Functional Implications of Dynamic DNA Methylation for the Developing, Aging and Diseased Brain

Geraldine Zimmer-Bensch

Contents

Abstract Epigenetic mechanisms of gene regulation as the interface between the genome and environment, control diverse processes in development, aging and disease. As proposed by increasing body of evidence, defects in epigenetic remodeling during brain development, function and aging seem central to diverse aspects of the pathophysiology of psychiatric and neurological diseases.

The discovery of active ways of DNA demethylation has paved the way to reconsider the functional implications of DNA methylation in the brain, where dynamic reconfiguration of the DNA methylation landscape has been observed during development and aging. High-throughput studies profiling global DNA methylation and transcriptional changes suggest that DNA methylation-dependent gene regulation is crucially involved in regulating neuronal differentiation and maturation processes, as well as in age-related declines of neuronal function. As DNA methylation and DNA methyltransferases (DNMTs) also influences the histone code, the crosstalk of these two mechanisms of epigenetic gene regulation in neuronal development and function

G. Zimmer-Bensch (✉)
Division of Functional Epigenetics in the Animal Model, Institute for Biology II, RWTH Aachen University, Aachen, Germany
e-mail: zimmer@bio2.rwth-aachen.de

S. Jurga, J. Barciszewski (eds.), *The DNA, RNA, and Histone Methylomes*, RNA Technologies, https://doi.org/10.1007/978-3-030-14792-1_6

has been started to be investigated. Here, an overview is provided about the currently known functional implications dynamic DNA methylation and the crosstalk with histone modifications have in neuronal development and aging, as well as in associated diseases. Further, we discuss the integration and applicability of animal models as tool to gain insights in human brain aging.

Keywords DNA methyltransferases · Cortical interneurons · Neuronal migration · Cell death · Neuropsychiatric diseases

1 Introduction

Epigenetic mechanisms of gene expression control a variety of processes during development, aging and disease. Similar to histone modifications, DNA methylation catalyzed by DNA methyltransferases (DNMTs) turned out as dynamic epigenetic mark, due to the discovery of active ways of DNA demethylation involving TET-mediated oxidation of 5-methylcytosines. Dynamic DNA methylation is evident during neuronal development and maturation (Lister et al. 2013; Sharma et al. 2008), and seems implicated in regulating adult neuronal functions (Meadows et al. 2015, 2016; Sweatt 2016) as well as age-associated processes (Akbarian et al. 2013; Barter and Foster 2018; Lardenoije et al. 2015). Moreover, altered DNA methylation emerged to be involved in the etiology of neuropsychiatric disorders, including major depressive disorder, autism spectrum disorder and schizophrenia (Akbarian et al. 2013). Besides, dynamic changes in the DNA methylation landscape were observed during brain aging (Barter and Foster 2018; Lardenoije et al. 2015).

In this chapter, we discuss the role of DNA methylation in the developing, aging and diseased brain with focus on the cerebral cortex.

2 The Cerebral Cortex

Neuronal circuitries in the six-layered cerebral cortex, the seat of higher cognitive functions in the mammalian brain, are established by excitatory glutamatergic principal neurons and inhibitory gamma-aminobutyric acid (GABA)-expressing interneurons. Excitatory projection neurons adopt layer-specific identities and form specific dendritic and axonal connections. Layer II/III neurons mainly project contra- or ipsilateral to other cortical areas, while neurons of layer V and VI project to subcortical targets, including the thalamus (layer VI neurons), midbrain, hindbrain and spinal cord (layer V neurons) (Merot et al. 2009). The inhibitory GABAergic neurons populate different cortical layers and act as local modulators of excitatory neurons. Although only representing about 20% of the overall neuronal population, inhibitory interneurons are critical for cortical information processing, learning and

memory formation (Hensch 2005; Letzkus et al. 2015). Due to their enormous morphological and physiological diversity, inhibitory interneuron subtypes have the capacity to selectively target different sub-cellular compartments of projection neurons (De Marco Garcia et al. 2011), enabling a dynamic inhibition-dependent regulation of input and output processing (Gidon and Segev 2012; Pouille et al. 2013). Parvalbumin (PV)-positive interneurons primarily target the soma and axon initial segments of glutamatergic neurons, while dendritic inhibition is achieved by somatostatin (SST)-expressing interneurons. Vasointenstinal peptide (VIP)-positive interneurons inhibit mainly other cortical interneurons (Druga 2009).

The relevance of inhibitory interneuron function for cortical information processing is reflected by diverse neurologic and psychiatric diseases which involve defects in the cortical inhibition (Marin 2012). For example, deficits of SST-positive cortical interneuron function including impaired GABAergic transmission and decreased *Sst* expression levels are suggested to be implicated in the pathophysiology of schizophrenia (Lin and Sibille 2013; Morris et al. 2008). Defects in SST interneurons were further observed in numerous other human psychiatric and neurological disorders such as major depressive disorder, bipolar disorder, Alzheimer's disease and Parkinson's disease (Lin and Sibille 2013). Various studies already provided evidence that impairments during development contribute to defective inhibition underlying such diseases (Marin 2012). Hence, the correct establishment of the cortical GABAergic system during development is crucial for proper cortical function.

3 Cerebral Cortex Development

The formation of the cerebral cortex is a highly sophisticated process requiring the precise interplay of several developmental steps. These include proliferation of neuronal stems cells, differentiation, migration from the proliferative zone to their cortical target layer, axonal and dendritic growth as well as establishment of synaptic contacts.

Cortical projection neurons arise exclusively from progenitors located within the dorsal pallium. From there they migrate radially out to form the different cortical layers in an "inside-out" fashion, with deep layer neurons born first and upper-layer neurons born later, migrating past earlier born ones (Merot et al. 2009).

Neuroepithelial cells (NECs) as the earliest progenitors of the cortex, are organized in a pseudostratified neuroepithelium resulting from the apico-basal movement of their nuclei during cell-cycle progression. After initial expansion of the progenitor pool by symmetric proliferative divisions, they divide asymmetrically generating radial glial cells (RGCs) that are located in the ventricular zone (VZ) und display similar bipolar morphology (Agirman et al. 2017). At the onset of neurogenesis, RGCs divide asymmetrically to generate post-mitotic neurons or intermediate, transient amplifying progenitor cells. These intermediate progenitors delaminate and translocate their cell bodies more basally, forming the subventricular zone and

dividing symmetrically to indirectly generate neurons. The transient amplifying progenitors are already present at early stages of neurogenesis and are suggested to contribute to the neuronal production of all cortical layers (Agirman et al. 2017; Merot et al. 2009). In addition to short neural precursor cells (SNPs), outer RGCs (oRGCs) are described in the murine cortex to appear as a minor population, whereas they are proportionally more important in the developing cortex of gyrencephalic mammals contributing to the folding of the cortex (Hansen et al. 2010; Nonaka-Kinoshita et al. 2013). They share common molecular features with RGCs but reside in the outer part of the SVZ lacking basal attachment. Alike RGCs, SNPs reside in the VZ. However, they are transcriptionally distinct from RGCs, lack basal attachment and are programmed to generate neurons via symmetric differentiative divisions (Agirman et al. 2017).

In contrast to excitatory cortical neurons that arise from the cortical proliferative zones, comparatively little is known about progenitor subtypes generating the diverse subsets of inhibitory GABAergic interneurons that are located in spatially distinct domains of the subpallium. These include the medial and caudal ganglionic eminences, abbreviated with MGE and CGE, respectively, as well as the pre-optic area (POA) (Druga 2009). The MGE generates parvalbumin (PV)-positive basket and chandelier cells, as well as Martinotti and multipolar somatostatin (SST)-expressing interneurons (Butt et al. 2005, 2008; Xu et al. 2003), whereby SST-interneuron generation precedes the PV-interneuron generation (Butt et al. 2005, 2008; Inan et al. 2012). The POA contributes to a diverse subset of cortical interneurons, including neuropeptide Y (NPY), reelin, PV, SST, CTIP2 positive interneurons and neurogliaform cells (Gelman et al. 2009, 2011; Symmank et al. 2019). Likewise, the CGE produces a large variety of cortical interneurons including reelin positive cells, vasointestinal peptide (VIP)/calretinin positive bipolar interneurons and VIP/cholecystokinin positive basket cells (Hu et al. 2017; Miyoshi et al. 2010; Murthy et al. 2014; Rubin and Kessaris 2013).

Upon becoming post-mitotic, the different interneuron subsets migrate along particular routes through the basal telencephalon up to the cortex (Corbin and Butt 2011). This long-range tangential migration to cortical target regions represents a critical step. Apart from the initiation of migration by adapting a migratory morphology and the maintenance of their motility throughout the migratory period, the directionality has to be strictly controlled to achieve successful migration to the cortex, to precisely distribute over cortical areas and layers, and to finally integrate appropriately into cortical circuits [reviewed in Metin et al. (2006); Zimmer-Bensch (2018)].

4 Dynamic DNA Methylation in Neuronal Development

4.1 Key Players of DNA Methylation and Demethylation

DNA methylation is accomplished by DNA methyltransferases that in eukaryotes catalyze the methylation of predominantly cytosines at the fifth carbon of the pyrimidine ring yielding in 5-methylcytosine (5mC). DNA methylation of cytosines that are followed by guanines is called CpG methylation. In brain tissue as well as in human embryonic stem cells non-CpG or CpH methylation (H refers to adenine, thymine or another cytosine) is further prevalent (Guo et al. 2014; Lee et al. 2017; Pinney 2014). DNA methylation can be associated with silencing or activation of transcription, dependent on the methylated genomic regions and the DNA methylation-interacting proteins. DNA methylation can result in blocking the binding of transcription factors or recruiting methyl-binding proteins involved in gene silencing, thereby causing repression of gene transcription (Zhu et al. 2016). In addition to methyl-binding proteins a battery of transcription factors lacking the methyl-binding domain was suggested to interact with methylated DNA through different motifs, whereby the physiological relevance remains to be elucidated (Zhu et al. 2016).

Hypermethylation of CpG sites located in promoter or enhancer regions is often correlated with transcriptional repression (Chodavarapu et al. 2010; Lister et al. 2009). However, a substantial proportion of DNA methylation sites appears to be positively correlated with gene expression. Besides gene body methylation, which can be associated with repression and activation of gene expression (Lister et al. 2013; Yang et al. 2011), methylation upstream of transcriptional start sites can lead to transcriptional activation (Irizarry et al. 2009). Methylated cytosines are also evident in intergenic regions that control the transcription of genes nearby (Jones 2012). In neurons, alterations in CpH methylation were also found to correlate with transcriptional changes (Guo et al. 2014; Lister et al. 2013), emphasizing the gene regulatory potential of CpH methylation.

In the developing and adult nervous system, DNA methylation is achieved by DNMT1, DNMT3a and DNMT3b (Jang et al. 2017). Whereas DNMT1 acts as maintenance enzyme in dividing progenitors due to its high affinity to hemimethylated DNA, DNMT3a and DNMT3b were described as de novo methyltransferases (Jin and Robertson 2013). However, DNMT1 is also expressed in non-dividing post-mitotic neurons (Kadriu et al. 2012), where DNMT1 and DNMT3a can exert partly redundant (Feng et al. 2010) but also distinctive functions (Morris et al. 2016).

The discovery of active ways of DNA demethylation by Ten-eleven translocation (TET) family enzyme- dependent mechanisms (Wu and Zhang 2017) initiated a re-thinking about the functional implications of DNA methylation in post-mitotic and differentiated neurons. In the central nervous system, the DNA methylation landscape is dynamically altered throughout the developmental time course (Lister and Mukamel 2015; Lister et al. 2013), which has been related to cell-type specific

development and maturation (Lister and Mukamel 2015; Lister et al. 2013; Mo et al. 2015; Sharma et al. 2016). In the adult brain, dynamic DNA methylation was suggested to be involved in synaptic plasticity and memory formation (Kennedy and Sweatt 2016; Sweatt 2016; Meadows et al. 2015, 2016; Zovkic et al. 2013), while upon aging a shift in CpG methylation and a continuous increase in CpH methylation was described (Ianov et al. 2017).

4.2 DNA Methylation and Neurogenesis

The establishment of neuronal circuits relies on the proper generation of its diverse neuronal composites. Neurons are generated by neuronal stem cells, which become progressively restricted to generate the different types of neurons first (neurogenesis) and glia cells afterwards (gliogenesis). In addition to this temporal restriction, a spatial determination occurs early in development mediated by patterning (Kiecker and Lumsden 2005). For example, the excitatory and inhibitory neurons of the cerebral cortex derive from progenitors located in the dorsal and ventral telencephalon, respectively (Hu et al. 2017; Martynoga et al. 2012). The sequential generation of the excitatory neurons fated for the distinct layers of the cerebral cortex relies on progressive fate restriction (Martynoga et al. 2012), whereas progenitors of distinct spatial domains are proposed to give rise to different cortical interneuron subtypes (Hu et al. 2017). Although, diverse transcriptional networks and cascades implicated in interneuron subtype generation are already described, comparatively little is clear yet about the mechanisms of cell fate restriction in cortical interneuron progenitors, which contemporaneously give rise to inhibitory interneurons destined for diverse telencephalon regions (Hu et al. 2017). However, cell fate determination of both, excitatory principal cortical neurons and inhibitory interneurons, is associated with setting up subtype-specific transcriptional programs, directing subsequent developmental steps like migration, targeting and morphological differentiation (Franco and Muller 2013; Hu et al. 2017). Increasing body of evidence proposes a close connection between the epigenetic machinery and such stage- and subtype-specific transcriptional programs during neuronal differentiation. For example, the Nkx-class homeobox transcription factor 2.1 (NKX2-1), which is on top of the hierarchical transcriptional cascade governing development of MGE-derived inhibitory cortical interneurons (Flandin et al. 2010; McKinsey et al. 2013; Nobrega-Pereira et al. 2008; Sandberg et al. 2016; van den Berghe et al. 2013) also affects the epigenome, as significant alterations in histone profiles were observed in *NKX2-1* conditional knockout animals (Sandberg et al. 2016).

Indeed, dynamic temporal changes in DNA methylation patterns have been observed alongside with the sequentially generated neuronal subtypes (Lister and Mukamel 2015; Lister et al. 2013; Mo et al. 2015; Sharma et al. 2016). However, whether the methylome defines cell identity by suppressing alternative fates and thereby promoting a certain lineage, or whether the emergence of particular DNA

methylation profiles is a consequence of fate restriction driving subtype-specific developmental programs, is not clear so far.

In support of a role for DNA methylation in cell fate restriction, DNMTs are found widely expressed in neuronal precursors of the central nervous system (Feng et al. 2005). DNMT1 is suggested to be crucial for driving the neuronal fate by inhibiting astroglial differentiation during the neurogenic period. In the spinal cord, *Dnmt1* deficiency at progenitor level causes precocious astroglial differentiation and hypomethylation of genes associated to the gliogenic JAK/STAT pathway (Fan et al. 2005). Likewise, *Dnmt1*-deficiency promotes the differentiation of neuronal stem cells into astrocytes in precursors of the dendate gyrus (Noguchi et al. 2016b). Moreover, TET1 was suggested to contribute to the neurogenesis onset by promoting the expression of neuronal markers (Kim et al. 2016). In contrast to these findings, no indications of cell fate changes were observed upon the loss of *Uhrf1* in neuronal stem cells as determined by RNA-sequencing experiments (Ramesh et al. 2016), acting as important adaptor for DNMTs (Berkyurek et al. 2014). Hence, further research is required to decipher the detailed role of DNA methylation in neuronal progenitors, especially as DNMTs are known to act non-canonically through interactions with histone modifications in developing neurons (Symmank et al. 2018).

4.3 DNA Methylation in Post-mitotic Neuronal Development

Upon becoming post-mitotic, immature neurons migrate to their target regions where they adopt subtype-specific features in regard to morphology, molecular properties, firing and connectivity patterns. In addition to migration and morphological maturation including axonal and dendritic growth, programmed cell death is another crucial aspect of post-mitotic maturation that has to be highly regulated, to remove unconnected neurons and to regulate final neuron number (Southwell et al. 2012).

The establishment of methods for high resolution and large-scale methylome profiling lead to the discovery of highly dynamic DNA methylation reconfiguration during neuronal maturation (Lister and Mukamel 2015; Lister et al. 2013; Mo et al. 2015; Sharma et al. 2016). Thereby, different cell types like glia cells and neurons, but also distinct neuronal subtypes like GABAergic interneurons and glutamatergic projection neurons of the cerebral cortex differ vastly in their DNA methylation profiles (Kozlenkov et al. 2014, 2016; Lister et al. 2013). This points to a role of DNA methylation in cell type-specific maturation programs, whereby cell type-specific DNA methylation patterns seem rather a consequence than the cause of lineage-specification (Sharma et al. 2016).

Many post-mitotic developmental processes require the coordinated remodeling of the cytoskeleton, for example during migration, dendritic and axonal growth, and branching. In migrating cortical interneurons, DNMT1-dependent DNA methylation is suggested to regulate cytoskeleton-associated genes, thereby promoting the

migratory morphology required for proper migration (Pensold et al. 2017). DNMT1 target genes were identified by correlative methylome and transcriptome analysis applying MeDIP and RNA sequencing of FACS-enriched embryonic *Dnmt1* wild-type and knockout interneurons (Pensold et al. 2017). Among them *Pak6*, a member of the p21-activated kinases (PAKs), was found up-regulated in expression in *Dnmt1*-deficient cells (Pensold et al. 2017). PAKs are known to be involved in cell survival regulation as well as cytoskeletal rearrangements (Kumar et al. 2017), and PAK6 in particular was already shown to promote neurite complexity in excitatory cortical neurons (Civiero et al. 2015). Consistently, forced expression of PAK6 induced by a PAK6-GFP expression construct caused a multipolar morphology of embryonic interneurons, reminiscent to the phenotype determined for migrating *Dnmt1*-deficient interneurons (Pensold et al. 2017). In contrast, siRNA-mediated *Pak6* depletion reduced neurite complexity and cell death (Pensold et al. 2017). Hence, *Pak6* represents a downstream target of DNMT1-dependent transcriptional repression involved in cytoskeleton and cell death regulation underlying proper cortical interneuron migration (Fig. 1). However, no changes in the DNA methylation level of the *Pak6* gene locus, neither upstream nor downstream was observed in *Dnmt1*-deficient embryonic interneurons (Pensold et al. 2017; Symmank et al. 2018). Hence, DNA methylation-independent actions of DNMT1 likely account for the transcriptional regulation of *Pak6*. Indeed, many genes found altered in expression between *Dnmt1*-deficient and wild-type embryonic interneurons were not in conjunction with respective changes in DNA methylation and vice versa, pointing to non-canonical actions of DNMT1 (Pensold et al. 2017; Symmank et al. 2018).

In addition to DNA-methylation, DNMTs can also interact with histone modifying complexes (Du et al. 2015), thereby modulating transcription. There is evidence that DNA methylation inhibits permissive and supports repressive histone methylation to ensure gene silencing (Hashimshony et al. 2003; Lande-Diner et al. 2007). This can be achieved by direct interactions between DNA methylating and histone modifying enzymes via specific binding domains, which modulate the recruitment of proteins to complexes and the catalytic activity of their binding partners (Clements et al. 2012; Smallwood et al. 2007; Vire et al. 2006). For DNMT1, an interaction with EZH2, the core enzyme of the polycomp repressor complex 2 (PRC2) executing repressive trimethylations on lysine 27 at the N-terminal amino acid tail of histone 3 (H3K27me3) (Margueron and Reinberg 2011), was described to occur in non-neuronal cells (Ning et al. 2015; Purkait et al. 2016; Vire et al. 2006). Moreover, DNMT1 affects H3K27 trimethylation by modulating *Ezh2* expression levels (Purkait et al. 2016; So et al. 2011).

In migrating cortical interneurons, a crucial role of DNMT1-dependent establishment of repressive H3K27me3 marks was suggested to negatively act on *Pak6* gene expression (Symmank et al. 2018) (Fig. 1). Transcriptional repression of *Pak6* is crucial to maintain the migratory morphology and to promote interneuron survival, as determined by knockout and forced expression experiments (Pensold et al. 2017). Inhibition of EZH2, the core enzyme of the PRC2 (Chittock et al. 2017), executing H3K27 trimethylation, causes similar effects on neuronal

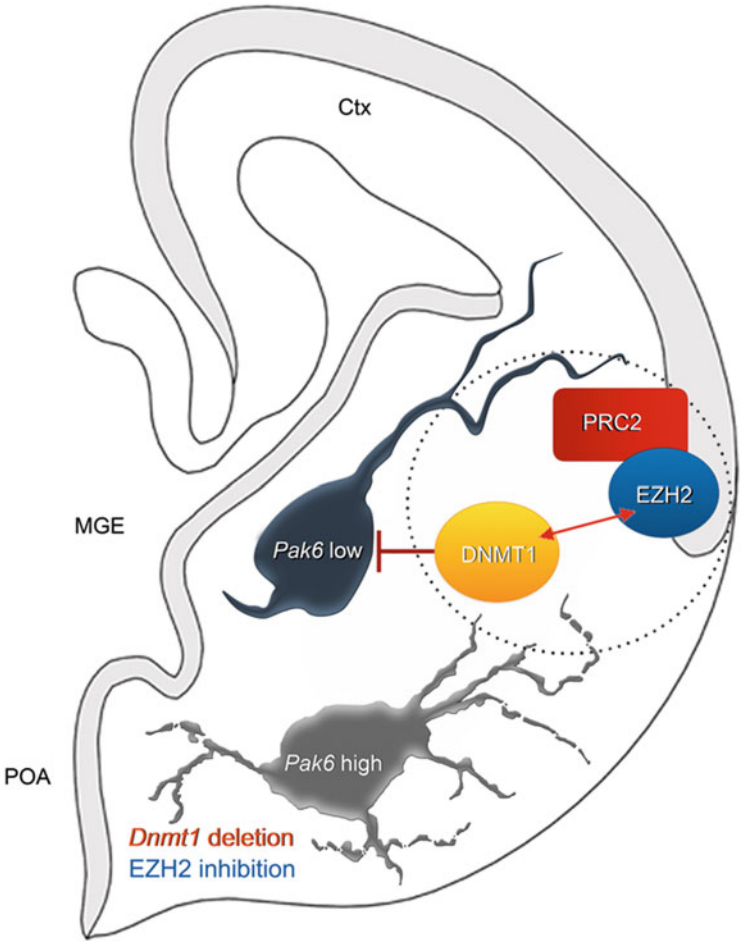

Fig. 1 Schematic view of a coronal section of one hemisphere of an embryonic mouse brain, illustrating a polarized migrating interneuron (dark blue) and a multipolar, degenerating interneuron (grey). DNMT1 promotes migration and survival by repressing *Pak6* expression, through interactions with EZH2 catalyzing the establishment of repressive H3K27me3 histone marks as the core enzyme of the polycomb repressor complex 2 (PRC2). In turn, *Dnmt1* deletion or EZH2 inhibition cause elevated *Pak6* expression levels and cellular complexity as well as cell death. *POA* preoptic area, *MGE* medial ganglionic eminence, *Ctx* cerebral cortex

complexity (Fig. 1), which are rescued by *Pak6* depletion (Symmank et al. 2018). Thereby, the DNMT1-dependent establishment of H3K27me3 marks were identified to rely on direct interactions of DNMT1 and EZH2 at protein level (Fig. 1) (Symmank et al. 2018).

DNMT1 has already been described to be critical for the post-mitotic maturation of other neuronal subtypes in vitro and in vivo (Chestnut et al. 2011; Fan et al. 2001; Hutnick et al. 2009; Rhee et al. 2012). DNMT1 promotes the morphological

maturation and refinement of cortical excitatory neurons (Feng et al. 2010; Hutnick et al. 2009), and is further crucial for the differentiation of dendate gyrus neurons (Noguchi et al. 2016b).

Another common role of DNMTs and DNA methylation during development of diverse neuronal subsets refers to cell death and survival regulation at post-mitotic level (Fan et al. 2001; Noguchi et al. 2016a; Pensold et al. 2017; Rhee et al. 2012). DNA hypomethylation perturbs the survival of neurons of the central nervous system (Fan et al. 2001) including retinal neurons (Rhee et al. 2012). *Dnmt1* deletion caused impaired survival of post-mitotic cortical interneurons (Pensold et al. 2017) and of newly generated hippocampal neurons in adult brains (Noguchi et al. 2015). While for retinal neuron survival DNMT1-dependent DNA methylation was proposed to be required (Rhee et al. 2012), non-canonical actions of DNMT1 through a crosstalk with histone modifications were suggested to contribute to the survival regulation in immature cortical interneurons (Pensold et al. 2017; Symmank et al. 2018).

The relevance of DNA methylation for survival regulation is further sustained by in vitro studies, showing an implication of TET2 function, involved in DNA demethylation, in cortical neuron survival (Mi et al. 2015). Consistently, *Tet1* deletion makes cerebellar granular cells more vulnerable towards oxidative stress-induced neuronal cell death (Xin et al. 2015).

Together, these studies emphasize a crucial role of DNA methylation as well as of non-canonical DNMT actions in post-mitotic neuronal development, including migration, morphological maturation, neuronal survival and cell death regulation.

5 DNA Methylation in the Aging Brain

5.1 Difficulties in Facing the Neurobiology of Aging

Aging causes structural, neurochemical and physiological alterations in the brain that lead to behavioral changes, memory decline and cognitive impairments (Rozycka and Liguz-Lecznar 2017). Cognitive aging depends on numerous factors and results in metabolic, hormonal and immune dysregulation, increased oxidative stress and inflammation, altered neurotransmission and reduced neurotrophic support of neurons (Rozycka and Liguz-Lecznar 2017). Thereby, different brain regions and neuronal cell types are distinctively affected by the aging process. In addition to reduced excitability and plasticity (Clark and Taylor 2011), and a decline of the inhibitory function (Cheng and Lin 2013; Shetty and Turner 1998; Stanley and Shetty 2004), a selective vulnerability of particular neuronal subtypes like inhibitory interneurons and GABAergic synapses (Rozycka and Liguz-Lecznar 2017) were observed in particular regions of aged brains.

However, observations like age-related changes in cell numbers differ between selected animal models and humans, and conflicting data even exist for the same species (Flood and Coleman 1988). Due to the important functions GABAergic

inhibitory interneurons have in cortical information processing, age-associated defects in inhibitory circuits appear as attractive hypothesis for cognitive decline and age-associated disorders (Rozycka and Liguz-Lecznar 2017). Indeed, several studies found reduced cell numbers of cortical interneuron subtypes across different species and brain regions (summarized in Table 1). Moreover, functional and structural changes of GABAergic synapses appear to occur in aged brains. These include loss of synaptic contacts, decreased neurotransmitter release, reduced post-synaptic responsiveness to neurotransmitters, suggested to contribute to the age-associated cognitive decline (Rozycka and Liguz-Lecznar 2017).

In agreement with reduced neurotransmitter release, major changes in the expression of genes related to neurotransmission and transcriptional repression especially of GABA-related transcripts have been reported for the human prefrontal cortex, which could however not be detected in non-primate mammals (Loerch et al. 2008). In contrast, to this, several studies described changes in transcripts related to GABAergic transmission across different species (summarized in Table 2).

Elevated neuroprotection-related gene expression and diminished expression of genes involved in general synaptic function at least appear as conserved features of mammalian brain aging (Ianov et al. 2016; Jiang et al. 2001; Loerch et al. 2008). Consistently, RNA sequencing of synaptosomes from cerebral cortices of aged mice moreover revealed changes in expression of synaptic transmission-related genes (Chen et al. 2017). Of note, in this study differential expression of diverse long non-coding RNAs were detected between young and old synaptosomes, proposed to be crucial for synaptic physiology.

Due to this heterogeneity in the reported structural, functional and transcriptional alterations in aged brains within one specie and between different species, approaching the functional implications of DNA methylation in brain aging is far from being a simple task. Comparative studies with more stringency in regard to the analysis of particular brain regions and individual cell types achieved by single cell methods enabling parallel single-cell based methylation and transcriptional analysis are needed to determine cell and species-specific age-related changes in DNA methylation and their transcriptional consequences.

Despite conflicting reports, murine models have called increased attention for investigating the neurobiology of aging and age-associated neurodegenerative diseases, due to the rapid evolution of mouse genetics and the comparatively short life span of mice (Bilkei-Gorzo 2014; Jucker and Ingram 1997).

5.2 The Implication of DNA Methylation Signatures for Brain Aging

Although DNA methylation signatures are altered upon aging in human and mouse brains (Lister et al. 2013; Siegmund et al. 2007), apparent region-specific differences impede general conclusions about their functional implications (Kraus et al. 2016;

Table 1 Summary of studies investigating age-associated alterations in cortical interneuron numbers across species

Species	Cortical area	Observations	Age of old species	References
Human	Ctx/Hc	Unchanged number of PV cells	>65 years	Bu et al. (2003)
Human	Visual Ctx and parahippocampal gyrus	Reduced density of CB-immunopositive cells	>65 years	Bu et al. (2003)
Human	Auditory Ctx	Reduced density of CCR-immunopositive cells	>65 years	Bu et al. (2003)
Cat	Visual Ctx	Reduced GABAergic interneurons	12 years	Hua et al. (2008)
Rat	Hc	Reduced GABAergic interneurons	26–30 months	Stanley et al. (2012)
Rat	Auditory Ctx	Decreased numbers of GAD65- and 67-immunoreactive neurons	30–35 months	Burianova et al. (2009)
Rat	Perirhinal Ctx	No differences in PV- or CR immunoreactivity	26 months	Moyer et al. (2011)
Rat	Auditory Ctx	Reduced CB interneuron numbers	>28 months	Ouda et al. (2012)
Rat	Auditory Ctx	Reduced numbers of PV interneurons	>28 months	Ouda et al. (2008)
Rat	Somatosensory and motor Ctx	Reduced numbers of PV interneurons	26 months	Miettinen et al. (1993)
Rat	Somatosensory and motor Ctx	Decreased SOM interneurons	26 months	Miettinen et al. (1993)
Rat	Hc	Decreased SOM interneurons	23 months	Stanley et al. (2012)
Rat	Hc	Reduced numbers of CB interneurons	25–30 months	Potier et al. (2006)
Rat	Sensory Ctx	Reduced numbers of VIP interneurons	20–29 months	Cha et al. (1997)
Rat	Frontal, occipital and temporal cortical areas, Hc	Reduced numbers of NPY neurons	20–29 months	Cha et al. (1997)
Rat	Auditory Ctx	Reduced numbers of NPY cells	25 months	Ouellet and de Villers-Sidani (2014)
Rat	Auditory Ctx	Decreased numbers of SOM and PV-interneurons	25 months	Ouellet and de Villers-Sidani (2014)

Ctx cortex, *Hc* hippocampus

Table 2 Summary of studies reporting age-related changes in mRNA or protein level of GABA-related genes across different species

Species	Cortical area	Observations	References
Primates	Ctx, Hc	Reduction of *Sst* mRNA	Hayashi et al. (1997)
Rat	Auditory Ctx	Reduced levels of *Gad1* and *Gad2* mRNAs	Ling et al. (2005)
Rat	Auditory Ctx	Decrease in the protein levels of GAD65 and GAD67	Burianova et al. (2009)
Monkey	Visual Ctx	Altered GABAergic gene expression	Liao et al. (2016)
Rat	Medial PFC	Reduction in GAT-1	Banuelos et al. (2014)
Human	Frontal Ctx	Reduction in GAT-1	Sundman-Eriksson and Allard (2006)
Rat	Hc	Decrease in the VGAT level	Canas et al. (2009)
Mouse	Barrel Ctx	Decreased *Vgat* mRNA and VGAT protein levels	Liguz-Lecznar et al. (2015)

PFC prefrontal cortex, *Ctx* cortex, *Hc* hippocampus

Numata et al. 2012). Another general challenge is the correlation of methylation marks with the transcriptional output to elucidate the physiological and biological relevance. Is the changed transcription a consequence of altered DNA methylation or do transcriptional alterations predispose for alterations in DNA methylation signatures?

As described above, the relationship between DNA methylation and expression depends on the genomic localization, with transcriptional potential being shown for DNA methylation within promoter regions, as well as within gene bodies, presumably at enhancer and silencer regions in introns and exons (Clermont et al. 2016; Kulis et al. 2013; Lee et al. 2015; Vinson and Chatterjee 2012). Hereinafter an overview about reported age-related changes in DNA methylation found for particular brain regions, genomic localizations and genes will be provided.

A decrease in CpG methylation upon aging was observed within repetitive sequences, including transposable elements (Ianov et al. 2017). Repressive DNA methylation contributes to genomic stability by preventing transposable elements from translocating in the DNA. Reduced DNA methylation causes increased transposon activity that has been related to diminished neuronal function and memory impairments during aging in Drosophila (Li et al. 2013).

In contrast to reduced methylation levels at CpG sites, non-CpG methylation, which can also causes gene silencing (Guo et al. 2014), continues to increase in the aging brain (Ianov et al. 2017; Lister et al. 2013). Interestingly, for aged cognitively impaired animals, hypermethylation of non-CpGs is enriched for synaptic genes suggesting that de novo methylation of non-CpGs is linked to the decrease in their expression (Ianov et al. 2017).

For activity and synapse-associated genes an increase in promoter methylation has further been reported (Haberman et al. 2012; Keleshian et al. 2013; Penner

et al. 2016). In contrast, promoter hypomethylation was detected for immune-related genes and seems associated with increased neuroinflammation (Mangold et al. 2017).

Hypermethylation in gene bodies of synaptic genes in conjunction with decreased expression was further reported for aged animals that display impaired PFC-dependent behavior (Ianov et al. 2017). CpG and non-CpG methylation of gene bodies and intergenic regions of synaptic plasticity genes can be modulated by environmental factors and correlate with respective changes in gene expression (Guo et al. 2011, 2014; Halder et al. 2016). These studies emphasize a potential relevance of gene body methylation of synapse-related gene expression during aging.

Among synapse-related genes found to be differentially methylated and expressed in orbital frontal cortices of aged human brains, many GABA-related genes were identified (McKinney et al. 2015), which is consistent with the age-associated alterations in the cortical GABAergic system observed across different species (Tables 1 and 2).

DNA methylation has been reported to be modulated by neuronal activity in the adult brain, which can be mediated by NMDA receptor activity (Guo et al. 2011; Penner et al. 2016). As many synapse and neuronal activity-related genes are altered in expression upon aging, subsequent physiological changes can act in turn on the DNA methylation landscape. For sure, more work needs to be done to dissect the function of DNA methylation in the aging brain.

Although different studies described DNA methylation as crucial for neuronal survival during development (Hutnick et al. 2009; Pensold et al. 2017; Rhee et al. 2012), evidence for direct survival regulation in the aging brain is still lacking. Support for potential functional implications of DNA methylation in neuronal cell death regulation arouse from patients diagnosed with Alzheimer's Disease, an age-related neurodegenerative disorder. In neurons of postmortem cortical tissue 5mC and 5hmC immunoreactivity was found globally altered compared to age-matched control individuals (Coppieters et al. 2014; Mastroeni et al. 2010). However, the age-related mechanisms that can culminate in neuronal death or neurodegeneration seem very diverse, involving oxidative stress, disturbed calcium homeostasis, chromosomal instability, impaired DNA repair, and the accumulation of nuclear and mitochondrial DNA damage. These can either contribute individually or in combination to age-associated cell death in the central nervous system. DNMT1 was already reported to function coordinately with the DNA damage repair in cancer (Jin and Robertson 2013), whereas potential involvements in regulating neuronal aging-related cell death still remain elusive and require further investigations.

Despite numerous open questions, the current data points to an implication of a drift of DNA methylation upon aging in influencing the regulation of long-term neuronal survival and the vulnerability towards age-associated neurodegenerative disorders.

6 DNA Methylation in Neuropsychiatric Diseases

Increasing body of evidence points to an epigenetic component in multifactorial neuropsychiatric disorders, to which genetic and environmental factors contribute. Epigenetic marks, which are sensitive to environmental insult, may account for the yet unexplained individual susceptibility and the variability in the course and etiology of diseases like schizophrenia, major depression disorder and autism.

DNA methylation turns out as a key epigenetic mechanism in major depression disorder (Pishva et al. 2017). Social psychological stress is proposed to cause methylation of genes relevant to the disease (McGowan et al. 2009; Oberlander et al. 2008), and DNA demethylation of neuronal cell death-related genes together with neuronal cell death were described to be associated with major depression disorder [reviewed in Symmank and Zimmer (2017)]. Moreover, DNA methylation of *Bdnf* causing reduced synthesis of BDNF, which is crucial for the development, survival and maintenance of neurons, has been linked to depression (Na et al. 2016; Roth et al. 2011).

A genome-wide methylation study has provided evidence for dysregulated DNA methylation profiles in cortical neurons in Autism Spectrum Disorder, whereby changes in DNA methylation affect genes involved in synaptic, neuronal and GABAergic processes (Nardone et al. 2017).

Altered DNA methylation in GABAergic interneurons seems further to be involved in the pathophysiology of schizophrenia. Increased *Dnmt1* expression and subsequently elevated DNA methylation levels are detected in cortical interneurons of patients diagnosed with schizophrenia (Costa et al. 2007; Ruzicka et al. 2007; Veldic et al. 2004). Site-specific analysis revealed that genes like *Reln* and *Gad1* relevant for GABAergic neurotransmission and interneuron function display elevated levels of DNA methylation (Costa et al. 2007; Ruzicka et al. 2007; Veldic et al. 2004). The altered methylation patterns correlate with reduced expression of these genes suggested to account for impaired interneuron function (Costa et al. 2007; Ruzicka et al. 2007; Veldic et al. 2004). Besides schizophrenia, disruption of GABAergic interneuron functionality has been associated with the pathophysiology of other psychological disorders including autism and epilepsy, whereby defects in cortical interneuron development might be of relevance (Marin 2012; Symmank and Zimmer 2017). In support of this, prenatal stress elevates *Dnmt1* and *Dnmt3a* expression in GABAergic interneurons and induces abnormalities in the DNA methylation network as well as behaviors indicative of a schizophrenia-like phenotype in offspring (Matrisciano et al. 2013).

In addition to the reported transcriptional changes caused by altered DNA methylation, a significant layer-specific loss of inhibitory interneurons was identified in postmortem studies of schizophrenia patients (Benes et al. 1991). In agreement with the cell loss, a death receptor pathway was recently shown to be implicated in the pathology of schizophrenia (Catts and Weickert 2012). However, similar to the ageing brain a direct link between cell death genes and DNA methylation is still lacking in the context of schizophrenia.

The transcriptional regulation by DNA methylation in cortical interneurons in disease-related contexts reported so far mostly refers to genes relevant for brain development and physiology including neuronal activity (Costa et al. 2007; Ruzicka et al. 2007; Veldic et al. 2004). The modulation of signal transmission, synaptic plasticity and membrane excitability by DNMT1 was also reported in cortical excitatory neurons under normal conditions (Feng et al. 2010; Levenson et al. 2006; Meadows et al. 2016). As neuronal activity is closely linked to neuron survival (Pfisterer and Khodosevich 2017; Rozycka and Liguz-Lecznar 2017), cell loss observed in diseased brains could be an indirect consequence of DNMT-dependent DNA methylation of genes involved in synaptic neurotransmission. Elevated *Dnmt1* expression in cortical interneurons is also related to the pathogenesis of mental impairments and psychosis due to neural injury and drug abuse (Guidotti et al. 2011; Lewis 2012; Veldic et al. 2005). Thus, the modulation of DNMT1 expression and function, particular in developing and adult cortical interneurons, appears crucial for proper circuitry and the functionality of the adult cerebral cortex, with potential impact on neuronal survival.

7 Conclusive Remarks

Epigenetic mechanisms of gene regulation like DNA methylation emerge as attractive mediators integrating external stimuli into the genome, as they appear sensitive towards environmental insults. The dynamic changes of DNA methylation signatures in the developing, adult and aging brain may account for the yet unexplained individual susceptibility and variability of age-related disorders as well as for neuropsychiatric diseases, which in part are developmental in their origin.

However, the implications of DNA methylation for discrete sub-cellular processes necessitate more detailed research. Besides deciphering cell subtype-specific effects, which can be addressed by innovative single cell sequencing approaches, the correlation with transcriptional changes represents a crucial aspect. Moreover, the crosstalk of DNA methylation with histone modifying mechanisms multiplies the spectrum of potential effects on gene transcription, and needs to be investigated context- and stage-specifically. Apart from that it is important to dissect, how context-dependent target-specificity of DNA methylation and demethylation is achieved during neuronal development and aging, and how environmental stimuli mechanistically act on DNA methylation.

Acknowledgments This work was funded by the DFG ZI 1224/4-1.

References

Agirman G, Broix L, Nguyen L (2017) Cerebral cortex development: an outside-in perspective. FEBS Lett 591:3978–3992

Akbarian S, Beeri MS, Haroutunian V (2013) Epigenetic determinants of healthy and diseased brain aging and cognition. JAMA Neurol 70:711–718

Banuelos C, Beas BS, McQuail JA et al (2014) Prefrontal cortical GABAergic dysfunction contributes to age-related working memory impairment. J Neurosci 34:3457–3466

Barter JD, Foster TC (2018) Aging in the brain: new roles of epigenetics in cognitive decline. Neuroscience 24:516–525

Benes FM, McSparren J, Bird ED et al (1991) Deficits in small interneurons in prefrontal and cingulate cortices of schizophrenic and schizoaffective patients. Arch Gen Psychiatry 48:996–1001

Berkyurek AC, Suetake I, Arita K et al (2014) The DNA methyltransferase Dnmt1 directly interacts with the SET and RING finger-associated (SRA) domain of the multifunctional protein Uhrf1 to facilitate accession of the catalytic center to hemi-methylated DNA. J Biol Chem 289:379–386

Bilkei-Gorzo A (2014) Genetic mouse models of brain ageing and Alzheimer's disease. Pharmacol Ther 142:244–257

Bu J, Sathyendra V, Nagykery N et al (2003) Age-related changes in calbindin-D28k, calretinin, and parvalbumin-immunoreactive neurons in the human cerebral cortex. Exp Neurol 182:220–231

Burianova J, Ouda L, Profant O et al (2009) Age-related changes in GAD levels in the central auditory system of the rat. Exp Gerontol 44:161–169

Butt SJ, Fuccillo M, Nery S et al (2005) The temporal and spatial origins of cortical interneurons predict their physiological subtype. Neuron 48:591–604

Butt SJ, Sousa VH, Fuccillo MV et al (2008) The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. Neuron 59:722–732

Canas PM, Duarte JM, Rodrigues RJ et al (2009) Modification upon aging of the density of presynaptic modulation systems in the hippocampus. Neurobiol Aging 30:1877–1884

Catts VS, Weickert CS (2012) Gene expression analysis implicates a death receptor pathway in schizophrenia pathology. PLoS One 7:e35511

Cha CI, Lee YI, Lee EY et al (1997) Age-related changes of VIP, NPY and somatostatin-immunoreactive neurons in the cerebral cortex of aged rats. Brain Res 753:235–244

Chen BJ, Ueberham U, Mills JD et al (2017) RNA sequencing reveals pronounced changes in the noncoding transcriptome of aging synaptosomes. Neurobiol Aging 56:67–77

Cheng CH, Lin YY (2013) Aging-related decline in somatosensory inhibition of the human cerebral cortex. Exp Brain Res 226:145–152

Chestnut BA, Chang Q, Price A et al (2011) Epigenetic regulation of motor neuron cell death through DNA methylation. J Neurosci 31:16619–16636

Chittock EC, Latwiel S, Miller TC et al (2017) Molecular architecture of polycomb repressive complexes. Biochem Soc Trans 45(1):193–205. https://doi.org/10.1042/bst20160173

Chodavarapu RK, Feng S, Bernatavichute YV et al (2010) Relationship between nucleosome positioning and DNA methylation. Nature 466(7304):388–392. https://doi.org/10.1038/nature09147

Civiero L, Cirnaru MD, Beilina A et al (2015) Leucine-rich repeat kinase 2 interacts with p21-activated kinase 6 to control neurite complexity in mammalian brain. J Neurochem 135 (6):1242–1256. https://doi.org/10.1111/jnc.13369

Clark BC, Taylor JL (2011) Age-related changes in motor cortical properties and voluntary activation of skeletal muscle. Curr Aging Sci 4(3):192–199

Clements EG, Mohammad HP, Leadem BR et al (2012) DNMT1 modulates gene expression without its catalytic activity partially through its interactions with histone-modifying enzymes. Nucleic Acids Res 40:4334–4346

Clermont PL, Parolia A, Liu HH et al (2016) DNA methylation at enhancer regions: novel avenues for epigenetic biomarker development. Front Biosci (Landmark edition) 21:430–446
Coppieters N, Dieriks BV, Lill C et al (2014) Global changes in DNA methylation and hydroxymethylation in Alzheimer's disease human brain. Neurobiol Aging 35:1334–1344
Corbin JG, Butt SJ (2011) Developmental mechanisms for the generation of telencephalic interneurons. Dev Neurobiol 71:710–732
Costa E, Dong E, Grayson DR, Guidotti A, Ruzicka W, Veldic M (2007) Reviewing the role of DNA (cytosine-5) methyltransferase overexpression in the cortical GABAergic dysfunction associated with psychosis vulnerability. Epigenetics 2(1):29–36
De Marco Garcia NV, Karayannis T, Fishell G (2011) Neuronal activity is required for the development of specific cortical interneuron subtypes. Nature 472:351–355
Druga R (2009) Neocortical inhibitory system. Folia Biol 55(6):201–217
Du J, Johnson LM, Jacobsen SE et al (2015) DNA methylation pathways and their crosstalk with histone methylation. Nat Rev Mol Cell Biol 16:519–532
Fan G, Beard C, Chen RZ et al (2001) DNA hypomethylation perturbs the function and survival of CNS neurons in postnatal animals. J Neurosci 21:788–797
Fan G, Martinowich K, Chin MH et al (2005) DNA methylation controls the timing of astrogliogenesis through regulation of JAK-STAT signaling. Development (Cambridge, England) 132:3345–3356
Feng J, Chang H, Li E et al (2005) Dynamic expression of de novo DNA methyltransferases Dnmt3a and Dnmt3b in the central nervous system. J Neurosci Res 79:734–746
Feng J, Zhou Y, Campbell SL et al (2010) Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat Neurosci 13:423–430
Flandin P, Kimura S, Rubenstein JL (2010) The progenitor zone of the ventral medial ganglionic eminence requires Nkx2-1 to generate most of the globus pallidus but few neocortical interneurons. J Neurosci 30:2812–2823
Flood DG, Coleman PD (1988) Neuron numbers and sizes in aging brain: comparisons of human, monkey, and rodent data. Neurobiol Aging 9:453–463
Franco SJ, Muller U (2013) Shaping our minds: stem and progenitor cell diversity in the mammalian neocortex. Neuron 77:19–34
Gelman DM, Martini FJ, Nobrega-Pereira S et al (2009) The embryonic preoptic area is a novel source of cortical GABAergic interneurons. J Neurosci 29:9380–9389
Gelman D, Griveau A, Dehorter N et al (2011) A wide diversity of cortical GABAergic interneurons derives from the embryonic preoptic area. J Neurosci 31:16570–16580
Gidon A, Segev I (2012) Principles governing the operation of synaptic inhibition in dendrites. Neuron 75:330–341
Guidotti A, Auta J, Chen Y et al (2011) Epigenetic GABAergic targets in schizophrenia and bipolar disorder. Neuropharmacology 60:1007–1016
Guo JU, Ma DK, Mo H et al (2011) Neuronal activity modifies the DNA methylation landscape in the adult brain. Nat Neurosci 14:1345–1351
Guo JU, Su Y, Shin JH et al (2014) Distribution, recognition and regulation of non-CpG methylation in the adult mammalian brain. Nat Neurosci 17:215–222
Haberman RP, Quigley CK, Gallagher M (2012) Characterization of CpG island DNA methylation of impairment-related genes in a rat model of cognitive aging. Epigenetics 7:1008–1019
Halder R, Hennion M, Vidal RO et al (2016) DNA methylation changes in plasticity genes accompany the formation and maintenance of memory. Nat Neurosci 19:102–110
Hansen DV, Lui JH, Parker PR et al (2010) Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature 464:554–561
Hashimshony T, Zhang J, Keshet I et al (2003) The role of DNA methylation in setting up chromatin structure during development. Nat Genet 34:187–192
Hayashi M, Yamashita A, Shimizu K (1997) Somatostatin and brain-derived neurotrophic factor mRNA expression in the primate brain: decreased levels of mRNAs during aging. Brain Res 749:283–289

Hensch TK (2005) Critical period plasticity in local cortical circuits. Nat Rev Neurosci 6:877–888
Hu JS, Vogt D, Sandberg M et al (2017) Cortical interneuron development: a tale of time and space. Development (Cambridge, England) 144:3867–3878
Hua T, Kao C, Sun Q et al (2008) Decreased proportion of GABA neurons accompanies age-related degradation of neuronal function in cat striate cortex. Brain Res Bull 75:119–125
Hutnick LK, Golshani P, Namihira M et al (2009) DNA hypomethylation restricted to the murine forebrain induces cortical degeneration and impairs postnatal neuronal maturation. Hum Mol Genet 18:2875–2888
Ianov L, Rani A, Beas BS et al (2016) Transcription profile of aging and cognition-related genes in the medial prefrontal cortex. Front Aging Neurosci 8:113
Ianov L, Riva A, Kumar A et al (2017) DNA methylation of synaptic genes in the prefrontal cortex is associated with aging and age-related cognitive impairment. Front Aging Neurosci 9:249
Inan M, Welagen J, Anderson SA (2012) Spatial and temporal bias in the mitotic origins of somatostatin- and parvalbumin-expressing interneuron subgroups and the chandelier subtype in the medial ganglionic eminence. Cerebral Cortex (New York, NY) 1991(22):820–827
Irizarry RA, Ladd-Acosta C, Wen B et al (2009) The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. Nat Genet 41:178–186
Jang HS, Shin WJ, Lee JE et al (2017) CpG and non-CpG methylation in epigenetic gene regulation and brain function. Genes 8:148
Jiang CH, Tsien JZ, Schultz PG et al (2001) The effects of aging on gene expression in the hypothalamus and cortex of mice. Proc Natl Acad Sci U S A 98:1930–1934
Jin B, Robertson KD (2013) DNA methyltransferases, DNA damage repair, and cancer. Adv Exp Med Biol 754:3–29
Jones PA (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 13:484–492
Jucker M, Ingram DK (1997) Murine models of brain aging and age-related neurodegenerative diseases. Behav Brain Res 85:1–26
Kadriu B, Guidotti A, Chen Y et al (2012) DNA methyltransferases1 (DNMT1) and 3a (DNMT3a) colocalize with GAD67-positive neurons in the GAD67-GFP mouse brain. J Comp Neurol 520:1951–1964
Keleshian VL, Modi HR, Rapoport SI et al (2013) Aging is associated with altered inflammatory, arachidonic acid cascade, and synaptic markers, influenced by epigenetic modifications, in the human frontal cortex. J Neurochem 125:63–73
Kennedy AJ, Sweatt JD (2016) Drugging the methylome: DNA methylation and memory. Crit Rev Biochem Mol Biol 51:185–194
Kiecker C, Lumsden A (2005) Compartments and their boundaries in vertebrate brain development. Nat Rev Neurosci 6:553–564
Kim H, Jang WY, Kang MC et al (2016) TET1 contributes to neurogenesis onset time during fetal brain development in mice. Biochem Biophys Res Commun 471:437–443
Kozlenkov A, Roussos P, Timashpolsky A et al (2014) Differences in DNA methylation between human neuronal and glial cells are concentrated in enhancers and non-CpG sites. Nucleic Acids Res 42:109–127
Kozlenkov A, Wang M, Roussos P et al (2016) Substantial DNA methylation differences between two major neuronal subtypes in human brain. Nucleic Acids Res 44:2593–2612
Kraus TF, Kilinc S, Steinmaurer M et al (2016) Profiling of methylation and demethylation pathways during brain development and ageing. J Neural Transm (Vienna) 123:189–203
Kulis M, Queiros AC, Beekman R et al (2013) Intragenic DNA methylation in transcriptional regulation, normal differentiation and cancer. Biochim Biophys Acta 1829:1161–1174
Kumar R, Sanawar R, Li X, Li F (2017) Structure, biochemistry, and biology of PAK kinases. Gene 605:20–31
Lande-Diner L, Zhang J, Ben-Porath I et al (2007) Role of DNA methylation in stable gene repression. J Biol Chem 282:12194–12200

Lardenoije R, Iatrou A, Kenis G et al (2015) The epigenetics of aging and neurodegeneration. Prog Neurobiol 131:21–64
Lee SM, Choi WY, Lee J et al (2015) The regulatory mechanisms of intragenic DNA methylation. Epigenomics 7:527–531
Lee JH, Park SJ, Nakai K (2017) Differential landscape of non-CpG methylation in embryonic stem cells and neurons caused by DNMT3s. Sci Rep 7:11295
Letzkus JJ, Wolff SB, Luthi A (2015) Disinhibition, a circuit mechanism for associative learning and memory. Neuron 88:264–276
Levenson JM, Roth TL, Lubin FD, Miller CA, Huang IC, Desai P, Malone LM, Sweatt JD (2006) Evidence that DNA (cytosine-5) methyltransferase regulates synaptic plasticity in the hippocampus. J Biol Chem 281(23):15763–15773
Lewis DA (2012) Cortical circuit dysfunction and cognitive deficits in schizophrenia--implications for preemptive interventions. Eur J Neurosci 35:1871–1878
Li W, Prazak L, Chatterjee N et al (2013) Activation of transposable elements during aging and neuronal decline in Drosophila. Nat Neurosci 16:529–531
Liao C, Han Q, Ma Y et al (2016) Age-related gene expression change of GABAergic system in visual cortex of rhesus macaque. Gene 590:227–233
Liguz-Lecznar M, Lehner M, Kaliszewska A et al (2015) Altered glutamate/GABA equilibrium in aged mice cortex influences cortical plasticity. Brain Struct Funct 220:1681–1693
Lin LC, Sibille E (2013) Reduced brain somatostatin in mood disorders: a common pathophysiological substrate and drug target? Front Pharmacol 4:110
Ling LL, Hughes LF, Caspary DM (2005) Age-related loss of the GABA synthetic enzyme glutamic acid decarboxylase in rat primary auditory cortex. Neuroscience 132:1103–1113
Lister R, Mukamel EA (2015) Turning over DNA methylation in the mind. Front Neurosci 9:252
Lister R, Pelizzola M, Dowen RH et al (2009) Human DNA methylomes at base resolution show widespread epigenomic differences. Nature 462:315–322
Lister R, Mukamel EA, Nery JR et al (2013) Global epigenomic reconfiguration during mammalian brain development. Science 341:1237905
Loerch PM, Lu T, Dakin KA et al (2008) Evolution of the aging brain transcriptome and synaptic regulation. PLoS One 3:e3329
Mangold CA, Masser DR, Stanford DR et al (2017) CNS-wide sexually dimorphic induction of the major histocompatibility complex 1 pathway with aging. J Gerontol A Biol Sci Med Sci 72:16–29
Margueron R, Reinberg D (2011) The polycomb complex PRC2 and its mark in life. Nature 469:343–349
Marin O (2012) Interneuron dysfunction in psychiatric disorders. Nat Rev Neurosci 13:107–120
Martynoga B, Drechsel D, Guillemot F (2012) Molecular control of neurogenesis: a view from the mammalian cerebral cortex. Cold Spring Harb Perspect Biol 4(10)
Mastroeni D, Grover A, Delvaux E et al (2010) Epigenetic changes in Alzheimer's disease: decrements in DNA methylation. Neurobiol Aging 31:2025–2037
Matrisciano F, Tueting P, Dalal I et al (2013) Epigenetic modifications of GABAergic interneurons are associated with the schizophrenia-like phenotype induced by prenatal stress in mice. Neuropharmacology 68:184–194
McGowan PO, Sasaki A, D'Alessio AC et al (2009) Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 12:342–348
McKinney BC, Lin CW, Oh H et al (2015) Hypermethylation of BDNF and SST genes in the orbital frontal cortex of older individuals: a putative mechanism for declining gene expression with age. Neuropsychopharmacology 40:2604–2613
McKinsey GL, Lindtner S, Trzcinski B et al (2013) Dlx1&2-dependent expression of Zfhx1b (Sip1, Zeb2) regulates the fate switch between cortical and striatal interneurons. Neuron 77:83–98
Meadows JP, Guzman-Karlsson MC, Phillips S et al (2015) DNA methylation regulates neuronal glutamatergic synaptic scaling. Sci Signal 8(382):ra61

Meadows JP, Guzman-Karlsson MC, Phillips S et al (2016) Dynamic DNA methylation regulates neuronal intrinsic membrane excitability. Sci Signal 9:ra83
Merot Y, Retaux S, Heng JI (2009) Molecular mechanisms of projection neuron production and maturation in the developing cerebral cortex. Semin Cell Dev Biol 20:726–734
Metin C, Baudoin JP, Rakic S et al (2006) Cell and molecular mechanisms involved in the migration of cortical interneurons. Eur J Neurosci 23:894–900
Mi Y, Gao X, Dai J et al (2015) A novel function of TET2 in CNS: sustaining neuronal survival. Int J Mol Sci 16:21846–21857
Miettinen R, Sirvio J, Riekkinen P et al (1993) Neocortical, hippocampal and septal parvalbumin- and somatostatin-containing neurons in young and aged rats: correlation with passive avoidance and water maze performance. Neuroscience 53:367–378
Miyoshi G, Hjerling-Leffler J, Karayannis T et al (2010) Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. J Neurosci 30:1582–1594
Mo A, Mukamel EA, Davis FP et al (2015) Epigenomic signatures of neuronal diversity in the mammalian brain. Neuron 86:1369–1384
Morris HM, Hashimoto T, Lewis DA (2008) Alterations in somatostatin mRNA expression in the dorsolateral prefrontal cortex of subjects with schizophrenia or schizoaffective disorder. Cereb Cortex 18:1575–1587
Morris MJ, Na ES, Autry AE et al (2016) Impact of DNMT1 and DNMT3a forebrain knockout on depressive- and anxiety like behavior in mice. Neurobiol Learn Mem 135:139–145
Moyer JR Jr, Furtak SC, McGann JP et al (2011) Aging-related changes in calcium-binding proteins in rat perirhinal cortex. Neurobiol Aging 32:1693–1706
Murthy S, Niquille M, Hurni N et al (2014) Serotonin receptor 3A controls interneuron migration into the neocortex. Nat Commun 5:5524
Na KS, Won E, Kang J et al (2016) Brain-derived neurotrophic factor promoter methylation and cortical thickness in recurrent major depressive disorder. Sci Rep 6:21089
Nardone S, Sams DS, Zito A et al (2017) Dysregulation of cortical neuron DNA methylation profile in autism spectrum disorder. Cereb Cortex 27:5739–5754
Ning X, Shi Z, Liu X et al (2015) DNMT1 and EZH2 mediated methylation silences the microRNA-200b/a/429 gene and promotes tumor progression. Cancer Lett 359:198–205
Nobrega-Pereira S, Kessaris N, Du T et al (2008) Postmitotic Nkx2-1 controls the migration of telencephalic interneurons by direct repression of guidance receptors. Neuron 59:733–745
Noguchi H, Kimura A, Murao N et al (2015) Expression of DNMT1 in neural stem/precursor cells is critical for survival of newly generated neurons in the adult hippocampus. Neurosci Res 95:1–11
Noguchi H, Kimura A, Murao N et al (2016a) Prenatal deletion of DNA methyltransferase 1 in neural stem cells impairs neurogenesis and causes anxiety-like behavior in adulthood. Neurogenesis (Austin) 3:e1232679
Noguchi H, Murao N, Kimura A et al (2016b) DNA methyltransferase 1 is indispensable for development of the hippocampal dentate gyrus. J Neurosci 36:6050–6068
Nonaka-Kinoshita M, Reillo I, Artegiani B et al (2013) Regulation of cerebral cortex size and folding by expansion of basal progenitors. EMBO J 32:1817–1828
Numata S, Ye T, Hyde TM et al (2012) DNA methylation signatures in development and aging of the human prefrontal cortex. Am J Hum Genet 90:260–272
Oberlander TF, Weinberg J, Papsdorf M et al (2008) Prenatal exposure to maternal depression, neonatal methylation of human glucocorticoid receptor gene (NR3C1) and infant cortisol stress responses. Epigenetics 3:97–106
Ouda L, Druga R, Syka J (2008) Changes in parvalbumin immunoreactivity with aging in the central auditory system of the rat. Exp Gerontol 43:782–789
Ouda L, Burianova J, Syka J (2012) Age-related changes in calbindin and calretinin immunoreactivity in the central auditory system of the rat. Exp Gerontol 47:497–506

Ouellet L, de Villers-Sidani E (2014) Trajectory of the main GABAergic interneuron populations from early development to old age in the rat primary auditory cortex. Front Neuroanat 8:40
Penner MR, Parrish RR, Hoang LT et al (2016) Age-related changes in Egr1 transcription and DNA methylation within the hippocampus. Hippocampus 26:1008–1020
Pensold D, Symmank J, Hahn A et al (2017) The DNA methyltransferase 1 (DNMT1) controls the shape and dynamics of migrating POA-derived interneurons fated for the murine cerebral cortex. Cereb Cortex 27:5696–5714
Pfisterer U, Khodosevich K (2017) Neuronal survival in the brain: neuron type-specific mechanisms. Cell Death Dis 8:e2643
Pinney SE (2014) Mammalian non-CpG methylation: stem cells and beyond. Biology 3:739–751
Pishva E, Rutten BPF, van den Hove D (2017) DNA methylation in major depressive disorder. Adv Exp Med Biol 978:185–196
Potier B, Jouvenceau A, Epelbaum J et al (2006) Age-related alterations of GABAergic input to CA1 pyramidal neurons and its control by nicotinic acetylcholine receptors in rat hippocampus. Neuroscience 142:187–201
Pouille F, Watkinson O, Scanziani M et al (2013) The contribution of synaptic location to inhibitory gain control in pyramidal cells. Phys Rep 1:e00067
Purkait S, Sharma V, Kumar A et al (2016) Expression of DNA methyltransferases 1 and 3B correlates with EZH2 and this 3-marker epigenetic signature predicts outcome in glioblastomas. Exp Mol Pathol 100:312–320
Ramesh V, Bayam E, Cernilogar FM et al (2016) Loss of Uhrf1 in neural stem cells leads to activation of retroviral elements and delayed neurodegeneration. Genes Dev 30:2199–2212
Rhee KD, Yu J, Zhao CY et al (2012) Dnmt1-dependent DNA methylation is essential for photoreceptor terminal differentiation and retinal neuron survival. Cell Death Dis 3:e427
Roth TL, Zoladz PR, Sweatt JD et al (2011) Epigenetic modification of hippocampal Bdnf DNA in adult rats in an animal model of post-traumatic stress disorder. J Psychiatr Res 45:919–926
Rozycka A, Liguz-Lecznar M (2017) The space where aging acts: focus on the GABAergic synapse. Aging Cell 16:634–643
Rubin AN, Kessaris N (2013) PROX1: a lineage tracer for cortical interneurons originating in the lateral/caudal ganglionic eminence and preoptic area. PLoS One 8:e77339
Ruzicka WB, Zhubi A, Veldic M et al (2007) Selective epigenetic alteration of layer I GABAergic neurons isolated from prefrontal cortex of schizophrenia patients using laser-assisted microdissection. Mol Psychiatry 12:385–397
Sandberg M, Flandin P, Silberberg S et al (2016) Transcriptional networks controlled by NKX2-1 in the development of forebrain GABAergic neurons. Neuron 91:1260–1275
Sharma RP, Tun N, Grayson DR (2008) Depolarization induces downregulation of DNMT1 and DNMT3a in primary cortical cultures. Epigenetics 3:74–80
Sharma A, Klein SS, Barboza L et al (2016) Principles governing DNA methylation during neuronal lineage and subtype specification. J Neurosci 36:1711–1722
Shetty AK, Turner DA (1998) Hippocampal interneurons expressing glutamic acid decarboxylase and calcium-binding proteins decrease with aging in Fischer 344 rats. J Comp Neurol 394:252–269
Siegmund KD, Connor CM, Campan M et al (2007) DNA methylation in the human cerebral cortex is dynamically regulated throughout the life span and involves differentiated neurons. PLoS One 2:e895
Smallwood A, Esteve PO, Pradhan S et al (2007) Functional cooperation between HP1 and DNMT1 mediates gene silencing. Genes Dev 21:1169–1178
So AY, Jung JW, Lee S et al (2011) DNA methyltransferase controls stem cell aging by regulating BMI1 and EZH2 through microRNAs. PLoS One 6:e19503
Southwell DG, Paredes MF, Galvao RP et al (2012) Intrinsically determined cell death of developing cortical interneurons. Nature 491:109–113
Stanley DP, Shetty AK (2004) Aging in the rat hippocampus is associated with widespread reductions in the number of glutamate decarboxylase-67 positive interneurons but not interneuron degeneration. J Neurochem 89:204–216

Stanley EM, Fadel JR, Mott DD (2012) Interneuron loss reduces dendritic inhibition and GABA release in hippocampus of aged rats. Neurobiol Aging 33:431 e431–431 e413

Sundman-Eriksson I, Allard P (2006) Age-correlated decline in [3H]tiagabine binding to GAT-1 in human frontal cortex. Aging Clin Exp Res 18:257–260

Sweatt JD (2016) Dynamic DNA methylation controls glutamate receptor trafficking and synaptic scaling. J Neurochem 137:312–330

Symmank J, Zimmer G (2017) Regulation of neuronal survival by DNA methyltransferases. Neural Regen Res 12(11):1768–1775

Symmank J, Bayer C, Schmidt C et al (2018) DNMT1 modulates interneuron morphology by regulating Pak6 expression through crosstalk with histone modifications. Epigenetics 13:536–556

Symmank J, Gölling V, Gerstmann K, Zimmer G (2019) The transcription factor LHX1 regulates the survival and directed migration of POA-derived cortical interneurons. Cereb Cortex 29(4):1644–1658

van den Berghe V, Stappers E, Vandesande B et al (2013) Directed migration of cortical interneurons depends on the cell-autonomous action of Sip1. Neuron 77:70–82

Veldic M, Caruncho HJ, Liu WS et al (2004) DNA-methyltransferase 1 mRNA is selectively overexpressed in telencephalic GABAergic interneurons of schizophrenia brains. Proc Natl Acad Sci U S A 101:348–353

Veldic M, Guidotti A, Maloku E et al (2005) In psychosis, cortical interneurons overexpress DNA-methyltransferase 1. Proc Natl Acad Sci U S A 102:2152–2157

Vinson C, Chatterjee R (2012) CG methylation. Epigenomics 4:655–663

Vire E, Brenner C, Deplus R et al (2006) The Polycomb group protein EZH2 directly controls DNA methylation. Nature 439:871–874

Wu X, Zhang Y (2017) TET-mediated active DNA demethylation: mechanism, function and beyond. Nat Rev Genet 18:517–534

Xin YJ, Yuan B, Yu B et al (2015) Tet1-mediated DNA demethylation regulates neuronal cell death induced by oxidative stress. Sci Rep 5:7645

Xu Q, de la Cruz E, Anderson SA (2003) Cortical interneuron fate determination: diverse sources for distinct subtypes? Cereb Cortex 13:670–676

Yang J, Ji WY, Qu YR et al (2011) DNA methylation and histone modification relate to RASSF1A gene deletion in laryngeal carcinoma tissues. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 46:308–312

Zhu H, Wang G, Qian J (2016) Transcription factors as readers and effectors of DNA methylation. Nat Rev Genet 17:551–565

Zimmer-Bensch G (2018) Diverse facets of cortical interneuron migration regulation – implications of neuronal activity and epigenetics. Brain Res 1700:160–169

Zovkic IB, Guzman-Karlsson MC, Sweatt JD (2013) Epigenetic regulation of memory formation and maintenance. Learn Mem 20:61–74