The molecular bases of the suicidal brain

Gustavo Turecki

Abstract | Suicide ranks among the leading causes of death around the world and takes a heavy emotional and public health toll on most societies. Both distal and proximal factors contribute to suicidal behaviour. Distal factors — such as familial and genetic predisposition, as well as early-life adversity — increase the lifetime risk of suicide. They alter responses to stress and other processes through epigenetic modification of genes and associated changes in gene expression, and through the regulation of emotional and behavioural traits. Proximal factors are associated with the precipitation of a suicidal event and include alterations in key neurotransmitter systems, inflammatory changes and glial dysfunction in the brain. This Review explores the key molecular changes that are associated with suicidality and discusses some promising avenues for future research.

Suicidality
This broad term encompasses all forms of suicidal behaviour and suicidal ideation.

Suicidal ideation
This term describes the wish to die, including thoughts of actively ending one's life.

Suicidal behaviour
This term describes behaviours that result in self-injury and is generally used to refer to suicide attempts and suicide completion.

Self-harm
This broad term includes suicidal behaviour and non-suicidal self-injurious behaviours.

McGill Group for Suicide Studies, McGill University, Montreal H4H 1R3, Canada.
e-mail: gustavo.turecki@mcgill.ca
doi:10.1038/nrn3839
Published online
30 October 2014

In most developed countries, suicide ranks among the leading causes of death for individuals of all ages and it is the leading or the second most common cause of death for young people (see Further information). Owing to the availability of effective treatments for suicidality and associated psychiatric disorders, death by suicide is often avoidable[1]. However, suicide remains, to a large degree, charged with prejudice and stigma, characteristics that do not encourage individuals who experience suicidal ideation or who show suicidal behaviour to reach out and seek help. Despite the extent of its public health effects, there is generally little awareness in society about suicide's toll, which has caused suicide to be referred to as "the quiet epidemic" (REF. 2).

Suicide completion is commonly regarded as the extreme end of a continuum that also includes suicide attempts and suicidal ideation[3]. For the purpose of this Review, suicidality will be used to refer to both suicidal ideation and suicidal behaviours, and suicidal behaviours will be considered as a single entity. However, there is a debate about the relationship between these phenotypes and disagreement about the inclusion of other presentations of self-harm, such as non-suicidal self-injurious behaviours, in this continuum[4]. There is considerable variability among and within suicidality categories in terms of the level of intent to self-harm, the severity of the self-harm outcome and the associated psychopathology, among other features. Indeed, most individuals presenting with suicidal behaviour are affected by a psychiatric disorder[5,6], an association which is strongest among suicide completers; approximately 90% of individuals who die by suicide are affected by a psychiatric disorder before death, mainly by major depressive disorder (MDD), schizophrenia, substance-related disorders and/or personality disorders[6]. Of note, suicidal behaviour disorder was recently proposed as a diagnosis for consideration for the fifth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[7].

The variability among and within suicidality categories probably reflects the underlying aetiological heterogeneity of suicidality and its related psychopathology. Other variables could also be used to partly explain the observed variability in each phenotypic suicidality group. Among suicide completers, differences in psychopathology, personality traits and suicide intent between individuals may be age related; for example, impulsive aggressive traits are more strongly linked to suicide in young subjects[8,9]. In addition, sex contributes to phenotypic variability in each category; male and female suicide attempters and suicide completers show clear differences in the methods used, their underlying psychopathology and comorbid diagnoses[10,11]. Thus, models of suicidal behaviours, including biological factors predisposing individuals to and mediating these phenotypes, will probably explain only a proportion of the total phenotypic variability that is observed. Nevertheless, they are helpful as they provide important conceptual frameworks that enhance our understanding of suicide-spectrum behaviours and can guide us towards new hypotheses.

There are many models of suicide risk[12] and most are conceptually related to the initial model that was proposed by Moscicki[13] and its refined version that was presented

Non-suicidal self-injurious behaviours
Deliberate self-injury, often in the form of superficial skin cuts that are made with the intent to decrease emotional pain rather than to die.

Suicidal behaviour disorder
This disorder has recently been proposed as a condition for further study in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) and is defined by the occurrence of at least one suicide attempt with some intent to die within the last 24 months. Conditions for further study in the DSM are disorders that should be investigated and considered for its future versions.

Suicidal crisis
Period when suicidal ideation becomes acute, which is often associated with emotional instability.

Distal risk factors
Predisposing factors that occur or are expressed temporally distant from the onset of the phenotype.

Early-life adversity
(ELA). Acts by a parent or caregiver that result in physical, sexual and/or psychological abuse of a child or that lead to neglect of essential physical or psychological needs of childhood.

Treatment-emergent suicidal events
This term describes suicidal ideation or suicidal behaviour that occurs in association with treatment and is a common term used in clinical trials of antidepressants.

Childhood sexual abuse
Any completed or attempted sexual act, or exposure to sexual interactions, with or without physical contact, with a child by a caregiver.

Childhood physical abuse
The intentional use of physical force against a child that results in, or has the potential to result in, physical injury.

Parental neglect
Failure to meet a child's basic physical, emotional, medical or dental, or educational needs, or a failure to ensure a child's safety.

by Mann[14]. These models are based on the premise that suicide results from the interaction of risk factors acting distally to the suicidal event, which increase predisposition to such an event, with those acting proximally to the suicidal event, which precipitate the suicidal crisis. In this Review, I describe the relationships between such distal and proximal factors, focusing on molecular events that predispose individuals to suicidality, that mediate suicidal ideation and behaviour, and that precipitate suicidal crises, rather than on the neural circuitry of suicidal behaviour.

Distal or predisposing factors

Distal factors confer risk of suicide but have a distant temporal relationship to the suicidal crisis. As such, these factors increase predisposition to suicide rather than precipitate suicidal crises. Among distal risk factors for suicide, family history, genetic variation and early-life adversity (ELA) are the best investigated.

Familial and genetic predisposition. Many studies examining family history of suicidal behaviour, including a recent large registry study that comprised the total population of Sweden[15], as well as studies directly interviewing relatives of probands with suicidal behaviour, have shown that suicidal behaviour runs in families, with relatives of suicide probands who have died by suicide estimated to have a 3–10-fold greater risk of suicidal behaviour than relatives of control individuals[15–17]. As suicidal behaviour is strongly associated with psychopathology (see below) and psychiatric disorders also run in families, considerable effort has been made to understand to what extent the liability to suicidal behaviour is different from the liability to psychiatric disorders. In contrast to suicidal behaviour, suicidal ideation does not seem to be as clearly linked to a family history of suicidal ideation[18–20], although a family history of suicide attempt or completion does predict a higher frequency of suicidal ideation (2.41–5.1-fold greater risk)[20,21]. Studies have shown that familial transmission of suicide and psychopathology, although partly overlapping, are distinct[19,22,23]. Thus, familial aggregation of suicide cannot be explained by the transmission of psychopathology[23].

Evidence that the familial aggregation of suicidal behaviour is at least partly due to genetic factors has been provided by several twin studies, which have yielded variable estimates of heritability but have consistently indicated an increased concordance of such behaviour in monozygotic twins compared with dizygotic twins[15,16]. Adoption studies have shown concordant results, finding that biological relatives of adoptees who had committed suicide have a higher rate of suicide than relatives of adoptees who had died by other causes[15,24], and that parental suicide confers a similar level of suicide risk on adopted-away and non-adopted offspring[25].

Genetic variation. Guided by the genetic epidemiological data suggesting that genes increase predisposition to suicidal behaviour, many molecular genetic studies have been carried out over the past two decades to identify the genetic variants that may be used to explain liability to suicide. As for other psychiatric phenotypes, most studies used a candidate gene association approach and have particularly focused on genes coding for components of the serotonergic system, given the evidence suggesting that suicidal behaviour is associated with decreased serotonergic neurotransmission[26,27]. In particular, the genes coding for the serotonin transporter tryptophan hydroxylase and monoamine oxidase A have been widely studied in molecular analyses owing to their well-defined roles in serotonin metabolism[26,28]. Unfortunately, given the lack of consistent findings between studies, the evidence from candidate gene-based association studies told us little about the contributions of specific genes.

Genome-wide association studies (GWASs) of suicidal behaviour or of treatment-emergent suicidal events have more recently been carried out to identify novel loci (see Supplementary information S1 (table))[29–34]. Although these have mostly produced inconclusive or inconsistent results, it is notable that such approaches facilitate an unbiased analysis of molecular pathways that are implicated in disease and are an important method to use. For example, a GWAS identified 14 single-nucleotide polymorphisms (SNPs) that were associated with treatment-emergent suicidal ideation in patients who had been treated with antidepressants[30], and another study examining suicide completers identified seven genes that were differentially expressed in suicide completers compared with control individuals but not in individuals with mood disorders[35]. However, most of the GWASs used fairly small samples that lacked the power to detect effects other than major gene effects and their results require confirmation. In many of the large-scale GWASs that have been carried out to date, SNPs that have been identified to be of interest in the discovery phase of studies have not been identified as such in replication cohorts[31–34,36].

Early-life adversity. Several lines of evidence support a strong relationship between ELA — such as experiences of childhood sexual abuse, childhood physical abuse and parental neglect — and lifetime risk of suicidal behaviour. This evidence comes from prospective cohort studies that have investigated epidemiologically representative samples[5,37], and retrospective cross-sectional studies that have investigated epidemiological and clinical samples[38,39]. Maltreatment during early development is also among the strongest predictors of psychiatric pathology and of a severe clinical course for psychiatric disorders, including early onset of illness, poor treatment response and increased comorbidity[40–42]. However, the specific association between ELA and lifetime risk of suicidal behaviour is robust and cannot solely be explained by the link between psychiatric disorders and childhood maltreatment[43,44].

Between 10% and 73% of individuals who manifest suicidal behaviour have a history of childhood abuse (the rate depends on the type and frequency of the abuse, and the form of suicidal behaviour)[37,45–47]. Thus, this association can only be used to explain a proportion, albeit a notable to substantial one, of the total variance in risk of suicidality. A few important factors determine the strength of the relationship between childhood abuse and

Attachment styles
Stereotypical interpersonal styles that are rooted in early-life interactions with caregivers.

Biological embedding
The effects of early-life experiences on the differential regulation of biological systems and development.

Mediators
Variables that can fully or partially explain the relationship between a predictor and a dependent variable.

suicidal behaviour, including the sex of the abused, the identity of the abuser and the frequency of the abuse. For example, the risk of suicidal behaviour is higher among individuals who have experienced abuse by close caregivers than among individuals who have experienced abuse by extended family members or unrelated individuals, and frequent abuse increases the risk of suicidal behaviour[44]. During development, immediate caregivers are responsible for the establishment of adequate attachment styles and appropriate emotional responses to environmental and stress-related stimuli throughout the life of the individual[48,49]. Attachment styles define the ways in which individuals relate to others and can be broadly divided into secure and insecure attachments, with insecure attachments being characterized by anxiety or avoidance[50]. Thus, frequent exposure to maltreatment by those who are supposed to provide protection and care may signal that the environment is hostile. These signals in turn may prompt the developing brain to adjust stress-response circuits to adapt to this hostile environment by increasing levels of alertness. In support of this hypothesis, studies suggest a relationship between poor parent–child attachment, maladjustment in the parental role and childhood abuse, and suicidal behaviour[37,50].

Biological systems affected by early-life adversity. Biological embedding refers to the effects of early-life experiences on the differential regulation of biological systems and development[51]. Important insight into systems and pathways that undergo biological embedding and the underlying molecular mechanisms responsible for this has come from animal studies that clearly suggest that variation in the early-life environment associates with stable expression changes in genes that are important for key behavioural and stress responses. Indeed, animal studies have shown that epigenetic processes, particularly DNA methylation of various genes in different brain structures, mediate biological embedding (BOX 1).

Findings from animal studies have led to a growing interest in the investigation of epigenetic factors — primarily DNA methylation — as possible mediators of biological embedding resulting from ELA in humans. Although theoretically appealing, studying methylation changes caused by ELA in humans and associating them with behavioural and emotional phenotypes poses several operational and logistical challenges. Epigenetic changes are tissue and cell-type specific but sampling brain tissue from living subjects is impossible. Thus, epigenetic studies on human brains are limited to post-mortem specimens. Although post-mortem studies are informative, it is difficult to establish temporal relationships between epigenetic marks and behavioural phenotypes from such studies. By contrast, peripheral samples are less challenging to obtain but the extent to which they are informative about processes occurring in the CNS is under debate. In spite of these challenges, several epigenetic studies have been carried out investigating CNS and peripheral samples from individuals who have been exposed to ELA.

Of particular interest in the context of suicidal behaviour is the regulation of the hypothalamus–pituitary–adrenal (HPA) axis. The activation of the HPA axis leads to cortisol release, which is a steroid hormone secreted in response to stress that promotes arousal and attention, among other physiological changes[52]. Cortisol also binds to the glucocorticoid receptor in the hippocampus to inhibit further stimulation of the HPA axis in a negative feedback loop. The HPA axis is overactive in individuals exposed to ELA, who are more likely than control individuals to show heightened stress responsiveness[53]. As such, nuclear receptor subfamily 3 group C member 1 (*NR3C1*), which encodes the glucocorticoid receptor, has been widely studied. Several animal studies have suggested that the early environment regulates *Nr3c1* through DNA methylation changes (BOX 2). The first evidence for an effect of ELA on the human epigenome indicated that childhood abuse or severe neglect in humans regulates both the patterns and the levels of *NR3C1* methylation in the hippocampus, which can lead to increased HPA axis reactivity in humans who have been exposed to childhood maltreatment[54]. Individuals who died by suicide and had histories of severe childhood abuse had a marked decrease in the levels of both total *NR3C1* mRNA and untranslated exon 1_F variant *NR3C1* mRNA compared with individuals who died by suicide and did not have a history of childhood abuse and non-suicide controls. This decreased mRNA expression was associated with increased methylation in the exon 1_F promoter, particularly in a region where the transcription factor nerve growth factor-induced protein A (NGFIA; also known as EGR1) binds, a finding that is in line with evidence from rats (BOX 1). Studies carried out *in vitro* suggested that this increased methylation that was observed in suicide completers with histories of abuse could interfere with NGFIA binding of the *NR3C1* promoter, resulting in decreased glucocorticoid receptor expression[54].

Box 1 | **Biological embedding through DNA methylation**

Biological embedding refers to the effects of early-life experience on the regulation of biological systems[51]. Animal studies have provided important insight into the mechanisms that can be used to explain biological embedding. One of the best-investigated models is the maternal care model in rats, which makes use of naturally occurring variations in quantifiable behaviours (for example, licking and grooming, and arched-back nursing) in nursing rat mothers. Variations in maternal care have marked and stable effects on endocrine and behavioural responses in the offspring; for example, increased tactile stimulation by mothers dampens the hormonal response to stress[202] and reduces behavioural fearfulness[103]. Other animal models, such as induced maternal abusive behaviours in rats[74] or early-life stress in mice[203], have shown similar effects. Subsequent studies in animals have shown that epigenetic processes, particularly DNA methylation involving different genes and brain structures, mediate biological embedding[204].

DNA methylation refers to the addition of a methyl group (CH_3) to the 5′ carbon of a cytosine base. DNA methylation occurs commonly, but not exclusively, at sequences composed of cytosines followed by guanines (known as CpG dinucleotides). Cytosine methylation is generally associated with transcriptional repression, which occurs by interference with the recruitment and binding of transcriptional machinery to gene regulatory regions[205]. However, in studies identifying DNA methylation within the gene body, it has been associated with transcriptional activation and alternative transcript selection[206]. DNA methylation is theoretically dynamic, but this is only the case for a minority of CpGs[207] and thus DNA methylation is generally a stable process that is involved in long-term gene silencing, including X chromosome inactivation, suppression of alternative promoters and tissue-specific suppression of gene regulation.

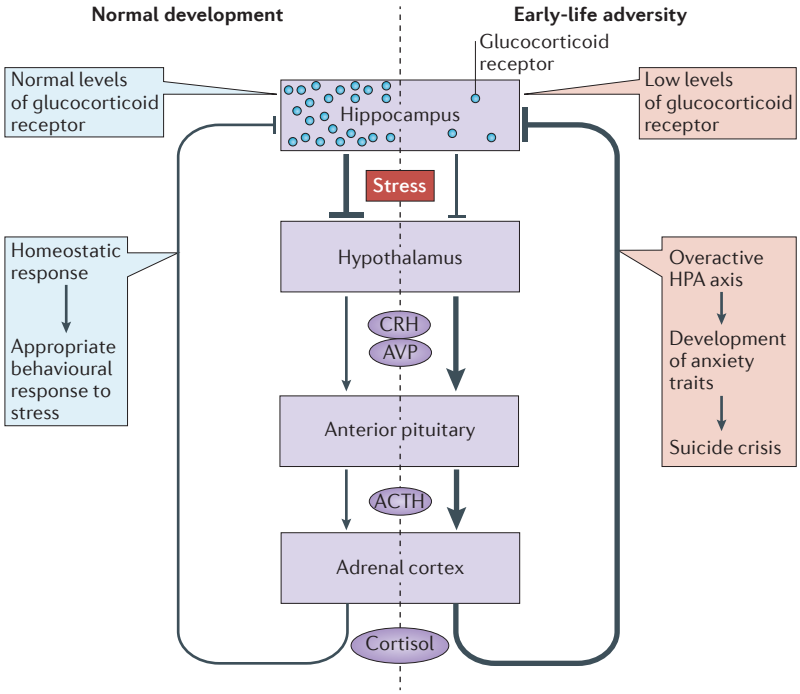
Since the initial study linking glucocorticoid receptor methylation in the brain with childhood abuse was published[54], several independent studies have been carried out to investigate the relationship between *NR3C1* exon 1 methylation, ELA and suicidal behaviour or psychopathology, including phenotypes that are associated with altered parental care[55–60] (see Supplementary information S2 (table)). Although these studies were carried out using different study designs, measures of adversity and tissue samples, there is a remarkable consistency

Box 2 | **Effect of early-life adversity on the hypothalamus–pituitary–adrenal axis**

The hypothalamus–pituitary–adrenal (HPA) axis is activated by stress (see the figure). Following activation, the hypothalamus secretes corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), which act on the anterior pituitary gland to trigger the release of adrenocorticotropic hormone (ACTH). This stimulates the adrenal cortex to produce cortisol, which heightens alertness. Cortisol-mediated activation of glucocorticoid receptors in the hippocampus exerts negative feedback on activity of the HPA axis. The glucocorticoid receptor-encoding gene, nuclear receptor subfamily 3 group C member 1 (*NR3C1*), is downregulated in the hippocampus of individuals who have been exposed to early-life adversity (ELA), which leads to ineffective inhibition of CRH secretion and an overactive HPA axis. This overactive HPA axis may be associated with the development of anxiety traits, which in turn are mediators of suicidal behaviour risk.

In humans, *NR3C1* mRNA may contain one of 11 untranslated splice variants of exon 1, which are encoded by seven different variants of exon 1, each with its own promoter, and each mRNA variant has a unique tissue-specific distribution[208]. There is remarkable homology between the structure of exon 1 in humans and rats[208,209], and mRNAs containing exon 1_7 (in rats) and 1_F (in humans) are highly expressed in the hippocampus[208]. CpG methylation levels in the exon 1_7 promoter region are markedly increased at almost all CpGs in offspring raised by low licking and grooming rat mothers, whose increased physical attention to pups results in attenuated stress responses (BOX 1) compared with offspring raised by high licking and grooming mothers. Importantly, one CpG nucleotide located in the 5′ end of the binding site of the transcription factor nerve growth factor-induced protein A (NGFIA) is methylated in almost all offspring raised by low licking and grooming mothers, whereas it is almost never methylated in offspring from high licking and grooming mothers[204].

Other animal studies investigating the effects of early-life environments on behavioural phenotypes have shown comparable results in genes encoding components of the HPA axis or other key gene systems. For example, in mice, maternal deprivation (that is, placement in a cleaned cage devoid of maternal odour for 3 hours per day for the first 10 days of life) produces sustained hyperactivity of the HPA axis that is characterized by increases in stress-induced corticosteroid secretion and in the expression of pro-opiomelanocortin (POMC) in the pituitary gland, and hypertrophy of the adrenal glands[203]. These changes in POMC expression are associated with sustained *Pomc* promoter hypomethylation[210]. These mice also present increased despair-like behaviour and memory deficits, phenotypes that are mediated through AVP signalling and epigenetic adaptations at an AVP enhancer locus. Accordingly, mice subject to early-life stress present a persistent increase in AVP expression in the hypothalamic paraventricular nucleus (PVN), which associates with decreased DNA methylation of an *Avp* enhancer element located in the intergenic region between *Avp* and the gene encoding oxytocin, where methyl-CpG-binding protein 2 (MECP2) binds. As a result, MECP2 occupancy of this *Avp* regulatory sequence is decreased, leading to sustained increased AVP expression[203]. Such overexpression of AVP can result in HPA axis hyperactivity and altered coping mechanisms that resemble depression in humans. In adult animals, decreased AVP promoter methylation in the amygdala is associated with active coping mechanisms after exposure to stressful stimuli[211], and AVP expression is associated with aggressive behaviour[212–214]. Collectively, animal studies on early-life environmental variation[74,204] indicate that the early environment epigenetically regulates diverse genomic loci that are important in the regulation of emotional and behavioural traits of relevance to depression and suicide.



in their findings, which mainly show increased levels of methylation in *NR3C1* exon 1_F among individuals exposed to less favourable environmental experiences during early life.

Epigenetic processes are essential in the differentiation of cells and tissues, as well as in the developmental regulation of genes. Thus, there is a predictably higher variability when comparing epigenetic patterns between tissues from the same individual than when comparing the same tissue from different individuals[61]. However, there is also evidence of within-individual epigenetic variant correlation across tissues[61]. The consistency in the *NR3C1* methylation findings observed using different tissues suggests that social adversity may activate systemic signals, such as steroids or other hormones, that may affect epigenetic patterns in multiple tissues.

A different line of evidence suggesting that ELA regulates the activity of the HPA axis through differential methylation comes from studies investigating FK506-binding protein 5 (FKBP5). This protein regulates the glucocorticoid receptor by decreasing its capacity to bind glucocorticoids, which hampers the ensuing translocation of the glucocorticoid receptor complex to the nucleus. Certain sequence variants of *FKBP5* put individuals at increased risk of depression and post-traumatic stress disorder[62,63], and of showing suicidal behaviour[64–66] or experiencing suicidal events in the course of antidepressant treatment[67,68]; the link with suicidality is particularly strong for subjects with a history of ELA[69,70]. Interestingly, one study reported that the interaction between the *FKBP5* genotype and ELA occurs through decreased methylation affecting functional glucocorticoid response elements located in intron 7 of *FKBP5*, which results in a downstream increase in FKBP5 expression and glucocorticoid receptor resistance[71].

ELA also differentially regulates genes and pathways involved in neuronal plasticity. For example, genome-wide methylation studies in hippocampal tissue from individuals who died by suicide and had histories of ELA indicated that ELA was associated with differential methylation of genes involved in neuronal growth and neuroprotection[57,72]. Studies in peripheral samples from living subjects also suggested that a history of ELA was linked to differential methylation of neuronal plasticity genes[73]. These findings are concordant with animal studies that support early-life environmental regulation of plasticity genes, such as the gene coding brain-derived neurotrophic factor (BDNF). Experiments carried out in rodents showed that when gestating females were put under conditions of stress (in this case, introduction to a new environment with inadequate bedding material), the expression of *Bdnf* was dysregulated. Specifically, *Bdnf* mRNA expression was decreased in the prefrontal cortex, and this decreased expression correlated with hypermethylation of the *Bdnf* promoters in exons 4 and 9 (REF. 74). Similar effects have been reported for the *Bdnf* exon 4 promoter in the dorsal hippocampus in an adult rat model of post-traumatic stress disorder[75].

Another neurotrophic factor, glial cell line-derived neurotrophic factor (GDNF), is also epigenetically regulated by environmental experience in rodents through chromatin modification and DNA methylation of its gene promoter, and these changes are associated with behavioural responses to chronic stress[76]. However, these methylation changes in *Gdnf* were observed in adult mice, which thus suggests that the epigenetic programming of neurotrophic factors such as GDNF may not be limited to developmental periods. It remains unclear whether the epigenetic changes that occur as a result of ELA are of a similar quality, intensity and stability if they occur during childhood or later in life (BOX 3).

Box 3 | **Stability of DNA methylation changes over time**

In well-characterized brain circuits, brain plasticity is considerably more pronounced during childhood than in later years[215], and clinical studies suggest that abusive experiences during early-life are more likely to result in pervasive behavioural phenotypes than similar events experienced in adulthood[216–218]. However, the relationship between timing of the stressor, epigenetic effect and behavioural consequences remains one of the most crucial questions in epigenetic studies of mental health phenotypes.

Recent publications have begun to explore longitudinal analyses of methylation in relation to the subject's environment. Although the stability of epigenetic changes over time has not yet been clearly shown, a study evaluating methylation at a wide range of loci indicates that DNA methylation is likely to be stable over long periods of time (11–20 years)[219]. Although this finding requires confirmation, it is interesting to note that the authors specifically examined genes involved in the stress response — nuclear receptor subfamily 3 group C member 1 (*NR3C1*) and corticotropin-releasing hormone (*CRH*) — and found their methylation status to be stable over time. In addition, emotional adversity during childhood effects long-term changes in methylation. In a study evaluating monozygotic twins at 5 and 10 years of age, Ouellet-Morin and colleagues[220] showed that children who had experienced bullying had increased methylation at the serotonin transporter (*SERT*) compared with their non-bullied monozygotic twin[220]. Interestingly, this study also showed that individuals with increased *SERT* methylation showed a decreased cortisol response to stressful situations, which suggests a link between the serotonergic response and the hypothalamus–pituitary–adrenal axis. Lasting changes in DNA methylation also seem to occur into adulthood, as shown by recent studies on post-stroke depression. In patients who had suffered a stroke, those experiencing post-stroke depression or worsening depression during the year following their stroke had increased methylation of the genes encoding brain-derived neurotrophic factor (*BDNF*) and solute carrier family 1 member 2 (*SLC6A4*)[221,222]. Such examples of long-lasting epigenetic changes help us to understand how distal events, such as early-life adversity, can contribute to increased suicide risk, and how proximal events, such as stressful life events, can precipitate suicidal acts by altering gene expression profiles in the brain that have a marked effect on behaviour.

BDNF and the gene that encodes its high-affinity receptor, tropomyosin-related kinase B (TRKB), have received considerable attention in human studies of suicidal behaviour, although most of these studies have not examined whether the participants had a history of ELA. Some of these studies have revealed that suicide completers have decreased mRNA and/or protein levels of BDNF, TRKB or both in the prefrontal cortex[77–80], temporal cortex[81] and hippocampus[77,82]. Moreover, others have reported differential methylation patterns in regulatory regions of *BDNF* and *TRKB*[79,82]. Specifically, in the Wernicke area of suicide completers, four CpG sites located downstream of transcription initiation site of the promoter in exon 4 of *BDNF* were more highly methylated in suicide completers than in control individuals, and this increased methylation was associated with markedly lowered BDNF expression, which is consistent with the expected repressive effects of methylation in promoter sequences on transcription[83]. In line with these findings, recent studies have reported that living patients with depression show differential methylation of the *BDNF* promoter in peripheral samples[84–86]. In addition, studies have revealed that suicide completers have differential methylation in the promoter[79] and in a transcript-specific 3′ untranslated region (UTR)[80] of *TRKB* in the prefrontal cortex.

In addition to hypothesis-driven studies, a growing number of genome-wide studies have investigated changes in the methylome that are associated with ELA and/or suicide. In spite of the inherent sources of variation when measuring methylation levels[87] and the relatively small sample sizes of these studies, particularly in comparison to those of GWASs, it is noteworthy that epigenome-wide association studies have identified methylation differences at several genomic loci. Studies using peripheral samples[73,88] and brain samples[57,72] have shown differential methylation in gene pathways involved in stress, neural plasticity and cognitive processes. Studies that investigated brain tissue and that carried out cell sorting[57,72] found that these differences were mostly accounted for by methylation changes in neuronal DNA. These strong associations between ELA and DNA methylation show long-lasting changes in the regulation of gene expression that are triggered by early environment experiences and these changes are strongly linked to suicidal behaviour[89,90].

Mediators of suicide risk

Distal factors affect biological systems and, in turn, the resulting changes in these systems can increase the risk of suicide. Thus, distal factors do not directly cause suicidal behaviour. Indeed, if they did, these factors would have been subject to selective pressures and progressively eliminated. Moreover, if distal factors had a direct link with suicide risk, ELA would have a more specific relationship with suicidal behaviour, instead of being linked to an increased risk of several negative mental health outcomes. Distal factors are therefore thought to act indirectly, through developmental traits, which may be intermediate phenotypes, or endophenotypes, for suicidality[91,92].

Although it is unclear which specific genes contribute to the total genetic variance increasing predisposition to suicidal behaviour, family studies have consistently indicated that familial aggregation for suicidal behaviour is partly explained by transmission of impulsive aggressive traits. Familial clustering of suicidal behaviour is increased in families in which probands have higher levels of impulsive aggressive traits than in those in which probands have lower levels of such traits[19]. Moreover, compared with control individuals, relatives of suicide completers have elevated levels of impulsive aggressive traits and are themselves more likely to have histories of suicidal behaviour[22,23]. In addition, impulsive aggressive traits show evidence of familial loading in families of suicide completers and mediate the relationship between family history and suicidal behaviour[23]. Thus, the transmission of behavioural traits (or endophenotypes[91]) such as impulsive aggressive behaviours is probably explained by familial, and possibly genetic, susceptibility for suicidal behaviour.

In studies examining the link between endophenotypes and suicidality, anxiety, impulsivity and aggressive traits correlate with suicidal behaviour[23,37,93]. These findings have been supported by clinical and epidemiological studies on samples that are representative and non-representative of the general population[94,95]. Although they are more difficult to carry out than cross-sectional studies, longitudinal studies using multiple observation points offer stronger evidence of causal relationships and allow temporal relationships between different variables to be examined. A series of longitudinal and trajectory studies have investigated predictors of suicidal behaviour[37,44,45,96–98]. Collectively, this work shows that high levels of childhood anxiety and disruptive behaviour (characterized by impulsivity, aggression, hyperactivity and oppositional behaviour) are linked to suicidal behaviour in adulthood[93]. Supporting these findings, cross-sectional studies and two-point longitudinal studies have shown that stable anxiety and impulsive aggressive behaviour are associated with suicidality in adulthood[99–101].

ELA is likely to function through developmental dysregulation of behavioural and emotional traits. In animal studies, changes in the early-life environment correlate with stable behavioural phenotypes[102,103]. For example, in non-human primates, social deprivation leads to sustained aggressive behaviour[102]. In humans, individuals with histories of ELA frequently show dysregulation of emotional and behavioural traits such as internalizing and externalizing disorders[104], and the personality trait phenotypes of individuals with histories of ELA share characteristics with those that are seen among individuals with suicidal behaviour[37,105]. However, this association is mitigated by other variables, such as sex and underlying disorders. High-anxiety trajectories fully mediate the relationship between ELA and suicide attempts among individuals with externalizing disorders, such as attention-deficit and hyperactivity and oppositional defiant and conduct disorders; among individuals without externalizing disorders, this mediation, although still marked, is only partial[90]. The relationships between these mechanisms may be used to explain the link between ELA and

Endophenotypes
Traits that associate with an illness in the population, are heritable, state independent, and co-segregate with the condition investigated and are present in non-affected family members of affected individuals at a higher rate than in the general population.

Impulsive aggressive behaviours
The tendency to react with animosity or overt hostility without consideration of the possible consequences when piqued or under stress.

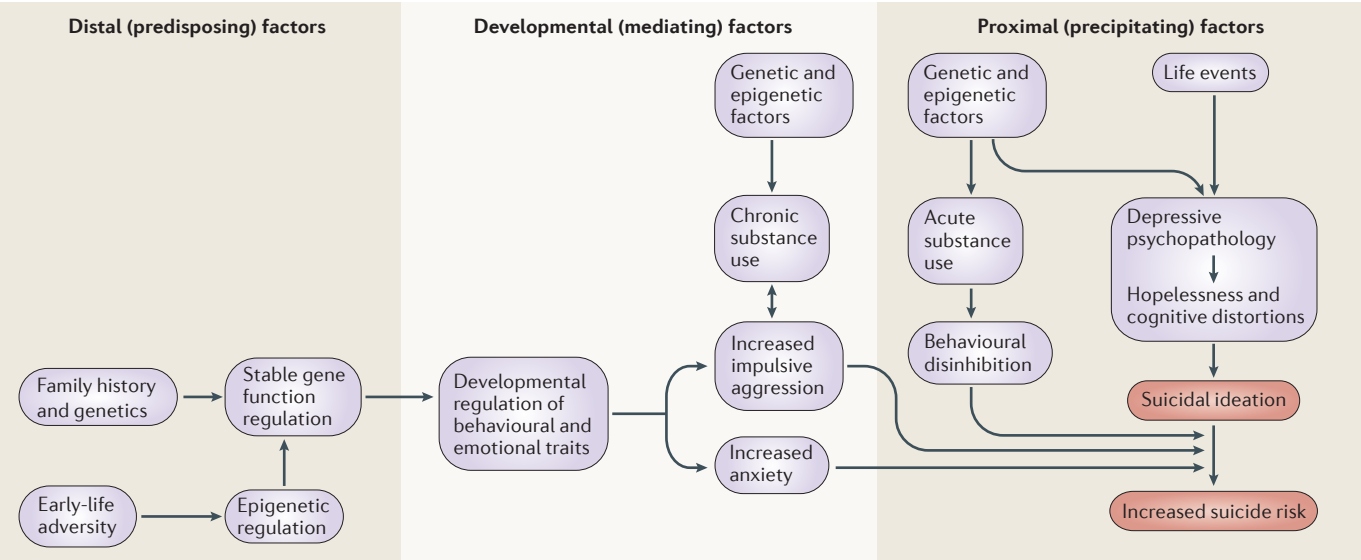


Figure 1 | **Overview of the contributors to suicidal behaviour.** Suicidal behaviour is regulated by several different factors that can be broadly categorized as distal (predisposing), developmental (mediating) or proximal (precipitating) factors. The model proposed in this Review takes into account the classic psychosocial and genetic risk factors for suicide and integrates newer findings on epigenetic changes associated with suicidality. Predisposing factors include family history of suicide and associated genetic predisposition, and early-life adversity and associated epigenetic changes. In both cases, predisposing factors lead to long-term effects on gene expression and regulation. Predisposing factors do not directly trigger suicidal events but are linked to increased suicide risk through the effects of mediating factors. Mediating factors — which may directly result from the gene changes that occur as a consequence of predisposing factors or which may be associated with other factors, such as chronic substance abuse — increase the risk of suicide by accentuating traits linked with suicidality. Specifically, family disposition and early-life events can shape behavioural and emotional traits such as impulsive aggressive behaviour and anxiety traits, increasing the risk of acting on suicidal ideation, which is a common feature of depressive psychopathology and hopelessness. Proximal risk factors, such as depressive psychopathology and acute substance abuse, can also be associated with genetic and epigenetic factors, and are often triggered by life events.

the development of high-anxiety trajectories (FIG. 1). Similarly to high-anxiety trajectories, highly disruptive trajectories, which are characterized by high levels of impulsive aggressive traits, hyperactivity and oppositional behaviour, partially mediate the relationship between ELA and suicide attempts among females but not among males[90].

Approximately 40% of individuals who die by suicide have lifetime histories of alcohol dependence or abuse and around 25% have histories of illicit substance dependence or abuse[8]. Exact rates vary globally and between studies[6], but there is a robust association between chronic substance use disorders and suicide. Impulsive traits strongly associate with substance use disorders and, although it is difficult to establish in human studies whether impulsivity is a cause or an effect of the chronic use of substances, animal experiments suggest that chronic substance use leads to increased impulsivity[33]. Interestingly, suicide cases that were associated with a high number of distal (predisposing) risk factors had particularly high levels of comorbidity with substance use disorders[106,107]. Thus, chronic use of substances may further increase the levels of impulsive aggressive traits among individuals at risk of suicide. Consistent with this hypothesis, suicide completers with comorbid substance use disorders had higher levels of impulsive aggressive behavioural traits than those without such disorders[108].

The molecular factors underlying or correlating with mediators have not been well characterized to date but there are a few promising leads. Impulsive aggressive behaviours are associated with low levels of serotonin[109], as discussed below, and suicidal behaviour is associated with low levels of cholesterol[24,110–112]. Overall, studies exploring the link between cholesterol levels and suicidal behaviour have indicated that subjects with low serum cholesterol levels are at higher risk of suicidal behaviour, increased impulsive aggressive behaviour, violence and violent methods of suicide attempt (reviewed in REF. 24). The mechanisms underlying these relationships are unclear but brain cholesterol is essential for continued neural plasticity and it is tempting to speculate that predatory behaviours such as impulsive aggression are modulated to some degree by dietary needs, which correlate with serum lipid levels.

Proximal or precipitating factors

Suicidal crises are typically triggered by recent life events[113] and are strongly associated with the onset of episodes of psychopathology. Thus, approximately 90% of individuals who die by suicide meet criteria for a psychiatric disorder in their last 6 months of life, according to a large number of retrospective, proxy-based studies (known as psychological autopsies)[6], epidemiological and registry-based studies[114], and studies in clinical

at-risk populations[115]. Although only an average of 50% of individuals who die by suicide meet the criteria for MDD (a proportion that increases with age) at the time of death[6], almost all individuals who are suicidal, regardless of their main psychiatric diagnosis, present some degree of suicidal ideation, hopelessness or other similar cognitive distortions that are characteristic of depressive states. When these cross-nosological depressive symptoms drastically alter the individual's problem-solving capacity and judgment, they may be interpreted as precipitants of suicidal crises. These depressive states are associated with molecular changes that are likely to underlie proximal risk factors for suicide and the most important of these are reviewed below.

Serotonergic alterations. Decreased serotonergic neurotransmission mediates depressive states. Almost four decades ago, Asberg and colleagues[116] suggested that, among patients with depression, those with lower cerebrospinal fluid (CSF) levels of the serotonin metabolite 5-hydroxyindoleacetic acid (5HIAA) are more likely to show suicidal behaviour[116]. Subsequently, important post-mortem studies indicated altered serotonergic binding in the frontal cortex of suicide completers[117,118] and, since then, researchers have extensively investigated serotonergic changes in suicide and related behaviours (reviewed in REF. 14). A detailed discussion of this extensive body of work is beyond the scope of this Review. One of the most consistent findings suggests that responses to challenges with fenfluramine, which is a potent serotonergic agonist, is blunted among living subjects who attempted suicide[109]. In addition, post-mortem work using the brain cortex of suicide completers revealed decreased availability of the serotonin transporter[117], and both post-mortem binding studies and *in vivo* studies using positron emission tomography (PET) have reported altered 5HT1A and 5HT2A receptor availability in suicidal brains, which suggests that insufficient serotonin in certain areas of the brain may be linked to suicidality[26,27].

Two important questions remain in relation to the association between serotonin and suicide. The first question is to what extent are serotonergic changes in suicide explained by depressive psychopathology? Although there is some evidence suggesting that serotonin alterations may be specific to suicidal behaviour[119,120], these alterations partly overlap with those observed in the depressed brain. For example, the more pronounced serotonergic changes observed in suicide might be a function of more intense hopelessness and suicidal ideation. This key point has not been adequately addressed, particularly in post-mortem brain studies, mainly because of the methodological challenges of assessing subjective and non-behavioural symptoms of depression by means of proxy-based interviews, which is the process whereby post-mortem brain studies obtain clinical information on the subjects they investigate. Another challenge is obtaining brain tissue from individuals who were affected by depression of equivalent severity to that presented by suicide completers, but who died from other causes. The second question is to what extent are serotonergic changes associated with suicide state markers of suicidality or depression, or trait markers of predisposition? There is evidence that lower serotonergic neurotransmission is also associated with behaviours that increase suicide risk, such as impulsive aggressive traits[121], and that serotonergic genes have both shared and unique contributions to the risk for depression and suicidal behaviour[122]. However, it is also clear that changes in serotonin levels are associated with the onset of depressive and suicidal states in euthymic individuals who had previous depressive episodes[123], and in individuals at risk for depression[124]. Thus, serotonergic changes underlie proximal depressive and suicide risk factors.

The polyamine stress response. Our group and others have studied changes in the polyamine stress response (PSR) system in the context of suicide. The PSR, like the HPA axis, is implicated in physiological responses to physical, emotional and hormonal stressors, including glucocorticoids (BOX 4). The mRNA and protein levels of several components of the polyamine system are altered in cortical and subcortical brain regions of suicide completers[125–128], as well as in peripheral samples from suicide attempters[129,130] and psychiatric patients[131]. In addition, non-human primate models of depression suggest that stress induces changes in gene expression that lead to altered brain polyamine levels[132]. Spermidine and spermine N1-acetyltransferase (SAT1), which is the rate-limiting enzyme in the catabolism of polyamines, is decreased in the cortex of suicide completers[125] and may even act as a peripheral biomarker of suicidality. Epigenetic regulation of some key polyaminergic genes, which involves different epigenetic processes in both biosynthetic and catabolic polyamine genes, has been reported in suicide cases[133–136]. Although the evidence suggests differential epigenetic regulation of several polyaminergic genes in suicide, further studies are necessary to understand the external validity of these findings, their functional effect on the PSR and their relationship to peripheral markers.

Glutamatergic and GABAergic alterations. Several studies have been carried out over the past decade investigating the genome-wide transcriptomic changes that are associated with depression and suicide[137,138]. These studies typically used mRNA microarrays to compare post-mortem brain tissue from individuals who were diagnosed with MDD and died by suicide with samples from individuals with good mental health who did not die by suicide. They reported dysregulation of genes involved in glutamatergic and GABAergic signalling in diverse cortical and subcortical regions. In particular, for GABAergic signalling, genes encoding GABA type A receptor subunits and their associated binding proteins were consistently found to be upregulated, notably in the prefrontal cortex, hippocampus and anterior cingulate[137,138]. Among genes related to glutamatergic signalling, those encoding AMPA and NMDA glutamatergic receptor subunits were upregulated in the anterior cingulate and dorsolateral prefrontal cortex, whereas glutamate-ammonia ligase (*GLUL*; also known as glutamine synthetase), which is implicated

Proximal risk factors
Precipitating factors that occur or are expressed temporally close to the onset of the phenotype.

State markers
Biological, psychological, behavioural or clinical markers associated with a given phenotype.

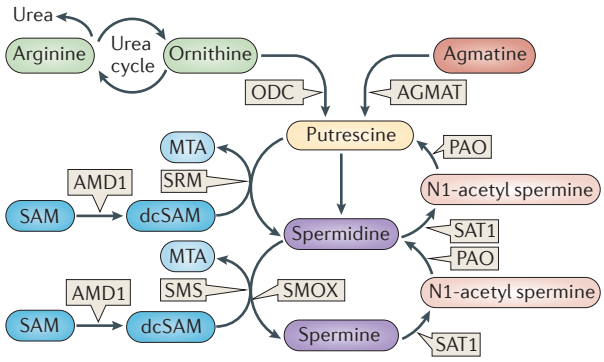
Trait markers
Biological, psychological, behavioural or clinical markers that indicate a predisposition to or risk of a given phenotype.

Box 4 | **The polyamine stress response system**

Polyamines are ubiquitous aliphatic molecules containing two or more amine (NH_2) groups. This group of molecules primarily comprises putrescine, spermidine, spermine and agmatine, the presence of which in mammalian brains was discovered relatively recently[223]. Polyamine synthesis is highly regulated, with several rate-limiting enzymes, such as ornithine decarboxylase (ODC), *S*-adenosylmethionine (SAM) decarboxylase (AMD1), and spermidine and spermine N1-acetyltransferase (SAT1). Their activities are tightly controlled by polyamine-mediated feedback loops. Other enzymes, such as spermidine synthase (SRM) and spermine synthase (SMS), may be induced in some conditions but otherwise have constant activity levels, whereas polyamine oxidase (PAO) activity is controlled by substrate availability[222] (see the figure). Ornithine is produced through the metabolism of arginine in the urea cycle, which is itself controlled by substrate-limited enzymes.

Polyamines have a multitude of functions, including the regulation of gene transcription and post-transcriptional modifications, in addition to modulating the activities of several proteins[224]. Polyamines regulate the expression or release of several neurotransmitters, including catecholamines[225], glutamate[226], GABA[227] and nitric oxide[228], and agmatine itself is thought to function as a neurotransmitter[229]. Polyamines also interact with several transmembrane channels and can thus influence the properties of excitable cells[226,230].

In the mammalian brain, stressful stimuli such as physical, emotional and hormonal stressors, including glucocorticoids, elicit the polyamine stress response (PSR)[231]. Activation of the PSR results in elevated levels of putrescine and agmatine in both the brain and the peripheral tissues. The magnitude of the PSR is related to the intensity of the stressor and correlates with levels of behavioural and physiological responsiveness under stressful conditions[231]. The PSR can be pharmacologically manipulated[232,233], and there is strong evidence showing that elevated agmatine and putrescine levels in the brain are beneficial; numerous animal studies have shown that these compounds have both anxiolytic and antidepressant effects[234], and polyamine depletion can produce altered emotional reactivity and anxiety-like behaviours[235]. Similarly, there are some encouraging pilot data in humans suggesting that agmatine has antidepressant properties[236] and, in agreement with these results, studies investigating the effects of antidepressants indicate a role for the polyamine system in the antidepressant response, particularly through allosteric binding of agmatine or putrescine to NMDA receptors[234]. AGMAT, agmatinase; dcSAM, decarboxylated *S*-adenosylmethionine; MTA, 5′ methylthioadenosine; SMOX, spermine oxidase.



in glutamate recycling, and the genes encoding the solute carriers solute carrier family 1 member 2 (SLC1A2) and SLC1A3 were downregulated in these cortical areas and in the amygdala[137,138]. GLUL and the solute carriers are of particular interest, as these are primarily expressed in glial cells, which are altered in depression and suicide (see below). Although glutamatergic receptors have multiple functions and these expression differences may indicate global expression changes that may not be specific to the underlying psychopathological processes taking place in the suicidal brain, they are consistent with growing evidence of the efficacy of glutamatergic agents as rapid-acting antidepressants that are useful in the treatment of both depressed mood[139] and acute suicidal ideation[140,141].

Inflammatory factors. Much of the data produced over the past few decades supports a relationship between inflammation and depressive states[142]. Notably, such states have been linked to increased levels of pro-inflammatory cytokines, particularly tumour necrosis factor (TNF) and interleukin-6 (IL-6)[143,146]. Furthermore, high levels of comorbidity have been observed between inflammatory autoimmune illnesses and depression, and a substantial proportion of patients undergoing therapy with cytokines, such as interferon-α (IFNα), have been found to develop depression[147–150]. In contrast to the clear evidence indicating an association between changes in inflammatory markers and depressive states, there is only sparse evidence linking these markers to suicidal behaviour, but the data seem to support an association. There are several reports of an association between suicidal behaviour — irrespective of depression status — and inflammatory cytokines (reviewed in REF. 151), with the most consistent results suggesting that patients with suicidal behaviour have an increase in levels of IL-6 and a decrease in levels of IL-2. Other inflammatory factors have been described to be altered in such individuals; for example, there have been reports of decreased levels of vascular endothelial growth factor (VEGF)[152], increased levels of quinolinic acid[153] and increased levels of kynurenic acid in patients with MDD who have a history of attempted suicide[154,155]. Although most studies investigating inflammatory markers in suicidal behaviour have used blood samples, some studies were carried out in CSF[153,156] or in post-mortem brain tissue[157–160], and the results of these studies have led to the suggestion that suicide is associated with low-grade inflammation in the brain. Glucocorticoids can blunt immune and inflammatory responses, but recent studies have shown that they can also stimulate immune responses in certain circumstances, such as during acute cellular damage or the death of brain tissue (reviewed

in REF. 161). Glucocorticoid-mediated regulation of the immune system could lead to lasting effects of ELA on immunomodulation, as has been suggested by others[162], and this is supported by the observation that victims of childhood maltreatment show heightened immune activation as adults[163].

Glial and astrocytic dysfunction. Psychiatric phenotypes have traditionally been understood to result from dysfunction in brain neurons, and glia have been primarily investigated for their role in supporting neuronal function. As neuroscientists have gained increased insight into the diversity of functions that glial cells carry out in the brain, more interest has been paid to glial cells, and particularly to astrocytes, in psychopathology. Studies investigating animal models of depression have reported glial cell alterations, such as impaired glial function[164,165], and post-mortem brain studies have suggested that glial cell counts are decreased in cortical grey matter from patients with depression[166], and that astrocytes are hypertrophied in cortical white matter from depressed individuals who died by suicide[167]. These results are in agreement with the findings from several mRNA expression studies using genome-wide microarrays in post-mortem brain tissue from depressed individuals who committed suicide, which consistently showed alterations in the expression of several astrocytic genes[137,168,169]. Among the most pronounced findings, these studies have indicated a marked downregulation of the expression of connexin 30 (*CX30*) and *CX43* — two genes encoding gap junction proteins that, in the brain, are almost exclusively expressed in astrocytes. Interestingly, $Cx30^{-/-}$ and $Cx43^{-/-}$ mice show altered reactivity to novel environments and important changes in brain neurotransmitters, including serotonin[170,171]. Other astrocyte-specific genes have been reported to be differentially expressed in suicide completers; for example, expression of the gene encoding the truncated splice variant of TRKB (TRKB.T1), which lacks the catalytic activity of the full-length protein[172], is reduced in the frontal cortex of suicide completers compared with control individuals[79]. This is consistent with results from work in mice indicating that mice overexpressing TRKB.T1 have an increased sensitivity to chronic social stress, which results in consistent social avoidance[173].

Neuroendocrine dysfunction in suicide. As discussed above, the HPA axis is dysregulated in individuals who have experienced ELA. In suicide attempters and completers, there is also evidence of HPA axis dysregulation, regardless of ELA history. Studies investigating suicide attempters have shown that HPA axis dysregulation correlates with the violence of the suicide attempt[174]. There is also evidence that HPA axis alterations may be more closely linked to suicidal behaviour than to individual psychopathologies, as the ability to suppress dexamethasone (which is a synthetic glucocorticoid used to measure the HPA axis response) predicts suicide completion[175] and suicidal behaviour in patients with MDD[176]. Post-mortem studies of suicide completers have revealed that such individuals have increased corticotropin-releasing hormone (CRH) activity in the paraventricular nucleus (PVN)[177–179], increased CRH expression in the CSF[180], fewer CRH-binding sites in the frontal cortex[181], decreased glucocorticoid receptor expression in the hippocampus[54] and increased pro-opiomelanocortin (POMC) in the pituitary[182], which indicates altered HPA axis function in the brains of suicide completers. Concordant with these findings is the observation that suicide completers have increased adrenal gland weight and adrenocortical hypertrophy[183,184], and relatives of suicide completers also show altered HPA axis responses in experimental evaluations of the stress response[185].

Considerations for future directions

Suicide is a complex behaviour that results from the interaction of different factors. This Review examines evidence focusing on molecular changes associated with distal factors that increase the lifetime predisposition to suicide; the molecular and behavioural changes that are associated with factors mediating suicide risk; and, finally, those changes that are associated with depressive states that function as precipitants of a suicidal crisis (FIG. 1). Although this Review discusses these factors as if suicidal behaviour were a single phenotype, this is not the case. There is only a partial overlap between suicide attempts and suicide completion, and there is clear clinical, phenomenological and probably aetiological heterogeneity in and between each of these phenotypes. Thus, aetiological models, such as the one discussed in this paper, are helpful as they provide theoretical relationships that can be tested, but they should not be taken at face value. In addition, many of the studies discussed in this Review focus on depression as a precursor to suicide but other psychiatric conditions, such as schizophrenia, bipolar disorder and personality disorders, are important contributors to suicidality, both in terms of suicidal ideation and suicidal behaviour[6,101]. In this regard, the recently proposed diagnosis of suicidal behaviour disorder should be considered in future research, as suggested in DSM-5. This practice will help us to more effectively separate the neurobiological factors that are associated with suicidal behaviour from those that are related to psychopathology.

One avenue of investigation that may shed some light on the molecular basis of suicidality is the study of the molecular basis of treatment-emergent suicidal events and the mechanisms of action of pharmacological agents that affect suicidality. For example, recent advances in clarifying the mechanism of action of lithium suggest that it functions by modulating dopaminergic, glutamatergic and GABAergic pathways, as well as by upregulating neuroprotective factors, such as BDNF and apoptosis regulator BCL-2, and by downregulating apoptotic factors[186]. Lithium is effective at reducing the risk of suicide in patients with mood disorders, an effect that seems to be independent of its mood-stabilizing effect[187], and it may also decrease aggression and impulsivity[188]. As such, further investigation of lithium activity in suicidal patients may identify new genes that are involved in suicidality. Similarly, studies investigating

dynamic molecular changes associated with treatment-emergent suicidal events or with fast cessation of suicidal ideation, such as those seen following treatment with rapid-acting glutamatergic agents[140], may indicate new molecular pathways of interest that will help us to understand suicide.

The studies discussed above have led to increased interest in the investigation of the epigenetic factors that are associated with both ELA and the molecular processes that mediate suicidality. However, before embarking on large sequencing-based studies, the field should consider several adjustments to the current approaches that could enhance the quality of the results generated. First, consortium-based initiatives, such as those seen in genetic variation studies of schizophrenia, should be promoted as they have recently produced encouraging results[189–191]. Second, alternative designs focusing on related phenotypes should be considered. For example, investigating simple Mendelian conditions such as Lesch–Nyhan disease[192], for which the genetic architecture is reasonably well understood and phenotypes of interest such as self-injurious behaviours are manifested, should help us to identify gene pathways of interest. Third, a major focus of upcoming research initiatives should be to improve the comparability of the data. Standardizing the way that methodologies are reported (for example, by including the exact sequences of DNA regions investigated, detailed descriptions of probes or specific stimuli and details of the parameters that were controlled for) will improve comparability and help to avoid some of the drawbacks linked to genetic variability.

Other methodological aspects to consider for studies of suicide are the use of peripheral tissues to study epigenetic changes linked to suicidality and the use of tissue homogenates. Although epigenetic studies carried out on peripheral tissues are feasible and cost-effective, their scientific value has not been well characterized and we do not know the extent to which epigenetic modifications in peripheral tissues are representative of those in the CNS. Epigenetic regulation is, to a large degree, cell- and tissue-specific[193–195], with greater epigenetic variability between different tissues of a single individual than between similar tissues of different individuals[61,196–198]. Therefore, there is a need to clearly establish the relationship between peripheral epigenetic marks and CNS counterparts, a task that should become easier once reference epigenomic maps are generated from different brain regions and cellular fractions and compared with similar reference maps from peripheral tissues. In addition, studying single cell types can be facilitated through the use of laser-capture microdissection[199] or fluorescence-activated cell sorting[200]. Alternatively, computational methods facilitating the estimation of cell populations within tissue homogenates[201] could be used to refine results from studies using whole-tissue homogenates. Finally, epigenetic studies in suicide research to date have mainly focused on DNA methylation. However, other mechanisms of epigenetic regulation have been uncovered and should be further explored in the future (BOX 5).

Such methodological improvements would allow us to confidently draw conclusions from studies on the epigenetic modifications linked to suicide and might accelerate the identification of useful biomarkers for increased risk of suicide. In the long-term, this would translate into better management of high-risk patients and improved health outcomes.

Box 5 | **Rethinking epigenetic mechanisms in suicidal behaviour**

Most epigenetic research investigating suicidal behaviours has so far focused on DNA methylation. As we gain insight into which different epigenetic marks are enriched in the brain, we should investigate the role of other epigenetic mechanisms that may be associated with suicidal behaviours, including the activity of intermediaries of cytosine methylation, histone modifications and the effects of non-coding RNAs.

Epigenetic modifications of cytosine residues lead to the formation of intermediate products of methylcytosine oxidation, such as hydroxymethylcytosine, formylcytosine and carbocytosine, all of which are enriched in brain tissue. These intermediaries are probably functional, and their potential contribution to the regulation of gene expression should be explored further. Cytosine methylation at sites other than CpG dinucleotides is also more prevalent in brain tissue than in other tissues. Most studies have so far focused on CpG methylation, and investigating the contribution of non-CpG methylation to the regulation of gene expression in brain tissue may be a promising avenue of research in suicide studies.

Histones are essential protein complexes that regulate the accessibility of DNA to transcriptional machinery by modulating the level of chromatin compaction. Post-translational modifications of the amino-terminal tails of histones change their effect on chromatin structure and activity. Specific genomic loci are linked to distinct histone modification profiles; for example, active promoters are associated with histone 3 lysine 4 (H3K4) dimethylation and trimethylation, and histone 3 lysine 27 (H3K27) acetylation and H3K4 monomethylation in enhancer regions, whereas repressed promoters are associated with H3K9 and H3K27 dimethylation and trimethylation. Specific contributions of histone modification to the emergence of suicide-related phenotypes have yet to be fully explored, but histone modification represents a ubiquitous mechanism for regulating gene transcription.

Another new aspect of epigenetic regulation of gene expression is the role of non-coding RNAs. A large proportion of the transcriptome is composed of regulatory RNAs that do not encode proteins and that instead regulate mRNA transcription, function and availability, and interact directly with DNA, regulatory proteins and enzymes. Among non-coding RNA species, long non-coding RNAs are of particular interest as they are highly expressed in the brain and are less evolutionarily conserved than other RNA species, and thus may associate with human brain processes that could be relevant to suicide research.

1. Mann, J. J. *et al.* Suicide prevention strategies: a systematic review. *JAMA* **294**, 2064–2074 (2005).
2. Duffy, A. M., The quiet epidemic. *The Ottawa Citizen, Supplement on Suicide* (2003).
3. Silverman, M. M. The language of suicidology. *Suicide Life Threat. Behav.* **36**, 519–532 (2006).
4. Kapur, N., Cooper, J., O'Connor, R. C. & Hawton, K. Non-suicidal self-injury v. attempted suicide: new diagnosis or false dichotomy? *Br. J. Psychiatry* **202**, 326–328 (2013).
5. Brezo, J. *et al.* Identifying correlates of suicide attempts in suicidal ideators: a population-based study. *Psychol. Med.* **37**, 1551–1562 (2007).
6. Arsenault-Lapierre, G., Kim, C. & Turecki, G. Psychiatric diagnoses in 3275 suicides: a meta-analysis. *BMC Psychiatry* **4**, 37 (2004).
7. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders* 5th edn (American Psychiatric Publishing, 2013).
8. Dumais, A. *et al.* Risk factors for suicide completion in major depression: a case-control study of impulsive and aggressive behaviors in men. *Am. J. Psychiatry* **162**, 2116–2124 (2005).
This study clearly shows that when depressive psychopathology is controlled for, impulsive aggressive traits are strongly associated with suicide, particularly among young suicide completers.
9. McGirr, A. *et al.* Impulsive-aggressive behaviours and completed suicide across the life cycle: a predisposition for younger age of suicide. *Psychol. Med.* **38**, 407–417 (2008).
10. Beautrais, A. L. Suicide and serious suicide attempts in youth: a multiple-group comparison study. *Am. J. Psychiatry* **160**, 1093–1099 (2003).
11. Dalca, I. M., McGirr, A., Renaud, J. & Turecki, G. Gender-specific suicide risk factors: a case-control study of individuals with major depressive disorder. *J. Clin. Psychiatry* **74**, 1209–1216 (2013).

12. O'Connor, R. C., Platt, S. & Gordon, J. (eds) *International Handbook of Suicide Prevention: Research, Policy and Practice* (John Wiley & Sons, 2011).
13. Moscicki, E. K. Gender differences in completed and attempted suicides. *Ann. Epidemiol.* **4**, 152–158 (1994).
14. Mann, J. J. Neurobiology of suicidal behaviour. *Nature Rev. Neurosci.* **4**, 819–828 (2003).
15. Tidemalm, D. *et al.* Familial clustering of suicide risk: a total population study of 11.4 million individuals. *Psychol. Med.* **41**, 2527–2534 (2011).
16. Baldessarini, R. J. & Hennen, J. Genetics of suicide: an overview. *Harv. Rev. Psychiatry* **12**, 1–13 (2004).
17. Turecki, G. Suicidal behavior: is there a genetic predisposition? *Bipolar Disord.* **3**, 335–349 (2001).
18. Brent, D. What family studies teach us about suicidal behavior: implications for research, treatment, and prevention. *Eur. Psychiatry* **25**, 260–263 (2010).
19. Brent, D. A., Bridge, J., Johnson, B. A. & Connolly, J. Suicidal behavior runs in families. A controlled family study of adolescent suicide victims. *Arch. Gen. Psychiatry* **53**, 1145–1152 (1996).
This is an important study that convincingly shows that familial aggregation of suicide is not exclusively explained by psychopathology.
20. Lieb, R., Bronisch, T., Hofler, M., Schreier, A. & Wittchen, H. U. Maternal suicidality and risk of suicidality in offspring: findings from a community study. *Am. J. Psychiatry* **162**, 1665–1671 (2005).
21. Blum, R., Sudhinaraset, M. & Emerson, M. R. Youth at risk: suicidal thoughts and attempts in Vietnam, China, and Taiwan. *J. Adolesc. Health* **50**, S37–S44 (2012).
22. Kim, C. D. *et al.* Familial aggregation of suicidal behavior: a family study of male suicide completers from the general population. *Am. J. Psychiatry* **162**, 1017–1019 (2005).
23. McGirr, A. *et al.* Familial aggregation of suicide explained by cluster B traits: a three-group family study of suicide controlling for major depressive disorder. *Am. J. Psychiatry* **166**, 1124–1134 (2009).
24. Ernst, C., Mechawar, N. & Turecki, G. Suicide neurobiology. *Prog. Neurobiol.* **89**, 315–333 (2009).
25. von Borczyskowski, A., Lindblad, F., Vinnerljung, B., Reintjes, R. & Hjern, A. Familial factors and suicide: an adoption study in a Swedish National Cohort. *Psychol. Med.* **41**, 749–758 (2011).
26. Mann, J. J. The serotonergic system in mood disorders and suicidal behaviour. *Phil. Trans. R. Soc. B* **368**, 20120537 (2013).
27. Bach, H. & Arango, V. in *The Neurobiological Basis of Suicide* (ed. Dwivedi, Y.) (CRC Press, 2012).
28. Brezo, J., Klempan, T. & Turecki, G. The genetics of suicide: a critical review of molecular studies. *Psychiatr. Clin. North Am.* **31**, 179–203 (2008).
29. Laje, G. *et al.* Genome-wide association study of suicidal ideation emerging during citalopram treatment of depressed outpatients. *Pharmacogenet. Genom.* **19**, 666–674 (2009).
30. Menke, A. *et al.* Genome-wide association study of antidepressant treatment-emergent suicidal ideation. *Neuropsychopharmacology* **37**, 797–807 (2012).
31. Perlis, R. H. *et al.* Genome-wide association study of suicide attempts in mood disorder patients. *Am. J. Psychiatry* **167**, 1499–1507 (2010).
32. Perroud, N. *et al.* Genome-wide association study of increasing suicidal ideation during antidepressant treatment in the GENDEP project. *Pharmacogenom. J.* **12**, 68–77 (2012).
33. Schosser, A. *et al.* Genomewide association scan of suicidal thoughts and behaviour in major depression. *PLoS ONE* **6**, e20690 (2011).
34. Willour, V. L. *et al.* A genome-wide association study of attempted suicide. *Mol. Psychiatry* **17**, 433–444 (2012).
35. Galfalvy, H. *et al.* A pilot genome wide association and gene expression array study of suicide with and without major depression. *World J. Biol. Psychiatry* **14**, 574–582 (2013).
36. Perlis, R. H., Ruderfer, D., Hamilton, S. P. & Ernst, C. Copy number variation in subjects with major depressive disorder who attempted suicide. *PLoS ONE* **7**, e46315 (2012).
37. Fergusson, D. M., Woodward, L. J. & Horwood, L. J. Risk factors and life processes associated with the onset of suicidal behaviour during adolescence and early adulthood. *Psychol. Med.* **30**, 23–39 (2000).
38. Angst, J., Degonda, M. & Ernst, C. The Zurich Study: XV. Suicide attempts in a cohort from age 20 to 30. *Eur. Arch. Psychiatry Clin. Neurosci.* **242**, 135–141 (1992).
39. Afifi, T. O. *et al.* Population attributable fractions of psychiatric disorders and suicide ideation and attempts associated with adverse childhood experiences. *Am. J. Publ. Health* **98**, 946–952 (2008).
40. Gilbert, R. *et al.* Burden and consequences of child maltreatment in high-income countries. *Lancet* **373**, 68–81 (2009).
41. Collishaw, S. *et al.* Resilience to adult psychopathology following childhood maltreatment: Evidence from a community sample. *Child Abuse Neglect* **31**, 211–229 (2007).
42. Lansford, J. E. *et al.* A 12-year prospective study of the long-term effects of early child physical maltreatment on psychological, behavioral, and academic problems in adolescence. *Arch. Pediatr. Adolescent Med.* **156**, 824–830 (2002).
43. Fanous, A. H., Prescott, C. A. & Kendler, K. S. The prediction of thoughts of death or self-harm in a population-based sample of female twins. *Psychol. Med.* **34**, 301–312 (2004).
44. Brezo, J. *et al.* Predicting suicide attempts in young adults with histories of childhood abuse. *Br. J. Psychiatry* **193**, 134–139 (2008).
45. Brezo, J. *et al.* Natural history of suicidal behaviors in a population-based sample of young adults. *Psychol. Med.* **37**, 1563–1574 (2007).
46. Lopez-Castroman, J. *et al.* Suicidal phenotypes associated with family history of suicidal behavior and early traumatic experiences. *J. Affect Disord.* **142**, 193–199 (2012).
47. Lopez-Castroman, J. *et al.* Early childhood sexual abuse increases suicidal intent. *World Psychiatry* **12**, 149–154 (2013).
48. Cole, P. M., Michel, M. K. & Teti, L. O. The development of emotion regulation and dysregulation: a clinical perspective. *Monogr. Soc. Res. Child Dev.* **59**, 73–100 (1994).
49. Malatesta, C. Z. The role of emotions in the development and organization of personality. *Nebr. Symp. Motiv.* **36**, 1–56 (1988).
50. Smith, P. N. *et al.* The relationships of attachment style and social maladjustment to death ideation in depressed women with a history of childhood sexual abuse. *J. Clin. Psychol.* **68**, 78–87 (2012).
51. Hertzman, C. Putting the concept of biological embedding in historical perspective. *Proc. Natl Acad. Sci. USA* **109** (Suppl. 2), 17160–17167 (2012).
52. Raison, C. L. & Miller, A. H. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *Am. J. Psychiatry* **160**, 1554–1565 (2003).
53. Heim, C., Shugart, M., Craighead, W. E. & Nemeroff, C. B. Neurobiological and psychiatric consequences of child abuse and neglect. *Dev. Psychobiol* **52**, 671–690 (2010).
54. McGowan, P. O. *et al.* Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nature Neurosci.* **12**, 342–348 (2009).
This study shows for the first time in humans that adversity in childhood can induce brain methylation changes in crucial genes.
55. Conradt, E., Lester, B. M., Appleton, A. A., Armstrong, D. A. & Marsit, C. J. The roles of DNA methylation of NR3C1 and 11β-HSD2 and exposure to maternal mood disorder *in utero* on newborn neurobehavior. *Epigenetics* **8**, 1321–1329 (2013).
56. Perroud, N. *et al.* Increased methylation of glucocorticoid receptor gene (*NR3C1*) in adults with a history of childhood maltreatment: a link with the severity and type of trauma. *Transl. Psychiatry* **1**, e59 (2011).
57. Labonte, B. *et al.* Genome-wide epigenetic regulation by early-life trauma. *Arch. Gen. Psychiatry* **69**, 722–731 (2012).
This study is the first genome-wide study that examines the effect of early-life trauma on methylation in the brains of suicide completers.
58. van der Knaap, L. J. *et al.* Glucocorticoid receptor gene (*NR3C1*) methylation following stressful events between birth and adolescence. The TRAILS study. *Transl. Psychiatry* **4**, e381 (2014).
59. Perroud, N. *et al.* Childhood maltreatment and methylation of the glucocorticoid receptor gene NR3C1 in bipolar disorder. *Br. J. Psychiatry* **204**, 30–35 (2014).
60. Melas, P. A. *et al.* Genetic and epigenetic associations of *MAOA* and *NR3C1* with depression and childhood adversities. *Int. J. Neuropsychopharmacol* **16**, 1513–1528 (2013).
61. Davies, M. N. *et al.* Functional annotation of the human brain methylome identifies tissue-specific epigenetic variation across brain and blood. *Genome Biol.* **13**, R43 (2012).
62. Binder, E. B. *et al.* Association of *FKBP5* polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA* **299**, 1291–1305 (2008).
63. Mehta, D. *et al.* Using polymorphisms in *FKBP5* to define biologically distinct subtypes of posttraumatic stress disorder: evidence from endocrine and gene expression studies. *Arch. Gen. Psychiatry* **68**, 901–910 (2011).
64. Willour, V. L. *et al.* Family-based association of *FKBP5* in bipolar disorder. *Mol. Psychiatry* **14**, 261–268 (2009).
65. Supriyanto, I. *et al.* Association of *FKBP5* gene haplotypes with completed suicide in the Japanese population. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **35**, 252–256 (2011).
66. Leszczynska-Rodziewicz, A. *et al.* Possible association between haplotypes of the *FKBP5* gene and suicidal bipolar disorder, but not with melancholic depression and psychotic features, in the course of bipolar disorder. *Neuropsychiatr. Dis. Treat.* **10**, 243–248 (2014).
67. Brent, D. *et al.* Association of *FKBP5* polymorphisms with suicidal events in the Treatment of Resistant Depression in Adolescents (TORDIA) study. *Am. J. Psychiatry* **167**, 190–197 (2010).
68. Perroud, N. *et al.* Clinical and genetic correlates of suicidal ideation during antidepressant treatment in a depressed outpatient sample. *Pharmacogenomics* **12**, 365–377 (2011).
69. Roy, A., Gorodetsky, E., Yuan, Q., Goldman, D. & Enoch, M. A. Interaction of FKBP5, a stress-related gene, with childhood trauma increases the risk for attempting suicide. *Neuropsychopharmacology* **35**, 1674–1683 (2010).
70. Roy, A., Hodgkinson, C. A., Deluca, V., Goldman, D. & Enoch, M. A. Two HPA axis genes, *CRHBP* and *FKBP5*, interact with childhood trauma to increase the risk for suicidal behavior. *J. Psychiatr. Res.* **46**, 72–79 (2012).
71. Klengel, T. *et al.* Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nature Neurosci.* **16**, 33–41 (2013).
72. Labonte, B. *et al.* Genome-wide methylation changes in the brains of suicide completers. *Am. J. Psychiatry* **170**, 511–520 (2013).
73. Weder, N. *et al.* Child abuse, depression, and methylation in genes involved with stress, neural plasticity, and brain circuitry. *J. Am. Acad. Child Adolesc. Psychiatry* **53**, 417–424 (2014).
74. Roth, T. L., Lubin, F. D., Funk, A. J. & Sweatt, J. D. Lasting epigenetic influence of early-life adversity on the *BDNF* gene. *Biol. Psychiatry* **65**, 760–769 (2009).
75. Roth, T. L., Zoladz, P. R., Sweatt, J. D. & Diamond, D. M. Epigenetic modification of hippocampal *BDNF* DNA in adult rats in an animal model of post-traumatic stress disorder. *J. Psychiatr. Res.* **45**, 919–926 (2011).
76. Uchida, S. *et al.* Epigenetic status of *Gdnf* in the ventral striatum determines susceptibility and adaptation to daily stressful events. *Neuron* **69**, 359–372 (2011).
77. Dwivedi, Y. *et al.* Altered gene expression of brain-derived neurotrophic factor and receptor tyrosine kinase B in postmortem brain of suicide subjects. *Arch. Gen. Psychiatry* **60**, 804–815 (2003).
78. Pandey, G. N. *et al.* Brain-derived neurotrophic factor and tyrosine kinase B receptor signalling in post-mortem brain of teenage suicide victims. *Int. J. Neuropsychopharmacol.* **11**, 1047–1061 (2008).
79. Ernst, C. *et al.* Alternative splicing, methylation state, and expression profile of tropomyosin-related kinase B in the frontal cortex of suicide completers. *Arch. Gen. Psychiatry* **66**, 22–32 (2009).
80. Maussion, G. *et al.* Functional DNA methylation in a transcript specific 3'UTR region of TRKB associates with suicide. *Epigenetics* **9**, 1061–1070 (2014).
81. Keller, S. *et al.* Increased BDNF promoter methylation in the Wernicke area of suicide subjects. *Arch. Gen. Psychiatry* **67**, 258–267 (2010).
82. Banerjee, R., Ghosh, A. K., Ghosh, B., Bhattacharyya, S. & Mondal, A. C. Decreased mRNA and protein expression of BDNF, NGF, and their receptors in the hippocampus from suicide: an analysis in human postmortem brain. *Clin. Med. Insights Pathol.* **6**, 1–11 (2013).

83. Zhang, X. *et al.* Genome-wide high-resolution mapping and functional analysis of DNA methylation in arabidopsis. *Cell* **126**, 1189–1201 (2006).
84. Fuchikami, M. *et al.* DNA methylation profiles of the brain-derived neurotrophic factor (BDNF) gene as a potent diagnostic biomarker in major depression. *PLoS ONE* **6**, e23881 (2011).
85. Kang, H. J. *et al.* BDNF promoter methylation and suicidal behavior in depressive patients. *J. Affect. Disord.* **151**, 679–685 (2013).
86. Kim, J. M. *et al.* Association of BDNF promoter methylation and genotype with suicidal ideation in elderly Koreans. *Am. J. Geriatr. Psychiatry* **22**, 989–996 (2014).
87. Michels, K. B. *et al.* Recommendations for the design and analysis of epigenome-wide association studies. *Nature Methods* **10**, 949–955 (2013).
This article outlines the key considerations for future genome-wide studies, focusing on epigenetic regulation; these recommendations will help to strengthen the data gathered from such large-scale studies.
88. Yang, B. Z. *et al.* Child abuse and epigenetic mechanisms of disease risk. *Am. J. Prev. Med.* **44**, 101–107 (2013).
89. Turecki, G., Ernst, C., Jollant, F., Labonte, B. & Mechawar, N. The neurodevelopmental origins of suicidal behavior. *Trends Neurosci.* **35**, 14–23 (2012).
90. Wanner, B., Vitaro, F., Tremblay, R. E. & Turecki, G. Childhood trajectories of anxiousness and disruptiveness explain the association between early-life adversity and attempted suicide. *Psychol. Med.* **42**, 2373–2382 (2012).
Through trajectory analyses, this study gathers longitudinal data to describe the behavioural phenotypes that link ELA to suicide attempts.
91. Courtet, P., Gottesman, I., Jollant, F. & Gould, T. D. The neuroscience of suicidal behaviors: what can we expect from endophenotype strategies? *Transl. Psychiatry* **1**, e7 (2011).
92. Mann, J. J. *et al.* Candidate endophenotypes for genetic studies of suicidal behavior. *Biol. Psychiatry* **65**, 556–563 (2009).
93. Brezo, J. *et al.* Childhood trajectories of anxiousness and disruptiveness as predictors of suicide attempts. *Arch. Pediatr. Adolesc. Med.* **162**, 1015–1021 (2008).
94. Brent, D. A. *et al.* Familial transmission of mood disorders: convergence and divergence with transmission of suicidal behavior. *J. Am. Acad. Child Adolesc. Psychiatry* **43**, 1259–1266 (2004).
95. Gureje, O. *et al.* Parental psychopathology and the risk of suicidal behavior in their offspring: results from the World Mental Health surveys. *Mol. Psychiatry* **16**, 1221–1233 (2010).
96. Brezo, J., Paris, J. & Turecki, G. Personality traits as correlates of suicidal ideation, suicide attempts, and suicide completions: a systematic review. *Acta Psychiatr. Scand.* **113**, 180–206 (2006).
97. Brezo, J. *et al.* Broad and narrow personality traits as markers of one-time and repeated suicide attempts: a population-based study. *BMC Psychiatry* **8**, 15 (2008).
98. Fergusson, D. M., Beautrais, A. L. & Horwood, L. J. Vulnerability and resiliency to suicidal behaviours in young people. *Psychol. Med.* **33**, 61–73 (2003).
99. Herba, C. M., Ferdinand, R. F., van der Ende, J. & Verhulst, F. C. Long-term associations of childhood suicide ideation. *J. Am. Acad. Child Adolesc. Psychiatry* **46**, 1473–1481 (2007).
100. Sourander, A. *et al.* Childhood predictors of completed and severe suicide attempts: findings from the Finnish 1981 Birth Cohort Study. *Arch. Gen. Psychiatry* **66**, 398–406 (2009).
101. Sareen, J. *et al.* Anxiety disorders and risk for suicidal ideation and suicide attempts: a population-based longitudinal study of adults. *Arch. Gen. Psychiatry* **62**, 1249–1257 (2005).
102. Suomi, S. J. Early stress and adult emotional reactivity in rhesus monkeys. *Ciba Found. Symposium* **156**, 171–183 (1991).
103. Caldji, C. *et al.* Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc. Natl Acad. Sci. USA* **95**, 5335–5340 (1998).
104. van der Vegt, E. J., van der Ende, J., Ferdinand, R. F., Verhulst, F. C. & Tiemeier, H. Early childhood adversities and trajectories of psychiatric problems in adoptees: evidence for long lasting effects. *J. Abnorm Child Psychol.* **37**, 239–249 (2009).
105. Johnson, J. G. *et al.* Childhood adversities, interpersonal difficulties, and risk for suicide attempts during late adolescence and early adulthood. *Arch. Gen. Psychiatry* **59**, 741–749 (2002).
106. Kim, C. *et al.* Patterns of co-morbidity in male suicide completers. *Psychol. Med.* **33**, 1299–1309 (2003).
107. Seguin, M. *et al.* Life trajectories and burden of adversity: mapping the developmental profiles of suicide mortality. *Psychol. Med.* **37**, 1575–1383 (2007).
108. Chachamovich, E., Ding, Y. & Turecki, G. Levels of aggressiveness are higher among alcohol-related suicides: results from a psychological autopsy study. *Alcohol* **46**, 529–536 (2012).
109. Coccaro, E. F. *et al.* Serotonergic studies in patients with affective and personality disorders. Correlates with suicidal and impulsive aggressive behavior. *Arch. Gen. Psychiatry* **46**, 587–599 (1989).
110. Freemantle, E., Chen, G. G., Cruceanu, C., Mechawar, N. & Turecki, G. Analysis of oxysterols and cholesterol in prefrontal cortex of suicides. *Int. J. Neuropsychopharmacol.* **16**, 1241–1249 (2013).
111. Lalovic, A. *et al.* Cholesterol metabolism and suicidality in Smith-Lemli-Opitz syndrome carriers. *Am. J. Psychiatry* **161**, 2123–2126 (2004).
112. Lalovic, A. *et al.* Cholesterol content in brains of suicide completers. *Int. J. Neuropsychopharmacol.* **10**, 159–166 (2007).
113. Heikkinen, M., Aro, H. & Lonnqvist, J. Recent life events and their role in suicide as seen by the spouses. *Acta Psychiatr. Scand.* **86**, 489–494 (1992).
114. Qin, P., Agerbo, E. & Bo Mortensen, P. Suicide risk in relation to family history of completed suicide and psychiatric disorders: a nested case-control study based on longitudinal registers. *Lancet* **360**, 1126–1130 (2002).
115. Angst, F., Stassen, H. H., Clayton, P. J. & Angst, J. Mortality of patients with mood disorders: follow-up over 34–38 years. *J. Affect. Disord.* **68**, 167–181 (2002).
116. Asberg, M., Thoren, P., Traskman, L., Bertilsson, L. & Ringberger, V. Serotonin depression — a biochemical subgroup within the affective disorders. *Science* **191**, 478–480 (1976).
This is the first study to suggest that there is a link between alterations in the serotonergic system and suicidal behaviour.
117. Stanley, M., Virgilio, J. & Gershon, S. Tritiated imipramine binding sites are decreased in the frontal cortex of suicides. *Science* **216**, 1337–1339 (1982).
118. Stanley, M. & Mann, J. J. Increased serotonin-2 binding sites in frontal cortex of suicide victims. *Lancet* **1**, 214–216 (1983).
119. Arango, V. *et al.* Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. *Neuropsychopharmacology* **25**, 892–903 (2001).
120. Miller, J. M. *et al.* Positron emission tomography quantification of serotonin transporter in suicide attempters with major depressive disorder. *Biol. Psychiatry* **74**, 287–295 (2013).
121. Yanowitch, R. & Coccaro, E. F. The neurochemistry of human aggression. *Adv. Genet.* **75**, 151–169 (2011).
122. Brezo, J. *et al.* Differences and similarities in the serotonergic diathesis for suicide attempts and mood disorders: a 22-year longitudinal gene–environment study. *Mol. Psychiatry* **15**, 831–843 (2010).
123. Smith, K. A., Fairburn, C. G. & Cowen, P. J. Relapse of depression after rapid depletion of tryptophan. *Lancet* **349**, 915–919 (1997).
124. Benkelfat, C., Ellenbogen, M. A., Dean, P., Palmour, R. M. & Young, S. N. Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch. Gen. Psychiatry* **51**, 687–697 (1994).
125. Sequeira, A. *et al.* Implication of SSAT by gene expression and genetic variation in suicide and major depression. *Arch. Gen. Psychiatry* **63**, 35–48 (2006).
126. Fiori, L. M. *et al.* Global gene expression profiling of the polyamine system in suicide completers. *Int. J. Neuropsychopharmacol.* **14**, 595–605 (2011).
127. Klempan, T. A. *et al.* Profiling brain expression of the spermidine/spermine N^1-acetyltransferase 1 (SAT1) gene in suicide. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **150B**, 934–943 (2009).
128. Chen, G. G. *et al.* Evidence of altered polyamine concentrations in cerebral cortex of suicide completers. *Neuropsychopharmacology* **35**, 1477–1484 (2010).
129. Guipponi, M. *et al.* Genetic and epigenetic analysis of SSAT gene dysregulation in suicidal behavior. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **150B**, 799–807 (2009).
130. Le-Niculescu, H. *et al.* Discovery and validation of blood biomarkers for suicidality. *Mol. Psychiatry* **18**, 1249–1264 (2013).
131. Dahel, K.-d., Al-Saffar, N. & Flayeh, K. Polyamine oxidase activity in sera of depressed and schizophrenic patients after ECT treatment. *Neurochem. Res.* **26**, 415–418 (2001).
132. Karssen, A. M. *et al.* Stress-induced changes in primate prefrontal profiles of gene expression. *Mol. Psychiatry* **12**, 1089–1102 (2007).
133. Fiori, L. M., Gross, J. A. & Turecki, G. Effects of histone modifications on increased expression of polyamine biosynthetic genes in suicide. *Int. J. Neuropsychopharmacol.* **15**, 1161–1166 (2012).
134. Fiori, L. M. & Turecki, G. Epigenetic regulation of spermidine/spermine N1-acetyltransferase (SAT1) in suicide. *J. Psychiatr. Res.* **45**, 1229–1235 (2011).
135. Gross, J. A., Fiori, L. M., Labonte, B., Lopez, J. P. & Turecki, G. Effects of promoter methylation on increased expression of polyamine biosynthetic genes in suicide. *J. Psychiatr. Res.* **47**, 513–519 (2013).
136. Lopez, J. P. *et al.* Regulatory role of miRNAs in polyamine gene expression in the prefrontal cortex of depressed suicide completers. *Int. J. Neuropsychopharmacol.* **17**, 23–32 (2014).
137. Sequeira, A. *et al.* Global brain gene expression analysis links glutamatergic and GABAergic alterations to suicide and major depression. *PLoS ONE* **4**, e6585 (2009).
138. Choudary, P. V. *et al.* Altered cortical glutamatergic and GABAergic signal transmission with glial involvement in depression. *Proc. Natl Acad. Sci. USA* **102**, 15653–15658 (2005).
139. Duman, R. S. Neurobiology of stress, depression, and rapid acting antidepressants: remodeling synaptic connections. *Depress. Anxiety* **31**, 291–296 (2014).
140. Larkin, G. L. & Beautrais, A. L. A preliminary naturalistic study of low-dose ketamine for depression and suicide ideation in the emergency department. *Int. J. Neuropsychopharmacol.* **14**, 1127–1131 (2011).
141. Price, R. B. *et al.* Effects of ketamine on explicit and implicit suicidal cognition: a randomized controlled trial in treatment-resistant depression. *Depress. Anxiety* **31**, 335–343 (2014).
142. Raison, C. L., Capuron, L. & Miller, A. H. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol.* **27**, 24–31 (2006).
143. Howren, M. B., Lamkin, D. M. & Suls, J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom. Med.* **71**, 171–186 (2009).
144. Dowlati, Y. *et al.* A meta-analysis of cytokines in major depression. *Biol. Psychiatry* **67**, 446–457 (2010).
145. Liu, Y., Ho, R. C. & Mak, A. Interleukin (IL)-6, tumour necrosis factor-α (TNFα) and soluble interleukin-2 receptors (sIL-2R) are elevated in patients with major depressive disorder: a meta-analysis and meta-regression. *J. Affect Disord.* **139**, 230–239 (2012).
146. Shelton, R. C. *et al.* Altered expression of genes involved in inflammation and apoptosis in frontal cortex in major depression. *Mol. Psychiatry* **16**, 751–762 (2011).
147. Capuron, L. & Miller, A. H. Cytokines and psychopathology: lessons from interferon-α. *Biol. Psychiatry* **56**, 819–824 (2004).
148. Musselman, D. L. *et al.* Paroxetine for the prevention of depression induced by high-dose interferon-α. *N. Engl. J. Med.* **344**, 961–966 (2001).
149. Raison, C. L. *et al.* Depression during pegylated interferon-α plus ribavirin therapy: prevalence and prediction. *J. Clin. Psychiatry* **66**, 41–48 (2005).
150. Constant, A. *et al.* Mood alterations during interferon-α therapy in patients with chronic hepatitis C: evidence for an overlap between manic/hypomanic and depressive symptoms. *J. Clin. Psychiatry* **66**, 1050–1057 (2005).
151. Serafini, G. *et al.* The role of inflammatory cytokines in suicidal behavior: a systematic review. *Eur. Neuropsychopharmacol.* **23**, 1672–1686 (2013).
152. Isung, J. *et al.* Low vascular endothelial growth factor and interleukin-8 in cerebrospinal fluid of suicide attempters. *Transl. Psychiatry* **2**, e196 (2012).
153. Erhardt, S. *et al.* Connecting inflammation with glutamate agonism in suicidality. *Neuropsychopharmacology* **38**, 743–752 (2013).

154. Sublette, M. E. *et al.* Plasma kynurenine levels are elevated in suicide attempters with major depressive disorder. *Brain Behav. Immun.* **25**, 1272–1278 (2011).
155. Carlborg, A., Jokinen, J., Jonsson, E. G., Erhardt, S. & Nordstrom, P. CSF kynurenic acid and suicide risk in schizophrenia spectrum psychosis. *Psychiatry Res.* **205**, 165–167 (2013).
156. Bay-Richter, C. *et al.* A role for inflammatory metabolites as modulators of the glutamate *N*-methyl-D-aspartate receptor in depression and suicidality. *Brain Behav. Immun.* http://dx.doi.org/10.1016/j.bbi.2014.07.012 (2014).
157. Pandey, G. N. *et al.* Proinflammatory cytokines in the prefrontal cortex of teenage suicide victims. *J. Psychiatr. Res.* **46**, 57–63 (2012).
158. Tonelli, L. H. *et al.* Elevated cytokine expression in the orbitofrontal cortex of victims of suicide. *Acta Psychiatr. Scand.* **117**, 198–206 (2008).
159. Torres-Platas, S. G., Cruceanu, C., Chen, G. G., Turecki, G. & Mechawar, N. Evidence for increased microglial priming and macrophage recruitment in the dorsal anterior cingulate white matter of depressed suicides. *Brain Behav. Immun.* http://dx.doi.org/10.1016/j.bbi.2014.05.007 (2014).
160. Steiner, J. *et al.* Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J. Psychiatr. Res.* **42**, 151–157 (2008).
161. Bellavance, M. A. & Rivest, S. The, H. P. A. - Immune axis and the immunomodulatory actions of glucocorticoids in the brain. *Front. Immunol.* **5**, 136 (2014).
162. Ehlert, U. Enduring psychobiological effects of childhood adversity. *Psychoneuroendocrinology* **38**, 1850–1857 (2013).
163. Danese, A. *et al.* Elevated inflammation levels in depressed adults with a history of childhood maltreatment. *Arch. Gen. Psychiatry* **65**, 409–415 (2008).
164. Czeh, B., Fuchs, E. & Flugge, G. Altered glial plasticity in animal models for mood disorders. *Curr. Drug Targets* **14**, 1249–1261 (2013).
165. Banasr, M. *et al.* Glial pathology in an animal model of depression: reversal of stress-induced cellular, metabolic and behavioral deficits by the glutamate-modulating drug riluzole. *Mol. Psychiatry* **15**, 501–511 (2010).
166. Rajkowska, G. Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. *Biol. Psychiatry* **48**, 766–777 (2000).
167. Torres-Platas, S. G. *et al.* Astrocytic hypertrophy in anterior cingulate white matter of depressed suicides. *Neuropsychopharmacology* **36**, 2650–2658 (2011).
168. Ernst, C. *et al.* Dysfunction of astrocyte connexins 30 and 43 in dorsal lateral prefrontal cortex of suicide completers. *Biol. Psychiatry* **70**, 312–319 (2011).
169. Bernard, R. *et al.* Altered expression of glutamate signaling, growth factor, and glia genes in the locus coeruleus of patients with major depression. *Mol. Psychiatry* **16**, 634–646 (2011).
170. Dere, E. *et al.* Connexin30-deficient mice show increased emotionality and decreased rearing activity in the open-field along with neurochemical changes. *Eur. J. Neurosci.* **18**, 629–638 (2003).
171. Frisch, C. *et al.* Mice with astrocyte-directed inactivation of connexin43 exhibit increased exploratory behaviour, impaired motor capacities, and changes in brain acetylcholine levels. *Eur. J. Neurosci.* **18**, 2313–2318 (2003).
172. Rose, C. R. *et al.* Truncated TRKB-T1 mediates neurotrophin-evoked calcium signalling in glia cells. *Nature* **426**, 74–78 (2003).
173. Razzoli, M. *et al.* A role for BDNF/TRKB signaling in behavioral and physiological consequences of social defeat stress. *Genes Brain Behav.* **10**, 424–433 (2011).
174. Roy, A. Hypothalamic–pituitary–adrenal axis function and suicidal behavior in depression. *Biol. Psychiatry* **32**, 812–816 (1992).
175. Coryell, W. & Schlesser, M. The dexamethasone suppression test and suicide prediction. *Am. J. Psychiatry* **158**, 748–753 (2001).
176. Pfeffer, C. R., Stokes, P. & Shindledecker, R. Suicidal behavior and hypothalamic–pituitary–adrenocortical axis indices in child psychiatric inpatients. *Biol. Psychiatry* **29**, 909–917 (1991).
177. Raadsheer, F. C. *et al.* Corticotropin-releasing hormone mRNA levels in the paraventricular nucleus of patients with Alzheimer's disease and depression. *Am. J. Psychiatry* **152**, 1372–1376 (1995).
178. Raadsheer, F. C., Hoogendijk, W. J., Stam, F. C., Tilders, F. J. & Swaab, D. F. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology* **60**, 436–444 (1994).
179. Wang, S. S., Kamphuis, W., Huitinga, I., Zhou, J. N. & Swaab, D. F. Gene expression analysis in the human hypothalamus in depression by laser microdissection and real-time PCR: the presence of multiple receptor imbalances. *Mol. Psychiatry* **13**, 786–799 (2008).
180. Nemeroff, C. B. *et al.* Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* **226**, 1342–1344 (1984).
181. Nemeroff, C. B., Owens, M. J., Bissette, G., Andorn, A. C. & Stanley, M. Reduced corticotropin releasing factor binding sites in the frontal cortex of suicide victims. *Arch. Gen. Psychiatry* **45**, 577–579 (1988).
182. Lopez, J. F. *et al.* Localization and quantification of pro-opiomelanocortin mRNA and glucocorticoid receptor mRNA in pituitaries of suicide victims. *Neuroendocrinology* **56**, 491–501 (1992).
183. Dumser, T., Barocka, A. & Schubert, E. Weight of adrenal glands may be increased in persons who commit suicide. *Am. J. Forens. Med. Pathol.* **19**, 72–76 (1998).
184. Szigethy, E., Conwell, Y., Forbes, N. T., Cox, C. & Caine, E. D. Adrenal weight and morphology in victims of completed suicide. *Biol. Psychiatry* **36**, 374–380 (1994).
185. McGirr, A. *et al.* Dysregulation of the sympathetic nervous system, hypothalamic-pituitary-adrenal axis and executive function in individuals at risk for suicide. *J. Psychiatry Neurosci.* **35**, 399–408 (2010).
186. Malhi, G. S., Tanious, M., Das, P., Coulston, C. M. & Berk, M. Potential mechanisms of action of lithium in bipolar disorder. Current understanding. *CNS Drugs* **27**, 135–153 (2013).
187. Goodwin, F. K. *et al.* Suicide risk in bipolar disorder during treatment with lithium and divalproex. *JAMA* **290**, 1467–1473 (2003).
188. Cipriani, A., Hawton, K., Stockton, S. & Geddes, J. R. Lithium in the prevention of suicide in mood disorders: updated systematic review and meta-analysis. *BMJ* **346**, f3646 (2013).
189. Ripke, S. *et al.* Genome-wide association analysis identifies 13 new risk loci for schizophrenia. *Nature Genet.* **45**, 1150–1159 (2013).
190. Fromer, M. *et al.* De novo mutations in schizophrenia implicate synaptic networks. *Nature* **506**, 179–184 (2014).
191. Purcell, S. M. *et al.* A polygenic burden of rare disruptive mutations in schizophrenia. *Nature* **506**, 185–190 (2014).
192. Lesch, M. & Nyhan, W. L. A. Familial disorder of uric acid metabolism and central nervous system function. *Am. J. Med.* **36**, 561–570 (1964).
193. Meissner, A. *et al.* Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* **454**, 766–770 (2008).
194. Mikkelsen, T. S. *et al.* Genome-wide maps of chromatin state in pluripotent and lineage-committed cells. *Nature* **448**, 553–560 (2007).
195. Lister, R. *et al.* Global epigenomic reconfiguration during mammalian brain development. *Science* **341**, 1237905 (2013).
This is an important study showing that during prenatal and postnatal brain development, neurons and non-neuronal cells undergo different patterns of dynamic methylation at CpG and non-CpG sequences, as well as in 5-hydroxymethylcytosine. This work shows that there is a clear link between these methylation changes and important brain plastic changes, such as synaptogenesis.
196. Liang, P. *et al.* Genome-wide survey reveals dynamic widespread tissue-specific changes in DNA methylation during development. *BMC Genomics* **12**, 231 (2011).
197. Xin, Y. *et al.* Genome-wide divergence of DNA methylation marks in cerebral and cerebellar cortices. *PLoS ONE* **5**, e11357 (2010).
198. Xin, Y. *et al.* Role of CpG context and content in evolutionary signatures of brain DNA methylation. *Epigenetics* **6**, 1308–1318 (2011).
199. Nakamura, N. *et al.* Laser capture microdissection for analysis of single cells. *Methods Mol. Med.* **132**, 11–18 (2007).
200. Jiang, Y., Matevossian, A., Huang, H. S., Straubhaar, J. & Akbarian, S. Isolation of neuronal chromatin from brain tissue. *BMC Neurosci.* **9**, 42 (2008).
201. Guintivano, J., Aryee, M. J. & Kaminsky, Z. A. A cell epigenotype specific model for the correction of brain cellular heterogeneity bias and its application to age, brain region and major depression. *Epigenetics* **8**, 290–302 (2013).
202. Liu, D. *et al.* Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science* **277**, 1659–1662 (1997).
This landmark study establishes a link between maternal grooming of pups and regulation of the HPA axis in offspring.
203. Murgatroyd, C. *et al.* Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nature Neurosci.* **12**, 1559–1566 (2009).
This important study in mice shows that ELA regulates DNA methylation of an intergenic region that regulates the activity of AVP and results in enduring hypersecretion of corticosterone and alterations in passive stress coping and memory.
204. Weaver, I. C. *et al.* Epigenetic programming by maternal behavior. *Nature Neurosci.* **7**, 847–854 (2004).
This ground-breaking study shows that maternal behaviour regulates the expression of the glucocorticoid receptor by inducing promoter methylation changes.
205. Klose, R. J. & Bird, A. P. Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.* **31**, 89–97 (2006).
206. Maunakea, A. K. *et al.* Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* **466**, 253–257 (2010).
207. Ziller, M. J. *et al.* Charting a dynamic DNA methylation landscape of the human genome. *Nature* **500**, 477–481 (2013).
This study shows that only a minority of CpG nucleotides have variable levels of methylation and that they are mostly located in regulatory gene elements, such as enhancers and transcription factor-binding sites.
208. Turner, J. D. & Muller, C. P. Structure of the glucocorticoid receptor (NR3C1) gene 5′ untranslated region: identification, and tissue distribution of multiple new human exon 1. *J. Mol. Endocrinol.* **35**, 283–292 (2005).
209. McCormick, J. A. *et al.* 5′-heterogeneity of glucocorticoid receptor messenger RNA is tissue specific: differential regulation of variant transcripts by early-life events. *Mol. Endocrinol.* **14**, 506–517 (2000).
210. Wu, Y., Patchev, A. V., Daniel, G., Almeida, O. F. & Spengler, D. Early-life stress reduces DNA methylation of the *Pomc* gene in male mice. *Endocrinology* **155**, 1751–1762 (2014).
211. Bowen, M. T. *et al.* Active coping towards predatory stress is associated with lower corticosterone and progesterone plasma levels and decreased methylation in the medial amygdala vasopressin system. *Horm. Behav.* **66**, 561–566 (2014).
212. Bester-Meredith, J. K., Young, L. J. & Marler, C. A. Species differences in paternal behavior and aggression in peromyscus and their associations with vasopressin immunoreactivity and receptors. *Horm. Behav.* **36**, 25–38 (1999).
213. Wersinger, S. R., Caldwell, H. K., Christiansen, M. & Young, W. S. 3rd. Disruption of the vasopressin 1b receptor gene impairs the attack component of aggressive behavior in mice. *Genes Brain Behav.* **6**, 653–660 (2007).
214. Wersinger, S. R., Ginns, E. I., O'Carroll, A. M., Lolait, S. J. & Young, W. S. 3rd. Vasopressin V1b receptor knockout reduces aggressive behavior in male mice. *Mol. Psychiatry* **7**, 975–984 (2002).
215. Barkat, T. R., Polley, D. B. & Hensch, T. K. A critical period for auditory thalamocortical connectivity. *Nature Neurosci.* **14**, 1189–1194 (2011).
216. Fisher, H. L. *et al.* The varying impact of type, timing and frequency of exposure to childhood adversity on its association with adult psychotic disorder. *Psychol. Med.* **40**, 1967–1978 (2010).
217. Jun, H. J. *et al.* Child abuse and smoking among young women: the importance of severity, accumulation, and timing. *J. Adolesc. Health* **43**, 55–63 (2008).
218. Blaauw, E., Arensman, E., Kraaij, V., Winkel, F. W. & Bout, R. Traumatic life events and suicide risk among jail inmates: the influence of types of events, time period and significant others. *J. Trauma Stress* **15**, 9–16 (2002).

219. Talens, R. P. *et al.* Variation, patterns, and temporal stability of DNA methylation: considerations for epigenetic epidemiology. *FASEB J.* **24**, 3135–3144 (2010).
220. Ouellet-Morin, I. *et al.* Increased serotonin transporter gene (SERT) DNA methylation is associated with bullying victimization and blunted cortisol response to stress in childhood: a longitudinal study of discordant monozygotic twins. *Psychol. Med.* **43**, 1813–1823 (2013).
221. Kim, J. M. *et al.* A longitudinal study of *SLC6A4* DNA promoter methylation and poststroke depression. *J. Psychiatr. Res.* **47**, 1222–1227 (2013).
222. Kim, J. M. *et al.* A longitudinal study of *BDNF* promoter methylation and genotype with poststroke depression. *J. Affect. Disord.* **149**, 93–99 (2013).
223. Fiori, L. M. & Turecki, G. Implication of the polyamine system in mental disorders. *J. Psychiatry Neurosci.* **33**, 102–110 (2008).
224. Pegg, A. E. & Casero, R. A. Jr. Current status of the polyamine research field. *Methods Mol. Biol.* **720**, 3–35 (2011).
225. Bastida, C. M. *et al.* Sexual dimorphism of ornithine decarboxylase in the mouse adrenal: influence of polyamine deprivation on catecholamine and corticoid levels. *Am. J. Physiol. Endocrinol. Metab.* **292**, E1010–E1017 (2007).
226. Williams, K. Modulation and block of ion channels: a new biology of polyamines. *Cell. Signal.* **9**, 1–13 (1997).
227. Brackley, P. *et al.* Spermine and philanthotoxin potentiate excitatory amino acid responses of *Xenopus* oocytes injected with rat and chick brain RNA. *Neurosci. Lett.* **114**, 51–56 (1990).
228. Galea, E., Regunathan, S., Eliopoulos, V., Feinstein, D. L. & Reis, D. J. Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. *Biochem. J.* **316**, 247–249 (1996).
229. Reis, D. J. & Regunathan, S. Is agmatine a novel neurotransmitter in brain? *Trends Pharmacol. Sci.* **21**, 187–193 (2000).
230. Doyle, K. M., Kirby, B. P., Murphy, D. & Shaw, G. G. Effect of L-type calcium channel antagonists on spermine-induced CNS excitation *in vivo*. *Neurosci. Lett.* **380**, 247–251 (2005).
231. Gilad, G. M. & Gilad, V. H. Overview of the brain polyamine-stress-response: regulation, development, and modulation by lithium and role in cell survival. *Cell. Mol. Neurobiol.* **23**, 637–649 (2003).
232. Hayashi, Y., Tanaka, J., Morizumi, Y., Kitamura, Y. & Hattori, Y. Polyamine levels in brain and plasma after acute restraint or water-immersion restraint stress in mice. *Neurosci. Lett.* **355**, 57–60 (2004).
233. Lee, M., Wynder, C., Schmidt, D., McCafferty, D. & Shiekhattar, R. Histone H3 lysine 4 demethylation is a target of nonselective antidepressive medications. *Chem. Biol.* **13**, 563–570 (2006).
234. Piletz, J. E. *et al.* Agmatine: clinical applications after 100 years in translation. *Drug Discov. Today* **18**, 880–893 (2013).
235. Gupta, N., Zhang, H. & Liu, P. Behavioral and neurochemical effects of acute putrescine depletion by difluoromethylornithine in rats. *Neuroscience* **161**, 691–706 (2009).
236. Shopsin, B. The clinical antidepressant effect of exogenous agmatine is not reversed by parachlorophenylalanine: a pilot study. *Acta Neuropsychiatr.* **25**, 113–118 (2013).

Acknowledgements
Preparation of this Review was supported by grants from the Canadian Institute of Health Research (CIHR), MOP119429 and MOP119430, and by the Fonds de Recherche du Québec – Santé (FRQS), through a Chercheur National salary award to the author and through support to the Réseau québécois sur le suicide, les troubles de l'humeur et les troubles associés (RQSHA). The author is indebted to S. Daniels for expert and essential help in the preparation of this Review.

Competing interests statement
The author declares no competing interests.

FURTHER INFORMATION
World Health Organization, Global Health Estimates summary tables: http://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.htm

SUPPLEMENTARY INFORMATION
See online article: S1 (table) | S2 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF