Effects of the social environment and stress on glucocorticoid receptor gene methylation: a systematic review

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Abstract

The early-life social environment can induce stable changes that influence neurodevelopment and mental health. Research focused on early-life adversity revealed that early-life experiences have a persistent impact on gene expression and behaviour through epigenetic mechanisms. The hypothalamus-pituitary-adrenal (HPA) axis is sensitive to changes in the early-life environment that associate with DNA methylation of a neuron-specific exon 1_7 promoter of the glucocorticoid receptor (GR; \textit{NR3C1}). Since Weaver et al published the initial findings in 2004, numerous reports have investigated GR gene methylation in relationship to early-life experience, parental stress and psychopathology. We conducted a systematic review of this growing literature, which identified 40 articles (13 animal and 27 human studies) published since 2004. The majority of these examined the GR exon variant 1_F in humans or the GR1_7 in rats, and 89% of human studies and 70% of animal studies of early-life adversity reported increased methylation at this exon variant. All the studies investigating exon 1_F/1_7 methylation in conditions of parental stress (one animal study and 7 human studies) also reported increased methylation. Studies examining psychosocial stress and psychopathology had less consistent results, with 67% of animal studies reporting increased exon 1_7 methylation and 17% of human studies reporting increased exon 1_F methylation. We found great consistency among studies investigating early life adversity and the effect of parental stress, even if the precise phenotype and measures of social environment adversity varied among studies. These results are encouraging and warrant further investigation to better understand correlates and characteristics of these associations.

Keywords

glucocorticoid receptor; epigenetics; DNA methylation; social environment; early-life adversity; systematic review
Introduction

There is substantial theoretical and empirical research supporting an association between early-life environmental adversity and poor lifetime mental health outcomes (1–12). A critical issue concerns the molecular mechanisms that account for such strong and long-lasting effects. There is evidence suggesting that early-life environmental influences induce changes in stable epigenetic states that regulate gene expression and ultimately, complex neural functions. Thus in both rodents and nonhuman primates the early-life environment, including the quality of maternal care, regulates hypothalamus-pituitary-adrenal (HPA) axis function in adulthood (13–15). Variations in the early social environment in rodents, modeled by maternal care, reveal profound and persistent alterations in gene expression and behaviour that are mediated through epigenetic mechanisms, including changes in DNA methylation (16). The offspring of mothers with an increased frequency of pup licking/grooming (i.e., high LG mothers) show increased hippocampal glucocorticoid receptor (GR; NR3CI) expression, greater negative feedback regulation over hypothalamic corticotropin releasing factor (CRF) and more modest responses to stress compared to the offspring of low LG mothers (16–18). Variations in maternal LG are linked to an epigenetic modification of a neuron-specific exon 17 GR promoter (16) such that increased maternal LG associates with decreased methylation of the exon 17 promoter and increased hippocampal GR expression.

Subsequent studies in humans have expanded on the findings in rats. Accordingly, evidence for a long-term effect of early-life adversity (ELA) on the epigenetic state of the human genome was observed while investigating the methylation state of the GR gene in the hippocampus of individuals who died by suicide and had histories of child abuse (19). ELA in humans reprograms the DNA methylation patterns of the GR gene exon 1F (GR1F; GR17 homologue in rats) promoter and decreases GR1F expression in the hippocampus of suicide completers with a history of child abuse compared to non-abused suicide completers and healthy controls (19). An earlier study reported that children born to mothers with depression, irrespective of SSRI use, exhibited higher GR1F methylation levels (20). Since these first reports, several studies have investigated the effect of environmental adversity, measured by ELA or exposure to parental stress, on GR gene methylation, using both animal models and human samples. These studies also used different designs, measures of adversity, and tissue samples, and investigated methylation of diverse GR gene sequences. A growing number of studies have also been investigating the relationship between psychological stress or psychopathology and GR methylation. We conducted a systematic review of the growing literature investigating the relationship between environmental experience, stress and GR gene methylation.

Methods

Study identification

We performed a search of association studies of the GR gene and DNA methylation. The primary search was carried out through the National Library of Medicine (NLM) PubMed and a replication search was conducted through the Web of Knowledge database. The search included publications from 2004 up to July 2014 using the Weaver et al. (16) study as a
starting point. The Medical Subject Headings (MeSH) terms used were ‘(“glucocorticoid receptor” OR NR3C1) AND (epigenetics OR “DNA methylation”)’. Additional articles were found by scanning the list of references of the original publications and review articles. Only articles in English and those investigating humans or other mammals were included.

Study selection

The studies included in this systematic review met the following criteria: (a) use of a case-control or cohort design; (b) use of at least one analysis investigating DNA methylation of the GR gene in response to a change or perturbation in social environment; and (c) inclusion of studies independent from one another. Analyses based on the same set of data were excluded. In such cases, only the larger or more representative sample was retained. Studies in which a control group was absent also were not included.

Data extraction

Information for each study was extracted based on nine variables: 1) Species (human or nonhuman), 2) Study (experimental) group; 3) Sex; 4) Sample size; 5) Methodology (DNA methylation assessment); 6) Tissue(s) investigated; 7) Subject age at tissue collection; 8) Region or first exon variant(s) investigated; and 9) Effect on methylation (see tables 1–3). Studies were then grouped according to a broad classification of the study criteria and attributed to tables 1, 2 or 3. Within each table, animal- and human-based studies were considered independently.

Results

There is a growing number of studies reporting changes in GR gene methylation in association with social environment and stress. Our search identified a total of 430 articles. Of these, 173 were review papers and were excluded. Another 210 articles were excluded due to lack of relevance to the topic of this review. Seven studies were removed because they were not independent, as they investigated samples for which results had been reported elsewhere. In all, 40 articles met all the specified criteria. These were then sorted based on whether they addressed GR gene methylation changes in response to ELA (22 articles; Table 1), parental stress (9 articles; Table 2), or psychological stress/psychopathology (11 articles; Table 3) (two studies were included in two tables because they addressed both ELA and psychological stress (21) or parental stress and psychological stress (22)). Within each table, the articles were further subdivided into animal studies, human studies using peripheral tissues, and human studies using CNS tissue.

Sample type: species and tissues studied

Among the animal studies included, all used either rat (8 out of 13 studies) or mouse (5 out of 13 studies). All animal studies examined brain tissue, and 1 compared brain tissue and fecal matter (23), while another compared brain tissue and adrenal tissue (24). The brain region studied varied, including cortical and subcortical regions.

In the human studies, the majority of articles reported on GR gene methylation in peripheral tissues, where the term peripheral refers to tissues other than the CNS. In the “human
peripheral studies” category, 21 of the 24 articles used blood tissue, while 2 used saliva (25, 26), 1 used buccal epithelial cells (27) and 1 used placental tissue (28). Of the 3 studies examining brain tissue (the “human central tissues” category), all examined tissues from the limbic and cortical regions (19, 29, 30), and focused in particular on the hippocampus. In addition, Labonté and colleagues examined the anterior cingulate cortex (ACC), while Alt and colleagues investigated the amygdala, inferior prefrontal gyrus, cingulate gyrus and nucleus accumbens.

**GR gene region examined**—The GR gene in humans and rodents consists of 11 exons including untranslated first exon variants (31). Nine untranslated first exon variants each possessing their own promoter region have been identified in humans (1A, 1D, 1J, 1E, 1B, 1F, 1C and 1H) and in rats (1, 4–11). We found significant heterogeneity in the reporting and identification of the specific regions of the first exon variants that were studied. Specifically, there was no consistency in how CpG sites were identified and labeled, making the determination of overlapping regions difficult. This made detecting consistency in findings at the sequence level among studies challenging. We compiled the sequence data that we were able to retrieve from information published in all the human studies and located the regions within the GR gene containing the first exon variants investigated (see supplemental figure 1).

**Early-life adversity**

**Experimental groups**—To ensure the maximum impact of the review and allow for the comparison of results, we carefully selected articles that used similar criteria to define ELA.

In animal models, early-life experience was characterized by the use of maternal care models in 6 studies (16, 32–35) or maternal separation models in 4 studies (36–38).

Human studies defined ELA as exposure to traumatic events in childhood, including emotional, physical or sexual abuse, neglect, early parental death and other traumatic events. All studies included subjects who had been exposed to childhood abuse, with variations in the type of abuse included. In addition, 3 studies included early parental death as a marker of ELA (25, 39, 40).

**GR region studied**—The majority of studies included in this review examined exon 1\(_{7}\) (10 out of 10 animal studies), or exon 1\(_{F}\) (10 out of 12 human studies). One study also examined exon 1\(_{D}\), while two studies also examined exon 1\(_{B}\) and 1\(_{C}\), and three studies examined exon 1\(_{H}\). Of the 10 studies examining exon 1\(_{7}\) and the 10 studies examining exon 1\(_{F}\), 9 and 6, respectively, included examination of the binding site for the transcription factor NGFI-A (a.k.a., Egr-1, Zyf-268; table 1 and supplemental figure 1), which regulates GR gene transcription (19, 41).

**Effect on methylation**—As the majority of studies reported on exon 1\(_{7}/1_{F}\), we focused our comparisons on this particular variant. In the ELA group, all 10 animal studies examining GR gene methylation investigated exon 1\(_{7}\), and 7 studies (70%) reported a significant increase in methylation in the exon 1\(_{7}\) promoter, while three studies reported no change in exon 1\(_{7}\) methylation status (22, 33, 36). Of the 10 human studies examining exon

_Biol Psychiatry._ Author manuscript; available in PMC 2017 January 15.
methylated. 9 reported increased promoter methylation with ELA (90%). Of these, one study reported increased methylation at CpG sites labelled by the authors as CpGs 3, 6, and 7 in the whole sample, while a socioeconomic-matched subsample exhibited a decrease in methylation level of a single CpG (CpG2), located before the NF1-A binding site (CpGs 3 & 4), in conjunction with increased methylation at CpGs 3, 5 and 6 (42). One study reported no change in GR1F methylation status in response to ELA (21) (figure 1). A single study reported on exon 1D methylation, and found decreased methylation in blood samples of adolescents with early-life stress (40). In addition, one study reported no change in methylation of exons 1B and 1C in peripheral tissues (21), while another found increased methylation in brain tissues of subjects with childhood abuse (29). Three studies reported on the methylation status of exon 1H, with one study reporting no change (21), one reporting increased methylation (40) and one reporting decreased methylation (29). Additionally, one study reported decreased GR gene methylation, but did not specify the region investigated (43).

Parental stress

Experimental groups—One animal study (44) used a variety of non pain-inducing, non-habituating stressors over the course of 7 days to stress mouse dams. Human studies examined the methylation status of children of women who had experienced anxiety and mood disorders (20, 28), pregnancy-related anxiety (45), violence (46) or war stress (47, 48) during pregnancy. Another study examined the effects of parental stress on GR gene methylation in adolescence (27), and one additional study studied the methylation patterns of individuals whose parents had experienced war, but not necessarily during pregnancy (49) (table 2).

GR gene region—Most studies in this group examined exon 17 (for the animal study) or exon 1F (7 of 8 human studies), and the sites analyzed spanned the NF1-A binding site. One study also examined exons 1D and 1B (45).

Effect on methylation—Interestingly, all 8 studies investigating exon 1F/17 (including the animal study) reported an increased methylation of exon 1F/17 in offspring of parentally-stressed individuals (figure 2). Of these, two studies also reported decreased methylation at specific CpG sites and in particular conditions. For example, Hompes and colleagues reported a decreased methylation at a position labelled by the authors as CpG 36 (near the NGF-A binding site) only during trimesters 1 and 2 in women reporting a fear of changes associated with their pregnancy (45). Additionally, in the study of the offspring of Holocaust survivors, maternal experience of Holocaust was associated with decreased methylation of exon 1F, while paternal experience of Holocaust was associated with increased exon 1F methylation (49). Of note, the study investigating Holocaust survivors recruited participants born after the end of World War II, and therefore did not necessarily include mothers who experienced stress during pregnancy, but rather before pregnancy. Only one study examined other exon 1 variants, reporting increased methylation of exon 1D in the children of women experiencing fear of delivery in all trimesters or fear of the integrity of the baby in the first trimester, and decreased methylation of exon 1B in the children of women with fear of
delivery (45). Finally, an additional study investigated a single CpG located downstream of the $1_H$ promoter (in the gene body) and found no change in methylation status (27).

**Psychological stress/Psychopathology**

**Experimental groups**—Three animal studies used acute or chronic stress models to assess the impact of social stressors on GR gene methylation (24, 50). In addition, 8 studies examined the effects of psychopathologies on human GR gene methylation. Specifically, studies included response to stress (51), borderline personality disorder (52), bulimia nervosa (21), post-traumatic stress disorder (PTSD) (53–55) and depression (30, 56) (table 3).

**GR gene region**—Most studies in this group examined exon 1$_F$ (6 of the 8 human studies). In addition, 3 studies examined exon 1$_B$ (21, 30, 55), 2 studies examined exons 1$_C$ and 1$_H$ (21, 55), and 1 study examined exons 1$_J$ and 1$_E$ (30). Of the 8 studies examining exon 1$_F$, 6 specified that they included examination of the binding site for NGFI-A.

**Effect on methylation**—Two of the three animal studies included in this group reported increased exon 1$_F$ methylation in stressed animals, but studies reporting on human psychological stress/psychopathology had more varied results. One study reported increased methylation of exon 1$_F$ (52), while three reported no change (21, 30, 53), and two reported decreased methylation (30, 54). Among the other exon variants examined, there was no consensus on the effects of psychological stress/psychopathology on methylation status. Exons 1$_J$ and 1$_E$ showed no change in methylation, exon 1$_B$ was unchanged in two studies (21, 30) and had decreased methylation in one study (54), exon 1$_C$ was hypermethylated in one study (21), hypomethylated in one study (55) and unchanged in one study (51). Finally, exon 1$_H$ was hypomethylated in one study (21) and unchanged in another (55).

**Conclusion**

Since the publication of Weaver et al. 2004, there has been a surge in interest in GR gene methylation changes associated with altered social environment and stress, as evidenced by the number of articles that have since been published on the subject. We conducted a systematic review of all studies that investigated GR gene methylation in relation to various psychological stressors, including parental stress, adverse early-life social environments in animals, such as interventions affecting early environment, ELA in humans, and psychological stress or psychopathology in adults.

Most of these studies investigated the GR exon variant 1$_F$ and studies considering early-life and in utero adversity mostly reported increased methylation at this exon. In particular, negative early-life social environments were associated with greater exon 1$_F$ methylation in the large majority of studies (70% of animal ELA studies and 90% of human ELA studies assessing exon 1$_F$; 100% of parental stress studies assessing exon 1$_F$). In studies of gestational stress, it is important to note that 4 studies collected tissue samples at birth (20, 28, 45, 47), and it is therefore unclear whether the observed changes would persist into adulthood. However, the other studies included used adolescent or adult subjects (27, 46, 48,
When combined with the ELA studies, these findings show a compelling consensus of increased exon 1F methylation in conjunction with stress in early life (16 out of 17 studies; human studies from Tables 1 and 2, combined, that investigated exon 1F).

The strength of the association between adverse postnatal social environments and GR1F methylation in humans is consistent with the original report from McGowan et al. (16). Inter-individual differences in DNA methylation can be tissue- and cell type-specific, yet we found multiple reports of associations between the quality of childhood experience and the methylation status of the exon 1F NR3C1 gene promoter in readily-accessible peripheral cells. Perroud et al. (57, 58) used peripheral blood lymphocytes to show that childhood maltreatment associates with increased exon 1F methylation and, importantly, that promoter methylation status was closely correlated with both the frequency and severity of maltreatment (also see (42)). Interestingly, Tyrka et al. (39) reported that increased methylation of the exon 1F NR3C1 gene promoter in leukocytes, associated with disruption of normal parent-offspring interactions or maltreatment, was linked to an attenuated cortisol response to the Dex/CRH test. In this study, and that of Melas et al. (25), childhood parental loss was associated with increased methylation of the exon 1F NR3C1 gene promoter (note the Melas et al. study used salivary DNA, which is primarily of leukocyte origin (59)).

These findings appear despite the significant evidence of tissue-specific DNA methylation profiles (60–63), leading to the question of how a ‘social adversity-related’ epigenetic signal might appear in cell populations as diverse as peripheral blood cells and CNS-derived cells. One possibility is that social adversity activates stress responses that include signals such as steroid hormones (e.g., glucocorticoids) or cytokines, which act in multiple cell types and might initiate a coordinated remodelling of the epigenome at specific sites. While this reasoning is a matter of speculation, it does suggest a pathway by which the epigenetic imprint associated with social adversity might appear in a range of cell types, thus enabling meaningful population analyses of the effects of childhood environmental conditions on the epigenome. This also suggests that the nature of specific epigenetic marks across multiple tissues might be context specific: environmental conditions of sufficient biological impact, such as social adversity, might lead to coordinated changes that would enhance the probability of detecting specific epigenetic states across multiple tissues. This might also support the inclusion of epigenetic analyses of peripheral samples within intervention programs targeting brain-based phenotypes. The merits of this approach will become apparent with future studies focusing on a broader range of genomic targets, including genome-wide analyses (e.g. (27)).

The stability of DNA methylation is also a point of interest (64). Recent evidence supports the hypothesis that epigenetic plasticity is sustained in the brain throughout adulthood, potentially as a mechanism to cope with the evolving demands of the environment, yet there are clear moments during development when plasticity is heightened, and these may be more strongly associated with the establishment of life-long epigenetic modifications (reviewed in (65)). Another important consideration is that the studies cited here report low overall levels of methylation. This is consistent with the fact that strong CpG promoters, such as those from the GR gene, have generally low levels of methylation (66).
There has been great consistency regarding the increased methylation of the NGFI-A binding site within exon1F. There are also reports of differential methylation at other sites of the GR promoter that are not associated with NGFI-A binding (tables 1–3). However, the functional implications of such differential methylation have not been empirically tested, and further investigation of these sites is warranted. Although NGFI-A is enriched in the brain, it appears to be expressed ubiquitously (67), and belongs to the early growth response family of proteins, which contain a zinc-finger motif, allowing for interactions with target DNA regions (68). NGFI-A is activated by a range of stimuli, including neurotransmitters and cellular stimulation, and is a key contributor to T lymphocyte proliferation (69). Therefore, blocking NGFI-A binding-sites in the GR gene promoter in peripheral tissues (blood and saliva, in which the majority DNA-contributing cells are lymphocytes) is likely to actively contribute to the regulation of GR expression.

The activity of the HPA axis is governed by corticotropin-releasing factor (CRF) and arginine vasopressin (AVP), both of which are subject to GR-mediated feedback regulation at multiple levels within the axis and inhibition from extra-hypothalamic sites. Hippocampal GR activation associates with the inhibition of hypothalamic CRF synthesis and dampened HPA activity (70). Studies with adults reveal that childhood maltreatment is associated with an increased HPA response to stress (71, 72). Subsequent statistical analyses revealed that childhood abuse was the strongest predictor of ACTH responsiveness, followed by the number of abuse events, adulthood traumas and depression. An interaction term of childhood and adulthood trauma proved to be the most potent predictor of ACTH responses, suggesting that a history of childhood abuse per se is related to increased stress reactivity, which is further enhanced when additional trauma occurs in adulthood (71, 73). Among women with no history of MDD, childhood trauma was similarly associated with and increased ACTH response to stress (71).

There is evidence for elevated CSF levels of CRF in adults associated with a history of childhood maltreatment (74, 75), poor quality of parental care (76) and childhood stressful experience (77). Heim et al. (74) showed that CSF CRF concentrations were correlated with the severity and duration of physical and sexual abuse, and high CRF may arise due to GR down-regulation and impaired negative feedback inhibition, as supported by early reports linking childhood abuse with higher cortisol response to the DEX/CRH challenge test in adults (71, 78). Recent work has shown that some subjects having experienced childhood abuse exhibit lower levels of cortisol, with marked differences depending on gender (79–82), time of cortisol sampling (79), source tissue (83), type of abuse (84, 85), and the presence of concurrent psychiatric (71, 78) or other health conditions (84). Importantly, decreased cortisol may not be exclusively linked to PTSD (83, 86), as has often been supposed (87, 88); rather, decreased cortisol production may reflect an adaptation to chronically stressful situations, whereas elevated cortisol production may prime individuals to react to unpredictable stressors, and these situations may both constitute ELA (81). Currently, it is difficult to draw conclusions on the overall impact of GR methylation variations on basal and reactive cortisol levels, as the majority of studies investigating GR promoter methylation did not measure cortisol levels.
These findings suggest that childhood adversity stably influences HPA responses to stress. Childhood adversity moderates the relation between stressful life events in adulthood and depression, with increased risk for depression or anxiety in response to moderately stressful circumstances among individuals with a history of childhood adversity (5, 10, 89). This is consistent with the idea that childhood maltreatment sensitizes neural and endocrine responses to stress, thus establishing a vulnerability for mood disorders.

Recent rodent studies suggest that epigenetic programming of HPA function occurs at multiple levels of the HPA axis in addition to effects on hippocampal GR expression. Environmental conditions that increase the frequency of LG in the rat are associated with decreased paraventricular CRF expression (18, 90–92). Avishai-Eliner et al. (93) showed that this maternally-regulated decrease in CRF expression is accompanied by an increased hypothalamic expression of the transcriptional repressor NRSF and NRSF binding to a 21 bp sequence within the regulatory region (intron) of the Crh gene (94). Korosi et al. (95) showed that augmented maternal care reduced the number of excitatory synapses onto CRF neurons. A study where CRF expression was increased through disruption of maternal care in the mouse (96) showed enhanced glutamatergic transmission to hypothalamic CRF neurons in the offspring (97). Moreover, prolonged periods of maternal separation alter the methylation state of the promoter of the avp gene, increasing hypothalamic AVP synthesis and HPA responses to stress (98). Maternal separation of neonatal mice also produces an enduring hypomethylation of the POMC gene, which encodes for the ACTH pro-hormone, proopiomelanocortin (99), increased POMC mRNA expression and increased basal and CRF-induced levels of ACTH. These findings extend previous studies of hippocampal GR regulation and reveal that the quality of postnatal maternal care in rodents epigenetically programs gene expression at multiple levels of the HPA axis to regulate both basal and stress-induced activity.

The initial reports of epigenetic regulation of hippocampal GR expression are now accompanied by reports of environmentally-regulated alterations in the methylation status of multiple genes directly implicated in HPA function. Likewise, in humans, childhood maltreatment associates with differential methylation of the FKB5 gene, which encodes for a functional regulator of GR signalling. FKB5 alters glucocorticoid receptor function by decreasing ligand binding and impeding translocation of the receptor complex to the nucleus. Childhood maltreatment produces an FKB5 genotype-specific demethylation of a distal enhancer, resulting in increased FKB5 expression, and decreased GR signalling (100). A remarkable feature of these findings is the co-ordinated epigenetic effects on multiple genes, in multiple tissues, that collectively serve to increase HPA responsivity to stress in response to early social adversity.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

The authors would like to thank Germaine Lowe, Dave Checknita and Sylvanne Daniels for their invaluable help retrieving and processing original papers for this review. GT is supported by grants from the Canadian Institute of

*Biol Psychiatry. Author manuscript; available in PMC 2017 January 15.*
Health Research MOP93775, MOP11260, MOP119429, and MOP119430; from the National Institutes of Health 1R01DA033684-01; and by the Fonds de Recherche du Québec - Santé through a Chercheur National salary award and through the Quebec Network on Suicide, Mood Disorders and Related Disorders.

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Biol Psychiatry. Author manuscript; available in PMC 2017 January 15.


Figure 1. Early-life adversity findings in animal and human studies

Significant findings reported from animal and human studies examining the effects of early-life adversity according to the NR3C1 first exon variant investigated. Publications are numbered according to their position in the reference list. Upward arrow indicates increased methylation while downward arrow indicates decreased methylation. Results were reported as mean methylation of the region investigated unless otherwise indicated by the footnotes.

a. GR gene CpG methylation undetectable in all conditions.

b. Hippocampus only at CpG 1 and 2, NGFI-A site; ↑ methylation correlated to decreased nursing frequency.

c. CpG 35 (NGFI-A site is CpG 37 & 38).

d. For 6 promoter-associated CpGs.

e. CpG 6 & 8, ↑ methylation of both abused and non-abused compared to controls; CpG11, ↓ methylation of both abused and non-abused compared to controls; hippocampus only.

f. CpGs 8, 9, 12 & 13; hippocampus only.

g. Hippocampus only.

h. Cerebellum; CpGs −127 & −10; single sex males and mixed-litter females compared to mixed-sex males.

i. CpG 13, 14, 17, DBA/2J only.

j. C57BL/6J, hippocampus and males only.

k. CpG 1 & 3 only (NGFI-A site is CpG 3 & 4).

l. Repeated exposure to non-physical, non-sexual abuse.

m. Single exposure to sexual abuse only.

n. ↓ CpG 2 in subset of subjects only; ↑ CpGs 3, 6 and 7 whole sample, 3, 5 & 6 in subset; NGFI-A site at CpG 3 & 4.

o. CpGs at −99 & −57 for all females in hippocampus; CpGs −118, −116, −114 in single-sex females in nucleus accumbens; CpG −57 in males vs. females in nucleus accumbens.

p. Reached significance with physical abuse, trend with emotional neglect.
Figure 2. Parental stress findings in animal and human studies

Significant findings reported from animal and human studies examining the effects of parental stress according to the *NR3C1* first exon variant investigated. Publications are numbered according to their position in the reference list. Upward arrow indicates increased methylation while downward arrow indicates decreased methylation. Results were reported as mean methylation of the region investigated unless otherwise indicated by the footnotes. 

b. indicates a sampling downstream of region 1H, within the gene body.  
c. CpG2, depression only.  
d. Early gestation group only; CpGs –523 & –496.  
e. CpG 12 & 13 with fear of delivery all trimesters; CpG 25 & 28 with fear of integrity of the baby T1.  
f. CpG 6 with fear of delivery.  
g. Site- and parameter-specific: CpG 38/39 (near NFGI-A site) ↓ with fear of changes T1 & 2; CpG 36 ↑ with fear of integrity T1 & 2; CpG 36 ↑ with fear of delivery T3.  
h. Total region for children; CpGs 1, 5 & 8 for mothers.  
i. ↑ methylation with paternal PTSD only in the absence of maternal PTSD.
Figure 3. Psychological stress/psychopathology findings in animal and human studies

Significant findings reported from animal and human studies examining the effects of psychological stress/psychopathology according to the NR3C1 first exon variant investigated. Publications are numbered according to their position in the reference list. Upward arrow indicates increased methylation while downward arrow indicates decreased methylation. Results were reported as mean methylation of the region investigated unless otherwise indicated by the footnotes.

a. CpGs 10 & 21; BN vs. no eating disorder. b. In BN+BPD vs. BN no BPD/no eating disorder. c. no detectable methylation on NR3C1 CpGs. d. Acute stress group – ↑ methylation at several CpG sites in hippocampus only; Chronic stress group – ↑ methylation in response to psychosocial stress in adrenal and pituitary, ↑ methylation in adrenal and ↓ methylation in the pituitary in response to restraint stress. e. No change over time and no difference between responders and non-responders. f. CpGs 3 & 4.
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<td>6 Henningsen et al. 2012 (33)</td>
<td>Maternal care model (LG-ABN)</td>
<td>Rat</td>
<td>100</td>
<td>4</td>
<td>Sodium bisulfite treatment (Epitext Bisulfite Kit, Beckman Coulter Genomics) + sequencing</td>
<td>Dentate gyrus</td>
<td>12-week-old</td>
<td>no Δ</td>
<td>Y</td>
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<tr>
<td>7 Liberman et al. 2012 (23)</td>
<td>Maternal care model (LG-ABN)</td>
<td>Mouse</td>
<td>45</td>
<td>22</td>
<td>Sodium bisulfite treatment (Epitext Bisulfite Kit, Beckman Coulter Genomics) + pyrosequencing (PyroMark MD system and Pyro Q-Cycler 1.0.9. (Pyro))</td>
<td>Hippocampus</td>
<td>P35</td>
<td>↑</td>
<td>Y</td>
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<tr>
<td>9 Kudakovic et al. 2013 (38)</td>
<td>Maternal separation (daily for 3h) from P1 to P14</td>
<td>Mouse</td>
<td>C57BL/6j: 46</td>
<td>6</td>
<td>Sodium bisulfite treatment (Epitext Bisulfite Kit, Beckman Coulter Genomics) + pyrosequencing (PyroMark Q24)</td>
<td>Prefrontal cortex</td>
<td>P40</td>
<td>↑</td>
<td>Y</td>
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<tr>
<td>10 Koster et al. 2014b (35)</td>
<td>Maternal care model (male only vs. mixed sex)</td>
<td>Rat</td>
<td>48</td>
<td>Mixed males: 20</td>
<td>Mixed females: 20</td>
<td>Male single-sex: 8</td>
<td>Female single-sex: 12</td>
<td>Sodium bisulfite treatment (PyroMark Q96 MD Pyrosequencing System, QIAGEN)</td>
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<td>P35</td>
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<td>12 Lombard et al. 2013 (102)</td>
<td>Maternal care model (LG-ABN)</td>
<td>Mouse</td>
<td>35</td>
<td>35</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + pyrosequencing (PSQ 96 Gold reagent kit, Biotech)</td>
<td>Hippocampus</td>
<td>P35</td>
<td>↑</td>
<td>Y</td>
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<td>Tissue(s)</td>
<td>Age at Sample Collection</td>
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<td>Ij</td>
<td>Ik</td>
<td>Ia</td>
<td>IF</td>
<td>NGFI-A site?</td>
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<td>12 Tyrka et al. 2012</td>
<td>MDD + PTSD: 27</td>
<td>41</td>
<td>99</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + pyrosequencing (Pyromark Software, Qiagen)</td>
<td>Blood</td>
<td>273 (mean)</td>
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<td>13 Steiger et al. 2013</td>
<td>Bulimia nervosa with childhood abuse 0</td>
<td>0</td>
<td>BN with childhood abuse: 32</td>
<td>Sodium bisulfite treatment (Episeq Bisulfite Kit, Qiagen) + EpiTYPER platform (Sequenom)</td>
<td>Blood</td>
<td>BN: 26.05 (mean) No eating disorder: 23.67 (mean)</td>
<td>no Δ</td>
<td>no Δ</td>
<td>Y</td>
<td>no Δ</td>
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<td>14 Melas et al. 2013</td>
<td>Depressed (current) with childhood adversity - early parental death 0</td>
<td>0</td>
<td>Depression: 93</td>
<td>Control: 83</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + EpiTYPER platform (Sequenom)</td>
<td>Saliva</td>
<td>Deprived: 55 (median) Control: 56 (median)</td>
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<td>15 Perroud et al. 2014</td>
<td>Bipolar disorder (currently treated) with childhood trauma 44</td>
<td>44</td>
<td>BD: 99</td>
<td>Sodium bisulfite treatment (Episeq Bisulfite Kit, Qiagen) + pyrosequencing (PyroQ-CpG Software, Biotage)</td>
<td>Blood</td>
<td>446 (mean)</td>
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<td>16 Van der Knapp et al. 2014</td>
<td>Early-life trauma 50</td>
<td>50</td>
<td>Initial study: 468 Confirmation study: 454</td>
<td>Sodium bisulfite treatment (EZ-96 DNA Methylation Kit, Zymo Research) + EpiTYPER platform (Sequenom)</td>
<td>Blood</td>
<td>163 (mean)</td>
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<td>17 Weder et al. 2014</td>
<td>Abused or neglected children removed from parental care 42</td>
<td>42</td>
<td>Maltreated: 94 Non-traumatized: 96</td>
<td>Sodium bisulfite treatment (EZ-96 DNA Methylation Kit, Zymo Research) + Illumina 450K Methylation BeadChip (Illumina) + EpiTYPER platform (Sequenom)</td>
<td>Saliva</td>
<td>5–14 (10.2 mean)</td>
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<td>18 Martin-Blanco et al. 2014</td>
<td>Borderline personality disorder with / without childhood abuse 15</td>
<td>15</td>
<td>281</td>
<td>Sodium bisulfite treatment (Episeq Bisulfite Kit, Qiagen) + pyrosequencing (PyroMark Q24, Qiagen)</td>
<td>Blood</td>
<td>29.4 (mean)</td>
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<td>19 Rensens et al. 2014</td>
<td>Abused or neglected children removed from parental care 54</td>
<td>54</td>
<td>Total: 56</td>
<td>Multitreatment: 38 Controls: 38</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + pyrosequencing (PSQ 96 MA, Qiagen)</td>
<td>Blood</td>
<td>11–14 (12.11 mean)</td>
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<tr>
<td>20 Guillomin et al. 2014</td>
<td>Chronic physical abuse in childhood 0</td>
<td>0</td>
<td>Control: 14 Abused: 5</td>
<td>Methyl-CpG binding (MCAP) with microarray (Biotage), Validation by qPCR (Roche); Sodium bisulfite treatment (EZ-96 DNA Methylation Kit, Zymo Research) + Infinium Human Methylation450 Bead Chip (Illumina)</td>
<td>Blood</td>
<td>Control: 24.19 Abused: 25</td>
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<tr>
<td>21 McGowan et al. 2009</td>
<td>Abused suicides 100</td>
<td>100</td>
<td>Abused suicides: 12 Non-abused suicides: 12 Controls: 12</td>
<td>Sodium bisulfite treatment + sequencing (Cequation 8800, Beckman Coulter)</td>
<td>Hippocampus</td>
<td>SA: 34.2 (mean) SNA: 33.8 (mean) Controls: 35.8 (mean)</td>
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**n Central**
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<tr>
<th>Study</th>
<th>Experimental Group</th>
<th>Sex(% male)</th>
<th>Sample size</th>
<th>DNA Methylation Assessment</th>
<th>Tissue(s)</th>
<th>Age at Sample Collection</th>
<th>I 0</th>
<th>I 1</th>
<th>I 2</th>
<th>I 3</th>
<th>NGFI-A site?</th>
<th>I C</th>
<th>I H</th>
<th>Other</th>
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<tr>
<td>Labonte et al. 2012 (29)</td>
<td>Abused suicides</td>
<td>100</td>
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<td>Sodium bisulfite treatment</td>
<td>Hippocampus, Anterior cingulate cortex (BA 24)</td>
<td>Hippocampus SA: 37.3 (mean) SNA: 39.8 (mean) BA 24 SA: 36.1 (mean) SNA: 37.3 (mean) Controls: 39.8 (mean)</td>
<td>$\downarrow$ $\uparrow$ $\uparrow$ $\uparrow$</td>
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</table>

**NR:** Not reported.

$a$ GR gene CpG methylation undetectable in all conditions.

$b$ CpG 13, 14, 17, DBA/2J only.

c Hippocampus only at CpG 1 and 2, NGFI-A site; $\uparrow$ methylation correlated to decreased nursing frequency.

d CpGs at -99 & -57 for all females in hippocampus; CpG-118, -116, -114 in single-sex females in nucleus accumbens; CpG-57 in males vs. females in nucleus accumbens.

e CS7BL/6J, hippocampus and males only.

f Cerebellum; CpG-127 & -10; single sex males and mixed-litter females compared to mixed-sex males.

g Due to small sample size, emotional neglect was not considered.

$h$ Cpg 1 & 3 only (NGFI-A site is CpG 3 & 4).

$i$ CpG 35 (NGFI-A site is CpG 37 & 38).

j Repeated exposure to non-physical, non-sexual abuse.

$k$ Single exposure to sexual abuse only.

$l$ For 6 promoter-associated CpGs.

$m$ Reached significance with physical abuse, trend with emotional neglect.

$n$ CpG 2 in subset of subjects only; $\uparrow$ CpGs 3, 5 & 6 in subset; NGFI-A site at CpG 3 & 4.

$o$ Sampling region not specified.

$p$ CpG 6&8, $\uparrow$ methylation of both abused and non-abused compared to controls; CpG 11, $\downarrow$ methylation of both abused and non-abused compared to controls; hippocampus only.

$q$ CpGs 8, 9, 12 & 13; hippocampus only.
Parental stress affects GR gene methylation

Table 2

<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental Group</th>
<th>Species</th>
<th>Sex (% male)</th>
<th>Sample size</th>
<th>DNA Methylation Assessment</th>
<th>Tissue(s)</th>
<th>Age at Sample Collection</th>
<th>I0</th>
<th>I1</th>
<th>I2</th>
<th>I3</th>
<th>I4</th>
<th>NGFI-A site?</th>
<th>Ic</th>
<th>Iq</th>
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<tbody>
<tr>
<td><strong>1</strong></td>
<td>Mueller et al. 2008 (44)</td>
<td>Offspring of 7-day stress-exposed dams (early, mid and late gestation)</td>
<td>Mouse</td>
<td>100</td>
<td>4-5 animals per group</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + pyrosequencing (PSQ HS96 Pyrosequencing system, Biotage)</td>
<td>Brain</td>
<td>4 months</td>
<td>↑</td>
<td>Y</td>
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<td><strong>2</strong></td>
<td>Oberlander et al. 2008 (20)</td>
<td>Third trimester maternal depressed/anxious mood</td>
<td>Mouse</td>
<td>44</td>
<td>SRI exposed: 44</td>
<td>Baseline treatment (Epitext Bisulfite Kit, Sigma) + pyrosequencing (Pyromark MD System, Biotage)</td>
<td>Cord blood</td>
<td>At birth</td>
<td>↑</td>
<td>Y</td>
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<td><strong>4</strong></td>
<td>Mulligan et al. 2011 (47)</td>
<td>Exposure to varying degree of maternal stressors - war stress</td>
<td>Mouse</td>
<td>44</td>
<td>SRI exposed: 33, Depressed, no SRI: 13, Control: 36</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + pyrosequencing (PSQ HS96 Pyrosequencing system, Biotage)</td>
<td>Cord blood</td>
<td>At birth</td>
<td>↑</td>
<td>Y</td>
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<td><strong>5</strong></td>
<td>Homps et al. 2013 (45)</td>
<td>Pregnancy-related anxiety over 3 trimesters</td>
<td>Mouse</td>
<td>44</td>
<td>SRI exposed: 33, Depressed, no SRI: 13, Control: 36</td>
<td>Sodium bisulfite treatment (MethylDetector Bisulfite Modification Kit, ActiveMotif) + EpiTYPER (Sequenom)</td>
<td>Cord blood</td>
<td>At birth</td>
<td>↑</td>
<td>Y</td>
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<td><strong>6</strong></td>
<td>Conidi et al. 2013 (28)</td>
<td>In utero exposure to maternal mood-disorder</td>
<td>Mouse</td>
<td>44</td>
<td>SRI exposed: 44, Non-SRI exposed: 40</td>
<td>Baseline treatment (Pyromark PCR Kit, Sigma) + pyrosequencing (Pyromark MD, Sigma)</td>
<td>Placenta</td>
<td>At birth</td>
<td>↑</td>
<td>Y</td>
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<td><strong>7</strong></td>
<td>Eiss et al. 2013 (27)</td>
<td>Childhood stress exposure</td>
<td>Mouse</td>
<td>44</td>
<td>SRI exposed: 44, Non-SRI exposed: 40</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + Illumina Human Methylation27 BeadChip Assay (Illumina)</td>
<td>Buscal epithelium</td>
<td>15.1 (mean)</td>
<td>↑</td>
<td>Y</td>
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<td><strong>8</strong></td>
<td>Perroud et al. 2014b (48)</td>
<td>Women exposed to the Tutsi genocide during pregnancy and their children</td>
<td>Mouse</td>
<td>44</td>
<td>SRI exposed: 44, Non-SRI exposed: 40</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + pyrosequencing (PSQ HS96 Pyrosequencing Kit, Biotage)</td>
<td>Blood</td>
<td>Mother: 39–52 (mean), Children: 17–18 (mean)</td>
<td>↑</td>
<td>Y</td>
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<td><strong>9</strong></td>
<td>Yehuda et al. 2014 (49)</td>
<td>Parental exposure to the Holocaust</td>
<td>Mouse</td>
<td>44</td>
<td>SRI exposed: 44, Non-SRI exposed: 40</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + pyrosequencing (PSQ HS96 Pyrosequencing Kit, Biotage)</td>
<td>Blood</td>
<td>None: 57.13 (mean), Paternal: 47.64 (mean)</td>
<td>↑</td>
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<td>Study</td>
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<td>Sample size</td>
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<td>Tissue(s)</td>
<td>Age at Sample Collection</td>
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<td>l₁</td>
<td>l₂</td>
<td>l₃</td>
<td>NGFI-A site?</td>
<td>l₄</td>
<td>l₅</td>
<td>l₆</td>
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<td>Maternal PTSD: 18</td>
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<td>Bi-paternal PTSD: 19</td>
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- a Early gestation group only; CpGs -523 & -496.
- b CpG 1–3, NGFI-A site.
- c CpG 12 & 13 with fear of delivery all trimesters; CpG 25 & 28 with fear of integrity of the baby T1.
- d CpG 6 with fear of delivery.
- e Site- and parameter-specific: CpG 38/39 (near NGFI-A site); with fear of changes T1 & 2; CpG 36 ↑ with fear of integrity T1 & 2; CpG 36 ↑ with fear of delivery T3.
- f CpG2, depression only.
- g Indicates a sampling downstream of region 1H, within the gene body.
- h Mother’s ages estimated based on age at birth (28±6) and the age of children at assessment.
- i Total region for children; CpGs 1, 5 & 8 for mothers.
- j ↑ methylation with paternal PTSD only in the absence of maternal PTSD.
Table 3

Psychological stress and psychopathology affect GR gene methylation

<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental Group</th>
<th>Species</th>
<th>Sex (% male)</th>
<th>Sample size</th>
<th>DNA Methylation Assessment</th>
<th>Tissue(s)</th>
<th>Age at Sample Collection</th>
<th>I0</th>
<th>I1</th>
<th>I2</th>
<th>I3</th>
<th>NGFIA site?</th>
<th>Ic</th>
<th>Ic'</th>
<th>Other</th>
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<tr>
<td>1</td>
<td>DeRooij et al. 2012 (51)</td>
<td>Mouse</td>
<td>NR</td>
<td>5 animals per group</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation Kit, Zymo Research) + sequencing (ABI Prism 3100 Genetic Analyzer, Applied Biosystems)</td>
<td>Hippocampus</td>
<td>NR</td>
<td>no Δa</td>
<td>Y</td>
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<td>2</td>
<td>Witzmann et al. 2012 (24)</td>
<td>Rat</td>
<td>100</td>
<td>6 animals per group</td>
<td>Sodium bisulfite treatment (EpiTect Bisulphite kit, Qiagen) + pyrosequencing (Pyromark ID using Pyrogold reagents, Biotage)</td>
<td>Paraventricular nucleus (PVN) Hippocampus Adrenal Pituitary</td>
<td>10–12-week-old</td>
<td>Yb</td>
<td>Yc</td>
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<td>3</td>
<td>Tran et al. 2013 (50)</td>
<td>Rat</td>
<td>100</td>
<td>6 animals per group</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation-Gold kit, Zymo Research) + pyrosequencing (EpigenDX)</td>
<td>Amygdala</td>
<td>Adult</td>
<td>↑</td>
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<td>4</td>
<td>DeRooij et al. 2012 (51)</td>
<td>Human</td>
<td>47</td>
<td>675</td>
<td>Methylation-sensitive enzymatic restriction (AciI and HinfI) + qPCR (SYBR Green, Sigma)</td>
<td>Blood</td>
<td>35–60</td>
<td>no Δs</td>
<td></td>
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<td>5</td>
<td>Dammann et al. 2011 (52)</td>
<td>Human</td>
<td>0</td>
<td>BN with childhood abuse: 32 BN without childhood abuse: 32</td>
<td>Sodium bisulfite treatment (EpiTest Bisulfite Kit, Quagen) + Epityper platform (Sequenom)</td>
<td>Blood</td>
<td>BN: 26.05 (mean) No eating disorder: 23.67 (mean)</td>
<td>no Δ</td>
<td>no Δ</td>
<td>Y</td>
<td>Yd</td>
<td>Yc'</td>
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<td>6</td>
<td>Stein et al. 2013 (21)</td>
<td>Human</td>
<td>88</td>
<td>Responders: 8 Non-responders: 8</td>
<td>Sodium bisulfite treatment (EpiTest Bisulfite Kit, Quagen) + sequencing (Genewiz)</td>
<td>Blood</td>
<td>Responders: 41 Non-responders: 58</td>
<td>no Δ</td>
<td>Y</td>
<td></td>
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<td>7</td>
<td>Yehuda et al. 2013 (53)</td>
<td>Human</td>
<td>88</td>
<td>Responders: 8 Non-responders: 8</td>
<td>Sodium bisulfite treatment (EpiTest Bisulfite Kit, Quagen) + sequencing (Genewiz)</td>
<td>Blood</td>
<td>Responders: 41 Non-responders: 58</td>
<td>no Δ</td>
<td>Y</td>
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<td>8</td>
<td>Labonte et al. 2014 (55)</td>
<td>Human</td>
<td>50</td>
<td>PTSD: 30 Controls: 16</td>
<td>Sodium bisulfite treatment (EpiTest Bisulfite Kit, Quagen) + Epityper (Sequenom)</td>
<td>Blood</td>
<td>PTSD: 3.4 Controls: 3.6</td>
<td>↓</td>
<td>↓</td>
<td>no Δ</td>
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<td>9</td>
<td>Yehuda et al. 2014 (54)</td>
<td>Human</td>
<td>100</td>
<td>PTSD: 61 Controls: 61</td>
<td>Sodium bisulfite treatment (EpiTest Bisulfite Kit, Quagen) + Sequencing (Genewiz)</td>
<td>Blood</td>
<td>PTSD: 3.4 Controls: 3.10</td>
<td>↓</td>
<td>Y</td>
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<td>10</td>
<td>Na et al. 2014 (56)</td>
<td>Human</td>
<td>MDD: 24 Controls: 29</td>
<td>Total: 117 MDD: 45 Controls: 72</td>
<td>Sodium bisulfite treatment (EZ DNA Methylation-Gold kit, Zymo Research)</td>
<td>Blood</td>
<td>MDD: 45.6 (median)</td>
<td>38–65</td>
<td>8–65</td>
<td>MDD: 41.6 (median)</td>
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<tr>
<td>Study</td>
<td>Experimental Group</td>
<td>Sex (% male)</td>
<td>Sample size</td>
<td>DNA Methylation Assessment</td>
<td>Tissue(s)</td>
<td>Age at Sample Collection</td>
<td>lD</td>
<td>lJ</td>
<td>lK</td>
<td>lF</td>
<td>NGFI-A site?</td>
<td>lE</td>
<td>lH</td>
<td>Other</td>
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<td>Human Central Studies</td>
<td>11 Alt et al. 2010 (30)</td>
<td>Lifetime diagnosis of major depressive disorder</td>
<td>MDD: 67 Controls: 50</td>
<td>MDD: 6 Controls: 6</td>
<td>Sodium bisulfite treatment (EpiTect Bisulfite Kit, Qiagen) + Pyrosequencing (Pyromark ID, Varionostic)</td>
<td>Amygdala Hippocampus Inferior prefrontal gyrus Cingulate gyrus Nucleus accumbens</td>
<td>Controls: 40.72 (median)</td>
<td>no Δs</td>
<td>no Δs</td>
<td>no Δs</td>
<td>no Δs</td>
<td>Y</td>
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<td>MDD: 75.5 Controls: 72.5</td>
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</table>

\( ^a \) no detectable methylation on NR3C1 CpGs.

\( ^b \) Acute stress group – ↑ methylation at several CpG sites in hippocampus only; Chronic stress group – ↑ methylation in response to psychosocial stress in adrenal and pituitary, ↑ methylation in adrenal and ↓ methylation in the pituitary in response to restraint stress.

\( ^c \) Acute stress – hippocampus only; Chronic stress – adrenal only.

\( ^d \) CpGs 10 & 21; BN vs. no eating disorder.

\( ^e \) In BN+BPD vs. BN no BPD/no eating disorder.

\( ^f \) No change over time and no difference between responders and non-responders.

\( ^g \) CpGs 3 & 4.