Acta Neuropsychiatrica

cambridge.org/neu

DNA methylation in stress and depression: from biomarker to therapeutics

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Review Article

Cite this article: Sales AJ, Guimarães FS, and Joca SRL. (2021) DNA methylation in stress and depression: from biomarker to therapeutics. *Acta Neuropsychiatrica* **33**:217–241. doi: 10.1017/neu.2021.18

Received: 17 February 2021
Revised: 7 June 2021
Accepted: 11 June 2021
First published online: 21 June 2021

Key words:
depression; DNA methylation; DNA methyltransferase inhibitors; stress ; epigenetics

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Abstract

Epigenetic mechanisms such as DNA methylation (DNAm) have been associated with stress responses and increased vulnerability to depression. Abnormal DNAm is observed in stressed animals and depressed individuals. Antidepressant treatment modulates DNAm levels and regulates gene expression in diverse tissues, including the brain and the blood. Therefore, DNAm could be a potential therapeutic target in depression. Here, we reviewed the current knowledge about the involvement of DNAm in the behavioural and molecular changes associated with stress exposure and depression. We also evaluated the possible use of DNAm changes as biomarkers of depression. Finally, we discussed current knowledge limitations and future perspectives.

Summations

- DNA methylation (DNAm) is an epigenetic mechanism with high specificity.
- Stress, depression, and antidepressant drugs modulate DNAm.
- DNAm is a potential biomarker of depression and antidepressant response.

Considerations

- Contradictory results are found in studies investigating the involvement of DNA methylation (DNAm) in stress, depression, and antidepressant responses.
- DNAm is a specific epigenetic mechanism influenced by diverse factors making difficult the interpretation of the data.
- The analyses differ across the studies, for example, investigating diverse tissue, region, sites, and methods.

Introduction

Environmental stressors contribute to the development and progression of several psychiatric disorders, including major depressive disorder (MDD or depression) (Kendler et al., 1999, Hammen, 2005, Heim et al., 2008, Slavich and Irwin, 2014). Epigenetic alterations have been proposed by several preclinical and clinical studies to mediate the association between these factors (Heim et al., 2008, Johnstone and Baylin, 2010, Sun et al., 2013, Slavich and Irwin, 2014, Turecki and Meaney, 2016, Pena and Nestler, 2018).

The term epigenetic refers to molecular mechanisms that change the gene expression patterns without any modification in the underlying DNA sequence (Allis and Jenuwein, 2016). The epigenetic modifications have been related with diverse conditions including the control of signal transduction pathways and cell lineage specification (Cortese et al., 2011, Cabrera-Licona et al., 2021, MacArthur and Dawlaty, 2021). One common epigenetic mechanism is DNA methylation (DNAm). Increased DNAm, a typically repressive epigenetic mechanism that results specially in gene silencing, has been associated with stress and depression (Oh et al., 2013, Klengel et al., 2014). Stress exposure causes specific DNAm modifications in genes associated with the neurobiology of depression, including the serotonin transporter (SLC6A4 or 5-HTT), brain-derived neurotrophic factor (BDNF), glucocorticoid receptor (NR3C1 or GR), mineralocorticoid receptor (NR3C2 or MR), FK506-binding protein 5 (FKBP5), and corticotrophin-releasing hormone receptor 1 (CRHR1) (Ding and Dai, 2019). These modifications induce long-lasting effects on the expression of these genes, leading to brain structural and functional changes and behavioural alterations. However, it is still unclear how stress induces these DNAm changes to cause its behaviour and molecular effects (Fig. 1).

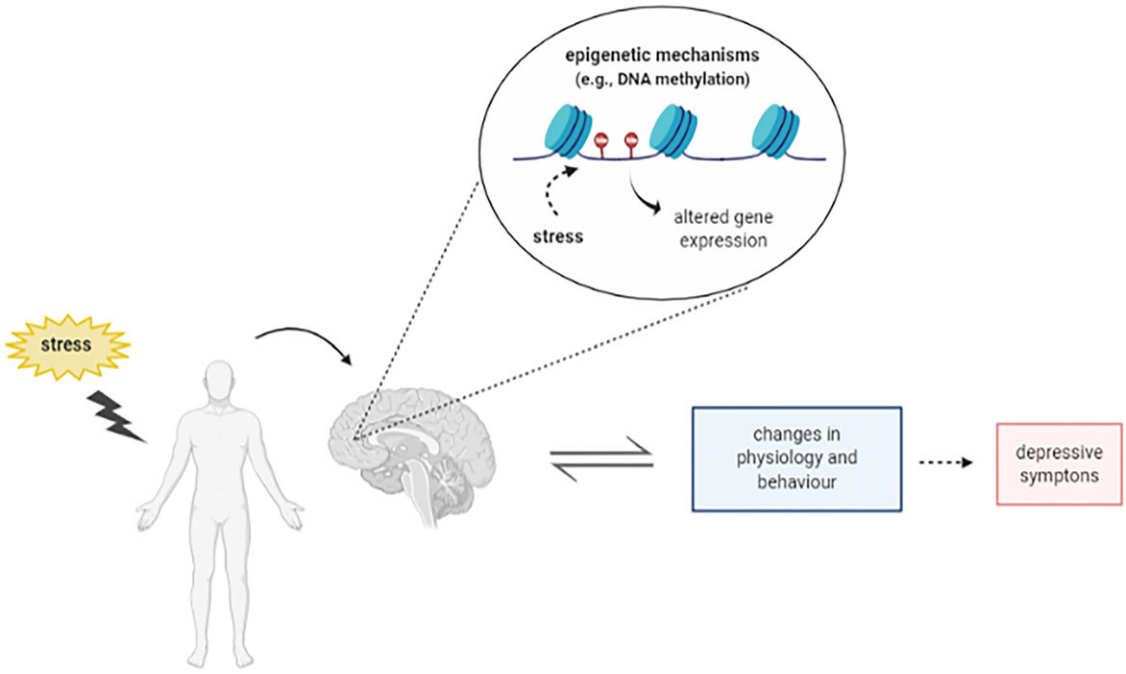

Figure 1. Schematic representation depicting the relation between stress, epigenetic mechanisms and depression. Figure designed using imagens from BioRender.com.

This review summarises our current knowledge on the links between DNAm, stress, and depression. It also discusses the current limitations to better understand these relationships and point to the pharmacological regulation of DNAm as a possible alternative target for the development of novel antidepressant medication.

Stress response and depression

Influence of environmental factors in depression

MDD is a complex, debilitating, and prevalent psychiatric disorder with a set of diverse symptoms including depressed mood, hopelessness, worthlessness, loss of interest in normally pleasurable activities (anhedonia), sleep or eating disturbances, low energy, reduced concentration, impaired cognition, excessive feeling of guilt or loss, and even suicidal thoughts, for at least two weeks (American Psychiatric Association, 2013, Schulz and Arora, 2015, Otte et al., 2016).

Depression affects on average 20% of the global population (Vigo et al., 2016) and is associated to high levels of morbidity and mortality (Fredman et al., 1988, Bijl and Ravelli, 2000, Kessler et al., 2006, Kessler and Bromet, 2013, Chirita et al., 2015, Laursen et al., 2016). It estimates that by 2030 depression will be the leading cause of disability in developed nations (WHO, 2017; 2018). However, its aetiology is still not fully understood.

Genetic factors increase in around 30% the risk of depression (Kendler and Gardner, 2016, Smoller, 2016, Shadrina et al., 2018, Pettersson et al., 2019). Epidemiologic studies have identified the important genetic contribution to MDD, expressed by the high concordance between monozygotic twins (Sullivan et al., 2000), and polymorphisms that increase the vulnerability to the development of this disorder (Fabbri et al., 2013).

Several lines of evidence indicate that genes are modulated by environmental factors, especially exposure to stress (Higuchi et al., 2011). Of note, studies have shown that the first episode of depression is preceded by a stressful event in about 60% of the cases (Post and Silberstein, 1994). In addition, a higher prevalence of stressful episodes is observed in the life of depressed compared to healthy individuals (Peyrot et al., 2013, Klengel and Binder, 2015, Lopizzo et al., 2015, Richter-Levin and Xu, 2018).

Although it has been possible to treat depression for decades, depressive symptoms are only reversed after chronic treatment (2–4 weeks) with the currently available antidepressant drugs. However, about 30% of the patients treated with conventional antidepressants do not respond to any medication (Berton and Nestler, 2006, Johnston et al., 2019). Even among those responsive, about 2/3 do not present complete remission (Rosenzweig-Lipson et al., 2007, Shelton et al., 2010). Overall, these data highlight the need for a better understanding of the neurobiology of depression and to develop faster and more effective treatments, reducing associated health costs, and patient suffering.

Neurobiology of depression and antidepressant action

The discovery that the first antidepressants act by blocking noradrenaline or serotonin reuptake led to the emergence of the monoaminergic theory of depression (Schildkraut, 1965, Coppen, 1972, Schildkraut, 1995, Castren, 2005). This theory postulated that monoaminergic neurotransmission is impaired in depressed individuals, and it is restored after chronic treatment with antidepressants. Even today, most of the antidepressants clinically used, such as tricyclic and selective serotonin reuptake inhibitors, facilitate this neurotransmission. However, the monoaminergic theory is overly simplistic since it does not explain the latency period for the therapeutic response (Castren, 2005, Papakostas and Ionescu, 2015, Otte et al., 2016, Ferrari and Villa, 2017).

To explain this latency, the molecular or neurotrophic hypothesis of depression assumes that the stress triggers intracellular signalling cascades that would result in reduced expression of genes important for plasticity in limbic structures, such as BDNF(Duman et al., 1997, Agid et al., 2007). Chronic administration of antidepressant drugs in stressed animals facilitates monoaminergic signalling, restores neurogenesis, and increases dendritic arborisation in the hippocampus and prefrontal cortex

https://doi.org/10.1017/neu.2021.18 Published online by Cambridge University Press

(Malberg and Duman, 2003, Rodrigues et al., 2009, Castren and Rantamaki, 2010, Kimpton, 2012, Liu et al., 2017). Therefore, gene expression regulation has been proposed as an important molecular mechanism mediating the long-term adaptations related to stress, depression, and antidepressant effect (Tsankova et al., 2007, Krishnan and Nestler, 2008, Harmer et al., 2017).

The recent discovery of fast-acting antidepressants such as ketamine, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, has renewed the hope in more rapid and effective therapies (Cipriani et al., 2018). The exact mechanisms behind ketamine effect are not fully understood, but evidence suggests that it activates the mechanistic target of rapamycin (mTOR) and BDNF signalling, increasing the expression of synaptic proteins (e.g. postsynaptic density protein-95, PSD-95, and synapsin, Syn) in the hippocampus and prefrontal cortex (Gerhard and Duman, 2018). These changes would result in a rapid, robust, and sustained antidepressant effects (Price et al., 2009, DiazGranados et al., 2010, Li et al., 2010, Li et al., 2011b, Duman and Voleti, 2012, Zhou et al., 2014, Pazini et al., 2016, Zhang et al., 2018, Abdallah et al., 2019). Currently, a great body of studies has investigated other compounds that might share similar fast-acting antidepressant action, including glutamatergic neuromodulators (riluzole, agmatine) and glycine-binding site ligands (GLYX-13 and rapastinel) (Zomkowski et al., 2002, Neis et al., 2016a, Neis et al., 2016b, Chen et al., 2018, Neis et al., 2018, Kadriu et al., 2019).

Brain areas related to depression

Several brain structures have been associated with stress circuits and the therapeutic action of antidepressants, including the amygdala, hypothalamus, ventral tegmental area, nucleus accumbens, hippocampus, prefrontal cortex, among others (Nestler et al., 2002, Berton and Nestler, 2006, Krishnan and Nestler, 2008, Price and Drevets, 2010). Plastic, neurochemical, and functional changes in these structures have been associated with the neurobiology of depression (Andrade and Rao, 2010).

Stress and MDD are associated with hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis and consequent release of corticotropin-releasing factor (CRF) from paraventricular neurons (PVN) of the hypothalamus, triggering the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary and leading to the releasing of glucocorticoids (cortisol in human and corticosterone in rodent) from the adrenal cortex into the blood. Glucocorticoids, through the glucocorticoid receptors (GRs), mediate negative feedback that regulates the HPA axis and stress endocrine responses (Heim and Binder, 2012). They influence the metabolic, physiological, and immunological functions regulated by several stress-associated brain structures such as the hypothalamus, amygdala, prefrontal cortex, and hippocampus (Gillespie and Nemeroff, 2005, Pariante and Lightman, 2008, Ulrich-Lai and Herman, 2009, Bonfiglio et al., 2011, Belda et al., 2015).

The amygdala is a limbic structure responsible for the regulation of emotions, behaviour, responses to stress, and memory formation (Bhatnagar et al., 2004, Li et al., 2012, Gilpin et al., 2015). MDD patients with low serotonin transporter binding potential present hyperactivation of the amygdala (Drevets, 2001, Sheline et al., 2001, Siegle et al., 2002, Parsey et al., 2006). It is intimately connected to other brain regions involved in depression such as hippocampus and cortices (Amaral and Insausti, 1992). Moreover, chronic mild stress increases the dendritic length and neurite density in this structure (Vyas et al., 2002, Khan et al., 2016a,b).

The hippocampus is a subcortical limbic brain structure, closely related to learning, memory, adaptation to stress, and depression. Depressed patients present reduced hippocampal volume (Sheline et al., 2003, Lorenzetti et al., 2009). In animal models, exposure to inescapable and chronic stress causes several hippocampal alterations such as a decrease in number and length of dendritic spines, synaptic loss, and impaired adult neurogenesis (Pittenger and Duman, 2008, Serafini, 2012), suggesting a decreased connectivity in this structure (Andrade and Rao, 2010). These changes are attenuated by chronic antidepressant treatment (Castren and Rantamaki, 2010, Kimpton, 2012). Recent studies suggest that the dorsal and ventral portions of hippocampus are molecularly and functionally distinct (Leonardo et al., 2006, Fanselow and Dong, 2010, Tanti and Belzung, 2013, Grigoryan and Segal, 2016, Diniz et al., 2018). This difference should be considered when investigating the role of the hippocampus in stress and depression.

Another structure closely related to the neurobiology of depression is the prefrontal cortex. The prefrontal cortex plays an important role in cognition, learning, memory, decision making, and modulation of behavioural, autonomic, and endocrine in stress responses (Barbas, 1995, Liston et al., 2006, Quirk et al., 2006, Resstel and Correa, 2006, Resstel et al., 2006, Girotti et al., 2018). Dysfunctions in this structure are related to the development of mental disorders associated with stress, such as depression and posttraumatic stress disorder – PTSD (Geuze et al., 2008, Pereira et al., 2013). Similar to the hippocampus, stressful insults result in plastic modifications in the prefrontal cortex such as apical dendritic atrophy and reductions of synaptogenesis and cortical volume. Antidepressant drugs reverse these morphological changes (Duncan et al., 1996, Cook and Wellman, 2004, Rocher et al., 2004, Brown et al., 2005, Czeh et al., 2008). In line with these data, depressed patients present functional and volume cortical changes, which are sensitive to chronic antidepressant treatment (Harmer et al., 2006, Fitzgerald et al., 2008, Koenigs et al., 2008, Yoshimura et al., 2010). In addition, the antidepressant effect of fast-acting drugs, such as ketamine, is associated with neuroplastic changes in the prefrontal cortex such as the rapid recovery of dendritic arborisation and synaptogenesis (Dwyer and Duman, 2013, Abdallah et al., 2016).

Recently, the lateral habenula nucleus has attracted interest as a relevant brain structure that is also associated with stress coping responses. Stressors led to hyperactivation of neurons in this structure following by negative effects on serotonergic function and an imbalance between glutamatergic and GABAergic signalling (Shabel et al., 2014, Metzger et al., 2017, Tchenio et al., 2017, Wang et al., 2017). These changes contribute to depressive-like behaviours (Aizawa et al., 2013, Li et al., 2013, Cui et al., 2014). The deep brain stimulation of the lateral habenula nucleus prevents depressive-like behaviour in animals (Li et al., 2011a) and alleviates depressive symptoms in patients (Sartorius et al., 2010).

Epigenetic

Epigenetic overview

The human genome encompasses about 20,000 genes containing essential information on the normal growth, development, and survival of the organism. The long DNA strand (2 m) is packed in the compact nucleus of eukaryotic cells (10 μm in diameter). This process depends on the association of the DNA with histone and non-histone proteins resulting in a highly complex and organised structure called chromatin. The fundamental and functional unit of chromatin is the nucleosome consisting of a 146 base pairs

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(bp) envelope of DNA surrounded by an octameric histone complex with four histone proteins (H2A, H2B, H3, and H4). Mechanisms of repair, replication, recombination, transcription, dynamic opening, and closure of the chromatin structure are fundamental for homoeostasis (Hauer and Gasser, 2017).

In the 1940s, Conrad Hal Waddington, a leading embryologist and geneticist, sought to explain how different cell types could be generated from cells containing the same common genome, thus generating different phenotypes that would result from the interaction between both genes and the environment. He believed that genes are important in determining the development of the organism, but environmental-induced modifications could occur and alter this development. He called this process causal embryology or "epigenetic" (Slack, 2002). The definition of epigenetics, a word that means "above the genome", has been modified over the years. It is now broadly understood as the process by which environmental factors can lead to stable changes in chromatin structure, altering gene expression but changing the primary sequence of bases in the DNA (Holliday, 2006, Allis and Jenuwein, 2016, Gayon, 2016). Epigenetic alterations have been related to the neurobiology of diverse illnesses, including psychiatric disorders (Tsankova et al., 2007, Mitchelmore and Gede, 2014, Cattaneo et al., 2015, Bartlett et al., 2017, Uchida et al., 2018, Bhandari et al., 2020, Forero and Gonzalez-Giraldo, 2020, Jackson, 2020).

The biological properties of chromatin have been extensively studied since the 19th century. In 1884, Albrecht Kossel identified the histones and determined that these basic proteins were associated with DNA. Thanks to this pivotal contribution, he received the Nobel Prize in Physiology and Medicine in 1910. Since then, a large number of studies have investigated the structure and function of the histones (Grayson and Guidotti, 2013). Transitions between euchromatin and heterochromatin are associated with active or inactive transcription, respectively, and are mediated by modifications in the structure of the histones that constitute the nucleosome (Jenuwein and Allis, 2001).

Histone modifications occur mainly in histones 3 (H3) and 4 (H4) and comprise phosphorylation, ubiquitination, acetylation and deacetylation, and methylation and demethylation (Mersfelder and Parthun, 2006, Bannister and Kouzarides, 2011). Regarding the amino acids present along the histone tails, lysine (K) and arginine (R) are preferentially subject to methylation (Jenuwein and Allis, 2001). Histones are acetylated in K-residues on the N-terminal branching as part of the genetic regulation, being catalysed by the histone acetyltransferases (HATs) enzymes (Fig. 2). In deacetylation, in turn, acetylated histones lose the acetyl group by the action of histone deacetylase enzymes (HDACs, (Grayson and Guidotti, 2013). Histone acetylation is primarily associated with decondensed chromatin followed by increased accessibility of DNA to transcriptional factors and gene activation, being able to facilitate the expression of genes important for cellular plasticity and involved with the neurobiology of depression and antidepressants effects (Strekalova et al., 2004, Krishnan and Nestler, 2008).

In addition to post-translational modifications in histone tails (e.g., methylation, acetylation, phosphorylation, and ubiquitination), epigenetic mechanisms encompass covalent modifications (e.g., DNAm, detailed below) and nontranslational mechanisms of gene modulation (e.g., microRNAs, miRNAs, ribonucleic acid) (Issler and Chen, 2015, Luoni and Riva, 2016, Ivanova et al., 2018). These mechanisms have been extensively discussed elsewhere (O'Carroll and Schaefer, 2013, Correia de Sousa et al., 2019, Tolsma and Hansen, 2019) and are not the scope of the current review.

Epigenetic mechanisms could promote long-lasting effects on mature neurons, interfering with complex neural mechanisms such as plasticity synaptic and neurogenesis (Tsankova et al., 2007, Karpova et al., 2017). The increase or decrease of chromatin condensation makes it more difficult or easier, respectively, the access of the transcriptional machinery to specific promoter regions, resulting in messenger RNA (mRNA) and protein levels changes (Tsankova et al., 2006, Tsankova et al., 2007, Krishnan and Nestler, 2008).

Although less understood, epigenetic mechanisms have recently been proposed to also play a crucial role in the rapid stress coping responses (Chandramohan et al., 2008, Saunderson et al., 2016, Mifsud et al., 2017) (Fig. 2). For example, stress and antidepressants modify cochaperones (mainly FK506-binding protein 51 and 52, FKBP51 and 52), which proteins that participate in the function of other chaperone proteins that act facilitating the folding proteins (Saibil, 2013). FKBP51 and 52 modulate the phosphorylation and activity of DNA methyltransferase (DNMT, an enzyme that catalyses DNAm), altering the DNAm levels (Gassen et al., 2015). Still, the exposure to acute stressful events results in DNA demethylation of c-Fos (FBJ murine osteosarcoma viral oncogene homolog), an immediate-early gene, leading to its increased expression in the dentate gyrus of the hippocampus (Saunderson et al., 2016).

DNA methylation

DNAm usually occurs by a covalent attachment of a methyl group from the S-adenosyl-L-methionine (SAMe) onto the C5 position of cytosine residue of DNA, primarily occurring on cytosine-phosphate-guanine (CpG) dinucleotides clusters, resulting in 5-methyl cytosine (5mC) (Fig. 3). While showing cell- and tissue-specific differences, in mammalian cells DNAm often occurs in cytosines located on the so-named CpGs islands (CGIs, regions with a high frequency of CpG sequences). These islands are typically found in or near promoter regions resulting in 70–80% of methylated CpGs during DNAm (Moore et al., 2013, Ziller et al., 2013, Li and Zhang, 2014).

Classically, DNAm in promoters containing CGIs is associated with transcriptional reduction (Antequera, 2003, Lorincz et al., 2004, Brenet et al., 2011). The methyl-CpG-binding domain (MBD) proteins bind preferentially to methylated CGIs. In addition, MBD proteins also form a complex with corepressor and chromatin-modifying proteins, resulting in gene silencing (Wajed et al., 2001, Jones, 2012). Moreover, the methyl binding can also mechanically block the access of transcription factors to promoter regions, also resulting in transcriptional repression followed by gene inactivation (Hark et al., 2000). Contrary to this, promoter hypermethylation has also been associated with increased transcriptional activity (reviewed in Smith et al., 2020), showing that the DNAm-mediated gene regulation is a very complex and unclear mechanism. It is possible that the DNAm may block the interaction between DNA and transcriptional repressors facilitating gene transcription (Nabilsi et al., 2009, Bahar Halpern et al., 2014, Jia et al., 2016). However, further studies are required for a better understanding of this mechanism.

Although DNAm occurs primarily in cytosine-guanine (CpG) dinucleotides, methylation also occurs in non-CpG sites, particularly in neuronal cells. The functions of these processes are unclear (Lister et al., 2013). Additionally, DNAm in non-promoter regions has variable and unpredictable effects on gene expression (Antequera, 2003, Lorincz et al., 2004, Hellman and Chess, 2007, Ball et al., 2009, Aran et al., 2011, Jones, 2012, Thomas et al., 2012, Kulis et al., 2013, Moore et al., 2013).

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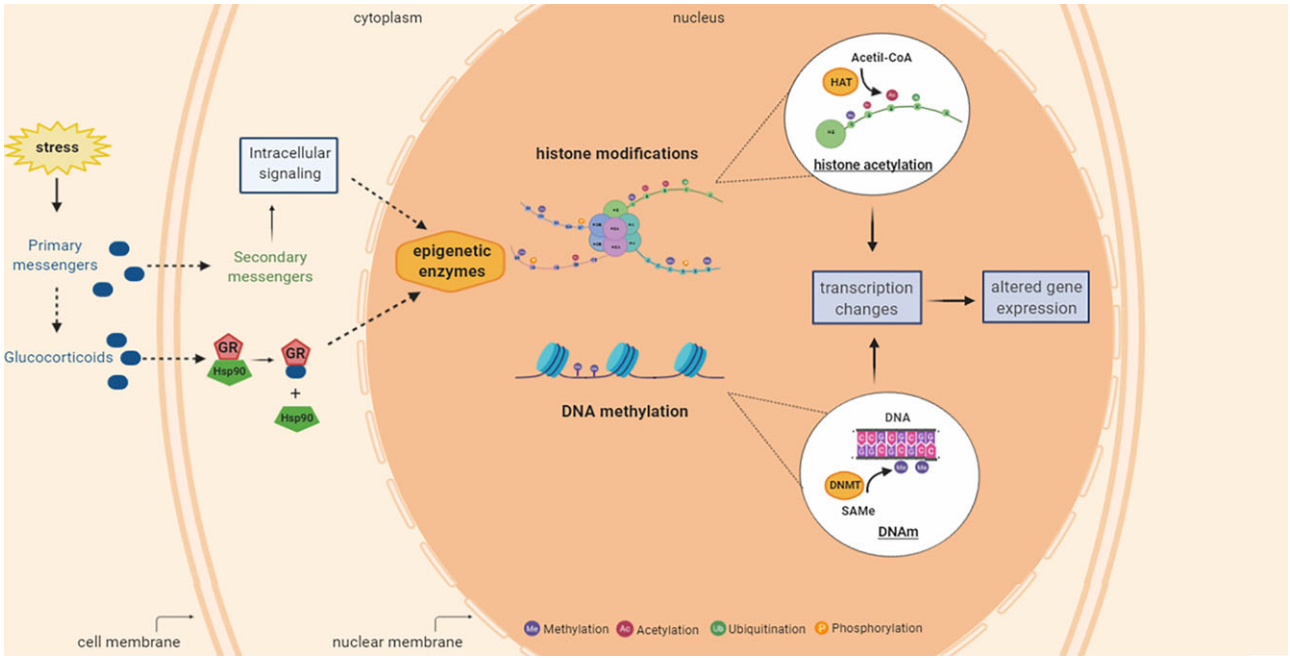

Figure 2. Summary of stress-induced epigenetic changes including histone acetylation and DNA methylation processes that can occur in mammalian tissues including brain. Stress is related with increased glucocorticoid levels and activation of signalling cascades that can result in the epigenetic enzymes modulation (activation or inhibition followed by alterations in the epigenetic mechanisms and transcription gene. Histone modifications can add various groups (methyl, acetyl, phosphoryl and ubiquitin) to the tails of the histone proteins. The histone acetyl transferase (HAT) enzyme catalyses the transfer of the acetyl group from acetyl-CoA to the histone protein. DNA methylation (DNAm) is the addition of methyl group from S-adenosyl-L-methionine (SAMe), catalysed by DNA methyltransferase (DNMT) enzymes, to the cytosine resulting in 5-methyl cytosine. These epigenetic mechanisms can affect the gene transcription and protein expression. H, histone protein; Acetyl-CoA, acetyl coenzyme A; HAT, histone acetyltransferase; SAMe, S-adenosyl-L-methionine; DNMT, DNA methyltransferase; DNAm, DNA methylation. Figure designed using imagens from BioRender.com.

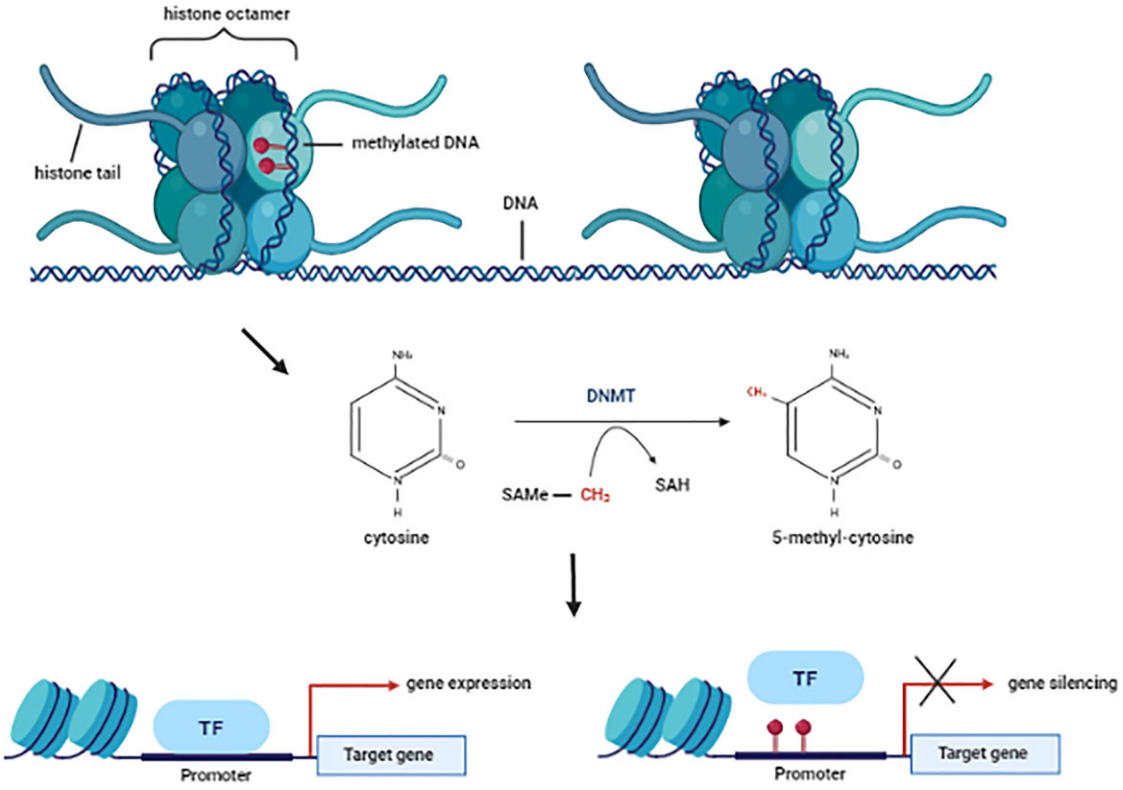

Figure 3. Schematic chromatin structure and DNA methylation mechanism. DNMTs catalyse the transfer of methyl group from S-adenosyl-L-methionine (SAMe) to the C5 position of the cytosine residue resulting in the S-adenosyl-L-homocysteine (SAH) and 5-methyl cytosine (5mC). This covalent modification is associated with low acessibily of the transcrition factors (TF) to the promoter gene following by gene silencing. SAMe, S-adenosyl-L-methionine; DNMT, DNA methyltransferase; SAH, S-adenosyl-L-homocysteine; TF, transcription fator. Figure designed using imagens from BioRender.com.

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The methylation reaction is catalysed by DNA methyltransferases (DNMTs) enzymes (Fig. 3), detailed below. The first suggestion that DNAm (or demethylation) plays an important biological role was done by Griffith and Mahler, in 1969. They proposed that this epigenetic mechanism could be the basis of long-term memory (Griffith and Mahler, 1969). In 1975, two other groups suggested that DNAm would change gene expression, resulting in the repression of diverse genes (Holliday and Pugh, 1975, Riggs, 1975). These studies also proposed that the methylation pattern was inherited through the action of a maintenance methylase enzyme capable of recognising hemimethylated DNA after replication, but unable to act on the unmethylated DNA.

DNAm has now been implicated in regulating gene activity in the adult brain under both normal and pathological conditions (Feng et al., 2010). Intracellular signal transduction events, among other functions, can activate or inhibit transcription factors. The regulation of transcriptional activity by DNA-binding transcription factors depends on the interaction between several of these factors, including co-activators or co-repressors proteins and the underlying structure of the chromatin. The synaptic activity can activate or repress gene expression through chromatin remodeling. This mechanism regulates the expression of genes important for neural activity, survival, morphology, integration, and behaviour regulation (Zuckerkandl, 1975, Whitehouse et al., 2007, Sweatt, 2016, Uchida et al., 2018).

DNMT enzymes comprise five subtypes (DNMT1, DNMT2, DNMT3a, DNMT3b, and DNMT3L). Three of these subtypes (DNMT1, DNMT3a, and DNMT3b) are functionally active and widely studied. DNMT1 plays a maintenance role by copying the preexisting methylation of the parent into the daughter cell DNA during the replication phase. DNMTs 3a and 3b, in turn, are responsible for the new methylations in unmethylated CpG sequences (Jeltsch, 2006). DNMT2 is usually inactive or with low activity in both *in vitro* and *in vivo* studies (Herman and Baylin, 2003, Liu et al., 2003). DNMT3L also lacks catalytic activity but is important for methylation by recruiting the active DNMTs (Bourc'his et al., 2001, Bourc'his and Bestor, 2004, Ooi et al., 2007, Liao et al., 2015, Long et al., 2017, Hervouet et al., 2018).

During cell proliferation, DNMT1 is located in replication loci and primarily methylates the unmethylated DNA of daughter cells, maintaining the pattern of parental methylation across generations. In divided cells, DNMT1 is concentrated in replication sites during the S phase in order to maintain DNAm profile after the DNA synthesis of the daughter cell. DNMT1 deletion in progenitor neural cells results in reduced DNAm levels in post-mitotic neurons (Feng et al., 2010, Hervouet et al., 2018). However, DNMT1 participates not only in maintenance but also in new methylations (Pradhan et al., 1999, Gowher et al., 2005, Hervouet et al., 2018).

DNMTs 3a and 3b (known as "*de novo*" DNMTs) are more actively involved in the methylation of unmethylated DNA, establishing new methylation patterns (Feng et al., 2010, Hervouet et al., 2018). DNMT 3b is widely expressed in the early stages of neurogenesis (Okano et al., 1999) but is also present in adults (Feng et al., 2005, Feng et al., 2010, Hervouet et al., 2018). DNMT 3a is present in both the mature and development brain, suggesting its involvement in embryonic development and adult neuronal function. The two enzymes are often co-localized and associated with heterochromatin regions independent of the cell cycle (Bachman et al., 2001), possibly maintaining DNAm in pre- and post-mitotic cells (Feng et al., 2010, Hervouet et al., 2018).

Pharmacological manipulation of DNA methylation

In the 1960s, Sorm et al. (1964) synthesised the first epigenetic drugs (or epi-drugs) with possible inhibitory action on DNMTs, 5-azacytidine (5-AzaC), and 5-Aza-2′-deoxycytidine (5-AzaD or decitabine; Sorm et al., 1964). Initially, these drugs were tested as antimetabolic nucleoside inhibitors for the treatment of acute myeloid leukemia. In 1968, Sorm and Veseley demonstrated that they cause a potent antileukemic effect in mice (Sorm and Vesely, 1968). The clinical studies began in the 1980s (Rivard et al., 1981, Momparler et al., 1985) and, corroborating the preclinical findings, indicated that the 5-AzaD treatment induces complete remission in leukemic patients (Richel et al., 1991). In 2004, the Food and Drug Administration (FDA) approved the first DNMT inhibitor (DNMTi) drug, 5-AzaC, and in 2006 the second, 5-AzaD, for clinical use in myelodysplastic syndromes. Their mechanisms are not fully understood but it is known that they inhibit DNAm and, at high doses, can be cytotoxic.

Aberrant promoter DNAm in tumour suppressor genes inhibits their expression and can contribute to tumourigenesis. The reactivation of these genes by inhibition of DNAm induced by DNMTi has potential antitumoural effects. Several studies in tumour cells have shown that genes involved in the development and progression of cancer are hypomethylated, whereas those associated with tumour suppression are hypermethylated (Stresemann and Lyko, 2008). DNMTi reversed this methylation pattern by reactivating tumour suppressor genes, such as p15 (Momparler, 2005, Yoo and Jones, 2006, Karahoca and Momparler, 2013).

DNMTi are divided into two groups, nucleoside and non-nucleoside, which inhibit DNMTs through different mechanisms. Nucleosidic DNMTi, such as 5-AzaC, 5-AzaD, zebularine, SGI-110, and CP-4200, are chemical analogues of cytidine, being integrated into the DNA molecule during replication (S-phase). They covalently bind to the DNMTs, causing an irreversible blockage of these enzymes and preventing DNAm (Stresemann and Lyko, 2008, Diesch et al., 2016). 5-AzaC and 5-AzaD are administered as prodrugs with low oral bioavailability (Zhang et al., 2013). They also have a short plasma half-life, approximately 30 minutes after intravenous administration (Daskalakis et al., 2010, Estey, 2013, Navada et al., 2014). In general, the cellular uptake of these prodrugs depends on diverse transporters, including nucleoside transporter proteins (SLC28 and SLCA29 gene families; Pastor-Anglada et al., 2004, Qin et al., 2009). These drugs are activated by uridine-cytidine kinase and deoxycytidine kinase and are inactivated by deamination (Pastor-Anglada et al., 2004, Qin et al., 2009, Daskalakis et al., 2010, Valencia et al., 2014). Moreover, nucleoside inhibitors are potentially nonspecific cytotoxic compounds with structural instability, which restricts their clinical therapy (Yoo and Jones, 2006, Stresemann and Lyko, 2008, Gros et al., 2012).

Non-nucleoside inhibitors, such as RG108, hydralazine, procaine, procainamide, IM25, and disulfiram, have a variety of action mechanisms. They are all independent of cell division. Their mechanisms include non-covalent inhibition in the DNA catalytic sites, prevention of their enzymatic activity (Lyko and Brown, 2005, Mai and Altucci, 2009), reduction of DNMTs affinity for the DNA (Zambrano et al., 2005, Castellano et al., 2008, Datta et al., 2009), inhibition of methyl donor proteins (Cui et al., 2006), or suppression of DNMTs expression (Pina et al., 2003). Usually, these drugs have a short plasma half-life (approximately 4 h for RG108 after subcutaneous administration) (Schneeberger et al., 2016) and reduced adverse effects (Xu et al., 2016). Additionally, natural compounds such as curcumin, genistein, EGCG, and

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resveratrol also act by indirect inhibition of DNMTs (revised in Lascano et al., 2018).

DNMTi drugs have a wide distribution in body fluids, crossing the blood-brain barrier (Marcucci et al., 2005, Schneeberger et al., 2016) and possibly reversing situations where there is increased methylation in diverse tissues including CNS.

DNA methylation in stress and depression

Preclinical studies

Preclinical studies have reported that stress modulates DNAm (Tables 1 and 2). Exposure to stress stimuli in the early developmental stages results in persistent epigenetic changes associated with long-lasting gene expression changes and influencing neural and behavioural functions in adulthood (Weaver et al., 2004, Tsankova et al., 2006). Diverse studies show stress-induced hypermethylation and subsequent reduced gene expression (Table 1). Gestational stress increases the expression of DNMT1 and DNAm on the BDNF promoter, resulting in decreased BDNF transcripts and protein levels in the hippocampus of the offspring (Zheng et al., 2016). Still, male mice of dams prenatally restraint stressed show increased DNAm in the promoter regions of glutamic decarboxylase 67 (Gad67), reelin (Reln), and BDNF (IX) (Dong et al., 2019). These changes in DNAm profile are associated with their reduced expression. Offspring prenatally exposed to the bisphenol A, considered an endocrine-disrupting chemical associated with long-term behavioural effects (Braun et al., 2011, Kundakovic and Champagne, 2011, Perera et al., 2012), shown long-lasting BDNF IV hypermethylation in the hippocampus and blood of mice (Kundakovic et al., 2015). Prenatal exposure to maternal depression increases neonatal DNAm of glucocorticoid receptor gene (NR3C1) in cord blood cells in humans (Oberlander et al., 2008). It also increases DNAm of BDNF IV promoter and decreases its mRNA in the amygdala and hippocampus of rodents (Boersma et al., 2014).

DNAm changes have been associated with maternal care. Low maternal care increased DNAm in the NR3C1 promoter region and decreased its gene expression in the hippocampus (Weaver et al., 2004, Szyf et al., 2007, Tsankova et al., 2007). Maternal separation stress was associated with hypermethylation in the rat hippocampus (McCoy et al., 2016). This stressor also increased DNMTs expression (Anier et al., 2014) and DNAm in the protein phosphatase 1 catalytic subunit (PP1C), a neuronal plasticity-related gene, in the nucleus accumbens, followed by its transcriptional downregulation (Anier et al., 2014). Similarly, DNAm in the nucleus accumbens but not in the prefrontal cortex is increased in the promoter of adenosine A2a receptor (A2AR) (Anier et al., 2014), which the upregulation is associated with the synaptic dysfunction observed in depression (Duman and Aghajanian, 2012, Domenici et al., 2019), suggesting that DNAm changes differ between brain regions.

In the prefrontal cortex, early maltreatment such as maternal separation increases DNMT activity, DNMT (1, 3a, and 3b) mRNA, and DNMT3a protein levels. These effects were correlated with the DNAm of the BDNF gene (exons IV and IX) and reduced BDNF mRNA in rats (Roth et al., 2009, Urb et al., 2019). This stressor also increased global DNAm in the dorsal hippocampus of adolescent male rats and DNAm of BDNF exon IV in the amygdala and ventral hippocampus of female rats (Doherty et al., 2016), suggesting sex-specific DNAm changes. In the medial prefrontal cortex of adolescent rats exposed to maltreatment in infancy, BDNF DNAm also increased in males (exon I) (Blaze et al., 2013).

In addition to the long-lasting effects induced in early development, stressful events in adult animals rapidly modulate DNAm. Footshocks stress increases global DNAm and DNMTs expression in the dorsal hippocampus and prefrontal cortex of rats subjected to the learned helplessness model. These molecular changes in the prefrontal cortex, but not in the dorsal hippocampus, were attenuated by chronic antidepressant treatment (Sales and Joca, 2018). Roth and coworkers (2011) found that predator exposure increases the DNAm of the BDNF promoter in the dorsal hippocampus (dentate gyrus and corn Ammonis, CA1, subregions) and reduces transcript levels of BDNF in the CA1 (Roth et al., 2011). Acute stress of forced swim also increased DNAm in NR3C1 gene and reduced GR mRNA levels in the hippocampus (dentate gyrus) (Mifsud et al., 2017).

Contradictory results including DNA hypomethylation induced by stress and direct correlation between DNAm and gene expression have been found in many studies (Table 2), suggesting that DNAm changes are dependent on multiple factors including stressor, sex, age, brain structure, gene, region, and site methylated. For example, maternal separation stress reduced global DNAm in the nucleus accumbens (Anier et al., 2014) and increased DNAm in NR3C1 and Syn I genes following by increased NR3C1 mRNA in hypothalamic neurons (Bockmuhl et al., 2015), Syn I mRNA, and protein levels in the amygdala (Park et al., 2014). Female mice submitted to the maternal exposure to predator odour show decreased DNAm in BDNF exon IV in hippocampus accompanied by elevated CRHR1 in the amygdala and reduced mRNA BDNF in the hippocampus (St-Cyr and McGowan, 2015). In the medial prefrontal cortex of adolescent rats exposed to maltreatment in infancy decreased BDNF DNAm was found in females (exon IV) (Blaze et al., 2013) while social defeat stress during early adolescence downregulated BDNF expression without altering DNAm of the BDNF IV promoter in adulthood (Xu et al., 2018). In adult rats, DNAm is reduced in both males and females (BDNF exon I) and increased in females (BDNF exon IV) submitted to the maternal maltreatment (Blaze et al., 2013). None BDNF mRNA expression change was observed (Blaze et al., 2013) suggesting that DNAm may not directly regulate gene transcription leading to an unclear understanding of its functional relevance.

DNAm, such as other epigenetic modifications, has been proposed as a form of genomic metaplasticity preparing the transcriptional response and subsequent neuronal reactivation (reviewed in (Baker-Andresen et al., 2013). Several studies support that DNAm mediates genomic metaplasticity in several ways including the regulation of alternative splicing among others (Oberdoerffer, 2012). Additionally, in early life, DNMT3a transiently binds across the genome catalysing the DNAm following by binding of methyl-DNA-binding protein MeCP2 in the mice brain. The DNAm within transcribed regions of genes is negatively regulated by gene transcription, and it occurs in a neuronal type-specific manner (Stroud et al., 2017). The density of DNAm in cytosine-adenine sequences across the genome increases in two specific neuronal subtypes, parvalbumin and vasoactive intestinal peptide expressing interneurons, and it is associated with reduced gene transcription in both neuronal subtypes. However, the increased gene transcription in vasoactive intestinal neuronal peptide and reduced in parvalbumin neurons result in lasting high methylated CA sequences within its transcribed regions in parvalbumin but not vasoactive intestinal peptide neurons (Stroud et al., 2017) suggesting the indirect gene transcription and DNAm interaction.

Acute stress of forced swim reduced DNAm at CpGs of the c-Fos gene in the hippocampus (Saunderson et al., 2016). Roth

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Table 1. Studies showing modulation and increased DNA methylation (DNAm) after stress

Stress measure	Animal specie	Sample size (n)	Tissue type	Gene	Description of gene	DNAm measure	Results	Reference
Gestational stress	Kumming mice (male)	10/group	Brain (hippocampus)	BDNF	Brain-derived neurotrophic factor	MeDIP and PCR	• Stress increased DNAm (exons I, IV, VI, and IX), DNMT1 (mRNA and protein), and reduced BDNF (mRNA and protein)	(Zheng *et al.*, 2016)
Utero bisphenol A exposure	BALB/c mice (female and male)	6/group	Brain (hippocampus) and blood	BDNF	Brain-derived neurotrophic factor	Bisulphite and Pyrosequencing	• Stress increased DNAm in the BDNF IV CpG sites 3 and 4 in the hippocampus and in the CpG4 in the blood of male mice	(Kundakovic *et al.*, 2015)
Prenatal stress	Sprague-Dawley rats (female and male)	5/group	Brain (amygdala and hippocampus)	BNDF	Brain-derived neurotrophic factor	Bisulphite and Pyrosequencing	• Stress increased DNAm in the BDNF IV and reduced mRNA BDNF	(Boersma *et al.*, 2014)
Maternal care	Long-Evans rats (male)	5/group	Brain (hippocampus)	NR3C1	Glucocorticoid receptor gene	Bisulphite and sequencing	• Low maternal care increased DNAm (exon 1_7) and reduced GR protein	(Weaver *et al.*, 2004)
Early maltreatmet	Long-Evans rats (female and male)	8–11/group	Brain (hippocampus)	Global	–	ELISA	• Stress increased DNAm in the dorsal hippocampus of adolescent male rats	(Doherty *et al.*, 2016)
Early maltreatment	Long-Evans rats (female and male)	7–11/group	Brain (prefrontal cortex)	BDNF	Brain-derived neurotrophic factor	Bisulphite and sequencing	• Stress increased DNAm (exons IV and IX) and reduced BDNF mRNA	(Roth *et al.*, 2009)
Early maltreatment	Long-Evans rats (female and male)	6–10/group	Brain (medial prefrontal cortex)	BDNF	Brain-derived neurotrophic factor	Bisulphite and sequencing	• Stress increased DNAm in adolescent male mice (exon I) • Stress increased DNAm (exon IV) in adult female mice	(Blaze *et al.*, 2013)
Early maltreatmet	Long-Evans rats (female and male)	8-11/group	Brain (amygdala and hippocampus)	BDNF	Brain-derived neurotrophic factor	Bisulphite and sequencing	• Stress increased DNAm (exon IV) in the amygdala and ventral hippocampus of adolescent female rats	(Doherty *et al.*, 2016)
Maternal separation	Wistar-Kyoto rats (male)	10/group	Brain (hippocampus)	Global	–	ELISA	• Stress increased DNAm	(McCoy *et al.*, 2016)
Maternal separation	Wistar rats (male)	6-7/group	Brain (nucleus accumbens)	PP1CA2AR	Protein phosphatase 1 catalytic subunit Adenosine A2a receptor	MeDIP, bisulphite and sequencing	• Stress increased specific DNAm and DNMTs mRNA • Stress reduced mRNA and increased protein levels	(Anier *et al.*, 2014)
Maternal separation	Sprague-Dawley rats (male)	5/group	Brain (amygdala)	Syn I	Synapsin 1	MeDIP and Microarray analysis	• Stress increased DNAm and reduced Syn I (mRNA and protein)	(Park *et al.*, 2014)
Forced swimming test	Swiss mice (male)	6-10/group	Brain (hippocampus)	global	–	ELISA	• Stress increased DNAm	(Sales *et al.*, 2016)
Forced swimming test	Wistar rats (male)	4-6/group	Brain (hippocampus)	NR3C1	Glucocorticoid receptor gene	Bisulphite and Pyrosequencing	• Stress increased DNAm and DNMT3a protein • Stress reduced GR mRNA (dentate gyrus)	(Mifsud *et al.*, 2017)
Learned helplessness	Wistar rats (male)	6-9/group	Brain (hippocampus and prefrontal cortex)	Global	–	ELISA	• Stress increased DNAm, DNMT3a, and DNMT3b protein in the dorsal hippocampus and prefrontal cortex	(Sales *et al.*, 2018)

Table 1. (*Continued*)

Predactor exposure	Sprague-Dawley rats (male)	6-8/group	Brain (hippocampus)	BDNF	Brain-derived neurotrophic factor	Bisulphite and sequencing	• Stress increased DNAm (exon IV) in the dorsal huppocampus (CA1 and dentate gyrus) • Stress reduced BDNF mRNA in the dorsal and ventral hippocampus (CA1)	(Roth *et al.*, 2011)
Chronic restraint stress	ICR mice (male)	5/group	Brain (hippocampus)	Npas4	Neuronal Per Arnt Sim domain 4	Bisulphite and Pyrosequencing	• Stress increased DNAm and reduced mRNA	(Furukawa-Hibi *et al.*, 2015)
Chronic mild repeated stress	Wistar rats (female and male)	5/group	Brain (hippocampus)	BDNFTrkB	Brain-derived neurotrophic factor Tyrosine kinase B	Methylation-sensitive restriction enzymes and qPCR	• Stress increased DNAm (BDNF IV and TrkB) and reduced its protein levels	(Niknazar *et al.*, 2016)
Chronic mild stress	Wistar Han rats (male)	6/group	Brain (hippocampus) and Blood (PBMCs)	Gpx1 SOD1 SOD2	Glutathione peroxidase Superoxide dismutase	Bisulphite and sequencing	• Stress increased Gpx1 DNAm (PBMCs) and SOD (hippocampus) • Stress reduced Gpx1mRNA in the PBMCs	(Wigner *et al.*, 2020)
Chronis unpredictable stress	Sprague-Dawley rats (male)	4/group	Brain (prefrontal cortex)	–	–	Bisulphite and sequencing	• Stress modulates DNAm in neuronal pathways • Stress reduced DNMT3a mRNA and protein levels • Stress reduced AMPA2 mRNA and protein levels	(Wei *et al.*, 2020)

Note: MeDIP, methylated DNA immunoprecipitation; PCR, polymerase chain reaction; DNAm, DNA methylation; DNMT, DNA methyltransferase; mRNA, messenger RNA; CpG, cytosine-phosphate-guanine; GR, glucocorticoid receptor; ELISA, enzyme-linked immunosorbent assay; CA, cornu ammonis; qPCR, quantitative polymerase chain reaction; PBMCs, peripheral mononuclear blood cells; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

and coworkers (2011) showed that predator exposure decreases DNAm of the BDNF promoter (CA3) and its mRNA (CA1) in the ventral hippocampus (CA3 subregion) (Roth et al., 2011). Still, the global hypomethylation and reduced expression of BDNF, GR, and MR are induced by repeated restrain stress in the rat hippocampus (Makhathini et al., 2017). Chronic stress also results in DNA hypomethylation, reduced DNMT1 RNAm, and protein levels in the lateral habenulae of rats (Shen et al., 2019). In rat prefrontal cortex, chronic unpredictable stress during 2 weeks results in reduced DNMT3a levels, while the overexpression of DNMT3a in stressed rats attenuated impaired stress-induced behaviour and improved the glutamatergic responses (Wei et al., 2020). A significant increase in DNAm of the promoter region of the serotonin 1A receptor (5-HT1A), a regulator of the brain serotonergic tone related to depression, associated with an increase in 5-HT1A mRNA and protein levels are observed in the prefrontal cortex of chronically stressed mice (Le Francois et al., 2015).

Stress also modulates DNAm and gene expression of glutathione peroxidase 1 (Gpx1) and superoxide dismutase1 and 2 (SOD1 and SOD2). The activity of these enzymes is reduced in depressed patients (Herken et al., 2007, Maes et al., 2011, Stefanescu and Ciobica, 2012, Rybka et al., 2013). Chronic mild stress increased the methylation of the Gpx1 promoter and reduced its expression in the blood, whereas DNAm in the SOD1 and SOD2 promoters increased in the hippocampus. The mRNA expression of these genes increased in the brain (hippocampus, amygdala, hypothalamus, midbrain, cortex, basal ganglia (Wigner et al., 2020). Increased mRNA and methylation of the promoter P11, a member of the S100 EF-hand family (Rescher and Gerke, 2008), considered a key neuronal modulator in depression and antidepressant response, were also observed in the prefrontal cortex of rats subjected to chronic stress and that are electroconvulsive stimulation (ECS) responsive (Neyazi et al., 2018).

Oxytocin (OXT) and its receptor (OXTR) are proposed to play a relevant role in emotional behaviours, depression, and stress (reviewed in Jurek and Neumann, 2018). OXTR gene promoter DNAm significantly decreased in the blood, but not in the brain (hippocampus, striatum, and hypothalamus), of rats reared with low licking compared to those exposed to high licking-grooming (Beery et al., 2016) suggesting that the tissue studied can modify the modulation of DNAm complicating its possible role biomarker in depressed individuals.

Clinical studies: DNAm as a possible biomarker for depression

Similarly to stress, depression, and antidepressant drugs have been associated with DNAm alterations (Tables 3 and 4). Genome-wide DNAm association studies are important tools for the identification of genetic associations with complex disorders including MDD, expanding the repertoire of genes, and the alterations related to epigenetic or genetic factors. So, genome-wide association promises significant progress in the understanding of MDD contributing to the identification of biological markers for MDD and response to antidepressant treatments and possible new therapeutic targets (Spreafico et al., 2020, Uffelmann and Posthuma, 2021). The first genome-wide of MDD was reported by Sullivan et al. (2009), and the first genome-wide DNAm scan in MDD, covering 3.5 million CpGs, was published in 2012 (Sabunciyan et al., 2012). Since then, an increasing number of studies about MDD and antidepressant response have identified DNAm differences in candidate regions (differentially methylated sites) for several genes (Numata et al., 2015), including neuronal development genes

https://doi.org/10.1017/neu.2021.18 Published online by Cambridge University Press

Table 2. Studies showing no change, decreased DNA methylation (DNAm), and complex gene expression modulation by DNAm after stress

Stress measure	Animal specie	Sample size (n)	Tissue type	Gene	Description of gene	DNAm measure	Results	Reference
Maternal exposure to predator odor	C57BL/6 mice (male)	5/group	Brain (amygdala and hippocampus)	BDNF	Brain-derived neurotrophic factor	Bisulphite and Pyrosequencing	• No alteration in the amygdala was observed • Stress reduced DNAm (exon IV) and mRNA BDNF in the hippocampus	(St-Cyr *et al.*, 2015)
Maternal care	Long-Evans rats (male)	6-7/group	Brain (hippocampus, striatum, hypothalamus) and blood	Oxtr	Oxytocin receptor gene	Bisulphite and Pyrosequencing	• Low maternal care reduced DNAm in the blood • No alteration was observed in the brain	(Beery *et al.*, 2016)
Early maltreatmet	Long-Evans rats (female and male)	8-11/group	Brain (amygdala)	Global	–	ELISA	• No alteration was observed	(Doherty *et al.*, 2016)
Early maltreatment	Long-Evans rats (female and male)	6–10/group	Brain (medial prefrontal cortex)	BDNF	Brain-derived neurotrophic factor	Bisulphite and sequencing	• Stress reduced DNAm in adolescent female mice (exon IV) • Stress reduced DNAm (exon I) in adult mice (female and male)	(Blaze *et al.*, 2013)
Early maltreatmet	Long-Evans rats (female and male)	8–11/group	Brain (amygdala and hippocampus)	BDNF	Brain-derived neurotrophic factor	Bisulphite and sequencing	• No alteration was observed in adolescent male rats	(Doherty *et al.*, 2016)
Maternal separation	Wistar rats (male)	6–7/group	Brain (nucleus accumbens)	Global PP1C A2AR	- Protein phosphatase 1 catalytic subunit Adenosine A2a receptor	ELISA MeDIP, bisulphite and sequencing	• Stress reduced global DNAm • Stress increased protein levels	(Anier *et al.*, 2014)
Maternal separation	C57BL/6N mice (male)	9–10/group	Brain (paraventricular nucleus)	NR3C1	Glucocorticoid receptor (GR) gene	Bisulphite and sequencing	• Stress increased DNAm and GR mRNA	(Bockmuhl *et al.*, 2015)
Adolescent social stress	C57BL/6J mice (male)	7–9/group	Brain (medial prefrontal cortex)	BDNF	Brain-derived neurotrophic factor	Bisulphite and sequencing	• No alteration in the DNAm was observed • Stress reduced BDNF mRNA	(Xu *et al.*, 2018)
FST	Swiss mice (male)	6–10/group	Brain (prefrontal cortex)	Global	–	ELISA	• Stress reduced DNAm levels	(Sales *et al.*, 2016)
FST	Wistar rats (male)	3–6/group	Brain (hippocampus)	c-FOS	FBJ murine osteosarcoma viral oncogene homolog	Bisulphite and Pyrosequencing	• Stress reduced DNAm (dentate gyrus)	(Saunderson *et al.*, 2016)
Repetitive restrain stress	Sprague-Dawley rats (male)	5/group	Brain (hippocampus)	Global	–	ELISA	• Stress reduced DNAm, BDNF, GR, and MR mRNA and protein	(Makhathini *et al.*, 2017)
Predactor exposure	Sprague-Dawley rats (male)	6–8/group	Brain (hippocampus)	BDNF	Brain-derived neurotrophic factor	Bisulphite and sequencing	• Stress reduced DNAm (exon IV) in the ventral HIP (CA3) • Stress reduced BDNF mRNA in the dorsal and ventral HIP (CA1)	(Roth *et al.*, 2011)
Chronic unpredictable mild stress	Wistar rats (male)	6/group	Brain (lateral habenulae)	Global	–	ELISA	• Stress reduced DNAm and DNMT1 (mRNA and protein)	(Shen *et al.*, 2019)
Chronic unpredictable mild stress	BALB/c mice (male)	7–9/group	Brain (prefrontal cortex)	5-HT1A	Serotonin 1A receptor	Bisulphite and sequencing	• Stress increased DNAm and 5-HT1A (mRNA and protein)	(Le Francois *et al.*, 2015)

Note: DNAm, DNA methylation; mRNA, messenger RNA; ELISA, enzyme-linked immunosorbent assay; MeDIP, methylated DNA immunoprecipitation; FST, forced swimming test; GR, glucocorticoid receptor; MR, mineralocorticoid receptor; CA, cornu ammonis; DNMT, DNA methyltransferase.

Table 3. Studies showing modulation and increased DNA methylation (DNAm) in depression

Gene	Description of gene	Sample size (n)	Tissue type	DNAm measure	Results	Reference
–	–	79 58	Peripheral blood monocytes Prefrontal cortex	Bisulphite and Illumina sequencing	• Genome-wide methylation shown 39 differentially DNA methylated regions of genes associated with lifetime history of MDD in monozygotic twin	(Zhu *et al.*, 2019)
–	–	53	Prefrontal cortex (Broadmann area 47, BA47)	Bisulphite and Illumina sequencing	• MDD suicides shown increased age-dependent DNAm in 140 CpG sites	(Haghighi *et al.*, 2014)
BDNF	Brain-derived neurotrophic factor	81	Cord blood	Bisulphite and pyrosequencing	• Maternal exposure to the bisphenol A increased BDNF promoter DNAm in two CpG sites (1A and 1B)	(Kundakovic *et al.*, 2015)
BDNF	Brain-derived neurotrophic factor	108	Peripheral blood	Bisulphite and pyrosequencing	• Suicidal ideation is associated with increased BDNF promoter DNAm	(Kang *et al.*, 2013)
BDNF	Brain-derived neurotrophic factor	77	Brain (Wernicke area)	Bisulphite and pyrosequencing	• Suicidal individuals shown increased promoter DNAm (exon IV) and reduced BDNF mRNA	(Keller *et al.*, 2010)
BDNF	Brain-derived neurotrophic factor	130	Whole blood	Bisulphite and pyrosequencing	• Patients with recurrent MDD shown increased promoter DNAm and reduced cortical thickness • Serum BDNF levels are decreased in MDD	(Na *et al.*, 2016)
TrkB	Tyrosine kinase B receptor	39	Frontal cortex	Bisulphite and sequencing	• Suicide individuals shown increased DNAm and reduced truncated variant of TrkB (TrkB.T1) expression	(Ernst *et al.*, 2009)
NR3C1 NR3C1	Glucocorticoid receptor Glucocorticoid receptor	57 82	Buccal swabs Cord blood mononuclear cells	Bisulphite and pyrosequencing Bisulphite and pyrosequencing	• Prenatal depression shown increased NR3C1 1F DNAm in male infants • Prenatal depression shown increased neonatal DNAm (exon 1F)	(Braithwaite *et al.*, 2015) (Oberlander *et al.*, 2008)
NR3C1	Glucocorticoid receptor	62	Peripheral blood	Bisulphite and pyrosequencing	• MDD patients shown increased DNAm (CpG7)	(Nantharat *et al.*, 2015)
NR3C1	Glucocorticoid receptor	67	Whole blood	Bisulphite and pyrosequencing	• MDD patients shown increased NR3C1 exon 1F DNAm	(Farrell *et al.*, 2018)
SLC6A4	Serotonin transporter	69	Blood	Pyrosequencing	• Higher childhood abuse is associated with increased DNAm	(Booij *et al.*, 2015)
SLC6A4	Serotonin transporter	57	Blood	Bisulphite and pyrosequencing	• Unmedicated patients with MDD shown increased DNAm and expression levels compared to healthy controls	(Iga *et al.*, 2016)
5-HTTLPR	Serotonin transporter gene-linked polymorphic region	133	Peripheral blood	Bisulphite and pyrosequencing	• Prenatal or early stress increased DNAm and reduced mRNA levels	(Wankerl *et al.*, 2014)
OXTR AVP	Oxytocin receptor Arginine vasopressin	218	saliva	Bisulphite and sequencing	• Mothers with persistent perinatal depression, but not their children, have increased OXTR DNAm and reduced AVP DNAm in the intergenic regions	(King *et al.*, 2017)
GFAP, ALDH1L1, SOX9, GLUL, SCL1A3, GJA1, GJB6	Glial fibrillary acidic protein, Aldehyde dehydrogenase 1 family member L1, SRY-Box transcription factor 9, Glutamine synthetase, Glial high-affinity transporters, Gap junction protein alpha 1, Gap junction protein beta 6	121	PFC –- left hemisphere (grey matter)	Bisulphite and sequencing	• Genome-wide methylation shown differentially methylated regions in the MDD (reduction in the majority)	(Nagy *et al.*, 2015)
P11	S100 calcium-binding protein A10 (S100A10)	65	Blood	Bisulphite and sequencing	• Refractory and pharmacoresistant (but eletroconvulsive therapy responsers) MDD patients shown increased promoter DNAm	(Neyazi *et al.*, 2018)

Note: MDD, major depressive disorder; DNAm, DNA methylation; CpG, cytosine-phosphate-guanine; mRNA, messenger RNA.

Table 4. Studies showing no change, decreased DNA methylation (DNAm), and complex gene expression modulation by DNAm in depression

Gene	Description of gene	Sample size (n)	Tissue type	DNAm measure	Results	Reference
–	–	39	Peripheral blood leukocytes	Bisulphite and Illumina sequencing	• 365 sites shown reduced DNAm in MDD patients	(Numata *et al.*, 2015)
–	–	274	Saliva cells	Bisulphite and Illumina sequencing	• No significant genome-wide association was found between maternal depressive symptoms and infant DNAm	(Wikenius *et al.*, 2019)
BDNF	Brain-derived neurotrophic factor	38	Peripheral blood	Bisulphite and SEQUENOM MassARRAY	• Patients with MDD shown DNAm changes in the BDNF I, but not IV	(Fuchikami *et al.*, 2011)
BDNF	Brain-derived neurotrophic factor	774	Saliva	Bisulphite and Illumina sequencing	• Highest MDD shown reduced DNAm in complete gene and exon I compared to lowest MDD	(Song *et al.*, 2014)
BDNF	Brain-derived neurotrophic fator	57	Buccal swabs	Bisulphite and pyro-sequencing	• Prenatal depression shown reduced BDNF IV DNAm in male and female infants	(Braithwaite *et al.*, 2015)
NR3C1	Glucocorticoid receptor	117	Peripheral blood	Bisulphite and pyro-sequencing	• Patients with MDD shown reduced DNAm at 2 CpG sites (CpG 3 and CpG4)	(Na *et al.*, 2014)
FKBP5	FK506-binding protein 5	67	Whole blood	Bisulphite and pyro-sequencing	• No alteration was observed	(Farrell *et al.*, 2018)
SLC6A4	Serotonin transporter	69	Blood	Pyrosequencing	• No significant association was found between MDD and DNAm	(Booij *et al.*, 2015)
SLC6A4	Serotonin transporter	57	Blood	Bisulphite and pyro-sequencing	• Unmedicated patients with MDD shown increased DNAm and expression levels compared to healthy controls	(Iga *et al.*, 2016)
SYN 1 SYN 2 SYN 3	Synapsin 1, Synapsin 2	32	Brain (PFC – Brodmann Area 10, BA10)	Bisulphite and sequencing	• Suicidal individuals with MDD shown reduced SYN 2 DNAm • No alteration was observed in SYN 1 and SYN 2 expression	(Cruceanu *et al.*, 2016)

Note: DNAm, DNA methylation; MDD, major depressive disorder; CpG, cytosine-phosphate-guanine.

(Sabunciyan et al., 2012, Weder et al., 2014) and plasticity (Weder et al., 2014), genes that encode cell adhesion molecules and neurotransmitter receptors (Oh et al., 2013), immune response-related genes (Nemoda et al., 2015), and others (Yang et al., 2013, Davies et al., 2014, Dempster et al., 2014, Cordova-Palomera et al., 2015, Ju et al., 2019). Although additional studies are needed, genome-wide analysis allows for the changes in specific genes, identifying the genomic and transcriptomic profiles for MDD, and thus contributing to the further understanding of pathways associated with complex psychiatric illnesses including depression. In this context, epigenetic studies in animals and humans suggest that the DNAm of specific genes could be a potential marker of several disorders, including depression. Integrative DNA methylome and transcriptome analysis found 39 differentially methylated regions (DMRs) and 30 differentially expressed genes (DEGs) in genes associated with signalling pathways related to stress responses, neuron apoptosis, the insulin receptor, mTOR, and nerve growth factor receptor signalling, in peripheral blood monocytes of monozygotic twin pairs with a lifetime history of MDD. These findings were replicated in the postmortem brain (dorsal lateral prefrontal cortex, BA9) of suicide individuals with MDD (Zhu et al., 2019). Additionally, the genome-wide analysis revealed 366 DMRs of genes associated with learning, memory, and behaviour in the hippocampus of suicide individuals (Labonte et al., 2013), suggesting the DNAm association and its potential biomarker role for depression.

Prenatal bisphenol A exposure results in disturbed emotional regulation, aggressive behaviour, and induces long-lasting BDNF DNAm alterations in the blood and brain (hippocampus) of mice. Still, increased DNAm in two CpG sites of BDNF IV was observed in the cord blood of humans exposed to high maternal bisphenol A levels in utero (Perera et al., 2012, Kundakovic et al., 2015). Mothers with persistent perinatal depression, but not their children, have increased OXTR DNAm in the saliva (King et al., 2017). However, individuals exposed to prenatal or early stress (child maltreatment) presented increased DNAm in four CpG sites of the serotonin transporter gene-linked polymorphic region (5-HTTLPR) promoter. This change was associated with decreased 5-HTTLPR mRNA levels in the peripheral blood cells (Wankerl et al., 2014).

A higher DNAm in the BDNF promoter region was associated with suicidal ideation and previous suicidal attempt history in MDD patients (Kang et al., 2013a). In the BDNF IV promoter, increased DNAm in the postmortem brain of suicide individuals was related to lower BDNF mRNA levels in the Wernicke area (Keller et al., 2010). MDD suicides also shown DNA hypermethylation in the cortex. The hypermethylation of TrkB promoter is associated with a lower TrkB expression in the frontal cortex (Ernst et al., 2009), and the increased DNAm in the prefrontal cortex (Brodmann Area 47, BA47) is positively correlated with age suggesting that DNAm alterations are age dependent (Haghighi et al., 2014).

Contradictory results in the modulation of DNAm in MDD have been shown in many studies (Table 4). Mothers with persistent perinatal depression have hypomethylation in intergenic regions of the arginine vasopressin (AVP) gene, a neuropeptide

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involved in maternal behaviour and stress regulation, in the saliva (Bachner-Melman and Ebstein, 2014, Bridges, 2015, King et al., 2017). None significant genome-wide association was observed between maternal depressive symptoms and infant DNAm (Wikenius et al., 2019). However, infants of mothers with MDD showed decreased BDNF IV DNAm (Braithwaite et al., 2015).

DNAm alterations also were found in peripheral tissues of MDD patients. Glucocorticoid receptor gene (NR3C1 exon 1F) was hypermethylated in the blood of MDD patients (Farrell et al., 2018). NR3C1 promoter hypermethylation was also observed in the blood of females, but not in males, MDD patients (Nantharat et al., 2015), indicating a gender influence.

Genome-wide analysis shown reduced DNAm in 365 CpG sites in the blood of MDD patients (Numata et al., 2015). DNA hypomethylated was observed in specific genes including NR3C1 and BDNF of patients with MDD. NR3C1 promoter was hypomethylated at two specific CpG sites in the peripheral blood of these patients (Na et al., 2014), and the BDNF hypomethylation was found in the complete gene (Song et al., 2014) and exon I promoter in the peripheral blood (Fuchikami et al., 2011) and saliva of persons with more severe MDD compared with a less severe disorder (Song et al., 2014). However, no correlation was observed between BDNF promoter methylation and its levels in the serum of MDD patients (Na et al., 2016). Additionally, Cruceanu and colleagues (2016) found significant DNA hypomethylation in the prefrontal cortex (BA10) of suicidal individuals with bipolar disorder or MDD compared with psychiatrically healthy individuals. Furthermore, the study showed an inverse correlation between the DNAm of the SYN2 gene and its mRNA expression (Cruceanu et al., 2016).

Candidate gene selection related to stress and depression based on prior knowledge is often used; however, genome-wide studies have reported controversial findings and a large number of associations in other genes (Sullivan et al., 2001, Sullivan, 2007, Sullivan, 2017, Border et al., 2019) including genes encoding lysophosphatidic acid receptor (LPAR2), related with diverse cellular activities (Fukushima et al., 2018), and adaptor-associated Kinase 1 (AAK1), associated with the intracellular trafficking of multiple viruses (Verdonck et al., 2019, Zhu et al., 2019). Additional studies are needed since the methylation rate of these genes may identify novel targets for antidepressant drugs, diverse signalling in stress-related pathologies, and display predictive function for evaluating the antidepressant response.

Effects of antidepressant drugs on DNAm

DNAm profile has been proposed as a factor influencing both the neurobiology of depression and antidepressant treatment response. In fact, DNAm changes at specific CpG sites have been documented for antidepressant drugs. Although few studies have investigated the potential of DNAm as a biomarker of treatment response, data support this hypothesis for antidepressants (Table 5).

Preclinical studies

Supporting the involvement of DNAm in the action of antidepressant, *in vitro* cortical astrocytes treated with antidepressant drugs shown reduced DNMT1 activity (Zimmermann et al., 2012). Other studies have shown that antidepressant drugs attenuate stress-induced hypermethylation in animals. For instance, results from an *in vivo* study identified that perinatal exposure to paroxetine, a selective serotonin reuptake inhibitor, leads to DNA hypomethylation in several genes including plasticity-related genes, and reduces DNMT3a mRNA in the hippocampus during the early of rats life (Glover et al., 2019). In Flinders Sensitive Line rats, a genetic model of depression, Melas and coworkers (2012) shown increased DNAm in the P11 promoter and decreased P11 levels in the prefrontal cortex, both reversed by chronic escitalopram treatment (Melas et al., 2012). Still, antidepressant drugs (fluoxetine, desipramine, and imipramine) attenuated stress-induced changes in DNAm levels in the hippocampus and prefrontal cortex of rodents (Sales and Joca, 2016, Sales and Joca, 2018). Footshocks stress increased total DNAm and DNMTs (DNMT 3a and DNMT 3b) levels in the dorsal hippocampus and prefrontal cortex while repeated imipramine treatment attenuated their changes only in the prefrontal cortex (Sales and Joca, 2018). In the prefrontal cortex of chronically stressed mice, the stress-induced 5-HT1A DNA hypermethylation and reduced 5-HT1A expression were reversed by chronic imipramine treatment (Le Francois et al., 2015). Chronic despair mouse model reduced mRNA and increased promoter DNAm of Homer1, a synaptic plasticity protein related with depression and action of antidepressant drugs (Serchov et al., 2015, Serchov et al., 2016) in the cortex and blood (Sun et al., 2020). However, chronic antidepressant treatment reduced Homer1 promoter DNAm and expression in the cortex (Sun et al., 2020), suggesting that the DNAm is brain region-specific and that the regulation of DNAm in the cortex is an important mechanism associated with the antidepressant effect.

Contradictory results demonstrate that antidepressant drugs can increased DNAm. Rat offspring exposed to fluoxetine during pregnancy and lactation showed decreased global DNAm in the hippocampus and increased DNAm in the cortex (Toffoli et al., 2014), suggesting long-lasting and region-specific DNAm changes induced by antidepressants. Contrary to this, the exposure to fluoxetine during gestation and lactation increased global DNAm in the hippocampus, reduced social interaction time, and decreased plasma corticosterone levels of male offspring subjected to the restraint stress (Silva et al., 2018). Still, increased BDNF expression is associated with increased DNMT1 activity in blood cells of MDD patients treated *ex vivo* with paroxetine (Gassen et al., 2015).

Clinical studies

No global DNAm difference was observed between MDD patients and healthy controls or between medicated and unmedicated individuals with MDD (Okada et al., 2014). However, genome-wide analyses revealed differentially methylated sites and expressed genes in the blood of MDD escitalopram responders (Ju et al., 2019). MDD individuals who best respond to the selective serotonin reuptake inhibitor, paroxetine, showed 623 CpG sites differently methylated (Takeuchi et al., 2017). Individual variations in the antidepressant responses were related to these DNAm changes in the blood (Takeuchi et al., 2017), suggesting that DNAm alterations at specific genes could be associated with antidepressant drugs being predictive and biomarker of therapeutic response.

Generally, in humans, the MDD-related hypermethylation is not attenuated by antidepressant treatment and curiously the antidepressant response can be associated with increased DNAm in specific genes. Hsieh and coworkers (2019) found that MDD is associated with lower serum BDNF mRNA and protein levels in the blood (Hsieh et al., 2019). In this study, patients with MDD showed different DNAm between the CpG sites of BDNF exon

https://doi.org/10.1017/neu.2021.18 Published online by Cambridge University Press

Table 5. Antidepressant´s effects on DNAm

Gene	Description of gene	Sample size (n)	Tissue type	DNAm measure	Results	Reference
Preclinical studies						
–	–	18 rat brains – (Sprague-Dawley rats)	Cortical astrocytes	–	• Antidepressants reduce DNMT1 activity	(Zimmermann *et al.*, 2012)
–	–	4-6/group (Male Wistar rats)	Brain (hippocampus, cortex, hypothalamus, periaqueductal grey area)	ELISA	• Early exposure to fluoxetine during gestation and lactation increased total DNAm in the hippocampus	(Silva *et al.*, 2018)
–	–	4-5/group (Male Sprague-Dawley rats)	Brain (hippocampus)	ELISA; MeDIP and Illumina sequencing	• Perinatal exposure to paroxetine results in reduced DNMT3a mRNA and DNAm	(Glover *et al.*, 2019)
–	–	7-9/group (Wistar rats)	Brain (prefrontal cortex and hippocampus)	ELISA	• Footshocks stress increased DNAm and DNMT (3a and 3b) • Chronic imipramine treatment reduced DNAm and DNMT (3a and 3b) expression in the prefrontal cortex • None alteration was observed by imipramine treatment in the hippocampus	(Sales *et al.*, 2018)
P11	S100 calcium-binding protein A10 (S100A10)	31	Brain (prefrontal cortex)	Bisulphite and pyrosequencing	• Flinders Sensitive Line rats shown increased DNAm and reduced P11 expression • Chronic treatment with escitalopram reduced DNAm and DNMT (1 and 3a) mRNA, and increased P11 expression	(Melas *et al.*, 2012)
Clinical studies						
–	–	68	Peripheral blood	Illumina sequencing	• 623 CpG sites differently DNA methylated in MDD patients who best responded to paroxetine compared to worst responders	(Takeuchi *et al.*, 2017)
–	–	176	Peripheral blood	Bisulphite and Illumina sequencing	• MDD individuals escitalopram responders shown differentially methylated sites and differentially expressed genes	(Ju *et al.*, 2019)
BDNF	Brain-derived neurotrophic factor	46	Leukocytes Neuroblastoma cell line SH-SY5Y	Bisulphite and sequencing	• None DNAm alterations was observed in the BDNF IV promoter of MDD individuals • ntidepressant non-response in MDD individuals is associated with lower DNAm in the CpG-87 and reduced plasma BDNF levels • Fluoxetine and venlafaxine increased the BDNF expression (48 h after incubation)	(Tadic *et al.*, 2014)
BDNF	Brain-derived neurotrophic factor	365	Peripheral blood	Bisulphite and Illumina sequencing	• MDD individuals shown inverse correlation between DNAm and FK506-binding protein 5 (FKBP5) expression • Cells of MDD individuals treated *ex vivo* with paroxetine showed increased BDNF levels and DNMT1 activity	(Gassen *et al.*, 2015)
BDNF	Brain-derived neurotrophic factor	544	Peripheral blood mononuclear cells	Bisulphite and qPCR	• MDD individuals shown increased DNAm in the BDNF exon I promoter • Increased DNAm is associated with antidepressant treatment	(Carlberg *et al.*, 2014)

Table 5. (*Continued*)

BDNF	Brain-derived neurotrophic factor	113	Peripheral blood	Bisulphite and pyrosequencing	• MDD individuals shown increased DNAm in the CpG site 217 • MDD individuals shown reduced DNAm in the CpG site 327 and CpG site 362 • MDD individuals shown reduced BDNF mRNA and protein levels • MDD antidepressant responders shown increased DNAm at CpG site 24 and CpG site324	(Hsieh *et al.*, 2019)
BDNF	Brain-derived neurotrophic factor	85 (MDD patients)	Peripheral blood	Bisulphite and Illumina sequencing	• DNA hypomethylation is associated with impaired escitalopram response	(Wang *et al.*, 2018b)
SLC6A4	Serotonin transporter	108	Peripheral blood leukocytes	Bisulphite and pyrosequencing	• MDD individuals shown increased DNAm • No significant association was observed between increased DNAm and antidepressant responses	(Kang *et al.*, 2013)
SLC6A4	Serotonin transporter	65	Peripheral blood	Bisulphite and SEQUENOM MassARRAY	• MDD individuals with better therapeutic responses shown increased DNAm in the CpG3 before antidepressant treatment	(Okada *et al.*, 2014)
SLC6A4	Serotonin transporter	94	Peripheral blood	Bisulphite and sequencing	• MDD individuals with better therapeutic responses shown increased DNAm in the promoter before escitalopram treatment	(Domschke *et al.*, 2014)
SLC6A4	Serotonin transporter	236	Whole blood	sequencing	• Promoter hypomethylation in MDD patients is related with antidepressant nonresponse and MDD nonremission	(Schiele *et al.*, 2020)
5-HT1A 5-HT1B	Serotonin 1A receptor Serotonin 1B receptor	86 (MDD patients)	Peripheral blood	Bisulphite and Illumina sequencing	• DNA hypomethylation is associated with impaired escitalopram response	(Wang *et al.*, 2018a)
5-HT1A 5-HT1B	Serotonin 1A receptor Serotonin 1B receptor	86 (MDD patients)	Peripheral blood	Bisulphite and Illumina sequencing	• DNA hypomethylation is associated with impaired escitalopram response	(Wang *et al.*, 2018a)
Homer1	Homer scaffold protein 1	40 (C57BL/6 Mice)	Cortex Peripheral blood	Bisulphite and pyrosequencing	• Chronic despair mouse model increased promoter DNAm in the cortex • Stress decreased Homer1a and Homer1b/c mRNA • Chronic imipramine and fluoxetine, such as acute ketamine, treatments increased Homer1a and Homer1b/c mRNA and reduced promoter DNAm in the cortex	(Sun *et al.*, 2020)

Note: qPCR, quantitative polymerase chain reaction; MDD, major depressive disorder; DNAm, DNA methylation; CpG, cytosine-phosphate-guanine; DNMT, DNA methyltransferase enzyme; ELISA, Enzyme-Linked Immunosorbent Assay; MEDIP, methylated DNA immunopreciptation; mRNA, messenger RNA.

IX promoter (Hsieh et al., 2019). BDNF promoter DNA hypermethylation was reported in the blood of patients with MDD (Carlberg et al., 2014), while antidepressant therapy further increased DNAm in MDD individuals compared with MDD individuals without antidepressant treatment (Carlberg et al., 2014). In fact, reduced BDNF DNAm is associated with impaired response of antidepressant escitalopram (Wang et al., 2018b), while the better antidepressant responses are related to DNA hypermethylation in two CpG sites of BDNF IX suggesting a site-dependent DNAm (Hsieh et al., 2019). In BDNF IV, the resistance to antidepressant response is related to reduced DNAm (CpG87) and lower BDNF expression in the blood of MDD patients (Domschke et al., 2014, Tadic et al., 2014). It is not clear, however, how these results can explain the well demonstrated association of low BDNF levels with depression (Lin and Huang, 2020, Notaras and van den Buuse, 2020).

The serotonin transporter gene (SLC6A4), responsible for serotonin reuptake, has been related to interindividual differences in antidepressant treatment responses (Taylor et al., 2010, Tolsma and Hansen, 2019). DNA hypermethylation at the SLC6A4 (promoter and CpG 3) found in patients with MDD before six weeks, but not twelve weeks (Kang et al., 2013b), of antidepressant treatment, was related with better therapeutic response possibility increasing serotonin levels in the synaptic cleft (Domschke et al., 2014, Okada et al., 2014, Schiele et al., 2020). These results suggest that DNAm levels in specific sites of SLC6A4 can be associated with antidepressant response during a specific time of treatment. Additionally, impaired escitalopram response is related to 5-HT1A and 5-HT1B DNA hypomethylation of two CpG sites (5-HT1a CpG 668 and 5-HTR1b CpG 1401) in the blood of MDD patients possibility increasing their expression and reducing 5-HT levels which might counteract the antidepressant effects of escitalopram (Wang et al., 2018a).

Together, these data indicate that DNAm can be influenced by antidepressant drugs in MDD patients, and this can be associated with its therapeutic response. However, more studies are needed in order to understand the predictive value of DNAm changes in depression and its potential use as a biomarker in antidepressant responses.

Effects of the pharmacological manipulation of DNAm in stress and depression

Supporting the involvement of DNAm in the stress responses, inhibition of DNAm with central or peripheral injections of DNMTi results in antidepressant-like effects. The acute systemic administration of two different and chemically unrelated DNMTi (5-AzaD and RG108) promoted stress-coping behaviour in mice exposed to the forced swim test and tail suspension test, smililary to other antidepressants (Sales et al., 2011, Sales and Joca, 2016). These effects were associated with increased BDNF levels in rat hippocampus (Sales et al., 2011). In another study, single injection of 5-AzaD and RG108 induces rapid (1h) and long-lasting (7 days after) antidepressant-like effects in rats exposed to the learned helplessness model (Sales et al., 2020). Interestingly, the RG108 treatment attenuated both increased DNAm and reduced BDNF IV and TrkB expression induced by stress in the prefrontal cortex. Moreover, the behavioural effects induced by RG108 were blocked by TrkB antagonism or mTOR inhibition in the medial prefrontal cortex (Sales et al., 2020), suggesting that the fast disinhibition of BDNF-TrkB-mTOR signalling can be associated with antidepressant-like effects of DNMTi. DNMTi (5-AzaD and RG108) microinjected intracerebroventricularly (Dong et al., 2019) or directly into the hippocampus (Sales et al., 2011) and nucleus accumbens (LaPlant et al., 2010) also induced antidepressant-like effects, thus indicating that the drugs themselves are promoting the antidepressant effect rather than some product of their metabolism.

Contrary to this, local 5-AzaD infusion into the ventrolateral orbital cortex (Xing et al., 2014) and lateral habenulae (Shen et al., 2019) resulted in depressive-like behaviours and decreased global DNAm. DNMT1 mRNA expression and protein levels also were reduced in the lateral habenulae (Shen et al., 2019). Still, the DNMTi-induced hypomethylation in the lateral habenulae is followed by reduced 5-HT and 5-HIAA levels in the dorsal raphe nucleus (Shen et al., 2019). Furthermore, unstressed rats treated with DNMTi shown similar effects to the chronically stressed rats including reduced AMPA receptor expression in the prefrontal cortex (Wei et al., 2020).

Such differences can reflect the differential expression of DNMT subtypes in the brain associated with the lack of selectivity of the drugs for the different DNMTs (Xu et al., 2016, Zhou et al., 2018). This is supported, for example, but studies in which conditional deletion of DNMT1 or DNMT3 produced different behavioural results, as shown by Morris and colleagues (Morris et al., 2016). In this study, conditional forebrain knockout DNMT1 mice presented anxiolytic and antidepressant-like behaviour in different animal models, whereas DNMT3a knockout mice did not show any significant behavioural changes in the same tests. This study highlights the importance of developing selective drugs that could allow the investigation of the differential participation of DNMTs subtypes as possible therapeutic targets in depression. Another possibility would be to use epigenome editing tools to selectively target the different DNMTs and therefore regulate DNAm in a more specific way. In a recent work developed by Lin et al. (2018), DNMT domains were fushioned to the nuclease-deficient clustered regularly interspaced short palindromic repeat (CRISPR) associated protein 9 (Cas9) as a way to regulate DNAm induced only by DNMTa. Similar approaches of gene therapy could represent a new perspective for targeting DNAm in stress-related psychiatric disorders, including depression.

Limitations

The data are consistent in the context of stress, depression, and antidepressant drugs modulate DNAm. However, contradictory results are currently found. It has been suggested that DNAm alterations are influenced by diverse factors, including the developmental stage, the methylated region of gene, tissue, region, cell type, sex, age, and stressor (type, duration, and intensity). To date, the majority of studies evaluate a limited number of regions and sites within a specific gene. Still, generally, studies investigating DNAm in the brain and peripheral blood use whole tissue and brain region, lysates, or only leukocytes without considering cell type-specific DNAm changes. In fact, methylation studies are typically performed in tissue containing multiple cell types. However, the analysis of cell-type specificity on DNAm is relevant for better interpretation of results, the understanding of stress responses and neurobiology of depression, and identifying new antidepressant targets since the neuronal diversity in the brain (Tasic et al., 2018) and microcircuit cell type specific related to stress and MDD (Tremblay et al., 2016) have confusing potential in the results of studies. Cell-type-specific methylome-wide association studies identified differences in sub-populations of neurons/

https://doi.org/10.1017/neu.2021.18 Published online by Cambridge University Press

glia and granulocytes/T-cells/B-cells/monocytes for the human brain and blood samples (Chan et al., 2020). The analyses in the T-cells, monocytes, and glia showed novel MDD-methylation associations in signalling involved in the immune system, nerve growth factor (NGF) and its receptor $p75^{NTR}$, and innate immune Toll-like receptors (TLR) in both blood and brain of MDD patients (Chan et al., 2020). Moreover, the methods for determining the DNAm profile differ across studies. These differential factors could help to explain some of the contradictory results in the literature.

Perspectives and conclusions

DNAm is a promising mechanism for the prediction of biomarkers of depression and antidepressant response. Additionally, DNAm is important for understanding of MDD neurobiology, and its modulation could be used for the development of new pharmacological tools in psychiatry. Although several findings support the relevance of DNAm, many contradictory results are found, possibly because of the high specificity of DNAm confusing the interpretation of the data. Therefore, new studies using genome-wide methylation analysis of specific cell types associated with the multi-omics approach could better point to more relevant changes in DNAm associated with stress and depression suggesting that cell-type specific DNAm analyses are relevant for biological knowing providing mechanistic insights into stress and depression. The biggest challenge has been the identification of causal mechanisms since DNAm changes in specific loci suggest the relevance for depression without direct relation to underlying biological function. Moreover, the use of genome editing tools, such as CRISPR-Cas9 based DNAm modifiers, could reveal the potential of targeting the epigenome in the search for better and more effective antidepressant treatments.

Acknowledgements. This work was supported by grants from FAPESP (2018/15896-3), CAPES, CNPq, and AUFF (AUFF-E-2020-7-19).

Disclosure statement. The authors declare no conflict of interest.

Author contributions. All authors wrote and agreed on the final version of the manuscript.

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