in childhood. A convergence of evidence from neurobiology and epidemiology. *Eur Arch Psychiatry Clin Neurosci* (2006) **256**:174–86. doi:10.1007/s00406-005-0624-4
87. Barber-Madden R, Cohn AH, Schloesser P. Prevention of child abuse: a public health agenda. *J Public Health Policy* (1988) **9**:167–76. doi:10.2307/3343003
88. Barnett D, Manly JT, Cicchetti D. Defining child maltreatment: the interface between policy and research. In: Toth DCSL, editor. *Child Abuse, Child Development, and Social Policy*. Norwood, NJ: Ablex (1993). p. 7–72.
89. Merrick J, Browne KD. Child abuse and neglect – a public health concern. *Public Health Rev* (1999) **27**:279–93.
90. Dawson G, Ashman SB, Carver LJ. The role of early experience in shaping behavioral and brain development and its implications for social policy. *Dev Psychopathol* (2000) **12**:695–712. doi:10.1017/S0954579400004089
91. Kendall-Tackett K. The health effects of childhood abuse: four pathways by which abuse can influence health. *Child Abuse Negl* (2002) **26**:715–29. doi:10.1016/S0145-2134(02)00343-5
92. De Kloet ER, Rosenfeld P, Van Eekelen JA, Sutanto W, Levine S. Stress, glucocorticoids and development. *Prog Brain Res* (1988) **73**:101–20. doi:10.1016/S0079-6123(08)60500-2
93. Heim C, Plotsky PM, Nemeroff CB. Importance of studying the contributions of early adverse experience to neurobiological findings in depression. *Neuropsychopharmacology* (2004) **29**:641–8. doi:10.1038/sj.npp.1300397
94. Kaufman J, Plotsky PM, Nemeroff CB, Charney DS. Effects of early adverse experiences on brain structure and function: clinical implications. *Biol Psychiatry* (2000) **48**:778–90. doi:10.1016/S0006-3223(00)00998-7
95. Stevens HE, Leckman JF, Coplan JD, Suomi SJ. Risk and resilience: early manipulation of macaque social experience and persistent behavioral and neurophysiological outcomes. *J Am Acad Child Adolesc Psychiatry* (2009) **48**:114–27. doi:10.1097/CHI.0b013e318193064c
96. Korosi A, Baram TZ. Plasticity of the stress response early in life: mechanisms and significance. *Dev Psychobiol* (2010) **52**:661–70. doi:10.1002/dev.20490
97. Lyons DM, Parker KJ, Schatzberg AF. Animal models of early life stress: implications for understanding resilience. *Dev Psychobiol* (2010) **52**:402–10. doi:10.1002/dev.20429
98. Franklin TB, Saab BJ, Mansuy IM. Neural mechanisms of stress resilience and vulnerability. *Neuron* (2012) **75**:747–61. doi:10.1016/j.neuron.2012.08.016
99. Rosenblum LA, Coplan JD, Friedman S, Bassoff T, Gorman JM, Andrews MW. Adverse early experiences affect noradrenergic and serotonergic functioning in adult primates. *Biol Psychiatry* (1994) **35**:221–7. doi:10.1016/0006-3223(94)91252-1
100. 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**:10171–9.
101. Andersen SL, Lyss PJ, Dumont NL, Teicher MH. Enduring neurochemical effects of early maternal separation on limbic structures. *Ann N Y Acad Sci* (1999) **877**:756–9. doi:10.1111/j.1749-6632.1999.tb09317.x
102. 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**:52–63. doi:10.1016/S0006-8993(02)02985-2
103. Brake WG, Zhang TY, Diorio J, Meaney MJ, Gratton A. Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *Eur J Neurosci* (2004) **19**:1863–74. doi:10.1111/j.1460-9568.2004.03286.x
104. Pryce CR, Dettling AC, Spengler M, Schnell CR, Feldon J. Deprivation of parenting disrupts development of homeostatic and reward systems in marmoset monkey offspring. *Biol Psychiatry* (2004) **56**:72–9. doi:10.1016/j.biopsych.2004.05.002
105. Tottenham N, Hare TA, Quinn BT, McCarry TW, Nurse M, Gilhooly T, et al. Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Dev Sci* (2010) **13**:46–61. doi:10.1111/j.1467-7687.2009.00852.x
106. Hostinar CE, Stellern SA, Schaefer C, Carlson SM, Gunnar MR. Associations between early life adversity and executive function in children adopted internationally from orphanages. *Proc Natl Acad Sci U S A* (2012) **109**(Suppl 2):17208–12. doi:10.1073/pnas.1121246109
107. Tottenham N. Human amygdala development in the absence of species-expected caregiving. *Dev Psychobiol* (2012) **54**:598–611. doi:10.3389/neuro.09.068.2009
108. Malter Cohen M, Jing D, Yang RR, Tottenham N, Lee FS, Casey BJ. Early-life stress has persistent effects on amygdala function and development in mice and humans. *Proc Natl Acad Sci U S A* (2013) **110**:18274–8. doi:10.1073/pnas.1310163110
109. Ladd CO, Huot RL, Thrivikraman KV, Nemeroff CB, Meaney MJ, Plotsky PM. Long-term behavioral and neuroendocrine adaptations to adverse early experience. *Prog Brain Res* (2000) **122**:81–103. doi:10.1016/S0079-6123(08)62132-9
110. Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinephrine release in the hypothalamic paraventricular nucleus. *J Neuroendocrinol* (2000) **12**:5–12. doi:10.1046/j.1365-2826.2000.00422.x
111. Roth TL, Sullivan RM. Memory of early maltreatment: neonatal behavioral and neural correlates of maternal maltreatment within the context of classical conditioning. *Biol Psychiatry* (2005) **57**:823–31. doi:10.1016/j.biopsych.2005.01.032
112. Fenoglio KA, Chen Y, Baram TZ. Neuroplasticity of the hypothalamic-pituitary-adrenal axis early in life requires recurrent recruitment of stress-regulating brain regions. *J Neurosci* (2006) **26**:2434–42. doi:10.1523/JNEUROSCI.4080-05.2006
113. Branchi I, D'Andrea I, Cirulli F, Lipp HP, Alleva E. Shaping brain development: mouse communal nesting blunts adult neuroendocrine and behavioral response to social stress and modifies chronic antidepressant treatment outcome. *Psychoneuroendocrinology* (2010) **35**:743–51. doi:10.1016/j.psyneuen.2009
114. Ivy AS, Rex CS, Chen Y, Dube C, Maras PM, Grigoriadis DE, et al. Hippocampal dysfunction and cognitive impairments provoked by chronic early-life stress involve excessive activation of CRH receptors. *J Neurosci* (2010) **30**:13005–15. doi:10.1523/JNEUROSCI.1784-10.2010
115. Daskalakis NP, Diamantopoulou A, Claessens SE, Remmers E, Tjalve M, Oitzl MS, et al. Early experience of a novel-environment in isolation primes a fearful phenotype characterized by persistent amygdala activation. *Psychoneuroendocrinology* (2014) **39**:39–57. doi:10.1016/j.psyneuen.2013.09.021
116. Butte JC, Kakihana R, Farnham ML, Noble EP. The relationship between brain and plasma corticosterone stress response in developing rats. *Endocrinology* (1973) **92**:1775–9. doi:10.1210/endo-92-6-1775
117. Henning SJ. Plasma concentrations of total and free corticosterone during development in the rat. *Am J Physiol* (1978) **235**:E451–6.
118. Guillet R, Saffran M, Michaelson SM. Pituitary-adrenal response in neonatal rats. *Endocrinology* (1980) **106**:991–4. doi:10.1210/endo-106-3-991
119. Walker SJ, Vrana KE. Pituitary corticotroph function during the stress hyporesponsive period in neonatal rats. *Neuroendocrinology* (1993) **57**:1003–10. doi:10.1159/000126464
120. Viau V, Sharma S, Meaney MJ. Changes in plasma adrenocorticotropin, corticosterone, corticosteroid-binding globulin, and hippocampal glucocorticoid receptor occupancy/translocation in rat pups in response to stress. *J Neuroendocrinol* (1996) **8**:1–8. doi:10.1111/j.1365-2826.1996.tb00680.x
121. Vazquez DM. Stress and the developing limbic-hypothalamic-pituitary-adrenal axis. *Psychoneuroendocrinology* (1998) **23**:663–700. doi:10.1016/S0306-4530(98)00029-8

122. Dallman MF. Moments in time – the neonatal rat hypothalamo-pituitary-adrenal axis. *Endocrinology* (2000) **141**:1590–2. doi:10.1210/endo.141.5.7527
123. Petersen HH, Andreassen TK, Breiderhoff T, Brasen JH, Schulz H, Gross V, et al. Hyporesponsiveness to glucocorticoids in mice genetically deficient for the corticosteroid binding globulin. *Mol Cell Biol* (2006) **26**:7236–45. doi:10.1128/MCB.00400-06
124. Levine S. The pituitary-adrenal system and the developing brain. *Prog Brain Res* (1970) **32**:79–85. doi:10.1016/S0079-6123(08)61521-6
125. Cote TE, Yasumura S. Effect of ACTH and histamine stress on serum corticosterone and adrenal cyclic AMP levels in immature rats. *Endocrinology* (1975) **96**:1044–7. doi:10.1210/endo-96-4-1044
126. Martin CE, Cake MH, Hartmann PE, Cook IF. Relationship between foetal corticosteroids, maternal progesterone and parturition in the rat. *Acta Endocrinol (Copenh)* (1977) **84**:167–76.
127. Walker CD, Perrin M, Vale W, Rivier C. Ontogeny of the stress response in the rat: role of the pituitary and the hypothalamus. *Endocrinology* (1986) **118**:1445–51. doi:10.1210/endo-118-4-1445
128. Walker CD, Sapolsky RM, Meaney MJ, Vale WW, Rivier CL. Increased pituitary sensitivity to glucocorticoid feedback during the stress nonresponsive period in the neonatal rat. *Endocrinology* (1986) **119**:1816–21. doi:10.1210/endo-119-4-1816
129. Levine S. The ontogeny of the hypothalamic-pituitary-adrenal axis. The influence of maternal factors. *Ann N Y Acad Sci* (1994) **746**:275–88. doi:10.1111/j.1749-6632.1994.tb39245.x
130. Grino M, Paulmyer-Lacroix O, Anglade G, Oliver C. Molecular aspects of the regulation of the hypothalamo-pituitary-adrenal axis during development in the rat. *Ann N Y Acad Sci* (1995) **771**:339–51. doi:10.1111/j.1749-6632.1995.tb44693.x
131. Ramsay DS, Lewis M. Developmental change in infant cortisol and behavioral response to inoculation. *Child Dev* (1994) **65**:1491–502. doi:10.2307/1131513
132. Gunnar MR, Brodersen L, Krueger K, Rigatuso J. Dampening of adrenocortical responses during infancy: normative changes and individual differences. *Child Dev* (1996) **67**:877–89. doi:10.2307/1131867
133. Gunnar MR. Integrating neuroscience and psychological approaches in the study of early experiences. *Ann N Y Acad Sci* (2003) **1008**:238–47. doi:10.1196/annals.1301.024
134. Gunnar MR, Fisher PA. Bringing basic research on early experience and stress neurobiology to bear on preventive interventions for neglected and maltreated children. *Dev Psychopathol* (2006) **18**:651–77. doi:10.1017/S0954579406060330
135. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* (2009) **10**:434–45. doi:10.1038/nrn2639
136. Sapolsky RM, Meaney MJ. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Res* (1986) **396**:64–76. doi:10.1016/0165-0173(86)90010-X
137. Nachmias M, Gunnar M, Mangelsdorf S, Parritz RH, Buss K. Behavioral inhibition and stress reactivity: the moderating role of attachment security. *Child Dev* (1996) **67**:508–22. doi:10.2307/1131829
138. Dettling AC, Parker SW, Lane S, Sebanc A, Gunnar MR. Quality of care and temperament determine changes in cortisol concentrations over the day for young children in childcare. *Psychoneuroendocrinology* (2000) **25**:819–36. doi:10.1016/S0306-4530(00)00028-7
139. Gunnar MR, Donzella B. Social regulation of the cortisol levels in early human development. *Psychoneuroendocrinology* (2002) **27**:199–220. doi:10.1016/S0306-4530(01)00045-2
140. Levine S. Regulation of the hypothalamic-pituitary-adrenal axis in the neonatal rat: the role of maternal behavior. *Neurotox Res* (2002) **4**:557–64. doi:10.1080/10298420290030569
141. Schoenfeld NM, Leathem JH, Rabii J. Maturation of adrenal stress responsiveness in the rat. *Neuroendocrinology* (1980) **31**:101–5. doi:10.1159/000123058
142. Walker CD, Scribner KA, Cascio CS, Dallman MF. The pituitary-adrenocortical system of neonatal rats is responsive to stress throughout development in a time-dependent and stressor-specific fashion. *Endocrinology* (1991) **128**:1385–95. doi:10.1210/endo-128-3-1385
143. Yi SJ, Baram TZ. Corticotropin-releasing hormone mediates the response to cold stress in the neonatal rat without compensatory enhancement of the peptide's gene expression. *Endocrinology* (1994) **135**:2364–8. doi:10.1210/endo.135.6.7988418
144. Hatalski CG, Guirguis C, Baram TZ. Corticotropin releasing factor mRNA expression in the hypothalamic paraventricular nucleus and the central nucleus of the amygdala is modulated by repeated acute stress in the immature rat. *J Neuroendocrinol* (1998) **10**:663–9. doi:10.1046/j.1365-2826.1998.00246.x
145. Dent GW, Okimoto DK, Smith MA, Levine S. Stress-induced alterations in corticotropin-releasing hormone and vasopressin gene expression in the paraventricular nucleus during ontogeny. *Neuroendocrinology* (2000) **71**:333–42. doi:10.1159/000054554
146. Meaney MJ, Sapolsky RM, McEwen BS. The development of the glucocorticoid receptor system in the rat limbic brain. II. An autoradiographic study. *Brain Res* (1985) **350**:165–8. doi:10.1016/0165-3806(85)90260-3
147. Alexis MN, Kitraki E, Spanou K, Stylianopoulou F, Sekeris CE. Ontogeny of the glucocorticoid receptor in the rat brain. *Adv Exp Med Biol* (1990) **265**:269–76. doi:10.1007/978-1-4757-5876-4_25
148. Stanton ME, Levine S. Inhibition of infant glucocorticoid stress response: specific role of maternal cues. *Dev Psychobiol* (1990) **23**:411–26. doi:10.1002/dev.420230504
149. Widmaier EP. Glucose homeostasis and hypothalamic-pituitary-adrenocortical axis during development in rats. *Am J Physiol* (1990) **259**:E601–13.
150. Arai M, Widmaier EP. Activation of the pituitary-adrenocortical axis in day-old rats by insulin-induced hypoglycemia. *Endocrinology* (1991) **129**:1505–12. doi:10.1210/endo-129-3-1505
151. Rosenfeld P, Van Eekelen JA, Levine S, De Kloet ER. Ontogeny of corticosteroid receptors in the brain. *Cell Mol Neurobiol* (1993) **13**:295–319. doi:10.1007/BF00711575
152. 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**:185–92. doi:10.1016/0165-3806(93)90022-3
153. Eghbal-Ahmadi M, Avishai-Eliner S, Hatalski CG, Baram TZ. Differential regulation of the expression of corticotropin-releasing factor receptor type 2 (CRF2) in hypothalamus and amygdala of the immature rat by sensory input and food intake. *J Neurosci* (1999) **19**:3982–91.
154. Hofer MA. Psychobiological roots of early attachment. *Curr Dir Psychol Sci* (2006) **15**:84–8. doi:10.1111/j.0963-7214.2006.00412.x
155. Baram TZ, Yi S, Avishai-Eliner S, Schultz L. Development neurobiology of the stress response: multilevel regulation of corticotropin-releasing hormone function. *Ann N Y Acad Sci* (1997) **814**:252–65. doi:10.1111/j.1749-6632.1997.tb46161.x
156. 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**:799–807. doi:10.1046/j.1365-2826.2001.00698.x
157. Avishai-Eliner S, Eghbal-Ahmadi M, Tabachnik E, Brunson KL, Baram TZ. Down-regulation of hypothalamic corticotropin-releasing hormone messenger ribonucleic acid (mRNA) precedes early-life experience-induced changes in hippocampal glucocorticoid receptor mRNA. *Endocrinology* (2001) **142**:89–97. doi:10.1210/en.142.1.89
158. Sanchez MM. The impact of early adverse care on HPA axis development: nonhuman primate models. *Horm Behav* (2006) **50**:623–31. doi:10.1016/j.yhbeh.2006.06.012
159. 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**:224–44. doi:10.1111/j.1469-7610.2007.01730.x
160. Howell BR, Sanchez MM. Understanding behavioral effects of early life stress using the reactive scope and allostatic load models. *Dev Psychopathol* (2011) **23**:1001–16. doi:10.1017/S0954579411000460
161. Bolhuis JJ, Honey RC. Imprinting, learning and development: from behaviour to brain and back. *Trends Neurosci* (1998) **21**:306–11. doi:10.1016/S0166-2236(98)01258-2
162. Ziabreva I, Poeggel G, Schnabel R, Braun K. Separation-induced receptor changes in the hippocampus and amygdala of *Octodon degus*: influence of maternal vocalizations. *J Neurosci* (2003) **23**:5329–36.
163. Insel TR, Young LJ. The neurobiology of attachment. *Nat Rev Neurosci* (2001) **2**:129–36. doi:10.1038/35053579
164. Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci* (1997) **20**:78–84. doi:10.1016/S0166-2236(96)10069-2

165. Sullivan RM, Landers M, Yeaman B, Wilson DA. Good memories of bad events in infancy. *Nature* (2000) **407**:38–9. doi:10.1038/35024156
166. Moriceau S, Sullivan RM. Maternal presence serves as a switch between learning fear and attraction in infancy. *Nat Neurosci* (2006) **9**:1004–6. doi:10.1038/nn1733
167. Shionoya K, Moriceau S, Bradstock P, Sullivan RM. Maternal attenuation of hypothalamic paraventricular nucleus norepinephrine switches avoidance learning to preference learning in preweanling rat pups. *Horm Behav* (2007) **52**:391–400. doi:10.1016/j.yhbeh.2007.06.004
168. Miller SS, Spear NE. Olfactory learning in the rat neonate soon after birth. *Dev Psychobiol* (2008) **50**:554–65. doi:10.1002/dev.20318
169. Sullivan RM, Holman PJ. Transitions in sensitive period attachment learning in infancy: the role of corticosterone. *Neurosci Biobehav Rev* (2010) **34**:835–44. doi:10.1016/j.neubiorev.2009.11.010
170. Leon M. Dietary control of maternal pheromone in the lactating rat. *Physiol Behav* (1975) **14**:311–9. doi:10.1016/0031-9384(75)90039-6
171. Pedersen PE, Williams CL, Blass EM. Activation and odor conditioning of suckling behavior in 3-day-old albino rats. *J Exp Psychol Anim Behav Process* (1982) **8**:329–41. doi:10.1037/0097-7403.8.4.329
172. Leon M. Neuroethology of olfactory preference development. *J Neurobiol* (1992) **23**:1557–73. doi:10.1002/neu.480231012
173. Kojima S, Alberts JR. Maternal care can rapidly induce an odor-guided huddling preference in rat pups. *Dev Psychobiol* (2009) **51**:95–105. doi:10.1002/dev.20349
174. Moriceau S, Roth TL, Sullivan RM. Rodent model of infant attachment learning and stress. *Dev Psychobiol* (2010) **52**:651–60. doi:10.1002/dev.20482
175. Landers MS, Sullivan RM. The development and neurobiology of infant attachment and fear. *Dev Neurosci* (2012) **34**:101–14. doi:10.1159/000336732
176. Singh PJ, Tobach E. Olfactory bulbectomy and nursing behavior in rat pups (Wistar DAB). *Dev Psychobiol* (1975) **8**:151–64. doi:10.1002/dev.420080207
177. Galef BG Jr, Kaner HC. Establishment and maintenance of preference for natural and artificial olfactory stimuli in juvenile rats. *J Comp Physiol Psychol* (1980) **94**:588–95. doi:10.1037/h0077693
178. Galef BG Jr. Acquisition and waning of exposure-induced attraction to a non-natural odor in rat pups. *Dev Psychobiol* (1982) **15**:479–90. doi:10.1002/dev.420150510
179. Alberts JR, May B. Nonnutritive, thermotactile induction of filial huddling in rat pups. *Dev Psychobiol* (1984) **17**:161–81. doi:10.1002/dev.420170207
180. Caza PA, Spear NE. Short-term exposure to an odor increases its subsequent preference in preweanling rats: a descriptive profile of the phenomenon. *Dev Psychobiol* (1984) **17**:407–22. doi:10.1002/dev.420170407
181. Duveau A, Godinot F. Influence of the odorization of the rearing environment on the development of odor-guided behavior in rat pups. *Physiol Behav* (1988) **42**:265–70. doi:10.1016/0031-9384(88)90080-7
182. Sullivan RM, Wilson DA, Wong R, Correa A, Leon M. Modified behavioral and olfactory bulb responses to maternal odors in preweanling rats. *Brain Res Dev Brain Res* (1990) **53**:243–7. doi:10.1016/0165-3806(90)90013-O
183. Johanson IB, Hall WG. Appetitive learning in 1-day-old rat pups. *Science* (1979) **205**:419–21. doi:10.1126/science.451612
184. Johanson IB, Teicher MH. Classical conditioning of an odor preference in 3-day-old rats. *Behav Neural Biol* (1980) **29**:132–6. doi:10.1016/S0163-1047(80)92596-0
185. Brake SC. Suckling infant rats learn a preference for a novel olfactory stimulus paired with milk delivery. *Science* (1981) **211**:506–8. doi:10.1126/science.7192882
186. Sullivan RM, Leon M. Early olfactory learning induces an enhanced olfactory bulb response in young rats. *Brain Res* (1986) **392**:278–82. doi:10.1016/0165-3806(86)90256-7
187. Sullivan RM, Wilson DA. Neural correlates of conditioned odor avoidance in infant rats. *Behav Neurosci* (1991) **105**:307–12. doi:10.1037/0735-7044.105.2.307
188. Moriceau S, Shionoya K, Jakubs K, Sullivan RM. Early-life stress disrupts attachment learning: the role of amygdala corticosterone, locus coeruleus corticotropin releasing hormone, and olfactory bulb norepinephrine. *J Neurosci* (2009) **29**:15745–55. doi:10.1523/JNEUROSCI.4106-09.2009
189. Levine S. Plasma-free corticosteroid response to electric shock in rats stimulated in infancy. *Science* (1962) **135**:795–6. doi:10.1126/science.135.3506.795-a
190. Haroutunian V, Campbell BA. Emergence of interoceptive and exteroceptive control of behavior in rats. *Science* (1979) **205**:927–9. doi:10.1126/science.472715
191. Camp LL, Rudy JW. Changes in the categorization of appetitive and aversive events during postnatal development of the rat. *Dev Psychobiol* (1988) **21**:25–42. doi:10.1002/dev.420210103
192. Otto T, Cousens G, Rajewski K. Odor-guided fear conditioning in rats: 1. Acquisition, retention, and latent inhibition. *Behav Neurosci* (1997) **111**:1257–64. doi:10.1037/0735-7044.111.6.1257
193. Paschall GY, Davis M. Olfactory-mediated fear-potentiated startle. *Behav Neurosci* (2002) **116**:4–12. doi:10.1037/0735-7044.116.1.4
194. Collier AC, Bolles RC. The ontogenesis of defensive reactions to shock in preweanling rats. *Dev Psychobiol* (1980) **13**:141–50. doi:10.1002/dev.420130206
195. Emerich DF, Scalzo FM, Enters EK, Spear NE, Spear LP. Effects of 6-hydroxydopamine-induced catecholamine depletion on shock-precipitated wall climbing of infant rat pups. *Dev Psychobiol* (1985) **18**:215–27. doi:10.1002/dev.420180303
196. Barr GA. Ontogeny of nociception and antinociception. *NIDA Res Monogr* (1995) **158**:172–201.
197. Fitzgerald M. The development of nociceptive circuits. *Nat Rev Neurosci* (2005) **6**:507–20. doi:10.1038/nrn1701
198. Fanselow MS, LeDoux JE. Why we think plasticity underlying Pavlovian fear conditioning occurs in the basolateral amygdala. *Neuron* (1999) **23**:229–32. doi:10.1016/S0896-6273(00)80775-8
199. Fendt M, Fanselow MS. The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev* (1999) **23**:743–60. doi:10.1016/S0149-7634(99)00016-0
200. Maren S. The amygdala, synaptic plasticity, and fear memory. *Ann N Y Acad Sci* (2003) **985**:106–13. doi:10.1111/j.1749-6632.2003.tb07075.x
201. Moriceau S, Wilson DA, Levine S, Sullivan RM. Dual circuitry for odor-shock conditioning during infancy: corticosterone switches between fear and attraction via amygdala. *J Neurosci* (2006) **26**:6737–48. doi:10.1523/JNEUROSCI.0499-06.2006
202. Johansen JP, Cain CK, Ostroff LE, Ledoux JE. Molecular mechanisms of fear learning and memory. *Cell* (2011) **147**:509–24. doi:10.1016/j.cell.2011.10.009
203. Sullivan RM. Unique characteristics of neonatal classical conditioning: the role of the amygdala and locus coeruleus. *Integr Physiol Behav Sci* (2001) **36**:293–307. doi:10.1007/BF02688797
204. Coopersmith R, Lee S, Leon M. Olfactory bulb responses after odor aversion learning by young rats. *Brain Res* (1986) **389**:271–7. doi:10.1016/0165-3806(86)90195-1
205. Wilson DA, Sullivan RM, Leon M. Single-unit analysis of postnatal olfactory learning: modified olfactory bulb output response patterns to learned attractive odors. *J Neurosci* (1987) **7**:3154–62.
206. McLean JH, Harley CW, Darby-King A, Yuan Q. pCREB in the neonate rat olfactory bulb is selectively and transiently increased by odor preference-conditioned training. *Learn Mem* (1999) **6**:608–18. doi:10.1101/lm.6.6.608
207. Yuan Q, Harley CW, Darby-King A, Neve RL, McLean JH. Early odor preference learning in the rat: bidirectional effects of cAMP response element-binding protein (CREB) and mutant CREB support a causal role for phosphorylated CREB. *J Neurosci* (2003) **23**:4760–5.
208. Zhang JJ, Okutani F, Inoue S, Kaba H. Activation of the cyclic AMP response element-binding protein signaling pathway in the olfactory bulb is required for the acquisition of olfactory aversive learning in young rats. *Neuroscience* (2003) **117**:707–13. doi:10.1016/S0306-4522(02)00962-4
209. Yuan Q, Mutoh H, Debarbieux F, Knopfel T. Calcium signaling in mitral cell dendrites of olfactory bulbs of neonatal rats and mice during olfactory nerve stimulation and beta-adrenoceptor activation. *Learn Mem* (2004) **11**:406–11. doi:10.1101/lm.75204
210. Yuan Q. Theta bursts in the olfactory nerve paired with beta-adrenoceptor activation induce calcium elevation in mitral cells: a mechanism for odor preference learning in the neonate rat. *Learn Mem* (2009) **16**:676–81. doi:10.1101/lm.1569309
211. Woo CC, Coopersmith R, Leon M. Localized changes in olfactory bulb morphology associated with early olfactory learning. *J Comp Neurol* (1987) **263**:113–25. doi:10.1002/cne.902630110

212. Johnson BA, Woo CC, Duong H, Nguyen V, Leon M. A learned odor evokes an enhanced Fos-like glomerular response in the olfactory bulb of young rats. *Brain Res* (1995) **699**:192–200. doi:10.1016/0006-8993(95)00896-X
213. Yuan Q, Harley CW, McLean JH, Knopfel T. Optical imaging of odor preference memory in the rat olfactory bulb. *J Neurophysiol* (2002) **87**:3156–9.
214. Shakhawat AM, Harley CW, Yuan Q. Olfactory bulb alpha 2-adrenoceptor activation promotes rat pup odor-preference learning via a cAMP-independent mechanism. *Learn Mem* (2012) **19**:499–502. doi:10.1101/lm.027359.112
215. Wilson DA, Leon M. Spatial patterns of olfactory bulb single-unit responses to learned olfactory cues in young rats. *J Neurophysiol* (1988) **59**:1770–82.
216. Sullivan RM, Wilson DA. Role of the amygdala complex in early olfactory associative learning. *Behav Neurosci* (1993) **107**:254–63. doi:10.1037/0735-7044.107.2.254
217. Wiedenmayer CP, Barr GA. Developmental changes in c-fos expression to an age-specific social stressor in infant rats. *Behav Brain Res* (2001) **126**:147–57. doi:10.1016/S0166-4328(01)00260-1
218. Thompson JV, Sullivan RM, Wilson DA. Developmental emergence of fear learning corresponds with changes in amygdala synaptic plasticity. *Brain Res* (2008) **1200**:58–65. doi:10.1016/j.brainres.2008.01.057
219. Sullivan RM, Hofer MA, Brake SC. Olfactory-guided orientation in neonatal rats is enhanced by a conditioned change in behavioral state. *Dev Psychobiol* (1986) **19**:615–23. doi:10.1002/dev.420190612
220. Shipley MT, Halloran FJ, De La Torre J. Surprisingly rich projection from locus coeruleus to the olfactory bulb in the rat. *Brain Res* (1985) **329**:294–9. doi:10.1016/0006-8993(85)90537-2
221. McLean JH, Shipley MT. Postnatal development of the noradrenergic projection from locus coeruleus to the olfactory bulb in the rat. *J Comp Neurol* (1991) **304**:467–77. doi:10.1002/cne.903040310
222. Nakamura S, Kimura F, Sakaguchi T. Postnatal development of electrical activity in the locus coeruleus. *J Neurophysiol* (1987) **58**:510–24.
223. Nakamura S, Sakaguchi T. Development and plasticity of the locus coeruleus: a review of recent physiological and pharmacological experimentation. *Prog Neurobiol* (1990) **34**:505–26. doi:10.1016/0301-0082(90)90018-C
224. Marshall KC, Christie MJ, Finlayson PG, Williams JT. Developmental aspects of the locus coeruleus-noradrenaline system. *Prog Brain Res* (1991) **88**:173–85. doi:10.1016/S0079-6123(08)63807-8
225. Rangel S, Leon M. Early odor preference training increases olfactory bulb norepinephrine. *Brain Res Dev Brain Res* (1995) **85**:187–91. doi:10.1016/0165-3806(94)00211-H
226. Winzer-Serhan UH, Leslie FM. Expression of alpha 2A adrenoceptors during rat neocortical development. *J Neurobiol* (1999) **38**:259–69. doi:10.1002/(SICI)1097-4695(19990205)38:2<259::AID-NEU8>3.0.CO;2-U
227. Moriceau S, Sullivan RM. Unique neural circuitry for neonatal olfactory learning. *J Neurosci* (2004) **24**:1182–9. doi:10.1523/JNEUROSCI.4578-03.2004
228. Sullivan RM, Zyzak DR, Skierkowski P, Wilson DA. The role of olfactory bulb norepinephrine in early olfactory learning. *Brain Res Dev Brain Res* (1992) **70**:279–82. doi:10.1016/0165-3806(92)90207-D
229. Sullivan RM, Wilson DA. The locus coeruleus, norepinephrine, and memory in newborns. *Brain Res Bull* (1994) **35**:467–72. doi:10.1016/0361-9230(94)90160-0
230. Sullivan RM, Wilson DA, Lemon C, Gerhardt GA. Bilateral 6-OHDA lesions of the locus coeruleus impair associative olfactory learning in newborn rats. *Brain Res* (1994) **643**:306–9. doi:10.1016/0006-8993(94)90038-8
231. Langdon PE, Harley CW, McLean JH. Increased beta adrenoceptor activation overcomes conditioned olfactory learning deficits induced by serotonin depletion. *Brain Res Dev Brain Res* (1997) **102**:291–3. doi:10.1016/S0165-3806(97)00090-4
232. Sullivan RM, Stackenwalt G, Nasr F, Lemon C, Wilson DA. Association of an odor with activation of olfactory bulb noradrenergic beta-receptors or locus coeruleus stimulation is sufficient to produce learned approach responses to that odor in neonatal rats. *Behav Neurosci* (2000) **114**:957–62. doi:10.1037/0735-7044.114.5.957
233. Fanselow MS, Gale GD. The amygdala, fear, and memory. *Ann N Y Acad Sci* (2003) **985**:125–34. doi:10.1111/j.1749-6632.2003.tb07077.x
234. Blozovski D, Cudennec A. Passive avoidance learning in the young rat. *Dev Psychobiol* (1980) **13**:513–8. doi:10.1002/dev.420130510
235. Myslivecek J. Inhibitory learning and memory in newborn rats. *Prog Neurobiol* (1997) **53**:399–430. doi:10.1016/S0301-0082(97)00036-1
236. Stanton ME. Multiple memory systems, development and conditioning. *Behav Brain Res* (2000) **110**:25–37. doi:10.1016/S0166-4328(99)00182-5
237. Sullivan RM. Developmental changes in olfactory behavior and limbic circuitry. *Chem Senses* (2005) **30**(Suppl 1):i152–3. doi:10.1093/chemse/bjh159
238. Moriceau S, Sullivan RM. Neurobiology of infant attachment. *Dev Psychobiol* (2005) **47**:230–42. doi:10.1002/dev.20093
239. Takahashi LK, Rubin WW. Corticosteroid induction of threat-induced behavioral inhibition in preweanling rats. *Behav Neurosci* (1993) **107**:860–6. doi:10.1037/0735-7044.107.5.860
240. Takahashi LK. Organizing action of corticosterone on the development of behavioral inhibition in the preweanling rat. *Brain Res Dev Brain Res* (1994) **81**:121–7. doi:10.1016/0165-3806(94)90074-4
241. Moriceau S, Sullivan RM. Corticosterone influences on mammalian neonatal sensitive-period learning. *Behav Neurosci* (2004) **118**:274–81. doi:10.1037/0735-7044.118.2.274
242. Moriceau S, Roth TL, Okotoghaide T, Sullivan RM. Corticosterone controls the developmental emergence of fear and amygdala function to predator odors in infant rat pups. *Int J Dev Neurosci* (2004) **22**:415–22. doi:10.1016/j.ijdevneu.2004.05.011
243. Barr GA, Moriceau S, Shionoya K, Muzny K, Gao P, Wang S, et al. Transitions in infant learning are modulated by dopamine in the amygdala. *Nat Neurosci* (2009) **12**:1367–9. doi:10.1038/nn.2403
244. Collier AC, Mast J, Meyer DR, Jacobs CE. Approach-avoidance conflict in preweanling rats: a developmental study. *Anim Learn Behav* (1979) **7**:514–20. doi:10.3758/BF03209712
245. Sullivan RM, Wilson DA, Leon M. Norepinephrine and learning-induced plasticity in infant rat olfactory system. *J Neurosci* (1989) **9**:3998–4006.
246. Sullivan RM, Wilson DA. The role of norepinephrine in the expression of learned olfactory neurobehavioral responses in infant rats. *Psychobiology (Austin, Tex)* (1991) **19**:308–12.
247. Sullivan RM, Moriceau S, Raineki C, Roth T. Ontogeny of infant fear learning and amygdala. In: Gazzaniga MS, editor. *Cognitive Neuroscience.* Cambridge: MIT Press (2009). p. 889–904.
248. Vogt JL, Coe CL, Levine S. Behavioral and adrenocorticoid responsiveness of squirrel monkeys to a live snake: is flight necessarily stressful? *Behav Neural Biol* (1981) **32**:391–405. doi:10.1016/S0163-1047(81)90826-8
249. Hennessy MB. Presence of companion moderates arousal of monkeys with restricted social experience. *Physiol Behav* (1984) **33**:693–8. doi:10.1016/0031-9384(84)90033-7
250. Kirschbaum C, Klauer T, Filipp SH, Hellhammer DH. Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. *Psychosom Med* (1995) **57**:23–31.
251. Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biol Psychiatry* (2003) **54**:1389–98. doi:10.1016/S0006-3223(03)00465-7
252. Taylor SE, Burklund LJ, Eisenberger NI, Lehman BJ, Hilmert CJ, Lieberman MD. Neural bases of moderation of cortisol stress responses by psychosocial resources. *J Pers Soc Psychol* (2008) **95**:197–211. doi:10.1037/0022-3514.95.1.197
253. Hostinar CE, Sullivan RM, Gunnar MR. Psychobiological mechanisms underlying the social buffering of the hypothalamic-pituitary-adrenocortical axis: a review of animal models and human studies across development. *Psychol Bull* (2013) **140**(1):256–82. doi:10.1037/a0032671
254. Stanton ME, Wallstrom J, Levine S. Maternal contact inhibits pituitary-adrenal stress responses in preweanling rats. *Dev Psychobiol* (1987) **20**:131–45. doi:10.1002/dev.420200204
255. Wiedenmayer CP, Magarinos AM, McEwen BS, Barr G. Mother lowers glucocorticoid levels of preweaning rats after acute threat. *Ann N Y Acad Sci* (2003) **1008**:304–7. doi:10.1196/annals.1301.038
256. DeVries AC, Glasper ER, Detillion CE. Social modulation of stress responses. *Physiol Behav* (2003) **79**:399–407. doi:10.1016/S0031-9384(03)00152-5
257. Kikusui T, Winslow JT, Mori Y. Social buffering: relief from stress and anxiety. *Philos Trans R Soc Lond B Biol Sci* (2006) **361**:2215–28. doi:10.1098/rstb.2006.1941
258. Hennessy MB, Kaiser S, Sachser N. Social buffering of the stress response: diversity, mechanisms, and functions. *Front Neuroendocrinol* (2009) **30**:470–82. doi:10.1016/j.yfrne.2009.06.001

259. Takahashi Y, Kiyokawa Y, Kodama Y, Arata S, Takeuchi Y, Mori Y. Olfactory signals mediate social buffering of conditioned fear responses in male rats. *Behav Brain Res* (2013) **240**:46–51. doi:10.1016/j.bbr.2012.11.017
260. Upton KJ, Sullivan RM. Defining age limits of the sensitive period for attachment learning in rat pups. *Dev Psychobiol* (2010) **52**:453–64. doi:10.1002/dev.20448
261. Gunnar MR, Brodersen L, Nachmias M, Buss K, Rigatuso J. Stress reactivity and attachment security. *Dev Psychobiol* (1996) **29**:191–204. doi:10.1002/(SICI)1098-2302(199604)29:3<191::AID-DEV1>3.3.CO;2-T
262. Eisenberg L. The biosocial context of parenting in human families. In: Krasnegor NAB Sr, editor. *Mammalian Parenting Biochemical, Neurobiological, and Behavioral Determinants.* New York, NY: Oxford University Press (1990). p. 9–24.
263. Field T. Child abuse in monkeys and humans: a comparative perspective. In: Reite M, Caine NG, editors. *Child Abuse: The Nonhuman Primate Data.* New York, NY: Alan R Liss, Inc. (1983). p. 151–74.
264. Schapiro S, Mitchel G. Infant-directed abuse in a seminatural environment: precipitating factors. In: Reite M, Caine NG, editors. *Child Abuse: The Nonhuman Primate Data.* New York, NY: Alan R Liss, Inc. (1983). p. 29–48.
265. Rice CJ, Sandman CA, Lenjavi MR, Baram TZ. A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology* (2008) **149**:4892–900. doi:10.1210/en.2008-0633
266. Pedersen CA, Caldwell JD, McGuire M, Evans DL. Corticotropin-releasing hormone inhibits maternal behavior and induces pup-killing. *Life Sci* (1991) **48**:1537–46. doi:10.1016/0024-3205(91)90278-J
267. Yeh KY. Corticosterone concentrations in the serum and milk of lactating rats: parallel changes after induced stress. *Endocrinology* (1984) **115**:1364–70. doi:10.1210/endo-115-4-1364
268. McEwen BS, Seeman T. Protective and damaging effects of mediators of stress. Elaborating and testing the concepts of allostasis and allostatic load. *Ann N Y Acad Sci* (1999) **896**:30–47. doi:10.1111/j.1749-6632.1999.tb08103.x
269. McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. *Brain Res* (2000) **886**:172–89. doi:10.1016/S0006-8993(00)02950-4
270. Weinstock M. The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain Behav Immun* (2005) **19**:296–308. doi:10.1016/j.bbi.2004.09.006
271. Cone RD, Low MJ, Elmquist JK, Cameron JL. Neuroendocrinology. 12th ed. In: Larsen PR, Melmed S, Polonsky KS, editors. *Williams Textbook of Endocrinology.* Philadelphia, PA: Saunders (2003). p. 81–176.
272. Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, et al. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol* (2003) **24**:151–80. doi:10.1016/j.yfrne.2003.07.001
273. Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr Rev* (1984) **5**:25–44. doi:10.1210/edrv-5-1-25
274. Dallman MF, Akana SF, Strack AM, Hanson ES, Sebastian RJ. The neural network that regulates energy balance is responsive to glucocorticoids and insulin and also regulates HPA axis responsivity at a site proximal to CRF neurons. *Ann N Y Acad Sci* (1995) **771**:730–42. doi:10.1111/j.1749-6632.1995.tb44724.x
275. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev* (2000) **21**:55–89. doi:10.1210/er.21.1.55
276. Seckl JR. Glucocorticoids, ageing and nerve cell damage. *J Neuroendocrinol* (2000) **12**:709–10.
277. Thierry AM, Tassin JP, Blanc G, Glowinski J. Selective activation of mesocortical DA system by stress. *Nature* (1976) **263**:242–4. doi:10.1038/263242a0
278. Herman JP, Guillonneau D, Dantzer R, Scatton B, Semerdjian-Rouquier L, Le Moal M. Differential effects of inescapable footshocks and of stimuli previously paired with inescapable footshocks on dopamine turnover in cortical and limbic areas of the rat. *Life Sci* (1982) **30**:2207–14. doi:10.1016/0024-3205(82)90295-8
279. Abercrombie ED, Jacobs BL. Single-unit response of noradrenergic neurons in the locus coeruleus of freely moving cats. I. Acutely presented stressful and nonstressful stimuli. *J Neurosci* (1987) **7**:2837–43.
280. Adell A, Garcia-Marquez C, Armario A, Gelpi E. Chronic stress increases serotonin and noradrenaline in rat brain and sensitizes their responses to a further acute stress. *J Neurochem* (1988) **50**:1678–81. doi:10.1111/j.1471-4159.1988.tb02462.x
281. Abercrombie ED, Keefe KA, Difrischia DS, Zigmond MJ. Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. *J Neurochem* (1989) **52**:1655–8. doi:10.1111/j.1471-4159.1989.tb09224.x
282. Deutch AY, Roth RH. The determinants of stress-induced activation of the prefrontal cortical dopamine system. *Prog Brain Res* (1990) **85**:367–402. doi:10.1016/S0079-6123(08)62691-6
283. Pei Q, Zetterstrom T, Fillenz M. Tail pinch-induced changes in the turnover and release of dopamine and 5-hydroxytryptamine in different brain regions of the rat. *Neuroscience* (1990) **35**:133–8. doi:10.1016/0306-4522(90)90127-P
284. Nisenbaum LK, Zigmond MJ, Sved AF, Abercrombie ED. Prior exposure to chronic stress results in enhanced synthesis and release of hippocampal norepinephrine in response to a novel stressor. *J Neurosci* (1991) **11**:1478–84.
285. Inoue T, Tsuchiya K, Koyama T. Regional changes in dopamine and serotonin activation with various intensity of physical and psychological stress in the rat brain. *Pharmacol Biochem Behav* (1994) **49**:911–20. doi:10.1016/0091-3057(94)90243-7
286. Finlay JM, Zigmond MJ, Abercrombie ED. Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: effects of diazepam. *Neuroscience* (1995) **64**:619–28. doi:10.1016/0306-4522(94)00331-X
287. Makino S, Schulkin J, Smith MA, Pacak K, Palkovits M, Gold PW. Regulation of corticotropin-releasing hormone receptor messenger ribonucleic acid in the rat brain and pituitary by glucocorticoids and stress. *Endocrinology* (1995) **136**:4517–25. doi:10.1210/en.136.10.4517
288. Inglis FM, Moghaddam B. Dopaminergic innervation of the amygdala is highly responsive to stress. *J Neurochem* (1999) **72**:1088–94. doi:10.1046/j.1471-4159.1999.0721088.x
289. Kaehler ST, Singewald N, Sinner C, Thurnher C, Philippu A. Conditioned fear and inescapable shock modify the release of serotonin in the locus coeruleus. *Brain Res* (2000) **859**:249–54. doi:10.1016/S0006-8993(00)01967-3
290. McEwen BS. Protective and damaging effects of stress mediators. *N Engl J Med* (1998) **338**:171–9. doi:10.1056/NEJM199801153380307
291. Herbert J, Goodyer IM, Grossman AB, Hastings MH, De Kloet ER, Lightman SL, et al. Do corticosteroids damage the brain? *J Neuroendocrinol* (2006) **18**:393–411. doi:10.1111/j.1365-2826.2006.01429.x
292. Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol* (2009) **5**:374–81. doi:10.1038/nrendo.2009.106
293. Nelson CA, Carver LJ. The effects of stress and trauma on brain and memory: a view from developmental cognitive neuroscience. *Dev Psychopathol* (1998) **10**:793–809. doi:10.1017/S0954579498001874
294. Toth SL, Cicchetti D. Remembering, forgetting, and the effects of trauma on memory: a developmental psychopathology perspective. *Dev Psychopathol* (1998) **10**:589–605. doi:10.1017/S0954579498001771
295. Matthews SG. Early programming of the hypothalamo-pituitary-adrenal axis. *Trends Endocrinol Metab* (2002) **13**:373–80. doi:10.1016/S1043-2760(02)00690-2
296. Tottenham N, Sheridan MA. A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing. *Front Hum Neurosci* (2009) **3**:68. doi:10.3389/neuro.09.068.2009
297. Pechtel P, Pizzagalli DA. Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology (Berl)* (2011) **214**:55–70. doi:10.1007/s00213-010-2009-2
298. Suomi SJ. Early stress and adult emotional reactivity in rhesus monkeys. *Ciba Found Symp* (1991) **156**:171–83.
299. Gunnar M, Vazquez D. Stress neurobiology and developmental psychopathology. In: Cicchetti DCD, editor. *Developmental Psychopathology: Developmental Neuroscience.* New York, NY: Wiley (2006). p. 533–77.
300. Andrews MH, Matthews SG. Programming of the hypothalamo-pituitary-adrenal axis: serotonergic involvement. *Stress* (2004) **7**:15–27. doi:10.1080/10253890310001650277
301. Rodrigues AJ, Leao P, Carvalho M, Almeida OF, Sousa N. Potential programming of dopaminergic circuits by early life stress. *Psychopharmacology (Berl)* (2011) **214**:107–20. doi:10.1007/s00213-010-2085-3
302. De Kloet ER, Rots NY, Cools AR. Brain-corticosteroid hormone dialogue: slow and persistent. *Cell Mol Neurobiol* (1996) **16**:345–56. doi:10.1007/BF02088100

303. Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci* (1993) **13**:3839–47.
304. Sullivan RM, Gratton A. Prefrontal cortical regulation of hypothalamic-pituitary-adrenal function in the rat and implications for psychopathology: side matters. *Psychoneuroendocrinology* (2002) **27**:99–114. doi:10.1016/S0306-4530(01)00038-5
305. Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry* (2005) **29**:1201–13. doi:10.1016/j.pnpbp.2005.08.006
306. Jankord R, Herman JP. Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. *Ann N Y Acad Sci* (2008) **1148**:64–73. doi:10.1196/annals.1410.012
307. Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci* (2009) **10**:397–409. doi:10.1038/nrn2647
308. Cunningham MG, Bhattacharyya S, Benes FM. Amygdalo-cortical sprouting continues into early adulthood: implications for the development of normal and abnormal function during adolescence. *J Comp Neurol* (2002) **453**:116–30.
309. Andersen SL, Teicher MH. Stress, sensitive periods and maturational events in adolescent depression. *Trends Neurosci* (2008) **31**:183–91. doi:10.1016/j.tins.2008.01.004
310. Teicher MH, Andersen SL, Polcari A, Anderson CM, Navalta CP. Developmental neurobiology of childhood stress and trauma. *Psychiatr Clin North Am* (2002) **25**:397–426, vii–viii. doi:10.1016/S0193-953X(01)00003-X
311. Meaney MJ. Plasticity and health: social influences on gene expression and neural development. In: Kessel F, Rosenfeld PL, Anderson NB, editors. *Expanding the Boundaries of Health and Social Science: Case Studies in Interdisciplinary Innovation*. New York, NY: Oxford University Press (2008). 147 p.
312. Loman MM, Gunnar MR. Early experience and the development of stress reactivity and regulation in children. *Neurosci Biobehav Rev* (2010) **34**:867–76. doi:10.1016/j.neubiorev.2009.05.007
313. Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, et al. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* (1984) **226**:1342–4. doi:10.1126/science.6334362
314. Gold PW, Calabrese JR, Kling MA, Avgerinos P, Khan I, Gallucci WT, et al. Abnormal ACTH and cortisol responses to ovine corticotropin releasing factor in patients with primary affective disorder. *Prog Neuropsychopharmacol Biol Psychiatry* (1986) **10**:57–65. doi:10.1016/0278-5846(86)90044-8
315. Young EA, Haskett RF, Murphy-Weinberg V, Watson SJ, Akil H. Loss of glucocorticoid fast feedback in depression. *Arch Gen Psychiatry* (1991) **48**:693–9. doi:10.1001/archpsyc.1991.01810320017003
316. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. *J Endocrinol* (1999) **160**:1–12. doi:10.1677/joe.0.1600001
317. Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, et al. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. *JAMA* (2000) **284**:592–7. doi:10.1001/jama.284.5.592
318. Heim C, Nemeroff CB. Neurobiology of early life stress: clinical studies. *Semin Clin Neuropsychiatry* (2002) **7**:147–59. doi:10.1053/scnp.2002.33127
319. Heim C, Newport DJ, Mletzko T, Miller AH, Nemeroff CB. The link between childhood trauma and depression: insights from HPA axis studies in humans. *Psychoneuroendocrinology* (2008) **33**:693–710. doi:10.1016/j.psyneuen.2008.03.008
320. Kraemer GW, Ebert MH, Schmidt DE, McKinney WT. A longitudinal study of the effect of different social rearing conditions on cerebrospinal fluid norepinephrine and biogenic amine metabolites in rhesus monkeys. *Neuropsychopharmacology* (1989) **2**:175–89. doi:10.1016/0893-133X(89)90021-3
321. Rosenblum LA, Andrews MW. Influences of environmental demand on maternal behavior and infant development. *Acta Paediatr Suppl* (1994) **397**:57–63. doi:10.1111/j.1651-2227.1994.tb13266.x
322. Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, et al. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. *Proc Natl Acad Sci U S A* (1996) **93**:1619–23. doi:10.1073/pnas.93.4.1619
323. Coplan JD, Trost RC, Owens MJ, Cooper TB, Gorman JM, Nemeroff CB, et al. Cerebrospinal fluid concentrations of somatostatin and biogenic amines in grown primates reared by mothers exposed to manipulated foraging conditions. *Arch Gen Psychiatry* (1998) **55**:473–7. doi:10.1001/archpsyc.55.5.473
324. Maestripieri D, Higley JD, Lindell SG, Newman TK, McCormack KM, Sanchez MM. Early maternal rejection affects the development of monoaminergic systems and adult abusive parenting in rhesus macaques (*Macaca mulatta*). *Behav Neurosci* (2006) **120**:1017–24. doi:10.1037/0735-7044.120.5.1017
325. Bremner JD, Krystal JH, Southwick SM, Charney DS. Noradrenergic mechanisms in stress and anxiety. I. Preclinical studies. *Synapse* (1996) **23**:28–38. doi:10.1002/(SICI)1098-2396(199605)23:1<28::AID-SYN4>3.3.CO;2-4
326. Bremner JD, Krystal JH, Southwick SM, Charney DS. Noradrenergic mechanisms in stress and anxiety. II. Clinical studies. *Synapse* (1996) **23**:39–51. doi:10.1002/(SICI)1098-2396(199605)23:1<28::AID-SYN4>3.3.CO;2-4
327. Heim C, Owens MJ, Plotsky PM, Nemeroff CB. The role of early adverse life events in the etiology of depression and posttraumatic stress disorder. Focus on corticotropin-releasing factor. *Ann N Y Acad Sci* (1997) **821**:194–207. doi:10.1111/j.1749-6632.1997.tb48279.x
328. Porsolt RD, Brossard G, Hautbois C, Roux S. Rodent models of depression: forced swimming and tail suspension behavioral despair tests in rats and mice. *Curr Protoc Neurosci* (2001) **Chapter 8**:Unit810A. doi:10.1002/0471142301.ns0810as14
329. Pollak DD, Rey CE, Monje FJ. Rodent models in depression research: classical strategies and new directions. *Ann Med* (2010) **42**:252–64. doi:10.3109/07853891003769957
330. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature* (1977) **266**:730–2. doi:10.1038/266730a0
331. McEwen BS. Early life influences on life-long patterns of behavior and health. *Ment Retard Dev Disabil Res Rev* (2003) **9**:149–54. doi:10.1002/mrdd.10074
332. Cicchetti D, Toth SL. Child maltreatment. *Annu Rev Clin Psychol* (2005) **1**:409–38. doi:10.1146/annurev.clinpsy.1.102803.144029
333. Pryce CR, Ruedi-Bettschen D, Dettling AC, Weston A, Russig H, Ferger B, et al. Long-term effects of early-life environmental manipulations in rodents and primates: potential animal models in depression research. *Neurosci Biobehav Rev* (2005) **29**:649–74. doi:10.1016/j.neubiorev.2005.03.011
334. Heim C, Shugart M, Craighead WE, Nemeroff CB. Neurobiological and psychiatric consequences of child abuse and neglect. *Dev Psychobiol* (2010) **52**:671–90. doi:10.1002/dev.20494
335. Hart J, Gunnar M, Cicchetti D. Altered neuroendocrine activity in maltreated children related to symptoms of depression. *Dev Psychopathol* (1996) **8**:201–14. doi:10.1017/S0954579400007045
336. 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**:1509–22. doi:10.1016/S0006-3223(99)00224-3
337. Costello EJ, Pine DS, Hammen C, March JS, Plotsky PM, Weissman MM, et al. Development and natural history of mood disorders. *Biol Psychiatry* (2002) **52**:529–42. doi:10.1016/S0006-3223(02)01372-0
338. Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci* (2008) **9**:947–57. doi:10.1038/nrn2513
339. Sevelinges Y, Moriceau S, Holman P, Miner C, Muzny K, Gervais R, et al. Enduring effects of infant memories: infant odor-shock conditioning attenuates amygdala activity and adult fear conditioning. *Biol Psychiatry* (2007) **62**:1070–9. doi:10.1016/j.biopsych.2007.04.025
340. Sevelinges Y, Sullivan RM, Messaoudi B, Mouly AM. Neonatal odor-shock conditioning alters the neural network involved in odor fear learning at adulthood. *Learn Mem* (2008) **15**:649–56. doi:10.1101/lm.998508
341. Moriceau S, Raineki C, Holman JD, Holman JG, Sullivan RM. Enduring neurobehavioral effects of early life trauma mediated through learning and corticosterone suppression. *Front Behav Neurosci* (2009) **3**:22. doi:10.3389/neuro.08.022.2009
342. Sevelinges Y, Mouly AM, Raineki C, Moriceau S, Forest C, Sullivan RM. Adult depression-like behavior, amygdala and olfactory cortex functions are restored by odor previously paired with shock during infant's sensitive period attachment learning. *Dev Cogn Neurosci* (2011) **1**:77–87. doi:10.1016/j.dcn.2010.07.005
343. Haynes-Seman C. Developmental origins of moral masochism: a failure-to-thrive toddler's interactions with mother. *Child Abuse Negl* (1987) **11**:319–30. doi:10.1016/0145-2134(87)90005-6

344. Zeanah CH, Keyes A, Settles L. Attachment relationship experiences and childhood psychopathology. *Ann N Y Acad Sci* (2003) **1008**:22–30. doi:10.1196/annals.1301.003
345. Bos KJ, Fox N, Zeanah CH, Nelson CA III. Effects of early psychosocial deprivation on the development of memory and executive function. *Front Behav Neurosci* (2009) **3**:16. doi:10.3389/neuro.08.016.2009
346. Anand KJ, Coskun V, Thrivikraman KV, Nemeroff CB, Plotsky PM. Long-term behavioral effects of repetitive pain in neonatal rat pups. *Physiol Behav* (1999) **66**:627–37. doi:10.1016/S0031-9384(98)00338-2

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 15 December 2013; paper pending published: 08 February 2014; accepted: 05 March 2014; published online: 21 March 2014.
*Citation: Rincón-Cortés M and Sullivan RM (2014) Early life trauma and attachment: immediate and enduring effects on neurobehavioral and stress axis development. Front. Endocrinol. **5**:33. doi: 10.3389/fendo.2014.00033*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
ENDOCRINOLOGY

MINI REVIEW ARTICLE
published: 15 November 2013
doi: 10.3389/fendo.2013.00178

Effects of an early experience involving training in a T-maze under either denial or receipt of expected reward through maternal contact

Antonios Stamatakis, Anastasia Diamantopoulou, Theofanis Panagiotaropoulos, Androniki Raftogianni and Fotini Stylianopoulou*

Biology-Biochemistry Laboratory, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece

Edited by:
Nikolaos P. Daskalakis, Mount Sinai School of Medicine, USA

Reviewed by:
Mathias V. Schmidt, Max Planck Institute of Psychiatry, Germany
Anne-Marie Mouly, Centre de Recherche en Neurosciences de Lyon, France

****Correspondence:***
Fotini Stylianopoulou, Biology-Biochemistry Laboratory, School of Health Sciences, National and Kapodistrian University of Athens, 123 Papadiamantopoulou Street, Athens 11527, Greece
e-mail: fstilian@nurs.uoa.gr

The mother is the most salient stimulus for the developing pups and a number of early experience models employ manipulation of the mother-infant interaction. We have developed a new model which in addition to changes in maternal behavior includes a learning component on the part of the pups. More specifically, pups were trained in a T-maze and either received (RER rats) or were denied (DER) the reward of maternal contact, during postnatal days 10–13. Pups of both experimental groups learn the T-maze, but the RER do so more efficiently utilizing a procedural-type of learning and memory with activation of the dorsal basal ganglia. On the other hand, the DER experience leads to activation of the hippocampus, prefrontal cortex, and amygdala in the pups. In adulthood, male DER animals exhibit better mnemonic abilities in the Morris water maze and higher activation of the hippocampus, while they have decreased brain serotonergic activity, exhibit a depressive-like phenotype and proactive aggressive behavior in the resident-intruder test. While male RER animals assume a reactive coping style in this test, and showed increased freezing during both contextual and cued memory recall following fear conditioning.

Keywords: neonatal learning, HPA axis, stress response, aggression, serotonergic system, hippocampus, amygdala, prefrontal cortex

A large wealth of data from both human and animal studies have documented well that early experiences have long-term effects thus influencing adult brain function and behavior. If the early experiences encompass adversity, they can lead later in life to psychopathology, particularly maladaptive stress responses, anxiety, and depression. In the animal models employed to study the effects of early experiences the major parameter modified is the care offspring receive, through altered maternal behavior (1–5). In our laboratory we have developed a novel experimental model of early experiences, which, in addition to altering maternal behavior, also involves a component of neonatal learning by the pups (6). More specifically, during postnatal days 10–13 pups are exposed to a T-maze, one arm of which leads to the mother-containing cage; one group of pups receives the expected reward (RER) of maternal contact by being allowed to be retrieved by the mother upon reaching her cage, while the other group is denied this reward (DER) by blocking access to the mother-containing cage. Interestingly, both groups of pups learn the T-maze, but – as expected – the RER do so more efficiently, by employing a procedural type of learning with activation of the dorsal basal ganglia, as shown by c-Fos immunoreactivity (6). On the other hand the DER experience leads to activation of the hippocampus and the prefrontal cortex, indicating increased vigilance and appraisal of environmental cues. Furthermore, the DER experience resulted in activation of the amygdala on the first day of training (postnatal day 10, PND10) and on that same day an increase in corticosterone was observed (7), which could mediate the early activation of the amygdala since it has been shown that circulating corticosterone controls the maturation of this brain area (8). The increase in corticosterone indicates that exposure to the DER experience for the first time is stressful for the pups and the stress-inducing factor is probably the denial of maternal contact. These results complement those of Sullivan et al. who established that the presence of the mother during early postnatal development acts to inhibit stress-induced corticosterone increase (9).

It should be noted that pups of both groups are returned to the home cage containing their mother and littermates immediately after the end of the exposure to the T-maze procedure: approximately 10 min. When back in the home cage, both RER and DER pups receive increased maternal care compared to the control (non-handled) pups (7), resembling in this respect animals subjected to the neonatal handling paradigm (1, 4, 5). Our model of early experience also bears resemblance to that of Sullivan et al. in that in both there is learning on the part of the pups, and the force motivating it is the mother; in our model the pups learn the position of the mother within the T-maze, while in that of Sullivan et al. the odor associated with the mother (10, 11).

Abbreviations: CRH, corticotrophin-releasing hormone; CRH-R1, type 1 CRH receptor; DER, denied expected reward; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal; MWM, Morris water maze; PND, postnatal day; PVN, paraventricular hypothalamic nucleus; RER, received expected reward.

One of the focuses of our studies was the effect of these two early experiences, one with a positive emotional valence (the RER), and the other of minor adversity (given that pups receive increased maternal care soon after exposure to the T-maze – the DER) on adult hypothalamic-pituitary-adrenal (HPA) axis function, since it is well known that it is a primary target of early experiences (12). Neither the RER nor the DER experience affected basal plasma corticosterone levels (13). However, the stress-induced corticosterone response of each group differed, depending on the characteristics of the stressful stimulus: when it was highly aversive within the natural behavioral repertoire of the animals, such as after exposure to an aggressive resident male in the resident-intruder test, there were no differences among the three groups (RER, DER, and Control) and all animals increased their corticosterone levels dramatically (14). Following a 15 min forced swim test, which is fairly aversive, but rodents are exposed to it only within the framework of laboratory experiments, all animals increased their plasma corticosterone, but the DER attained higher levels than either the RER or the controls, which did not differ between them (13). However the stress-induced corticosterone response of the DER animals cannot be characterized as "pathological," since it was contained, reaching basal levels by 2 h, just like that of the other two groups. Interestingly, when animals were exposed to a stressful stimulus of a very short duration (1 s electric shock of 0.5 mA) within the setting of a fear conditioning paradigm, both RER and DER animals attained lower corticosterone levels than the controls (7), indicating that both early experiences programed the HPA axis to give a blunted response to temporally transient stressful stimuli, a characteristic which could be of adaptive value.

Notably, other components of the HPA axis, such as glucocorticoid receptors (GRs) in the hippocampus, corticotrophin-releasing hormone (CRH) levels in the paraventricular hypothalamic nucleus (PVN) and amygdala, and type 1 CRH receptors (CRH-R1) in the hippocampus and the amygdala were affected only by the DER experience (13).

More specifically GR levels in the hippocampus were higher in the DER animals than either the control or the RER (whose GRs were at the control levels), in spite of the fact that both DER and RER animals received the same, increased maternal care. This finding indicates that the elevated hippocampal GR levels induced by increased maternal care, documented using other early experience models (neonatal handling, high licking and grooming mothers) (15, 16), can be modulated by other factors possibly related to the pups' behavior.

Another key player in the stress response is the CRH system (CRH and its type 1 receptor). The DER animals had lower CRH-R1 levels in the hippocampus and the amygdala, areas known to participate in the control of stress-induced CRH release (17). On the other hand, although the DER experience did not affect basal CRH levels in the PVN or amygdala, following the stressful stimulus of forced swimming CRH levels remained high 2 h after the termination of the stress in the DER animals (13). The sustained CRH expression in the DER animals following stress could be a reflection of a less efficient feedback control circuit of CRH release, mediated by the lower CRH-R1 levels in brain areas, such as the hippocampus and amygdala, participating in this circuit.

Animals receiving increased maternal care as neonates have improved cognitive abilities as adults, as shown in studies using neonatal handling or the high licking and grooming mothers (2, 18–21). Based on this, we assessed the abilities for learning and memory of the adult male animals subjected as neonates to the RER or DER experience. We employed two different tests: the Morris water maze (MWM), which measures spatial reference learning and memory and is hippocampus dependent, and fear conditioning, which has a strong emotional component and is dependent on both the amygdala and the hippocampus. Interestingly, the DER animals had better mnemonic abilities in the MWM test (22), while the RER in the fear conditioning (7). The improved memory of the DER animals was accompanied by increased levels of the transcription factor pCREB in the hippocampus, indicating increased activation and plasticity related processes in this brain area. It should be remembered that the DER experience resulted in increased activation of the hippocampus in the neonatal period (6). It is thus possible that this early activation of the hippocampus programed its function rendering it more efficient in adulthood. On the behavioral level the DER experience involves an element of "delayed" reward, since the pups are being denied maternal contact during the T-maze training, but do receive it soon after, upon return to the nest. This experience could prepare them to deal efficiently with situations in adulthood when an expected reward is not delivered, such as in the MWM memory trial, where the platform (learned "reward") is missing. On the other hand the RER neonatal experience, which lacks elements of adversity, renders the animals as adults more prone to remember emotionally negative events such as those eliciting fear. The above described results of the early experiences of our model are in agreement with the match-mismatch hypothesis, which states that if two events during critical periods of life "match" in being mildly, and not severely, stressful, their interaction can be beneficial resulting in a more adaptive phenotype (23).

The serotonergic system of the brain is strongly affected by early experiences. The DER neonatal experience of our model resulted in decreased serotonergic activity as assessed by the lower levels of serotonin in the prefrontal cortex and amygdala, of 5-HT1A receptors in the hippocampus, and of increased serotonin transporter in the amygdala of the adult brain (14).

Serotonergic neurotransmission is intimately involved in the etiopathogenesis of depression and the control of aggression. Interestingly, the adult DER animals were more vulnerable to express depressive-like behavior as assessed by increased immobility time in the forced swim test (13). It is worth mentioning that immobility time has been considered an expression of a passive coping strategy, which could be "adaptive" in the face of an inescapable stressful situation. Even in humans, depression has been viewed as an adaptive response developed through evolution to enable efficient management of complex challenging situations (24). The DER neonatal experience also affected aggressive behavior in both adolescence and adulthood. During adolescence the DER animals displayed play behavior with strong aggressive characteristics (14), similarly to children that have been maltreated or neglected (25). Relevantly, adolescent aggressive-like play is considered as a lack of abilities to integrate into peer groups and could

Table 1 | Effects of the DER and RER neonatal experience of our model in male rats – compared to controls.

DER	RER
NEONATAL LIFE (PND10–13)	
Activation of hippocampus, prefrontal cortex, and amygdala	Activation of basal ganglia
Increased vigilance and appraisal of environmental cues	Procedural type of T-maze learning
Increased plasma corticosterone on PND10	
Receive increased maternal care	Receive increased maternal care
ADOLESCENCE	
Play behavior with strong aggressive characteristics	
ADULT LIFE	
No effect on basal plasma corticosterone levels	No effect on basal plasma corticosterone levels
Higher, but temporally restrained, forced swim stress-induced increase in corticosterone levels	
Lower foot shock-induced increase in corticosterone levels	Lower foot shock-induced increase in corticosterone levels
Higher GR levels in the hippocampus	
Lower CRH-R1 levels in the hippocampus and the amygdala	
Sustained forced swim stress-induced increase in PVN and amygdalar CRH levels	
Better mnemonic abilities in the MWM test	Enhanced fear memory
Increased levels of pCREB in the hippocampus following MWM training	
Lower levels of serotonin in the prefrontal cortex and amygdala	
Lower levels of 5-HT1A receptors in the hippocampus	
Increased levels of serotonin transporter in the amygdala	
Increased immobility time in the forced swim test	
More proactive behaviors in the resident-intruder test	

be a prognostic factor for inappropriate social behaviors in adulthood. Moreover, as adults the DER male animals showed more proactive behaviors in the resident-intruder test, indicating a maladaptive response in the face of defeat (14). It has been shown by others that the proactive coping style is associated with increased offensive aggression, impulsivity, and behavioral inflexibility (26, 27), and it is the impulsive form of aggression which is increased by early life adversity (28, 29). Studies in humans have shown that early childhood trauma is often associated with enhanced aggression, which correlates with increased suicide attempts (30). Furthermore, clinically a strong relationship between depression and aggression is generally observed (31, 32).

A wealth of data has been accumulating lately that the consequences of early experiences are effected through epigenetic changes, which include DNA methylation and histone modifications, resulting in altered expression in relevant genes (33–35). Within this framework, it has been shown that the DER experience results in higher levels of phospho(Ser10)-acetyl(Lys14)-Histone-3 in the amygdala of the adult animals (7), while preliminary work has revealed differences in DNA methylation induced by the RER and DER experience throughout areas of the limbic system. It is thus evident that this line of research is highly promising as to the possibility of unraveling the molecular mechanisms underlying the behavioral and neurochemical effects of the early experiences of our model.

The characteristics of the DER neonatal experience, which involves a short separation from the mother and denial of contact with her, in spite of her presence, are reminiscent of human situations of maternal neglect. Furthermore, as presented throughout the text (see **Table 1**), the behavioral phenotype of the DER animals bears a strong resemblance with that of human adolescents and adults which have been neglected as children. Thus, the DER neonatal experience could provide a good animal model in which to study the long-term effects of early life experiences of mild adversity, which is important, since they are more frequent than those of severe negative valence, such as child abandonment or physical or sexual abuse.

ACKNOWLEDGMENTS

This work has been supported by the John S. Latsis Public Benefit Foundation. The sole responsibility for the content lies with its authors.

REFERENCES

1. Fenoglio KA, Chen Y, Baram TZ. Neuroplasticity of the hypothalamic-pituitary-adrenal axis early in life requires recurrent recruitment of stress-regulating brain regions. *J Neurosci* (2006) **26**:2434–42. doi:10.1523/JNEUROSCI.4080-05.2006
2. Liu D, Diorio J, Day JC, Francis DD, Meaney MJ. Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat Neurosci* (2000) **3**:799–806. doi:10.1038/77702
3. Macri S, Mason GJ, Wurbel H. Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. *Eur J Neurosci* (2004) **20**:1017–24. doi:10.1111/j.1460-9568.2004.03541.x
4. Pryce CR, Bettschen D, Feldon J. Comparison of the effects of early handling and early deprivation on maternal care in the rat. *Dev Psychobiol* (2001) **38**:239–51. doi:10.1002/dev.1018
5. Smotherman WP. Mother-infant interaction and the modulation of pituitary – adrenal activity in rat pups after early stimulation. *Dev Psychobiol* (1983) **16**:169–76. doi:10.1002/dev.420160303

6. Panagiotaropoulos T, Diamantopoulou A, Stamatakis A, Dimitropoulou M, Stylianopoulou F. Learning of a T-maze by rat pups when contact with the mother is either permitted or denied. *Neurobiol Learn Mem* (2009) **91**:2–12. doi:10.1016/j.nlm.2008.09.007
7. Diamantopoulou α, Raftogianni α, Stamatakis α, Tzanoulinou S, Oitzl MS, Stylianopoulou F. Denial or receipt of expected reward through maternal contact during the neonatal period differentially affect the development of the rat amygdala and program its function in adulthood in a sex-dimorphic way. *Psychoneuroendocrinology* (2013) **38**:1757–71. doi:10.1016/j.psyneuen.2013.02.012
8. Moriceau S, Roth TL, Okotoghaide T, Sullivan RM. Corticosterone controls the developmental emergence of fear and amygdala function to predator odors in infant rat pups. *Int J Dev Neurosci* (2004) **22**:415–22. doi:10.1016/j.ijdevneu.2004.05.011
9. Moriceau S, Sullivan RM. Maternal presence serves as a switch between learning fear and attraction in infancy. *Nat Neurosci* (2006) **9**:1004–6. doi:10.1038/nn1733
10. Moriceau S, Wilson DA, Levine S, Sullivan RM. Dual circuitry for odor-shock conditioning during infancy: corticosterone switches between fear and attraction via amygdala. *J Neurosci* (2006) **26**:6737–48. doi:10.1523/JNEUROSCI.0499-06.2006
11. Sullivan RM, Stackenwalt G, Nasr F, Lemon C, Wilson DA. Association of an odor with activation of olfactory bulb noradrenergic beta-receptors or locus coeruleus stimulation is sufficient to produce learned approach responses to that odor in neonatal rats. *Behav Neurosci* (2000) **114**:957–62. doi:10.1037/0735-7044.114.5.957
12. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* (2001) **49**:1023–39. doi:10.1016/S0006-3223(01)01157-X
13. Diamantopoulou A, Raftogianni R, Stamatakis A, Oitzl MS, Stylianopoulou F. Effects of denial of reward through maternal contact in the neonatal period on adult hypothalamic-pituitary-adrenal axis function in the rat. *Psychoneuroendocrinology* (2013) **38**:830–41. doi:10.1016/j.psyneuen.2012.09.007
14. Diamantopoulou A, Raftogianni R, Stamatakis A, Alikaridis F, Oitzl MS, Stylianopoulou F. Denial of reward in the neonate shapes sociability and serotonergic activity in the adult rat. *PLoS One* (2012) **7**(3):e33793. doi:10.1371/journal.pone.0033793
15. Avishai-Eliner S, Eghbal-Ahmadi M, Tabachnik E, Brunson KL, Baram TZ. Down-regulation of hypothalamic corticotropin-releasing hormone messenger ribonucleic acid (mRNA) precedes early-life experience-induced changes in hippocampal glucocorticoid receptor mRNA. *Endocrinology* (2001) **142**:89–97. doi:10.1210/en.142.1.89
16. Weaver IC, Diorio J, Seckl JR, Szyf M, Meaney MJ. Early environmental regulation of hippocampal glucocorticoid receptor gene expression: characterization of intracellular mediators and potential genomic target sites. *Ann N Y Acad Sci* (2004) **1024**:182–212. doi:10.1196/annals.1321.099
17. Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuropsychopharmacol Biol Psychiatry* (2005) **29**:1201–13. doi:10.1016/j.pnpbp.2005.08.006
18. Escorihuela RM, Tobena A, Fernandez-Teruel A. Environmental enrichment reverses the detrimental action of early inconsistent stimulation and increases the beneficial effects of postnatal handling on shuttlebox learning in adult rats. *Behav Brain Res* (1994) **61**:169–73. doi:10.1016/0166-4328(94)90157-0
19. Fenoglio KA, Brunson KL, Avishai-Eliner S, Stone BA, Kapadia BJ, Baram TZ. Enduring, handling-evoked enhancement of hippocampal memory function and glucocorticoid receptor expression involves activation of the corticotropin-releasing factor type 1 receptor. *Endocrinology* (2005) **146**:4090–6. doi:10.1210/en.2004-1285
20. Stamatakis A, Pondiki S, Kitraki E, Diamantopoulou A, Panagiotaropoulos T, Raftogianni A, et al. Effect of neonatal handling on adult rat spatial learning and memory following acute stress. *Stress* (2008) **11**:148–59. doi:10.1080/10253890701653039
21. Wong R, Jamieson JL. Infantile handling and the facilitation of discrimination and reversal learning. *Q J Exp Psychol* (1968) **20**:197–9. doi:10.1080/14640746808400149
22. Diamantopoulou A, Stamatakis A, Panagiotaropoulos T, Stylianopoulou F. Reward or its denial during the neonatal period affects adult spatial memory and hippocampal pCREB levels of both the neonatal and adult rat. *Neuroscience* (2011) **181**:89–99. doi:10.1016/j.neuroscience.2011.03.002
23. Schmidt MV. Animal models for depression and the mismatch hypothesis of disease. *Psychoneuroendocrinology* (2011) **36**:330–8. doi:10.1016/j.psyneuen.2010.07.001
24. Andrews PW, Thomson JA Jr. The bright side of being blue: depression as an adaptation for analyzing complex problems. *Psychol Rev* (2009) **116**:620–54. doi:10.1037/a0016242
25. Kotch JB, Lewis T, Hussey JM, English D, Thompson R, Litrownik AJ, et al. Importance of early neglect for childhood aggression. *Pediatrics* (2008) **121**:725–31. doi:10.1542/peds.2006-3622
26. Coppens CM, de Boer SF, Koolhaas JM. Coping styles and behavioural flexibility: towards underlying mechanisms. *Philos Trans R Soc Lond B Biol Sci* (2010) **365**:4021–8. doi:10.1098/rstb.2010.0217
27. Koolhaas JM, de Boer SF, Coppens CM, Buwalda B. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. *Front Neuroendocrinol* (2010) **31**:307–21. doi:10.1016/j.yfrne.2010.04.001
28. Ethier LS, Lemelin JP, Lacharite C. A longitudinal study of the effects of chronic maltreatment on children's behavioral and emotional problems. *Child Abuse Negl* (2004) **28**:1265–78. doi:10.1016/j.chiabu.2004.07.006
29. Fonagy P. Early-life trauma and the psychogenesis and prevention of violence. *Ann N Y Acad Sci* (2004) **1036**:181–200. doi:10.1196/annals.1330.012
30. Sarchiapone M, Jaussent I, Roy A, Carli V, Guillaume S, Jollant F, et al. Childhood trauma as a correlative factor of suicidal behavior – via aggression traits. Similar results in an Italian and in a French sample. *Eur Psychiatry* (2009) **24**:57–62. doi:10.1016/j.eurpsy.2008.07.005
31. Carver CS, Johnson SL, Joormann J. Serotonergic function, two-mode models of self-regulation, and vulnerability to depression: what depression has in common with impulsive aggression. *Psychol Bull* (2008) **134**:912–43. doi:10.1037/a0013740
32. Painuly N, Sharan P, Mattoo SK. Relationship of anger and anger attacks with depression: a brief review. *Eur Arch Psychiatry Clin Neurosci* (2005) **255**:215–22. doi:10.1007/s00406-004-0539-5
33. Chen J, Evans AN, Liu Y, Honda M, Saavedra JM, Aguilera G. Maternal deprivation in rats is associated with corticotrophin-releasing hormone (CRH) promoter hypomethylation and enhances CRH transcriptional responses to stress in adulthood. *J Neuroendocrinol* (2012) **24**:1055–64. doi:10.1111/j.1365-2826.2012.02306.x
34. Meaney MJ, Ferguson-Smith AC. Epigenetic regulation of the neural transcriptome: the meaning of the marks. *Nat Neurosci* (2010) **13**:1313–8. doi:10.1038/nn1110-1313
35. Zhang TY, Labonté B, Wen XL, Turecki G, Meaney MJ. Epigenetic mechanisms for the early environmental regulation of hippocampal glucocorticoid receptor gene expression in rodents and humans. *Neuropsychopharmacology* (2013) **38**:111–23. doi:10.1038/npp.2012.149

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 03 October 2013; paper pending published: 17 October 2013; accepted: 31 October 2013; published online: 15 November 2013.
*Citation: Stamatakis A, Diamantopoulou A, Panagiotaropoulos T, Raftogianni A and Stylianopoulou F (2013) Effects of an early experience involving training in a T-maze under either denial or receipt of expected reward through maternal contact. Front. Endocrinol. **4**:178. doi: 10.3389/fendo.2013.00178*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.

frontiers in
ENDOCRINOLOGY

ORIGINAL RESEARCH ARTICLE
published: 04 July 2014
doi: 10.3389/fendo.2014.00103

Maternal age at Holocaust exposure and maternal PTSD independently influence urinary cortisol levels in adult offspring

Heather N. Bader[1,2], Linda M. Bierer[1,2], Amy Lehrner[1,2], Iouri Makotkine[1,2], Nikolaos P. Daskalakis[1,2] and Rachel Yehuda[1,2]*

[1] *James J. Peters VA Medical Center, Bronx, NY, USA*
[2] *Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, USA*

Edited by:
Pierrette Gaudreau, Centre Hospitalier de l'Université de Montréal, Canada

Reviewed by:
Jacques Epelbaum, Université Paris Descartes, France
José A. Morais, McGill University, Canada

****Correspondence:***
Rachel Yehuda, Department of Psychiatry, Traumatic Stress Studies Division, Mental Health Care Center, James J. Peters Veterans Affairs Medical Center, Icahn School of Medicine at Mount Sinai, 526 OOMH PTSD 116/A, 130 West Kingsbridge Road, Bronx, NY 10468, USA
e-mail: rachel.yehuda@va.gov

Background: Parental traumatization has been associated with increased risk for the expression of psychopathology in offspring, and maternal posttraumatic stress disorder (PTSD) appears to increase the risk for the development of offspring PTSD. In this study, Holocaust-related maternal age of exposure and PTSD were evaluated for their association with offspring ambient cortisol and PTSD-associated symptom expression.

Method: Ninety-five Holocaust offspring and Jewish comparison subjects received diagnostic and psychological evaluations, and 24 h urinary cortisol was assayed by RIA. Offspring completed the parental PTSD questionnaire to assess maternal PTSD status. Maternal Holocaust exposure was identified as having occurred in childhood, adolescence, or adulthood and examined in relation to offspring psychobiology.

Results: Urinary cortisol levels did not differ for Holocaust offspring and comparison subjects but differed significantly in offspring based on maternal age of exposure and maternal PTSD status. Increased maternal age of exposure and maternal PTSD were each associated with lower urinary cortisol in offspring, but did not exhibit a significant interaction. In addition, offspring PTSD-associated symptom severity increased with maternal age at exposure and PTSD diagnosis. A regression analysis of correlates of offspring cortisol indicated that both maternal age of exposure and maternal PTSD were significant predictors of lower offspring urinary cortisol, whereas childhood adversity and offspring PTSD symptoms were not.

Conclusion: Offspring low cortisol and PTSD-associated symptom expression are related to maternal age of exposure, with the greatest effects associated with increased age at exposure. These effects are relatively independent of the negative consequences of being raised by a trauma survivor. These observations highlight the importance of maternal age of exposure in determining a psychobiology in offspring that is consistent with increased risk for stress-related pathology.

Keywords: maternal, PTSD, risk, cortisol, intergenerational, Holocaust, offspring, trauma

INTRODUCTION

Parental posttraumatic stress disorder (PTSD) has been identified as a salient contributor to the transgenerational transmission of risk for low cortisol trait and the development of PTSD in offspring (1–4). Furthermore, parental traumatization has been associated with increased risk for offspring depression and anxiety disorders (5, 6). Recent studies have noted the specific association of maternal, rather than paternal PTSD with lifetime expression of offspring PTSD has been previously noted (7) and linked maternal PTSD and even depression following exposure to the World trade center attacks to increased emotional reactivity in child offspring (8). The majority, but not all, of the studies have been done in Holocaust offspring (8). The Holocaust offspring population offers numerous advantages for the study of intergenerational effects including the opportunity to evaluate consequences of age of exposure, which has not previously been the focus of study. Because the Holocaust was a discrete historical event, persons of different ages were exposed simultaneously, allowing an investigation of the effect of age of trauma exposure on offspring symptoms and biology. Thus, although maternal PTSD has been identified as a strong contributor to hypothalamic–pituitary–adrenal (HPA) axis sensitivity and offspring psychopathology (7–10), the impact of maternal age at Holocaust exposure on offspring biology has not been sufficiently investigated as a potential contributor. In this report, we examine urinary cortisol levels in Holocaust survivor offspring and comparison subjects in relation to maternal age of Holocaust exposure and PTSD by presenting a reanalysis of previously reported data (1), with the addition of new subjects. The

Holocaust offspring in this study was conceived after, and in some cases well after, direct parental exposure to the Holocaust. Thus, this is an optimal sample in which to evaluate effects in offspring related to age of maternal exposure, as well as to maternal PTSD.

We hypothesized lower cortisol and greater PTSD symptomatology in offspring of mothers who were adults during their Holocaust exposure based on previous observations that older survivors had greater distressing thoughts and nightmares, contributing to greater PTSD symptoms than younger survivors (11). This would cause greater glucocorticoid disturbances in the survivor and potentially also a more adverse post-natal environmental effect on the offspring.

MATERIALS AND METHODS

SUBJECTS

Ninety-five participants, containing Holocaust survivor offspring ($n = 69$) and Jewish comparison subjects ($n = 26$), were included in the current study. Data from 31 of the Holocaust survivor offspring and 13 controls was previously published (1). Participants were recruited primarily through advertisements seeking Jewish volunteers and through Holocaust survivors who had previously participated in related research and were made aware of the current study as previously described (1, 2). Participant written, informed consent, and Icahn School of Medicine at Mount Sinai Institutional Review Board approval were received for all study procedures.

Holocaust survivor offspring was defined as having been born after World War II (1944 or later) and raised through adolescence by birth parents that were both exposed to the Nazi Holocaust. Comparison subjects were demographically comparable Jewish persons born to parents unexposed to the Holocaust, generally from the United States or Canada. Participants with psychotic illness, bipolar disorder, obsessive compulsive disorder, or a medical illness or medication that might interfere with HPA axis function were not studied.

Psychological evaluations were administered to assess the presence of any current or lifetime psychiatric disorders, including PTSD, according to DSM-IV criteria using the Structured Clinical Interview for the DSM-IV (12) and Clinician-Administered PTSD Scale [CAPS; (13)], respectively. Other self-rating instruments assessed depression and anxiety severity [Beck Depression Inventory (BDI); (14)] and Spielberger State-Trait Anxiety Inventory (STAI); (15), and severity of childhood adversity [Childhood Trauma Questionnaire (CTQ); (16)]. Additionally, Holocaust offspring was asked to rate parental symptoms using the Parental PTSD Questionnaire (PPQ), an instrument that has been previously developed in our laboratory and has demonstrated high concordance between offspring and clinician-rated evaluations of Holocaust survivors (17). The scale further inquires about potential consequences of being raised by a Holocaust survivor; for instance, becoming more or less harm avoidant, experiencing "psychological scars," or developing increased "stress sensitivity" (17). Other information about parents such as year of birth, age at Holocaust exposure, type of Holocaust exposure (e.g., concentration camp, hiding), and age at offspring birth, was also determined. The Holocaust offspring group was then further subcategorized based on maternal age of exposure and the presence or absence of maternal PTSD, as recently described (18).

URINE COLLECTION AND CORTISOL ASSAY

Participants were asked to collect 24-h urinary samples at home on a day anticipated to be relatively unstressful and in which vigorous exercise was avoided. Completeness of collection was monitored by asking participants about missed collections as well as by assessing urinary creatinine concentrations. The laboratory personnel who performed the assays were blind to whether the sample came from a Holocaust survivor offspring or comparison subject as well as any demographical or clinical information about the subjects who provided the samples. More detailed procedures for 24-h urine collections, storage, and cortisol determination have been described previously (2).

STATISTICAL METHODS

The purpose of the analyses was to compare Holocaust offspring to controls, or to examine differences between Holocaust offspring subgroups based maternal age of exposure (11 years or younger, 12–18 years of age, 18 years or older) and/or presence or absence of maternal PTSD. Other analyses examined associations between clinical and descriptive measures. Chi-square analyses were used to compare categorical variables, and analyses of variance and covariance (ANOVA, ANCOVA) were used to compare descriptive data across groups. Covariates were detected by performing correlations between urinary cortisol and other outcome measures with body mass index (BMI), presence/absence of current depressive disorder diagnosis, medication usage, and other variables known to influence the HPA axis; variables were included as covariates in analyses involving outcome measures with which they were significantly correlated. Age and gender are used as covariates in analyses including all subjects as there is an established biological basis for their influence on urinary cortisol (19, 20). Significance was set at $p < 0.05$ and trend level significance was set at $p < 0.10$. Bonferroni *post hoc* testing was performed when applicable.

RESULTS

COMPARISON OF HOLOCAUST OFFSPRING AND CONTROLS

Table 1 compares demographical and clinical characteristics, as well as urinary cortisol levels, for Holocaust offspring and Jewish comparison subjects. The groups differed significantly on age, presence of current and lifetime anxiety disorder as well as self-ratings of anxiety. There were trends for differences in self-reported and diagnosis of current depression and lifetime PTSD diagnosis, as well as childhood trauma. Furthermore, there was no significant difference between comparison subjects and Holocaust offspring urinary cortisol levels ($F_{1,91} = 0.72$, ns; covaried for age and gender).

HOLOCAUST OFFSPRING ACCORDING TO MATERNAL AGE AT EXPOSURE

Table 2 compares demographical and clinical characteristics for Holocaust offspring according to maternal age at exposure. As expected because the Holocaust occurred during a circumscribed period in history, the groups differed significantly on offspring age and maternal age at offspring birth. There were also significant differences between offspring groups on self-reported psychological

Table 1 | Comparison of Holocaust offspring and comparison subjects.

	Offspring ($n = 69$)	Control ($n = 26$)	F_{df}, p, or χ^2_{df}, p
Age	47.9 ± 7.4	42.5 ± 10.7	$F_{1,93} = 7.70$, $p = 0.007$
Gender (% males)	23 (33.3%)	12 (46.2%)	$\chi^2_1 = 1.33$, ns
Years of education	17.2 ± 3.1	16.9 ± 2.2	$F_{1,93} = 0.17$, ns
Body mass index (kg/m^2)	24.6 ± 4.3	24.1 ± 4.0	$F_{1,93} = 0.25$, ns
Beck Depression Inventory	8.1 ± 6.9	4.4 ± 5.6	$F_{1,64} = 3.96$, ($p = 0.051$)
Spielberger Trait Anxiety[a]	21.4 ± 10.9	15.1 ± 11.5	$F_{1,60} = 4.21$, $p = 0.044$
Spielberger State Anxiety[a]	16.8 ± 12.6	9.4 ± 8.8	$F_{1,64} = 5.09$, $p = 0.028$
CTQ total score[b]	41.2 ± 13.6	35.9 ± 12.1	$F_{1,87} = 2.88$, ($p = 0.094$)
CAPS total score – current[c]	14.8 ± 20.8	8.7 ± 19.7	$F_{1,85} = 1.46$, ns
CAPS total score – lifetime[c]	24.5 ± 26.5	15.9 ± 25.7	$F_{1,86} = 1.86$, ns
Depressive disorder – current[d]	13 (18.8%)	1 (3.8%)	$\chi^2_1 = 3.38$, ($p = 0.066$)
Depressive disorder – lifetime[d]	32 (46.4%)	8 (30.8%)	$\chi^2_1 = 1.89$, ns
Anxiety disorder – current[d]	22 (31.9%)	3 (11.5%)	$\chi^2_1 = 4.03$, $p = 0.045$
Anxiety disorder – lifetime[d]	32 (46.4%)	5 (19.2%)	$\chi^2_1 = 5.85$, $p = 0.016$
PTSD – current[e]	4 (5.8%)	1 (4.0%)	$\chi^2_1 = 0.12$, ns
PTSD – lifetime[e]	13 (18.8%)	1 (4.0%)	$\chi^2_1 = 3.19$, ($p = 0.074$)
Urinary cortisol (RIA; μg/day)	51.3 ± 27.5	57.6 ± 31.4	$F_{1,93} = 0.90$, ns

[a] Spielberger State-Trait Anxiety Inventory (STAI); [b] Childhood Trauma Questionnaire (CTQ); [c] Clinician-Administered PTSD Scale (CAPS) ratings for PTSD-related symptom severity; [d] Diagnoses according to DSM-IV criteria based on clinical interview; [e] Diagnoses based on CAPS criteria for current and lifetime PTSD.

Table 2 | Holocaust offspring variables according to maternal age at exposure.

	Maternal age at Holocaust exposure			F_{df}, p, or χ^2_{df}, p
	Child ($n = 22$)	Adolescent ($n = 30$)	Adult ($n = 17$)	
Offspring age	43.0 ± 8.0	48.6 ± 6.1	52.8 ± 4.4	$F_{2,66} = 11.47$, $p < 0.0005$[a,b]
Maternal age at offspring birth	26.6 ± 5.5	29.4 ± 4.6	35.0 ± 4.7	$F_{2,61} = 12.50$, $p < 0.0005$[b,c]
Gender (% males)	6 (27.3%)	10 (33.3%)	7 (41.2%)	$\chi^2_2 = 0.83$, ns
Years of education	17.5 ± 3.4	16.9 ± 3.2	17.3 ± 2.5	$F_{2,66} = 0.20$, ns
Body mass index (kg/m^2)	23.9 ± 3.3	25.0 ± 4.5	24.7 ± 5.4	$F_{2,66} = 0.35$, ns
Psychological scars and stress sensitivity[d]	4.9 ± 2.9	5.9 ± 2.5	7.4 ± 2.6	$F_{2,61} = 3.63$, $p = 0.032$[b]
Beck Depression Inventory	8.7 ± 6.7	7.0 ± 5.8	9.3 ± 9.1	$F_{2,45} = 0.52$, ns
Spielberger STAI-T[e]	20.4 ± 9.7	21.6 ± 10.4	22.5 ± 13.9	$F_{2,41} = 0.11$, ns
Spielberger STAI-S[e]	16.5 ± 11.4	15.5 ± 13.7	19.2 ± 12.8	$F_{2,45} = 0.31$, ns
CTQ total score[f]	41.1 ± 15.0	41.2 ± 12.7	41.4 ± 14.5	$F_{2,61} = 0.00$, ns
CAPS total score – current[g]	8.0 ± 15.6	14.0 ± 20.7	25.3 ± 24.1	$F_{2,61} = 3.23$, $p = 0.046$[b]
CAPS total score – lifetime[g]	14.5 ± 21.2	23.8 ± 25.3	39.2 ± 29.9	$F_{2,61} = 4.11$, $p = 0.021$[b]
Depressive disorder – current[h]	5 (22.7%)	4 (13.3%)	4 (23.5%)	$\chi^2_2 = 1.06$, ns
Depressive disorder – lifetime[h]	8 (36.4%)	15 (50.0%)	9 (52.9%)	$\chi^2_2 = 1.34$, ns
Anxiety disorder – current[h]	6 (27.3%)	11 (36.7%)	5 (29.4%)	$\chi^2_2 = 0.58$, ns
Anxiety disorder – lifetime[h]	7 (31.8%)	16 (53.3%)	9 (52.9%)	$\chi^2_2 = 2.75$, ns
PTSD – current[i]	1 (4.5%)	1 (3.3%)	2 (11.8%)	$\chi^2_2 = 1.51$, ns
PTSD – lifetime[i]	3 (13.6%)	5 (16.7%)	5 (29.4%)	$\chi^2_2 = 1.73$, ns
Urinary cortisol (RIA; μg/day)	57.8 ± 25.3	52.7 ± 28.2	40.6 ± 27.5	$F_{2,66} = 1.98$, ns [j]

[a] Significant post hoc difference between offspring of child and adolescent survivors; [b] Significant post hoc difference between offspring of child and adult survivors; [c] Significant post hoc difference between offspring of adolescent and adult survivors; [d] Composite score of two self-report items on the Parental PTSD Questionnaire (PPQ); [e] Spielberger State-Trait Anxiety Inventory (STAI); [f] Childhood Trauma Questionnaire (CTQ); [g] Clinician-Administered PTSD Scale (CAPS) ratings for PTSD-related symptom severity; [h] Diagnoses according to DSM-IV criteria based on clinical interview; [i] Diagnoses according to CAPS criteria for current and lifetime PTSD.
[j] Comparison without covariates as used in text.

scars and stress sensitivity, and PTSD severity, which were confirmed by *post hoc* testing to reflect significant differences between offspring with maternal exposures as children versus adults.

Figure 1 illustrates urinary cortisol levels for controls and offspring grouped by maternal age at Holocaust exposure. The main effect of group ($F_{3,89} = 3.09$, $p = 0.031$; covaried for age, and gender) resulted from a significant difference between offspring whose mothers were adults and those whose mothers were children at time of exposure ($p = 0.028$) and a trend level difference between offspring whose mothers were exposed during adulthood and controls ($p = 0.069$). There was no significant difference between offspring with mothers exposed during childhood compared to those with mothers exposed during adolescence or between controls, between either of the latter two groups, or between adult and adolescent maternally exposed offspring. **Figure 2** illustrates that strength of the association between maternal age at exposure and urinary cortisol levels when age was expressed as a continuous variable for Holocaust offspring only, covaried for age, gender, and current depressive disorder ($r = -0.35$, $df = 59$, $p = 0.006$). This relationship also remained significant when additionally accounting for offspring subjective assessment of childhood adversity ($r = -0.29$, $df = 59$, $p = 0.006$).

The observed relationship of offspring urinary cortisol with maternal age of exposure was not driven by maternal age at offspring birth. The relationship between urinary cortisol and maternal age at offspring birth in Holocaust offspring was not significant ($r = -0.206$, $df = 59$, $p = 0.112$, controlling for offspring age, gender, and current depressive disorder) and this relationship was unchanged when maternal age at Holocaust was added as a covariate ($r = 0.162$, $df = 58$, $p = 0.217$). Maternal age at offspring

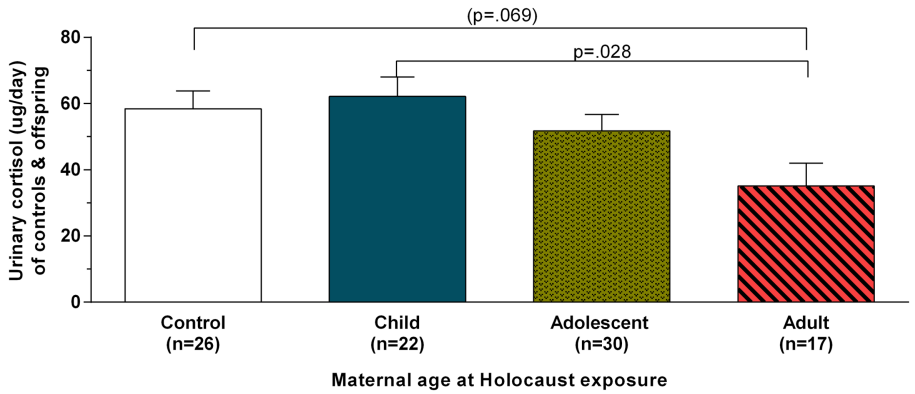


FIGURE 1 | Twenty-four-hour urinary cortisol excretion based on maternal age at Holocaust exposure in control subjects and Holocaust offspring. Estimated marginal means ± SEM for urinary cortisol levels (microgram per day) are presented for comparison subjects (white bar) and Holocaust survivor offspring grouped according to whether offspring's mother was a child (age 0–11; solid blue bar), adolescent (age 12–18; speckled gold bar), or adult (diagonally striped red bar) at Holocaust exposure (age 18 or older). Statistical significance was set at $p < 0.05$.

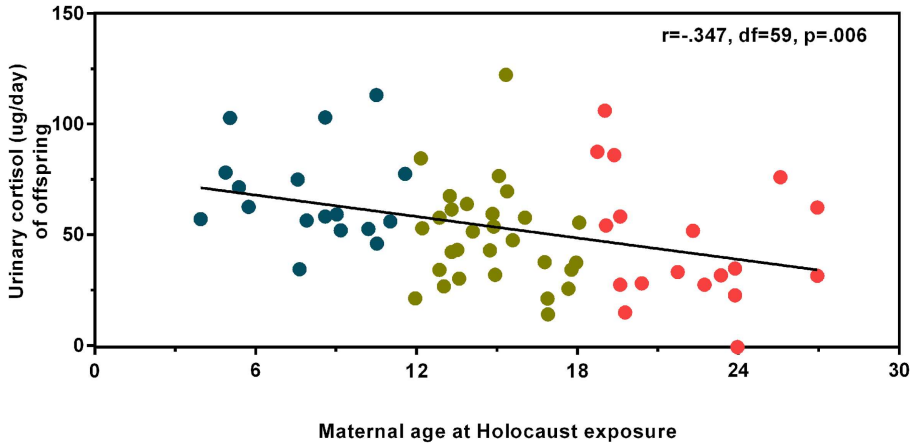


FIGURE 2 | Relationship of maternal age at Holocaust exposure with offspring 24-h urinary cortisol excretion. A partial correlation between maternal age at Holocaust exposure and Holocaust offspring cortisol level controlling for offspring age, gender, and current depressive disorder diagnosis ($r = -0.347$, $df = 59$, $p = 0.006$) is depicted using linear regression and unstandardized residuals that were added to the raw values. Data points are colored for ease of interpretation to associate with the child (blue), adolescent (gold), and adult (red) maternal age of exposure groups. The total number of subjects included in this analysis is 64. The correlation coefficients are denoted and the statistical significance was set at $p < 0.05$.

birth was also not significantly associated with urinary cortisol levels in the control group ($r = 0.274$, df = 15, $p = 0.287$).

HOLOCAUST OFFSPRING ACCORDING TO MATERNAL PTSD

Table 3 shows demographic and clinical characteristics of Holocaust offspring only by maternal PTSD status. As previously reported for a subset of this group, in this larger sample, offspring with maternal PTSD reported significantly greater psychological scars and stress sensitivity, were diagnosed more frequently with lifetime PTSD, and showed higher levels of anxiety and PTSD-associated symptoms. Offspring with maternal PTSD also demonstrated trends for increased self-ratings of depression, state anxiety, and childhood trauma exposure. There was a significant main effect of maternal PTSD on Holocaust offspring urinary cortisol ($F_{1,63} = 6.35$, $p = 0.014$; covaried for age, gender, and current depressive disorder).

OFFSPRING CORTISOL IN RELATION TO MATERNAL AGE AT EXPOSURE AND PTSD

As both maternal age at exposure and PTSD were associated with offspring urinary cortisol, these predictors were entered as main effects in a single ANCOVA including only Holocaust offspring, controlling for offspring age, gender, and current depressive disorder. Both main effects were significant (maternal age at exposure: $F_{2,59} = 4.76$, $p = 0.012$; maternal PTSD: $F_{1,59} = 5.85$, $p = 0.019$) but their interaction was not ($F_{2,59} = 1.12$, ns). All covariates were significant in this model (age, $p = 0.015$; gender, $p = 0.014$; current depressive disorder, $p = 0.008$). These data are depicted in **Figure 3A**. Adding childhood trauma as a covariate, reduced the main effect of maternal PTSD to a trend. **Figure 3B** demonstrates that a similar analysis examining lifetime offspring PTSD-associated symptom severity also showed a significant effect of maternal age at exposure ($F_{2,54} = 8.46$, $p = 0.001$) and maternal PTSD status ($F_{2,54} = 9.87$, $p = 0.003$); covaried for offspring age, gender, and current depressive disorder. Offspring whose mothers were children during their Holocaust exposure had significantly lower PTSD-associated symptom severity than those whose mothers were adults ($p < 0.0005$) or adolescents ($p = 0.029$). Adding childhood trauma severity as a covariate reduced the effect of maternal PTSD such that it was no longer significant. Similar results were found for current offspring PTSD-related symptom severity and PPQ assessment of perceived psychological scars and stress sensitivity.

IMPACT OF MATERNAL AND OFFSPRING CHARACTERISTICS ON URINARY CORTISOL EXCRETION

A regression analysis was performed to examine the relative contributions of maternal and offspring characteristics on Holocaust offspring cortisol. As a first step, age, gender, and presence of current depressive disorder were entered. Maternal age at Holocaust exposure, presence or absence of maternal PTSD, childhood trauma severity, and offspring PTSD-associated symptom severity (lifetime CAPS scores) were added as a second step. After controlling for variables in the first step, and each

Table 3 | Holocaust offspring variables according to maternal PTSD status.

	Maternal PTSD status		F_{df}, p or χ^2_{df}, p
	PTSD+ ($n = 42$)	PTSD− ($n = 26$)	
Offspring age	47.6 ± 7.5	48.7 ± 7.1	$F_{1,66} = 0.41$, ns
Maternal age at offspring birth	29.6 ± 5.2	29.7 ± 6.5	$F_{1,61} = 0.01$, ns
Gender (males)	16 (38.1%)	7 (26.9%)	$\chi^2_1 = 0.90$, ns
Number of years of education	17.2 ± 2.7	17.1 ± 3.7	$F_{1,66} = 0.04$, ns
Body mass index (kg/m^2)	24.7 ± 4.1	24.4 ± 4.9	$F_{1,66} = 0.07$, ns
Psychological scars and stress sensitivity[a]	6.8 ± 2.7	4.6 ± 2.4	$F_{1,62} = 11.70$, $p = 0.001$
Beck Depression Inventory	9.6 ± 7.9	6.3 ± 5.2	$F_{1,46} = 2.88$, $p = 0.097$
Spielberger STAI-T[b]	25.5 ± 10.2	16.1 ± 9.7	$F_{1,42} = 9.77$, $p = 0.003$
Spielberger STAI-S[b]	19.6 ± 12.8	13.1 ± 11.7	$F_{2,46} = 3.20$, $p = 0.080$
CTQ total score[c]	46.8 ± 13.9	32.2 ± 7.1	$F_{1,61} = 22.64$, $p < 0.0005$
CAPS total score – current[d]	19.6 ± 24.5	6.7 ± 9.7	$F_{1,61} = 6.04$, $p = 0.017$
CAPS total score – lifetime[d]	31.7 ± 29.5	11.9 ± 14.6	$F_{1,61} = 9.42$, $p = 0.003$
Depressive disorder – current[e]	10 (23.8%)	3 (11.5%)	$\chi^2_1 = 1.56$, ns
Depressive disorder – lifetime[e]	23 (54.8%)	9 (34.6%)	$\chi^2_1 = 2.62$, ns
Anxiety disorder – current[e]	10 (23.8%)	11 (42.3%)	$\chi^2_1 = 2.67$, ns
Anxiety disorder – lifetime[e]	17 (40.5%)	14 (53.8%)	$\chi^2_1 = 1.16$, ns
PTSD – current[f]	4 (9.5%)	0 (0.0%)	$\chi^2_1 = 2.63$, ns
PTSD – lifetime[f]	12 (28.6%)	1 (3.8%)	$\chi^2_1 = 6.35$, $p = 0.012$
Urinary cortisol (RIA; μg/day)	46.7 ± 23.7	59.3 ± 32.1	$F_{1,66} = 3.42$, ($p = 0.069$)[g]

[a] *Composite score of two self-report items on the Parental PTSD Questionnaire (PPQ);* [b] *Spielberger State-Trait Anxiety Inventory (STAI);* [c] *Childhood Trauma Questionnaire (CTQ);* [d] *Clinician-Administered PTSD Scale (CAPS) ratings for PTSD-related symptom severity;* [e] *Diagnoses according to DSM-IV criteria based on clinical interview;* [f] *Diagnoses according to CAPS criteria for current and lifetime PTSD;* [g] *Comparison made without covariates as in text.*

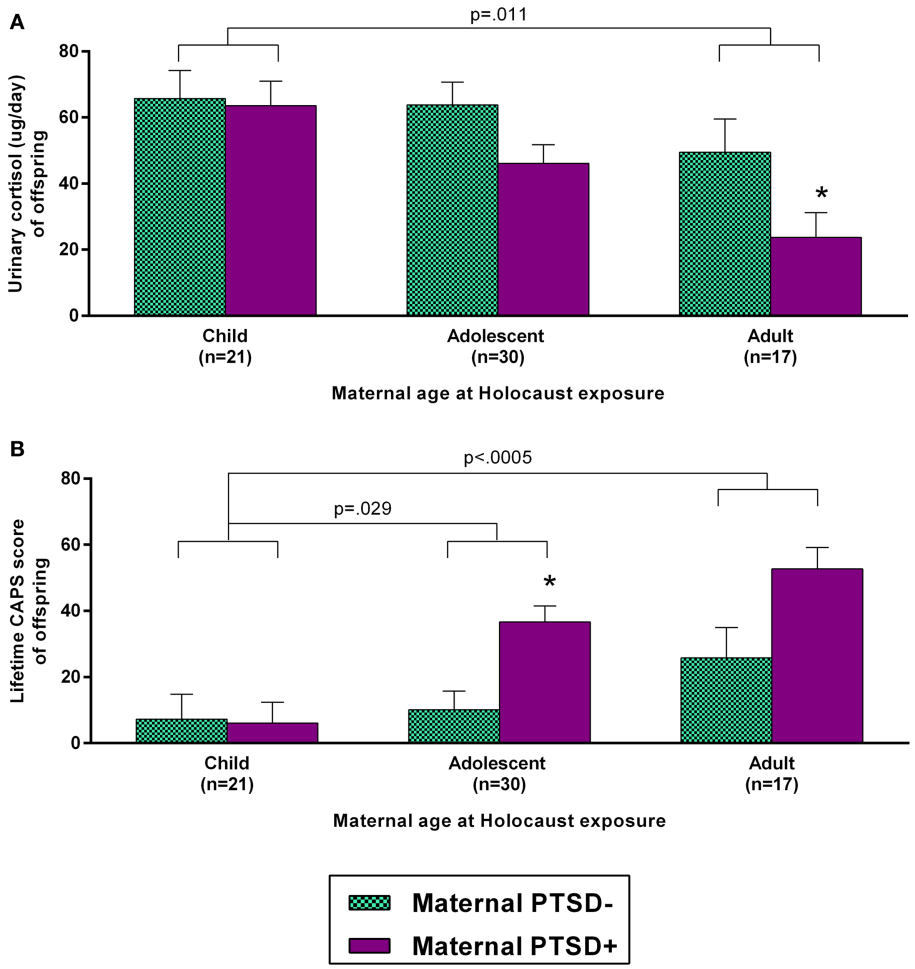


FIGURE 3 | Influence of maternal age at Holocaust exposure and maternal PTSD on offspring 24-h urinary cortisol excretion and lifetime PTSD-related symptom severity. Offspring cortisol **(A)** and offspring lifetime clinician-administered PTSD scale (CAPS) total score **(B)** are depicted based on maternal age at Holocaust and maternal PTSD status. Maternal PTSD − offspring are depicted with light blue, checkered bars and maternal PTSD + offspring are represented with solid purple bars. Data were adjusted for age, gender, and diagnosis of current depressive disorder and are represented as estimated marginal means ± SEM. *, versus PTSD – was statistically significant. Statistical significance was set at $p < 0.05$.

of the four variables in the second step, only maternal age at Holocaust exposure ($\beta = -0.562$, $p = 0.001$) and maternal PTSD ($\beta = -0.317$, $p = 0.020$) remained significant (adjusted $R^2 = 0.361$, adjusted $\Delta R^2 = 0.218$), but offspring PTSD and childhood trauma were not significant.

DISCUSSION

The results of the study replicate and extend previous findings by our group (1, 2, 9, 21) regarding cortisol levels in Holocaust offspring. It has been previously established that parental, and specifically maternal, PTSD associate with lower cortisol levels in offspring even after accounting for offspring's own traumatization and PTSD (1, 2, 5, 7, 9). This study adds that the low cortisol levels in association with maternal PTSD are additionally associated with maternal age at Holocaust exposure. In fact, maternal age at Holocaust exposure appears to exert a parallel effect on cortisol in offspring that is not a result of maternal PTSD or an interaction with maternal PTSD. The lowest levels of urinary cortisol were present in offspring of mothers with PTSD who survived the Holocaust as adults.

In parallel, maternal age at Holocaust exposure and presence of maternal PTSD were found to be similarly associated with psychological characteristics, particularly increased PTSD-related symptoms reflected by the CAPS total score, which may also reflect perceived psychological scars and increased stress sensitivity as a result of being raised by Holocaust survivors. This conclusion is supported by literature that demonstrates that offspring of trauma survivors have increased vulnerability to distress (22). The influence of maternal PTSD was also associated with increased scores on both of these psychological measures. However, when all variables, including childhood adversity, which in offspring is in part a function of having Holocaust survivor parents (with or without PTSD), were included in a regression analysis, maternal age at exposure, and maternal PTSD appeared to be independent contributors to offspring cortisol and symptoms. These maternal variables were more strongly

associated with offspring urinary cortisol excretion than were the offspring's own PTSD-associated symptoms or the related feelings of being victimized by their parents' exposures, interpreted as being psychologically scarred or having been sensitized to stress.

In that low cortisol has been associated with the PTSD diagnosis in Holocaust survivors (23), and indeed in PTSD related to other exposures (24–28), it is noteworthy that offspring's own PTSD symptom severity was less associated with low cortisol levels than the parental risk factors described above. We have previously demonstrated lower cortisol levels in offspring samples with no PTSD and have suggested that low cortisol levels may be related to PTSD vulnerability, potentially due to the presence of parental PTSD acting as a risk factor (1, 2, 5, 7, 9). In this sample, only 12/69 offspring met diagnostic criteria for lifetime PTSD and only 4 met diagnostic criteria for current PTSD. Thus, the assessment of PTSD severity using the CAPS score reflects relatively low levels of symptom severity in this sample. Possibly, this accounts for the relative lack of association of low cortisol with PTSD once other risk factors are considered.

The association of low cortisol in offspring with maternal age of exposure in addition to maternal PTSD implies an intergenerational effect on offspring that does not result from maternal PTSD symptoms *per se*. Studies of other populations have provided information that the "intergenerational effects" on cortisol are manifest in offspring at a very young age, including during infancy, as was demonstrated in infants of mothers with PTSD exposed to the 9/11 World Trade Center attacks while pregnant (29). The significant trimester effect observed in that study suggest some role for the intrauterine environment in transmission (30). In the current study, all offspring were born after the Holocaust had ended, and any potential intrauterine effects were not a result of direct *in utero* exposure.

The question raised by the data concern potential differences in intergenerational transmission depending on maternal developmental or age-related factors during trauma exposure. A previous study demonstrated that Holocaust survivors tended to show slightly different symptom profiles depending on whether they were children or adults during the war. Holocaust survivors who were younger at the age of Holocaust exposure had a higher prevalence of hypervigilance, psychogenic amnesia, and emotional detachment, while survivors who were older at time of exposure experience more distressing intrusive thoughts and nightmares (11). It stands to reason that if symptom profiles associate with age of exposure, biological characteristics might also associate with age of exposure.

In fact, the activity of kidney 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD-2) enzyme, which converts active cortisol to inert cortisone, was found to be higher in older Holocaust survivors (31), who displayed lower cortisol reactivity (32). Interestingly, in the current Holocaust offspring sample, we observed that 11β-HSD-2 activity was higher in offspring whose mothers were younger at the time of exposure (18). Thus, in that the intergenerational effect on 11β-HSD-2 was in the opposite direction in offspring compared with survivors, the effect in offspring may have in some measure obviated the consequences associated with PTSD risk in the form of low cortisol and symptoms. Thus, the current finding of lower cortisol and greater symptoms in association with older maternal exposure may reflect the absence of a protective epigenetic accommodation associated with the experience of extreme trauma in childhood, a particularly vulnerable period for developmental programing (30, 33, 34), in the Holocaust survivor parent.

The results of this study have a broad and enduring relevance, as they demonstrate not only potential psychological effects, but also a biological effect of maternal trauma exposure on unexposed offspring. Thus, distinct phenotypes may be observed in the offspring based not only on maternal experiences and the development of PTSD as a consequence, but also on the time of life during which a mother endured traumatic experiences. The study underscores prior observations that distinct phenotypes may result from trauma exposure at different ages (11), and further notes the potential for differential consequences for transmission in the next generation. Additional investigation is required to explore the possibility that these effects may span generations beyond the direct offspring of trauma survivors and explore potential mechanisms of this transmission.

AUTHOR CONTRIBUTIONS

Rachel Yehuda designed the study. Rachel Yehuda and Linda M. Bierer supervised the project and data collection. Linda M. Bierer supervised the clinical assessments, and Rachel Yehuda supervised the biological collection and protocol. Iouri Makotkine performed the biological assays and quality control. Heather N. Bader did primary analyses and drafted the manuscript. Nikolaos P. Daskalakis and Amy Lehrner assisted with literature review, manuscript preparation and editing. Rachel Yehuda and Linda M. Bierer edited the final manuscript. All the authors discussed the results and commented on the final version of the manuscript.

ACKNOWLEDGMENTS

This work was supported by 1RC1MH088101-01 "identification of an epigenetic risk marker for PTSD" and NIMH R01 MH 64675-01 "biology of risk and PTSD in Holocaust survivor offspring" and in part by the Grant Number #UL1TR000067 from the National Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of Health (NIH). The NIMH and NIH had no further role in the study design; in the collection, analysis, and interpretation of the data; in the writing of the report; or in the decision to submit the paper for publication.

REFERENCES

1. Yehuda R, Halligan SL, Bierer LM. Cortisol levels in adult offspring of Holocaust survivors: relation to PTSD symptom severity in the parent and child. *Psychoneuroendocrinology* (2002) **27**(1–2):171–80. doi:10.1016/S0306-4530(01)00043-9
2. Yehuda R, Bierer LM, Schmeidler J, Aferiat DH, Breslau I, Dolan S. Low cortisol and risk for PTSD in adult offspring of Holocaust survivors. *Am J Psychiatry* (2000) **157**(8):1252–9. doi:10.1176/appi.ajp.157.8.1252
3. Yehuda R, Teicher MH, Seckl JR, Grossman RA, Morris A, Bierer LM. Parental posttraumatic stress disorder as a vulnerability factor for low cortisol trait in offspring of Holocaust survivors. *Arch Gen Psychiatry* (2007) **64**(9):1040–8. doi:10.1001/archpsyc.64.9.1040
4. Nugent NR, Ostrowski S, Christopher NC, Delahanty DL. Parental posttraumatic stress symptoms as a moderator of child's acute biological response and subsequent posttraumatic stress symptoms in pediatric injury patients. *J Pediatr Psychol* (2007) **32**(3):309–18. doi:10.1093/jpepsy/jsl005

5. Yehuda R, Halligan SL, Bierer LM. Relationship of parental trauma exposure and PTSD to PTSD, depressive and anxiety disorders in offspring. *J Psychiatr Res* (2001) **35**(5):261–70. doi:10.1016/S0022-3956(01)00032-2
6. Jovanovic T, Smith A, Kamkwalala A, Poole J, Samples T, Norrholm SD, et al. Physiological markers of anxiety are increased in children of abused mothers. *J Child Psychol Psychiatry* (2011) **52**(8):844–52. doi:10.1111/j.1469-7610.2011.02410.x
7. Yehuda R, Bell A, Bierer LM, Schmeidler J. Maternal, not paternal, PTSD is related to increased risk for PTSD in offspring of Holocaust survivors. *J Psychiatr Res* (2008) **42**(13):1104–11. doi:10.1016/j.jpsychires.2008.01.002
8. Chemtob CM, Nomura Y, Rajendran K, Yehuda R, Schwartz D, Abramovitz R. Impact of maternal posttraumatic stress disorder and depression following exposure to the September 11 attacks on preschool children's behavior. *Child Dev* (2010) **81**(4):1129–41. doi:10.1111/j.1467-8624.2010.01458.x
9. Lehrner A, Bierer LM, Passarelli V, Pratchett LC, Flory JD, Bader HN, et al. Maternal PTSD associates with greater glucocorticoid sensitivity in offspring of Holocaust survivors. *Psychoneuroendocrinology* (2014) **40**:213–20. doi:10.1016/j.psyneuen.2013.11.019
10. Yehuda R, Bierer LM. Transgenerational transmission of cortisol and PTSD risk. *Prog Brain Res* (2008) **167**:121–35. doi:10.1016/S0079-6123(07)67009-5
11. Yehuda R, Schmeidler J, Siever LJ, Binder-Brynes K, Elkin A. Individual differences in posttraumatic stress disorder symptom profiles in Holocaust survivors in concentration camps or in hiding. *J Trauma Stress* (1997) **10**(3):453–63. doi:10.1023/A:1024860430725
12. Spitzer RL, Williams JBW, Gibbon M. *Structured Clinical Interview for DSM-IV (SCID)*. New York, NY: New York State Psychiatric Institute Biometrics Research (1995).
13. Blake DD, Weathers FW, Nagy LM, Kaloupek DG, Gusman FD, Charney DS, et al. The development of a clinician-administered PTSD scale. *J Trauma Stress* (1995) **8**(1):75–90. doi:10.1002/jts.2490080106
14. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry* (1961) **4**:561–71. doi:10.1001/archpsyc.1961.01710120031004
15. Spielberger CD. *The State-Trait Anxiety Inventory (S T A I): Test Manual for Form X*. Palo Alto: Consulting Psychologists Press (1968).
16. Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, et al. Initial reliability and validity of a new retrospective measure of child abuse and neglect. *Am J Psychiatry* (1994) **151**(8):1132–6.
17. Yehuda R, Labinsky E, Tischler L, Brand SR, Lavin Y, Blair W, et al. Are adult offspring reliable informants about parental PTSD? A validation study. *Ann N Y Acad Sci* (2006) **1071**:484–7. doi:10.1196/annals.1364.047
18. Bierer LM, Bader HN, Daskalakis NP, Lehrner A, Makotkine I, Seckl JR, et al. Elevation of 11β-hydroxysteroid dehydrogenase type 2 in Holocaust survivor offspring: evidence for an intergenerational effect of maternal trauma exposure. *Psychoneuroendocrinology* (2014) **48**:1–10. doi:10.1016/j.psyneuen.2014.06.001
19. Van Cauter E, Leproult R, Kupfer DJ. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J Clin Endocrinol Metab* (1996) **81**(7):2468–73. doi:10.1210/jcem.81.7.8675562
20. Nakamura J, Yakata M. Age- and sex-related differences in urinary cortisol level. *Clin Chim Acta* (1984) **137**(1):77–80. doi:10.1016/0009-8981(84)90314-0
21. Yehuda R, Blair W, Labinsky E, Bierer LM. Effects of parental PTSD on the cortisol response to dexamethasone administration in their adult offspring. *Am J Psychiatry* (2007) **164**(1):163–6. doi:10.1176/appi.ajp.164.1.163
22. Brom D, Kfir R, Dasberg H. A controlled double-blind study on children of Holocaust survivors. *Isr J Psychiatry Relat Sci* (2001) **38**(1):47–57.
23. Yehuda R, Kahana B, Binder-Brynes K, Southwick SM, Mason JW, Giller EL. Low urinary cortisol excretion in Holocaust survivors with posttraumatic stress disorder. *Am J Psychiatry* (1995) **152**(7):982–6.
24. Glover DA, Poland RE. Urinary cortisol and catecholamines in mothers of child cancer survivors with and without PTSD. *Psychoneuroendocrinology* (2002) **27**(7):805–19. doi:10.1016/S0306-4530(01)00081-6
25. Mason JW, Giller EL, Kosten TR, Ostroff RB, Podd L. Urinary free-cortisol levels in posttraumatic stress disorder patients. *J Nerv Ment Dis* (1986) **174**(3):145–9. doi:10.1097/00005053-198603000-00003
26. Kanter ED, Wilkinson CW, Radant AD, Petrie EC, Dobie DJ, McFall ME, et al. Glucocorticoid feedback sensitivity and adrenocortical responsiveness in posttraumatic stress disorder. *Biol Psychiatry* (2001) **50**(4):238–45. doi:10.1016/S0006-3223(01)01158-1
27. Bremner D, Vermetten E, Kelley ME. Cortisol, dehydroepiandrosterone, and estradiol measured over 24 hours in women with childhood sexual abuse-related posttraumatic stress disorder. *J Nerv Ment Dis* (2007) **195**(11):919–27. doi:10.1097/NMD.0b013e3181594ca0
28. Yehuda R, Flory JD, Bierer LM, Henn-Haase C, Lehrner A, Desarnaud F, et al. Lower methylation of glucocorticoid receptor gene promoter 1(F) in peripheral blood of veterans with posttraumatic stress disorder. *Biol Psychiatry* (2014). doi:10.1016/j.biopsych.2014.02.006
29. Yehuda R, Engel SM, Brand SR, Seckl J, Marcus SM, Berkowitz GS. Transgenerational effects of posttraumatic stress disorder in babies of mothers exposed to the world trade center attacks during pregnancy. *J Clin Endocrinol Metab* (2005) **90**(7):4115–8. doi:10.1210/jc.2005-0550
30. Yehuda R, Seckl J. Minireview: stress-related psychiatric disorders with low cortisol levels: a metabolic hypothesis. *Endocrinology* (2011) **152**(12):4496–503. doi:10.1210/en.2011-1218
31. Yehuda R, Bierer LM, Andrew R, Schmeidler J, Seckl JR. Enduring effects of severe developmental adversity, including nutritional deprivation, on cortisol metabolism in aging Holocaust survivors. *J Psychiatr Res* (2009) **43**(9):877–83. doi:10.1016/j.jpsychires.2008.12.003
32. van der Hal-Van Raalte EA, Bakermans-Kranenburg MJ, van Ijzendoorn MH. Diurnal cortisol patterns and stress reactivity in child Holocaust survivors reaching old age. *Aging Ment Health* (2008) **12**(5):630–8. doi:10.1080/13607860802343134
33. Essex MJ, Boyce WT, Hertzman C, Lam LL, Armstrong JM, Neumann SM, et al. Epigenetic vestiges of early developmental adversity: childhood stress exposure and DNA methylation in adolescence. *Child Dev* (2013) **84**(1):58–75. doi:10.1111/j.1467-8624.2011.01641.x
34. Denver RJ, Crespi EJ. Stress hormones and human developmental plasticity lessons from tadpoles. *Neoreviews* (2006) **7**(4):e183–8. doi:10.1542/neo.7-4-e183

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 31 March 2014; accepted: 16 June 2014; published online: 04 July 2014.
*Citation: Bader HN, Bierer LM, Lehrner A, Makotkine I, Daskalakis NP and Yehuda R (2014) Maternal age at Holocaust exposure and maternal PTSD independently influence urinary cortisol levels in adult offspring. Front. Endocrinol. **5**:103. doi: 10.3389/fendo.2014.00103*
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology.