Curr Psychiatry Rep (2016) 18: 98
DOI 10.1007/s11920-016-0736-8

A Slice of the Suicidal Brain: What Have Postmortem Molecular Studies Taught Us?

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Published online: 26 September 2016

Abstract Suicide ranks amongst the leading causes of death worldwide. Contemporary models of suicide risk posit that suicide results from the interaction of distal and proximal factors, including neurobiological, psychological/clinical, and social factors. While a wealth of neurobiological studies aimed at identifying biological processes associated with suicidal behaviour have been conducted over the last decades, the more recent development of arrays and high-throughput sequencing methods have led to an increased capacity and interest in the study of genomic factors. Postmortem studies are a unique tool to directly investigate genomic processes that may be dysregulated in the suicidal brain. In this review, we discuss postmortem literature investigating functional genomic studies of suicide, particularly focusing on epigenetic mechanisms.

Keywords Suicide · Postmortem studies · Epigenetics · Genomics

Introduction

According to the WHO, every year more than 800,000 people die by completing suicide, and as such, suicide ranks amongst the leading causes of death worldwide. Suicide occurs throughout the lifespan and is the second leading cause of death among 15–29-year-olds across the globe. While suicide represents a serious global concern, it is tainted with prejudice and stigma. Thus even though suicide is largely preventable due in part to the availability of effective prevention programs and evidence-based interventions, we remain unable to substantially decrease suicide rates. There have been many models constructed to explain suicide risk [1, 2•] based often on the premise that distal and proximal factors interact to increase suicide risk. Briefly, distal factors comprise family history and genetic loading, early life adversity, personality, and cognitive styles. While those proximal to the suicide crisis are represented by conditions acting as precipitants, including psychiatric/physical disorders, recent acutely stressful life events, availability of means, and psychosocial crises. We refer the reader to Turecki and Brent [3•] for a more comprehensive review of these factors. Sociodemographic variables must also be considered as they moderate the effect of other risk factors; these include gender, education, spiritual beliefs, family structure, income and employment, social support in the community, overall social environment, and age [4]. While numerous clinical markers such as differences in psychopathology, personality traits, suicide intent, hopelessness/helplessness, and impulsive-aggressive traits have been described in these models, none have the power of individually precisely identifying personal suicide risk [5, 6]. As such, scientists have turned to neurobiological and genomic studies aimed at identifying biological processes underlying suicidal behaviour. An increasing interest in these factors has been coupled with the development and refinement of methods in functional genomics, including arrays and high-throughput sequencing technologies. A powerful way to study the biological underpinnings of suicide and how one′s biological makeup interacts with the environment is by the investigation of epigenetics. Epigenetics refer to the collection of chemical and physical

This article is part of the Topical Collection on *Genetic Disorders*

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processes that regulate the genome; examples of epigenetic mechanisms include DNA methylation, hydroxymethylation, and histone modifications. They contribute to cerebral and genomic plasticity while being responsive to developmental, physiological, and environmental cues. In addition, epigenetic marks mediate biological adaptations in gene expression over extended periods of time [7, 8]. Postmortem studies are thus essential for the direct investigation of neuroepigenetic correlates of suicide. In this review, we survey recent advances in postmortem literature centered on the molecular basis of the suicidal brain. We refer the reader to Turecki [9•] for a more comprehensive review of molecular changes that may not be covered here but have been associated with suicidality. Emphasis is placed on discrete signalling cascades, and where applicable, how they may interact. Importantly, as we focus primarily on molecular studies, this review does not discuss work exploring serotonergic alterations in suicidal behaviour. Although this work is central to understanding the neurobiology of suicide, it was primarily the result of lines of investigation other than genomic. Furthermore, we draw attention to the intersection between one's environment and biological make-up by considering how childhood maltreatment might act to perturb stress signalling in the suicidal brain. We end with insight into important future directions for the field.

Polyamine System

The polyamines are a group of ubiquitous aliphatic molecules comprising putrescine, agmatine, spermidine, and spermine. In mammals, polyamines play essential roles in various physiological functions, including cell proliferation and apoptosis, immunity, cellular signalling, neurotransmission, adult brain neurogenesis, and stress regulation both at the cellular level (oxidative stress) and behavioural level (in response to psychosocial adversity) (as reviewed in Ramani et al. [10]). The majority of brain polyamines are stored in astrocytes and synaptic vesicles suggesting a structural distribution that allows for their functional regulation over a variety of receptors located on the surface of glia and neurons [11]. A key regulator of the polyamine stress response includes the rate-limiting enzyme spermidine/spermine *N [1]*-acetyltransferase 1(SAT1) [10]. Using gene expression arrays in postmortem suicide brain, Sequeira and colleagues [12] showed a reduction in the primary protein-encoding transcript of SAT1 (SSAT) when compared to controls. Furthermore, a sequence (rs6526342) variant located in the SAT1 polyamine-responsive element (PRE) regulatory region showed a significant effect of genotype on brain expression. In a sample of 181 male suicide completers and 80 controls, the authors observed a higher frequency of the rs6526342 C allele among suicides. Findings of decreased SSAT expression were confirmed by Guipponi et al. [13]; however, their data did not support an association between rs6526342 and variation in SSAT expression or suicidal behaviour. Employing whole-gene sequencing of SAT1 in bipolar disorder subjects with versus those without a history of suicide attempts, Monson et al. [14] found no evidence of an association between genetic variation and suicide attempt. Regardless of reports describing insignificant findings, reduced SAT1 expression remains a robust characteristic of the depressed suicide brain. In fact, using an array-based approach, Fiori et al. [15•] found that in extension to downregulation of SAT1 in the brain of depressed suicides, 13 new targets related to the polyamine system and its metabolism were identified across 22 brain regions. These results are suggestive of global dysregulation of polyamines in the depressed suicide brain [15•]. A major question, however, is whether or not SAT1 misexpression is specific to an underlying depressive state or related to the behavioural act of suicide per se. Klempan et al. [16] observed a reduction in SAT1 expression across five cortical regions in depressed individuals who died by suicide in comparison to matched sudden death controls. Importantly, these results were depression specific since the authors did not observe differences in SAT1 expression between non-depressed suicides and controls [16]. Reduced SAT1 expression was also replicated in an independent sample of German subjects who were depressed and died by suicide. Furthermore, using a microarray approach to investigate expression patterns in ventral regions of the prefrontal cortex, Klempan et al. [17] found lower SAT1 expression in depressed suicides versus non-depressed suicides. In a recent study by Pantazatos and colleague [18], the dorsal prefrontal cortex of depressed subjects who did not die by suicide showed a significant reduction in SAT1 expression in comparison to controls and was no different from those who were depressed and died in a suicide crisis. This later study also investigated whether all or only specific isoforms expressed by SAT1 are/is involved in the pathophysiology of depression. At the isoform level, reductions in depressed suicides were most pronounced for four transcripts, while reductions in MDD only subjects were pronounced in three transcripts, one of which was reduced in MDD exclusively, relative to MDD suicides. The authors did not find strong evidence to support differential exon usage (i.e., splicing) as a mechanism underlying depression and suicide. One exception, however, was isoform 3 which was significantly lower in MDD only, in comparison to MDD suicides, and this isoform showed trend level evidence for differential exon usage.

Studies investigating epigenetic regulation of the SAT1 promoter in postmortem brain have found that CpG methylation negatively correlates with expression. While total or site-specific methylation differences between suicides and controls were not observed by Fiori et al. [19], the rs6526342 SNP within the promoter region showed a trend for an increase in methylation in suicide completers carrying the C allele. No significant suicide driven findings were observed in H3K27me3 across this region [19]. More recently, using target

prediction analysis Lopez et al. [20] identified several miRNAs targeting the 3′UTR of SAT1 and SMOX, and profiled their expression to determine whether post-translational modifications explain lower expression levels of these genes in suicide. Several miRNAs revealed a significant upregulation in the prefrontal cortex of suicides; moreover these miRNA significantly correlated with the expression levels of both SAT1 and spermine oxidase (SMOX). Evidence for differential miRNA expression was however not observed in Pantazatos et al. [18], who did not detect group differences between depressed suicides and controls, nor did they observe any correlations between these miRNAs with SAT1 isoform expression. Following up their upregulated expression of arginase II (ARG2), S-adenosylmethionine decarboxylase (AMD1), and ornithine decarboxylase antizymes 1 and 2 (OAZ1 and OAZ2) in Fiori et al. [15•], in another study, the authors found increased H3K4me3 levels in the promoter region of OAZ1 in suicide and that this mark was correlated with the expression of OAZ1 and ARG2 in Brodmann area 44 [21]. Continuing to investigate epigenetic regulation over these genes, Gross et al. [22] observed both site-specific and overall differences in methylation between groups for all genes; however, only site-specific DNA methylation in the promoter region of ARG2 and AMD1 showed a relationship to their respective levels of expression in suicides. Thus, there appears to be significant evidence that suggests a role for polyamine dysregulation in suicidality. Moreover, while differences in polyamine expression characteristic of the suicidal brain may be in part explained by associated psychopathology such as depression, additional work is necessary to better separate them.

Astroglia

Astrocytes are multifaceted cells with numerous distinct functions within the CNS, including development of the nervous system, maintenance of neuronal functioning, synaptic communication and plasticity, regulation of cerebral blood flow, and immune regulation [23, 24]. While historically glial cells have been largely ignored by neuropsychiatric research, a number of independent studies have recently suggested astrocytic dysfunction in the depressed and suicide brain [25–27]. However, little is known about the underlying molecular mechanisms.

One way in which these cells engage in long-range glial communication is through calcium wave propagation. This function requires the multigene family of connexin cell membrane proteins whose cellular role is to form channels that allow molecules under 1 kDa to pass freely between neighboring cells. The misexpression of astrocytic connexins may thus play a role in the pathophysiology of mood disorders. In fact, connexins 30 and 40 have been shown to be downregulated in the dorsal lateral prefrontal cortex of depressed suicides, and this may be in part due to the transcription factor SOX9, which was also reduced in these subjects [28].

In a study investigating epigenetic factors, Nagy et al. [29•] measured the expression of seven glial specific genes in Brodmann areas 8/9 and 10 of 76 individuals who died by suicide and 45 sudden-death matched controls. Expression of each gene was significantly reduced in suicide completers; however, in order to better understand epigenetic regulation of glial cells, the authors choose cases with the most perturbed astrocytic molecular pathology. This was done by selecting suicide completers with the lowest mRNA expression levels of seven astrocyte genes used for screening and defining extreme cases by expression levels in the bottom quartile for at least five of seven genes. These subjects along with healthy controls were used to generate a genome-wide methylation map unique to altered astrocyte-associated depressive psychopathology and suicide. Results yielded 115 differentially methylated regions (DMRs) across the genome, most of which were characterized by hypomethylation in cases. In silico analysis revealed enrichment for 37 ENCODE regulatory elements in hypomethylated regions. Amongst intragenic DMRs, those found in GRIK2 (glutamate receptor ionotropic kainate 2) and BEGAIN (brain-enriched guanylate kinase-associated protein) were most significant and were inversely correlated with gene expression. Specifically, GRIK2 showed higher levels of expression in cases while BEGAIN was characterized by the opposite pattern. Neuronal and non-neuronal cell-sorted fractions were investigated and demonstrated an important non-neuronal contribution to BEGAIN methylation status. Functional cell assays revealed promoter and enhancer-like properties for regions of differential methylation in BEGAIN that were markedly decreased by methylation. The group of low expressers described by Nagy et al. [29•] was then used to interrogate glial fibrillary acidic protein (GFAP) expression and protein levels in various cortical and subcortical regions [30]. A downregulation of GFAP mRNA and protein levels was observed only in the mediodorsal thalamus and caudate nucleus of depressed suicides compared with controls. Interestingly, these subcortical regions showed on average 11- to 15-fold greater expression levels when compared to the cerebellum and neocortex, respectively. In extension to this, astrocytic morphology in these two regions was characterized by larger cell bodies and more ramified processes that extended across larger domains, features that have not been observed in cortical astrocytes [23]. Astrocytic abnormalities are thus likely specific to cortical and subcortical neural networks implicated in mood disorders. There is also evidence to support differences in astrocytic abnormalities for both neuroanatomical subfields [31] and across grey versus white matter [32•]. For instance, by using immunostaining for GFAP in the hippocampus of depressed subjects, most of which died by suicide, Cobb et al. [31] observed that the density of astrocytes

in the hilus, but not CA1 or CA2/3, was significantly decreased in antidepressant free cases. A sex difference emerged where GFAP immunoreactivity was significantly decreased in the dentate gyrus of women but not men with a diagnosis of depression. In the subclass of suicides, GFAP immunoreactivity was inversely correlated with duration of depression but only in CA2/3 of the hippocampus. While a characterization of astrocytes in the anterior cingulate cortex of depressed suicides by Torres-Platas et al. [32•] revealed that fibrous astrocytes adjacent to white matter, but not protoplasmic grey matter astrocytes, had significantly larger cell bodies, as well as longer, more ramified processes. More importantly, values for these parameters were approximately twice as high as those measured in controls. The authors argue that a hypertrophy of fibrous astrocytes may reflect local inflammation in white matter and support neuroinflammatory theories of depression and suicide [33]. In extension to supporting a major hypothesis in depression research, results in Torres-Platas et al. [32•] advance the need to understand cell-type specific dysregulation in suicide. In fact, laser capture microdissection has been employed, as a cell separation technique for postmortem brain, to show astrocyte specific decreases in the expression of two glutamate-related genes (SLC1A3 and SLC1A2) and GFAP in the locus coeruleus of depressed males who died by suicide [34]. Overall, GFAP immunoreactivity, as well as the density of GFAP-labelled astrocytes, was significantly reduced in depressed subjects in comparison to matched controls. No significant differences, however, were found in oligodendrocytes localized to the locus coeruleus.

Neuroinflammation

The inflammatory theory of depression stands as one of the main hypotheses explaining depressive psychopathology, primarily due to the large amount of data produced over the last few decades [35•, 36–39]. Evidence in support of this hypothesis is based on the findings that (i) depressive states are associated with increased proinflammatory cytokines, such as tumour necrosis factor (TNF) α and interleukin (IL) [40, 41•, 42, 43]; (ii) conversely, inflammatory illnesses and autoimmune diseases are associated with increased prevalence of depression [44–47]; and (iii) patients undergoing therapy for diverse illnesses with cytokines, such as interferon-α (IFN-α), have substantial chances of developing depression as a result of the treatment [48–51]. Recent postmortem work has been used to shed light on the inflammatory hypothesis of depression and suicide. For instance, significant increases in the expression of proinflammatory cytokines IL-1β and IL-6, and TNF-α have been observed in Brodmann area 10 of teenage suicides [52]. Sex differences have also emerged for proinflammatory cytokines such that female suicides show elevated levels of IL-4 in Brodmann area 11, while in men, the elevated cytokine was IL-1 [53]. In a more recent study, Devorak et al. [54] focused their analysis on cellular/molecular inflammatory profiles in the choroid plexus (ChP) of depressed suicides. The choroid plexus is a particularly unique structure in that it is a highly vascularized tissue that produces cerebral spinal fluid (CSF) and lacks a blood-brain barrier; it thus represents the interface between peripheral and central immune responses. Surprisingly, the authors noted a significant downregulation of IL-1β, a factor implicated in immune cell trafficking in the choroid plexus (ICAM1), and a marker of monocytes/macrophages (Iba1). No differences emerged in the density of Iba1+ macrophages present in the epithelial cell layer of the ChP. While more data is needed to confirm this hypothesis, the authors argue that the molecular profiles of the ChP might reflect a compensatory mechanism that attenuates the detrimental effects of chronically altered pro-inflammatory signalling observed both peripherally and centrally in depression.

Although these studies have increased our confidence in the validity of using pro-inflammatory cytokines as biomarkers that are reflective of the CNS, they have not addressed potential molecular mechanisms through which pro-inflammatory cytokines are elevated in mood disorders. The design of specific biological interventions targeting the pathophysiology of suicidal behaviour requires that we understand factors earlier in the immune activation cascade. A potential starting point involves innate immune receptors, known as toll-like receptors (TLRs), whose activation leads to the production of cytokines in the brain. In fact, there is increasing evidence that these receptors are crucial for the ability of the CNS to organize innate immune responses during systemic infection and neuronal injury [55]. Given the role that TLRs play in cytokine production, findings that the mRNA expression of TLR3 and TLR4 are significantly increased in DLPFC of both depressed suicides and depressed non-suicides align well with previous literature [56]. More significantly, protein expression of these receptors was driven by suicide and not underlying psychopathology. These findings not only inform our understanding of upstream mechanisms in neuroinflammation, but also speak to the fact that cytokine abnormalities may differ between suicidal and non-suicidal subjects irrespective of underlying depressive psychopathology. One interpretation is that a gradient of immune dysfunction may correspond to the severity of mood disorder symptomology, with suicidal behaviour as an extreme end of the spectrum. Support for this interpretation comes from results in the periphery where increased levels of IL-6 and TNF-α, as well as decreased IL-2 concentrations, were found in suicide attempters but not in depressed patients without a history of suicide attempt [57].

The next logical question in this pathway is what CNS cell types might be responsible for initiating neuroinflammation in suicide. Focusing again on TLRs, recent evidence suggests that the response of astrocytes to TLR2 and TLR3 agonists

is greatly enhanced by, while the response to TLR4 agonists is completely dependent on, the presence of functional microglia [58]. These results are not surprising since central immune responses are mainly modulated by microglia and astrocytes, which generally play inflammatory and anti-inflammatory roles, respectively [59]. There is now accumulating evidence to suggest that microglial cells are in part responsible for neuroimmune profiles of depression and suicide. For instance, ventral prefrontal white matter of suicides has been characterized as having a greater density of activated microglia; whereas in the absence of suicide, the same pattern was observed in the dorsal pole [60]. In the same study, the authors found that the density of perivascular cells within or in contact to blood vessel walls of the dorsal white matter was significantly higher in suicides versus non-suicides. Since these cells were not well defined in this study, they may be a combination of juxtavascular microglia, perivascular macrophages, pericytes, or some mixture of the aforementioned [60]. It may however be reasoned that perivascular cells play a role in blood-brain barrier properties characteristic of individuals who died by suicide. Using a more refined approach to macrophage identification via Iba1 immunostaining, Torres-Platas et al. [61•] observed that the ratio of primed over ramified ("resting") microglia was significantly increased in depressed suicides. Importantly, these results were obtained in the dorsal anterior cingulate cortex (dACC), which given the findings of hypertrophic fibrous astrocytes by Torres-Platas [32•], fits well within our understanding of the roles each of these glial cell types play in neuroinflammation [59]. Further adding to their story, the authors found that the proportion of blood vessels surrounded by a high density of macrophages was significantly increased in cases. Expression of Iba1, monocyte chemoattractant protein-1 (MCP-1), and a marker enriched in perivascular macrophages, namely CD44, was significantly upregulated in cases. Together, these histological and molecular data suggest a recruitment of monocytes in the dACC white matter of depressed suicides and might explain increases in pro-inflammatory cytokines as low-grade cerebral neuroinflammation. To further corroborate the relationship between central and peripheral inflammation, Schiavone et al. [62] found a suicide driven increase in a cortical marker of oxidative stress (8-hydroxy-2'deoxyguanosine), an enzyme complex involved in its generation (NADPH oxidase 2), and IL-6 immunoreactivity. The authors argue that these results are likely due to neuroinflammation, since oxidative stress has been linked to this pathway. The results of immune induced changes in oxidative stress may lead to blood-brain barrier permeability that allows for cross-talk between central and peripheral inflammatory responses. Evidence for this comes from Ventorp and collegues [63], who have recently shown an increase in extracellular matrix components specific to the CNS in the CSF of suicide attempters. Increases were specifically in glycosaminoglycan hyaluronic acid (HA) and matrix metalloproteinases (MMP9). CSF levels of HA correlated with blood-brain barrier permeability, as measured by an increase in CSF/serum albumin ratio; while MMP9 levels correlated with the soluble version of a cell-adhesion molecule known to associate with HA (CD44).

Trophic Factors

As a member of the neurotrophin family, brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin-related kinase B receptor (TrkB) play a key role in neuronal development, neurite outgrowth, synthesis of differentiating factors, and morphological plasticity [64]. On the other hand, nerve growth factor (NGF) is a prototypical growth factor secreted from a neuron's target cell; its signalling through TrkA is critical for the survival and maintenance of sympathetic and sensory neurons [65]. The former has been related to neural homeostasis and processes involved in neuronal plasticity and circuit connectivity, while the later appears to play a role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis-mediated stress response [64, 65]. Given the crucial role that stress and neuroplasticity play in the pathophysiology of neuropsychiatric disorders, these factors are interesting candidates for postmortem studies of suicide. Both mRNA and protein levels of BDNF and TrkB have been found to be downregulated in the hippocampus and prefrontal cortex of suicides [66]. In another study, mRNA levels of TrkA and TrkC were reduced in the hippocampus of suicides, while in the prefrontal cortex only TrkA was significantly decreased [67]. Interestingly, expression of pan75 neurotrophin receptor mRNA and protein levels were increased in both brain areas. Activity of Trks, as measured by protein phosphorylation, were decreased for all receptors in the hippocampus, but only Trk A and B in the PFC. An increased expression ratio was observed between pan75 neurotrophin receptor to Trks in both brain areas of suicides [67]. Results in the hippocampus for BDNF, TrkB, and TrkA have been replicated and extended to NGF mRNA and protein expression [68]. On the other hand, in the teenage suicide brain, BDNF and TrkB mRNA expression were reduced in both the hippocampus and prefrontal cortex. While a downregulation of BDNF protein levels was observed in the PFC but not the hippocampus of teenage suicides, TrKB levels were downregulated in both brain areas [69]. Together, these findings suggest a suboptimal activation of pro-survival pathways in depression and suicide in exchange for a pro-apoptotic signalling cascade. They also inform the need to consider and investigate circuit specific dysregulation for trophic factors and their receptors.

Previous work suggests that these results may, however, be sex specific since it has been shown that BDNF protein levels are reduced in the prefrontal cortex of female, but not male-depressed suicides, while the opposite sex difference was

observed in the hippocampus of these same subjects [70]. One important question that remains unanswered is whether or not differences in trophic factors are the result of underlying psychopathology or suicidal behaviour. Arguments for the former have come from observations that mRNA levels of key elements involved in retinoid signalling, BDNF, and TrkB are significantly reduced in the dorsolateral prefrontal cortex/anterior cingulate cortex of elderly depressed individuals who did not die by suicide [71]. In vitro assays in this study showed that retinoic acid receptor-α (RAR-α) is able to bind to and transactivate the TrkB promoter via a putative RA response element [71]. Thus, the finding that RAR-α and TrKB immunopositive cells demonstrated a positive correlation between the mRNA levels of these two factors in controls, but not depressed patients, suggests that the homeostatic interaction with each other might be a potential mechanism underlying the pathophysiology of depression. Since promoter sequences for all neurotrophin receptors have been well characterized and multiple transcription factors are implicated in the regulation of the expression of these receptors, an interesting question has been the potential role of transcription factors and/or epigenetic mechanisms in the differential regulation of the expression of these genes in depression and suicide [72, 73].

While studies such as Ernst et al. [74] have shown that DNA methylation may be partially responsible for the regulation of factors involved in BDNF signalling, this study focused on DNA methylation differences in promoter regions. Growing evidence, however, has since implicated intergenic and gene body methylation in the regulation of gene functions. For instance, while investigating methylation levels of the TrkB-T1 3′ UTR region in BA8/9 of low expressers that died by suicide, Maussion et al. [75] found four hypermethylated CpG sites in these subjects. A significant correlation was drawn between methylation levels at these sites and TrkB-T1 expression, a finding that was confirmed using in vitro luciferase assays. Other gene regulation mechanisms, such as microRNA's, have also been shown to partially explain low levels of TrkB-T1 expression through their action at the genes 3′ UTR. For example, the microRNA Hsa-miR-185* has been found to be differentially expressed and inversely correlated with TrkB-T1 expression in the frontal cortex of suicide completers [76]. Interestingly, this transcript variant is highly expressed in astrocytes, which as discussed previously, have been shown to associate with suicide [28, 29•, 30, 31, 32•]. Results of miRNA dysregulation are consistent with a report revealing a significant reduction in global miRNA expression in the prefrontal cortex of depressed suicides [77]. Downregulated miRNAs were predicted to target transcripts involved in cellular growth, differentiation, and pre-/post-synaptic proteins involved in neurotransmission and synaptic plasticity. In addition to this, a set of 29 miRNAs showed a high degree of co-regulation in the depressed suicide brain but not that of healthy controls [77]. Therefore, widespread changes in miRNA networks and their associated targets likely participate in the pathogenesis of major depression and/or suicide and should be further investigated in post-mortem samples. These epigenetic mechanisms are likely the result of cross-talk between many molecular systems interacting with and regulating trophic signalling. For instance, Pandya et al. [78] discovered that acute corticosterone exposure induced upregulation of TrkB protein expression in primary cortical neurons, a finding that was dependant on glucocorticoid receptors. Chronic treatment of cortical neurons led to significant decreases in both TrkB and c-Cbl protein levels, the latter factor playing a critical role in mediating stabilization and corticosterone-induced TrkB levels. In mature neurons, acute treatment failed to induce any significant effects, while chronic exposure reduced TrkB levels. In an in vivo rodent model, chronic exposure induced a downregulation of c-Cbl in the frontal cortex and hippocampus. Reduced c-Cbl mRNA expression levels were also observed in the prefrontal cortex of suicide completers.

HPA-Axis and Epigenetics

As molecular endocrine studies have accumulated over the years, those investigating the pathophysiology of mood disorders and suicide have found strong evidence for HPA-axis dysregulation in cortisol signalling. Evidence exists in support of both underactive and overactive baseline cortisol levels, and responses to stress challenges; with differences likely being age-dependent [79–85]. Regardless of levels of circulating cortisol, there have been many postmortem molecular studies pointing to an overall reduction in glucocorticoid receptor (GR) expression in the depressed and suicide brain [86–91]. The importance of these findings being that GR's play a crucial role in negative feedback inhibition of the HPA-axis [92]. Various reports have also shown both genetic and epigenetic influences over their expression [93••, 94–97]. Amongst these reports are studies investigating the effects of adverse experiences faced in childhood, and their impact on the development of adult psychopathology [93••, 95]. Since brain plasticity is considerably more pronounced during childhood, a phenomenon commonly referred to as a sensitive period, these experiences lead to long-term epigenetic changes [98]. McGowan et al. [93••] provided the first evidence of an association between childhood abuse and epigenetic changes in the brain. While investigating GR expression in the hippocampus, they found a significant reduction in individuals with histories of childhood abuse. Altered expression levels were in part due to hypermethylation at two distinct CpG sites in exon 1F of GR gene. In vitro experiments revealed that hypermethylation at these genomic loci prevented the transcription factor NGF1-A from binding and potentiating promoter activity governing GR gene expression. In a related study, Labonte and colleagues [95] observed a reduction in total GR and GR splice variants 1(B), 1(C), and 1(H) expression in the hippocampus of suicide completers with a history of childhood

abuse. Methylation of GR 1(B) and 1(C) promoter sequences negatively correlated with both total and variant specific GR expression. Since these reports were published, a large number of studies have investigated GR gene methylation patterns in peripheral and brain tissue in relationship to experiences of trauma or psychopathology. Recently, Turecki and Meaney [99•] conducted a systematic review of these findings; of the 27 articles investigating childhood maltreatment and GR DNA methylation in human peripheral samples, 89 % reported an early life adversity driven increased in methylation at the exon 1F variant. The authors interpreted these findings as suggestive of the stable influences that childhood maltreatment exerts on the HPA axis. Consolidating these findings into a developmental framework, an overactive stress response may be adaptive during the years of adversity; however, once the child develops out of these experiences, the HPA axis remains imprinted with an epigenetic memory biasing its activity towards maladaptive molecular and hormonal cascades characteristic of adult psychopathology [100, 101]. In fact, many studies have shown dysregulation of stress-related genes in the suicide brain with many of these results being specific to neuroanatomical networks implicated in mood disorders [86–91]. As with results observed in abused suicides [93••, 95], homeostatic mechanisms employing specific gene networks involved in stress regulation are perturbed in the neurobiology of mood disorders and suicide.

Concluding Remarks and Future Directions

In this review, we have summarized some of the major biological systems that have been implicated in studies investigating molecular changes associated with suicide. What is clear from the literature is that these systems are interdependent and involve perturbations in shared gene pathways. Our hypothesis is that these gene networks are regulated through coordinated epigenetic mechanisms. As such, epigenome and transcriptome-wide studies, such as Sequeira et al. [86], Labonté et al. [102•], and Smalheiser et al. [103], are needed in brain tissue investigations of suicide so as to better understand its molecular neurobiology. However, much of the current findings are based on studies that investigated whole tissue homogenates. Yet, transcriptomic and epigenetic changes are, to a large degree, cell type and tissue specific [104, 105, 106••]. In fact, there is greater epigenetic variability between different tissues of a single individual than between similar tissues of different individuals [107–110]. Since neuronal diversity arises in part through spatiotemporal regulation of gene expression by regulatory regions such as promoters and enhancers, it is reasonable to hypothesize that epigenomic landscapes should mirror neuronal diversity. Both animal and postmortem human studies have employed various methodologies to show clear regions of differential CpG and non CpG methylation in CpG island shores, gene bodies, and intergenic regions [111, 112•, 113]. These differences are observed when not only comparing between glia and neurons, but within subclasses of neurons as well [111, 112•, 113]. Thus, technologies such as fluorescence-activated cell sorting and laser capture microdissection are needed to understanding cell-type specific perturbations in epigenetic regulation over gene networks relevant to the neurobiology of suicide.

Compliance with Ethical Standards

Conflict of Interest Daniel Almeida and Gustavo Turecki declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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