Neuroscience and Biobehavioral Reviews 102 (2019) 337–344

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

TET enzymes in neurophysiology and brain function

Cláudia Antunes[a,b], Nuno Sousa[a,b], Luisa Pinto[a,b,*], C. Joana Marques[c,d,*]

[a] Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar, Braga 4710-057, Portugal
[b] ICVS/3B's-PT Government Associate Laboratory, Braga/Guimarães, Portugal
[c] Department of Genetics, Faculty of Medicine, University of Porto, 4200-319 Porto, Portugal
[d] i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, 4200-135 Porto, Portugal

ARTICLE INFO

Keywords:
Neuronal plasticity
Behavior
Epigenetics
TET enzymes
5hmC

ABSTRACT

The dynamic nature of epigenetic DNA modifications is crucial for regulating gene expression in an experience-dependent manner and, thus, a potential mediator of neuronal plasticity and behavior. The discovery of the involvement of 5-hydroxymethylcytosine (5hmC) and Ten Eleven Translocation (TET) family of enzymes in the demethylation pathway uncovered a potential link between neuronal TET protein function and cognitive processes. In this review, we provide an overview on how profile of 5hmC and TET enzymes are powerful mechanisms to explain neuronal plasticity and long-term behaviors, such as cognition. More specifically, we discuss how the current knowledge integrates the function of each TET enzyme in neurophysiology and brain function.

1. Introduction

Given the post-mitotic nature of mature differentiated neurons, which are long-lived cells, there is a constant challenge to maintain genomic stability within a context of high plasticity that permits adaptation to diverse stimuli. Long-lasting changes in synaptic plasticity are dynamic processes which regulate higher functions, such as learning and memory, that require a tight regulation of gene expression. Epigenetic marks, consisting of chemical modifications on the DNA and histone tails, regulate the binding of transcription factors by modulating their accessibility to genomic regulatory regions. At the DNA level, site-specific modifications catalyzed by DNA methyltransferases (DNMTs) and TET enzymes subsequently affect the assembly of proteins that recognize methylated/demethylated bases (Pastor et al., 2013). Hence, epigenetic mechanisms and the neuronal epigenome constitute a valuable tool for marking past, current and future actions.

One of the most well studied epigenetic modifications is DNA methylation, which occur at the 5-carbon position of cytosine (C) residues and is located mainly at CpG dinucleotides. CpG sites are usually methylated but when occurring in CG-dense regions, termed CpG islands and associated with gene promoters, are largely resistant to DNA methylation (Smith and Meissner, 2013); nevertheless, methylation at these regions is usually associated with gene repression, acting to lock in the silent state (Deaton and Bird, 2011). DNA Methylation is also observed, albeit less frequently, in non-CpG contexts, known as CpH dinucleotides (H = A/T/C), and the function of non-CpG methylation is suggested to be repressive as well (Guo et al., 2014). Importantly, neurons (and embryonic stem cells) have a high amount of non-CpG methylation compared with other tissues, but the specific meaning of this fact remains to be clarified (Shin et al., 2014). However, Guo and colleagues showed that non-CpG methylation supports nearby CpG methylation by the recruitment of methyl-binding proteins and consequent suppressing of transcriptional activities *in vivo* (Guo et al., 2014).

DNA methylation is distributed throughout the mammalian genome and plays a crucial role in various biological functions, such as transposon silencing, genomic imprinting and X-chromosome inactivation, amongst others (Bird, 2002). Although DNA methylation is regarded as a stable feature, 5-methylcytosine (5mC) can be converted to 5-hydroxymethylcytosine (5hmC) by the Ten-Eleven Translocation (TET) family of enzymes, a process thought to be involved in the DNA demethylation process (Branco et al., 2011). Importantly, DNA methylation and hydroxymethylation have been implicated in neurophysiological processes, but also in neuropathology (Day and Sweatt, 2011; Sweatt, 2013). While 5mC levels are similar in the brain and other organs, the 5hmC modification is singularly enriched in the CNS, being up to ten times more abundant in the CNS than in peripheral tissues (Globisch et al., 2010; Kriaucionis and Heintz, 2009; Munzel et al., 2010; Szwagierczak et al., 2010). Mature neurons seem to be the major

* Corresponding authors at: Department of Genetics, Faculty of Medicine of Porto, Alameda Professor Hernâni Monteiro, 4200-319 Porto, Portugal and Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus Gualtar, Braga 4710-057, Portugal.
E-mail addresses: luisapinto@med.uminho.pt (L. Pinto), cmarques@med.up.pt (C.J. Marques).

https://doi.org/10.1016/j.neubiorev.2019.05.006
Received 5 November 2018; Received in revised form 8 May 2019; Accepted 8 May 2019
Available online 09 May 2019

contributors for 5hmC brain levels, since 5hmC levels are higher in neuronal than in non-neuronal cell types (Cadena-del-Castillo et al., 2014; Szulwach et al., 2011). Consistently, 5hmC levels in the brain greatly increase after birth, a time when synaptogenesis and neuronal maturation occurs, with no concomitant 5mC decrease (Song et al., 2011; Szulwach et al., 2011). 5hmC enrichment at promoters and gene bodies is positively correlated with gene expression levels, being particularly relevant during the postnatal period in the brain (Hahn et al., 2013; Song et al., 2011; Szulwach et al., 2011). Furthermore, 5hmC was shown to increase during development and with aging, in the mouse cerebellum and hippocampus brain regions (Szulwach et al., 2011). In parallel, several brain regions also show elevated levels of *Tet* transcripts (Szwagierczak et al., 2010). Proper synaptic function requires tight regulation of many genes involved in synaptic formation and plasticity (Azpurua and Eaton, 2015). Manipulation of TET enzymes have been shown to interfere with expression and methylation levels of some of these genes, suggesting they can influence synaptic activity through their catalytic demethylating action (Campbell and Wood, 2019). Thus, the abundance and dynamic profile of 5hmC and TET enzymes have been suggested as a powerful mechanism to explain neuronal plasticity and long-term behaviors.

2. TET enzymes in brain function

The TET family of enzymes consists of TET1, TET2 and TET3, all dependent on α-ketoglutarate (α-KG, also called 2-oxoglutarate) and Fe (II) and sharing the ability to convert 5mC to 5hmC (Ito et al., 2010; Pastor et al., 2013). TETs also mediate the oxidation of 5hmC to 5-formylcytosine (5fC) and subsequently to 5-carboxylcytosine (5caC) (Ito et al., 2011), adding another layer of complexity in the efforts to uncover the specific function of these enzymes in the brain. 5fC and 5caC bases are suggested to be intermediates in the DNA demethylation process, since these bases can be subjected to deamination by the glycosylase-dependent excision, mediated by thymine DNA glycosylase (TDG) and consequent repair by base excision repair (BER), resulting in unmodified cytosines (Branco et al., 2011). Also, 5mC and 5hmC bases can be converted to thymine and 5-hydroxymethyluracil (5hmU) respectively, by the action of activation-induced cytidine deaminase/apolipoprotein B mRNA editing enzyme, catalytic polypeptide (AID/APOBEC) cytosine deaminases. TET-induced oxidation is not limited to 5mC but thymine is also a substrate that gives 5hmU at least in mouse embryonic stem cells (Pfaffeneder et al., 2014). Thymine and 5hmU can be further the substrate for DNA glycosylases, such as TDG, strand-selective monofunctional uracil-DNA glycosylase 1 (SMUG), and methyl-CpG- binding domain protein 4 (MBD4) and ultimately, repaired by base excision repair (BER), resulting in unmodified cytosines (Fig. 1). 5hmU base has been reported to affect protein-binding to DNA and may also be an important intermediate in the generation of site-specific mutations (Kawasaki et al., 2017). In the brain, the 5hmC derivatives 5caC and 5fC have also been detected, although at much lower levels than 5hmC (a ratio of ~10,000:11:1 in human brain and ~4700:12:1 in mouse brain, for 5hmC, 5fC and 5caC respectively, was reported) (Liu et al., 2013), their relevance still being largely unknown. Structurally, the three TET enzymes share a conserved C-terminal catalytic domain that contains the metal-binding residues indispensable for the oxidation reaction and a less conserved N-terminal region (Kohli and Zhang, 2013). TET1 and TET3 also contain a N-terminal zinc finger cysteine-X-X-cysteine (CXXC) domain, that binds to methylated and unmethylated CpGs (Xu et al., 2011, 2012; Zhang et al., 2010), and recruits chromatin-modifying activities to CGI elements (Long et al., 2013). Contrarily, TET2 does not possess a CXXC domain but partners with IDAX, an independent CXXC-containing protein (Ko et al., 2013). In fact, human TET enzymes only share 18–24% sequence identity, raising the possibility of a non-redundant role between the three TETs (UniProt Consortium, 2015; Fasolino et al., 2017). Although all TET enzymes present highly conserved catalytic and Cys-rich domains, the CXXC region exhibits differences between proteins. This might suggest specific roles for each TET enzyme according to the DNA sequence context and genomic regions. Biochemical analyses showed that the CXXC domain of TET1 binds unmodified C or 5mC- or 5hmC-modified CpG-rich DNA, suggesting that TET1 also prevents DNA methyltransferase activity at CpG-rich regions (Xu et al., 2011). On the other hand, TET3 CXXC domain binds to both non-CpG and CpG DNA oligos; additionally, the TET3 CXXC domain strongly binds to CmCGG (Xu et al., 2012). Indeed, TET1 primarily regulates 5hmC levels at gene promoters and transcription start sites (TSSs), whereas TET2 mainly regulates 5hmC levels in gene bodies. Interestingly, the TSS localization of TET1 is thought to promote transcriptional activation, supported by its genomic localization primarily at regions with high levels of histones modifications associated with permissive chromatin (Williams et al., 2011; Wu et al., 2011). TET3 ChIP-seq data in NPCs shows that TET3-binding sites also cluster close to TSSs, suggesting that TET1 and TET3 may have similar functions, despite their distinct temporal expression patterns (Li et al., 2016). Therefore, besides their functionally redundant roles in the generation of 5hmC, TET-family members also display distinct roles, in part because they are expressed in different cellular locations or at different developmental stages and regulated 5hmC levels at different genomic locations (Li et al., 2016).

All *Tet* transcripts are present in the brain, with *Tet3* being the most abundant, at least in the cerebellum, cortex, and hippocampus, followed by *Tet2* and *Tet1* (Szwagierczak et al., 2010). All TETs exhibit strong co-localization with the neuronal marker NeuN (Kaas et al., 2013; Li et al., 2014; Mi et al., 2015), suggesting that its abundance is mainly attributed to neuronal cells, which is in line with 5hmC

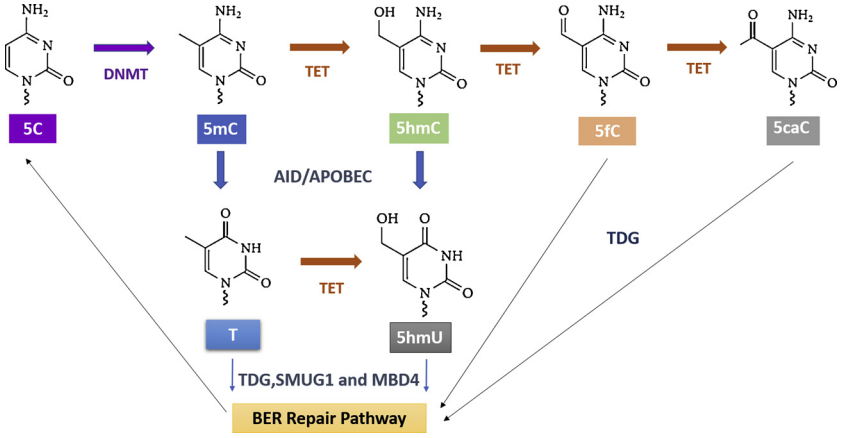

Fig. 1. Potential pathways for **TET-mediated active DNA demethylation cycle -** DNMTs convert unmodified C to 5mC. 5mC can be converted back to unmodified cytosine by TET mediated oxidation to 5hmC, 5fC and 5caC, followed by excision of 5fC or 5caC mediated by TDG coupled with BER. 5mC and 5hmC can be deaminated by AID/APOBEC, giving rise to T and 5hmU respectively, that are recognized by DNA glycosylases, producing an abasic site that is then repaired by the BER machinery. It was shown in mouse ES cells that TET enzymes can also convert T into 5hmU (Pfaffeneder et al., 2014). C, cytosine; 5-mC, 5-methylcytosine; 5-hmC, 5-hydroxymethylcytosine; 5fC, 5-formylcytosine; 5-caC, 5-carboxylcytosine; 5hmU, 5-hydroxymethyluracil; T, Thymine; DNMT, DNA methyltransferase; TET, Ten-eleven translocation enzyme; TDG, thymine DNA glycosylase; BER, base excision repair; AID/APOBEC, activation-induced cytidine deaminase/apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; SMUG1, strand-selective monofunctional uracil-DNA glycosylase 1; MBD4, methyl-CpG- binding domain protein 4.

Table 1
Phenotypes of full or conditional knockout (cKO) and knockdown (KD) mouse models of TET enzymes in neuronal plasticity and behavior.

Enzyme	Type of deletion	Region/Cell type	Phenotype	Molecular alterations	References
TET1	KO (*in vivo*)	Constitutive	Impairment in memory extinction; enhanced long-term depression (LTD)	Decreased expression of *Arc*, *Npas4*, *c-Fos*; hypermethylation of *Npas4* in the hippocampus and cortex	(Rudenko et al., 2013)
	KO (*in vivo*)	Constitutive	Impairment in spatial learning and short- term memory	Decreased expression and hypermethylation of *Gal*, *Cspg4* and *Ngb* in TET1 KO NPCs.	(Zhang et al., 2013)
	KO (*in vivo*)	Constitutive	Enhancement in memory consolidation and long-term storage	Decreased expression of *Arc*, *Egr1*, *Npas4* and *c-Fos*; increased expression of *Creb1*, *Bdnf*, *Calcineurin*, *Cdk5*, *Nr4a2* in the hippocampus CA1 region.	(Kumar et al., 2015)
	KD (*in vivo*)	Dorsal Hippocampus	Enhancement of spatial memory for object location	No analyzes were performed	(Kumar et al., 2015)
	KD (*in vitro*)	Neurons	Increased mEPSC amplitudes	No analyzes were performed	(Yu et al., 2015)
TET2	cKO (*in vivo*)	Adult neural progenitor cells	Impairment of short and long-term learning and memory	No analyzes were performed	(Gontier et al., 2018)
	KD (*in vitro*)	Neurons	Increased mEPSC amplitudes	No analyzes were performed	(Yu et al., 2015)
TET3	KD (*in vivo*)	ILPF cortex	Impairment in fear extinction memory	Inhibition of the increase of expression and 5hmC gain of *Gephyrin* locus in the ILPFC after extinction training	(Li et al., 2014)
	KO (*in vivo; CRISPR-mediated*)	Constitutive (Tet3-mutant chimeras)	Increased mEPSC frequency in CA1 and cortex layer 2/3 neurons and reduced mIPSC frequency and amplitudes	Slight hypermethylation (and decrease in expression) of *Bdnf* IV, IX and *Wfdc2*	(Wang et al., 2017)
	KD (*in vitro*)	Neurons	Increased mEPSC amplitudes	Increased expression of *Glur1* and decreased expression and hypermethylation of *Bdnf* IV	(Yu et al., 2015)

enrichment in these cells (Szulwach et al., 2011); nevertheless, it remains unclear what are the levels of expression in other non-neuronal cells of the CNS. To date, only one report shows TET1 expression in the soma of glial fibrillary acidic protein (GFAP) positive cells, hence identified as astrocytes, in the adult mouse hippocampus (Kaas et al., 2013). Regarding oligodendrocytes, expression of all TET enzymes was detected in the corpus callosum, from embryonic development until P30 (Zhao et al., 2014). The expression from that moment until the adult stage remains to be investigated, as well as its expression in other brain regions.

Since 2011, many studies have shown the importance of TET enzymes in neuronal function, which are summarized in Table 1 and described in detail for each TET enzyme, in the following sections, organized by neurophysiological and behavioral findings.

2.1. TET1

TET1, the first enzyme described as being capable of catalyzing the conversion of 5mC into 5hmC (Tahiliani et al., 2009), is the best-studied TET family member in the brain.

Regarding **neurophysiology**, there are two main studies reporting how TET1 is regulated in basal physiology. Kaas and collaborators observed that *Tet1* transcript levels are downregulated by neuronal activity either *in vitro,* when primary hippocampal neurons were incubated with KCl, resulting in cellular depolarization, or *in vivo,* in the dorsal CA1 subregion, after flurothyl-induced seizures or after fear conditioning (Kaas et al., 2013). All these approaches resulted in a significant reduction in *Tet1* mRNA levels compared to controls, while the transcripts of *Tet2* and *Tet3* did not consistently respond to stimulation using any of these activity-inducing paradigms. On the other hand, Yu and collaborators did not observe changes in *Tet1* (and *Tet2*) transcript levels when hippocampal neurons in culture were treated with bicuculline, a $GABA_A$ receptor antagonist commonly used to induce a robust increase in neuronal firing and synaptic activity, or with Tetrodotoxin (TTX), which decreases global synaptic activity (Yu et al., 2015). Additionally, TET1 KO mice exhibited normal basal synaptic transmission and presynaptic excitability in hippocampal slices (Kumar et al., 2015; Rudenko et al., 2013).

In terms of synaptic plasticity in the Schaffer collateral-CA1 pathway, it was observed that long-term depression (LTD) was significantly increased in the TET1 KO mouse (Rudenko et al., 2013), whereas hippocampal long-term potentiation (LTP) remained normal (Kumar et al., 2015; Rudenko et al., 2013). Considering that LTD is regulated by AMPA receptor trafficking and *Arc* modulates the trafficking of AMPA-type glutamate receptors (AMPARs) (Clem and Huganir, 2010; Liu and Cull-Candy, 2000), the observed downregulation of *Arc* (Rudenko et al., 2013) may affect proper function of various components of LTD machinery. Additionally, previous studies demonstrated a connection between LTD and memory extinction (Dalton et al., 2008; Kim et al., 2011; Ryu et al., 2008; Tsetsenis et al., 2011). Additionally, overexpression of either the catalytic active or inactive forms of TET1 peptide did not lead to any significant effects in LTP either (Kumar et al., 2015). In terms of basal electrophysiology findings, *in vitro* work showed that *Tet1* knockdown (KD) in primary hippocampal neurons leads to increased miniature excitatory postsynaptic current (mEPSC) amplitudes (Yu et al., 2015).

The TET1 KO mouse model was used to unravel a potential connection between TET1 protein function and **behavior/cognitive processes**. In terms of learning and memory, there are conflicting results (Rudenko et al., 2013; Zhang et al., 2013). Zhang and colleagues addressed the putative involvement of TET1 in neural plasticity using hippocampal-dependent cognitive tasks, such as spatial memory (Broadbent et al., 2004). Both WT and TET1 KO mutants exhibited similar escape latency and swim path to the visible platform, suggesting comparable vision and motivation between the two groups. However, when short term memory retention was tested (24 h after the 5-day

training), the mutant group showed significant deficiency in reaching the virtual platform, measured by both the platform crossing and the time spent in the target quadrant, indicating that *Tet1* deficiency can lead to **impairment in spatial learning and short-term memory.** The brain structure was analyzed but no obvious morphological or developmental brain abnormalities were observed (Zhang et al., 2013), similarly to what was observed by other authors (Rudenko et al., 2013). Considering adult neurogenesis implication in spatial learning and memory, a potential link between memory impairment and the lack of TET1 was further explored. Using Nestin-GFP transgenic mice, the authors observed that, when TET1 was ablated in neural precursor cells, the number of GFP-positive cells in the subgranular zone of the hippocampal dentate gyrus (DG) in adult mice was dramatically reduced, by 45%, compared to WT animals (Zhang et al., 2013). Analysis of gene expression and methylation changes in TET1 KO mice revealed decreased expression of a cohort of genes involved in neurogenesis, including *Galanin* (*Gal*), *Ng2* (*Cspg4*) and *Neuroglobin* (*Ngb*). Methylation analysis using gene-specific bisulfite sequencing showed that the promoter regions of these genes were hypermethylated, suggesting that TET1 positively regulates adult neurogenesis through the oxidation of 5mC to 5hmC in these genes (Zhang et al., 2013).

In contrast to the results by Zhang and collaborators, Rudenko and colleagues reported normal short-term memory and spatial learning, but impaired memory extinction of both contextual fear memory and spatial reference memory (Rudenko et al., 2013). The authors observed normal locomotor behavior and no changes in anxiety and depressive-like behaviors, as well as no difference was also observed in contextual learning and cued fear memory acquisition. However, regarding memory extinction, the authors reported impaired memory extinction in TET1 KO mice, both after contextual fear conditioning and for hippocampus-dependent spatial reference memory, using the Morris water maze (MWM) test. Several neuronal activity-regulated genes were found to be downregulated, namely *Arc*, *Npas4* and *c-Fos*, in the cortex and hippocampus. Hypermethylation of the *Npas4* promoter region was observed in the cortex and in the hippocampus of both naïve TET1 KO mice and after extinction training. *Npas4* is a transcription factor highly expressed in the brain which regulates the formation and maintenance of inhibitory synapses in response to excitatory synaptic activity; it was shown to be a key regulator of transcriptional programs involving neural activity-regulated genes and essential for contextual memory formation and regulation of cognitive and social functions (Coutellier et al., 2012; Ramamoorthi et al., 2011).

These results might indicate independent epigenetic programs being activated during memory acquisition *versus* memory extinction. Nonetheless, the discrepancy between TET1 role in spatial learning and memory could also be explained by the differences in the TET1 KO mouse models, with distinct exons being targeted (exon 4 in the study by Rudenko et al., resulting in an unstable truncated form; and exons 11–13 in the study by Zhang et al., which are part of the catalytic domain). Moreover, no other learning and memory tasks were used beyond MWM in the study by Zhang and colleagues, whereas Rudenko and colleagues used Pavlovian fear conditioning showing that TET1 mutant mice have normal memory acquisition.

Additionally, a curious finding was the observation of memory enhancement in TET1 KO animals, namely threat recognition learning, long-term memory and remote memory consolidation (Kumar et al., 2015). Consistent with a previous study (Rudenko et al., 2013), this group found normal threat memory acquisition and short-term fear memory in TET1 KO mice. However, an enhancement in memory consolidation and long-term storage was observed in TET1 KO, using contextual and cued fear conditioning tests. These are apparent opposing results when compared with Zhang and colleagues work, which showed an impairment in spatial learning (Zhang et al., 2013). Kumar and colleagues suggested that these might be attributed to the behavior test used since MWM and fear conditioning are both hippocampal-dependent tasks, but MWM may involve stronger and more aversive motivational factors than fear conditioning and occurs over many more training trials of longer duration (Kumar et al., 2015). These differences might account for differential susceptibilities to effects of TET1 knockout in the water maze *versus* fear conditioning behavioral tests.

Using a virally mediated knockdown of *Tet1* mRNA in the dorsal hippocampus, they also observed an enhancement in hippocampus-dependent long-term spatial memory for object location (Kumar et al., 2015). Zhang and colleagues reported that TET1 KO impairs hippocampal-dependent spatial short-term memory, using the MWM test (Zhang et al., 2013). Hence, distinct roles for TET1 in different memory types can explain these differences. At the molecular level, Kumar and colleagues also found that TET1 ablation resulted in altered expression of numerous neuronal activity-regulated genes, such as increased expression of *Bdnf* and decreased levels of *Arc*, *Fos* and *Npas4*, as previously observed by others (Rudenko et al., 2013). Interestingly, a compensatory upregulation of *Tet2* and *Tet3* was reported, together with increased transcript levels of other genes involved in the active DNA demethylation pathway, such as *Gadd45b*, *Smug*, *Apobec1* and *Tdg*. Intriguingly, a strong upregulation was also observed for DNA methyltransferases *Dnmt1*, *Dnnmt3a* and *Dnmt3b*, suggesting coordination of the epigenetic regulators transcriptional network in the CNS (Kumar et al., 2015).

In addition to loss-of-function studies, the discovery that TET1 expression is downregulated in the dorsal CA1 of mice after fear learning motivated gain-of-function studies: TET1 overexpression (OE) in the dorsal hippocampus did not affect exploratory or anxiety-like behavior but impaired long-term, but not short-term, memory in the contextual fear conditioning (CFC) test (Kaas et al., 2013). This deficit in long-term memory formation was observed for both catalytically active and inactive forms of TET1, suggesting that TET1's role in memory formation is independent of its catalytic activity but may rely on an allosteric mechanism and contribute to explain non-redundancy between TET enzymes. Importantly, the authors found the same set of genes (*Fos*, *Nr4a2*, *Bdnf*, *Homer1*) upregulated by overexpression of TET1 and TET1m, suggesting that TET1 regulates the expression of these genes, at least in part independently of 5mC to 5hmC conversion, and that these genes might be responsible for the observed memory dysfunction. Another gain-of-function study has shown that overexpression of either the catalytically active or the catalytically inactive TET1 peptide did not lead to any significant effect on LTP compared with control, and basal synaptic transmission also remained constant (Kumar et al., 2015).

Additionally, TET1 overexpression, but not TET1m, led to an increase in 5hmC levels in the microdissected CA1 area, concomitant with a decrease in global 5mC levels, suggesting an increase in global 5mC to 5hmC conversion (Kaas et al., 2013). Furthermore, TET1 OE resulted in upregulation of many neuronal activity-related genes such as *c-Fos*, *Bdnf*, *Arc*, *Egr1* (Kaas et al., 2013), whereas TET1 KO resulted in downregulation of some of these genes (Rudenko et al., 2013). Therefore, considering the downregulation of Immediate Early Genes (IEGs) in TET1 KO mice and their upregulation in TET1 OE in hippocampal regions, these studies suggest that *Tet1* bidirectionally regulates IEGs levels. Similarly, Guo and collaborators performed overexpression of TET1, and TET1m, in the adult mouse dentate gyrus and observed that OE of TET1, but not TET1m, led to an increase in the levels of 5hmC by 43% (Guo et al., 2011). Concerning methylation levels at specific neuronal-genes, namely *Bdnf* and *Fgf1*, the authors reported that overexpression of TET1, but not TET1m, led to significant decreases in CpG methylation levels at promoter IX of *Bdnf* and brain-specific promoter of *Fgf1*. On the other hand, *Tet1* knockdown in the adult dentate gyrus completely abolished electroconvulsive stimulation (ECS)-induced demethylation of both *Bdnf*IX and *Fgf1B*, suggesting that *Tet1* is required for neuronal activity-induced, region-specific, active DNA demethylation and gene expression in the adult brain (Guo et al., 2011).

Together, these findings support that TET1 contributes to basal neuronal 5hmC levels, and this interferes with the regulation of

important neuronal regulatory genes. However, the behavioral effects of TET1 should still motivate further investigation, considering the discrepant results in short-term memory and spatial learning.

2.2. TET2

TET2 is the least characterized TET enzyme member in the brain, despite its high level of expression (Szwagierczak et al., 2010). Whilst brain defects have not been described in TET2 KO mouse model (Ko et al., 2011; Li et al., 2011), a behavioral characterization was missing.

Regarding **neurophysiology**, *in vitro* studies using hippocampal neurons did not show changes in *Tet2* mRNA levels after global synaptic activity increase or decrease, induced by bicuculline or tetrodotoxin, respectively. However, association of this enzyme with **basal** synaptic transmission has been observed since hippocampal neurons with decreased *Tet2* expression exhibited increased mEPSC, similarly to what was observed in *Tet1* KD (Yu et al., 2015).

Additionally, a role for TET2 in neurogenesis was firstly proposed by Hahn and collaborators, as the double knockdown of *Tet2* and *Tet3* in the mouse embryonic cortex led to defects in the differentiation of the cells migrating from the subventricular zone to the cortical plate (Hahn et al., 2013). More recently, another work using a TET2 KO mouse model showed that depletion of TET2 leads to increased adult neural stem cell proliferation, but reduced differentiation capacity *in vitro* and *in vivo* (Li et al., 2017). Mechanistically, the authors show that *Tet2* physically interacts with forkhead box O3 (*Foxoa3*) and regulates expression of genes related to neural stem cell proliferation. *Foxoa3* is a mammalian forkhead family member, well known to regulate gene expression and help preserve an intact pool of neural stem cells, at least in part by negatively regulating neuronal differentiation (Rafalski and Brunet, 2011). To overcome the limitations of a constitutive full knockout model, a more recent work used a conditional model ablating *Tet2* in adult Neural Precursor Cells (NPCs) and demonstrated that the specific deletion of this enzyme in adult NPCs is sufficient to impair the neurogenic process, translated by a significant decrease in the number of Doublecortin (Dcx)-positive newly-born neurons, Bromodeoxyuridne (BrdU)-positive cells and BrdU/NeuN-positive mature differentiated neurons (Gontier et al., 2018). The authors also observed that decreased levels of *Tet2* expression, achieved by shRNA injection in the hippocampal neurogenic niche, resulted in a significant decrease in the number of NPCs and newly-born neurons, as observed by conditional deletion in NPCs.

Additionally, for the first time, a **behavioral** evaluation was performed, showing that reducing *Tet2* levels in the hippocampus impairs cognitive function, namely hippocampal-dependent learning and memory which were assessed using radial arm water maze (RAWM) and contextual fear-conditioning (CFC) paradigms (Gontier et al., 2018). Both the animals presenting a global abrogation of TET2 in the Dentate Gyrus (known as the adult hippocampal neurogenic niche) and mice carrying a conditional deletion of TET2 in adult NPCs showed worse performance in finding the platform location during both short-term and long-term learning and memory probes. When measuring the freezing time after fear conditioning training, both TET2 ablation models showed decreased freezing time during contextual, but not cued, memory testing. Thus, TET2 decreased levels in the adult neurogenic niche or specifically in adult NPCs resulted in impaired long-term hippocampal-dependent spatial learning and memory and associative fear memory acquisition. Interestingly, the authors also observed that restoration of TET2 levels in the aged brain was sufficient to rescue age-related regenerative decline as observed by the increased number of NPCs and newly-born neurons, the similar learning capacity in RAWM performance and an increased freezing time during contextual memory test when comparing animals under this rescue with the control group (Gontier et al., 2018). These findings suggest an important role for TET2 in the regulation of neurogenesis and cognitive functions, and a key molecular mediator of neurogenic rejuvenation.

2.3. TET3

The most highly expressed TET enzyme member in the brain, TET3, was also described as an essential enzyme in neuronal differentiation, including maintenance of NPCs *in vitro* (Li et al., 2015) and *in vivo* during early neocortical development (Lv et al., 2014).

Regarding **neurophysiology**, TET3 was described as a synaptic activity sensor, since TET3 levels are sensible to neuronal activity, and this enzyme reacts to it, mediating homeostatic synaptic transmission (Yu et al., 2015). Synaptic activity bi-directionally regulates neuronal *Tet3* expression, and consequently *Tet3* controls glutamatergic synaptic transmission through regulation of target genes, namely glutamate receptor 1 (*GluR1*) levels (Yu et al., 2015). Neurons with *Tet3* knockdown exhibited substantially larger miniature glutamatergic excitatory post-synaptic current (mEPSC) amplitudes whereas *Tet3* overexpression decreased this parameter. It should be noted that although both *Tet1* and *Tet2* knockdowns also increase mEPSC amplitudes, the effects are less pronounced. Furthermore, when DNA demethylation was inhibited through the blocking of the two major components of the BER pathway, the poly (ADP-ribose) polymerase or the apurinic/apyrimidinic endonuclease, the mEPSC amplitudes were also increased, resembling the *Tet3* KD (Yu et al., 2015). These results suggest that excitatory synaptic transmission in neurons is regulated through DNA oxidation *via* TET and, subsequently, BER.

Additionally, it was shown that *Tet3* is required for homeostatic synaptic plasticity. Both *Tet3* KD and BER inhibition elevated mEPSC amplitudes linearly across the spectrum under basal conditions, which was comparable to the scaling-up effect induced by TTX treatment in normal neurons. Thus, downregulation of *Tet3* signaling appears to be sufficient to induce scaling-up. On the other hand, neurons overexpressing *Tet3* exhibited reduced mEPSC amplitudes linearly across the spectrum, resembling bicuculline-induced scaling-down in normal neurons. Hence, the authors suggested that global synaptic activity modulates *Tet3* expression and DNA demethylation activity, which in turn mediate homeostatic synaptic scaling-up or scaling-down (Yu et al., 2015).

A key cellular mechanism regulating both basal glutamatergic synaptic transmission and homeostatic scaling is the control of surface levels of glutamate receptors. Yu and colleagues have shown that *Tet3* regulates basal excitatory synaptic transmission *via* regulating surface *GluR1* levels (Yu et al., 2015). Also, *Tet3* knockdown was sufficient to elevate surface *GluR1* levels and prevented further changes induced by TTX or bicuculline treatments. Regulation of *Arc* levels appears to explain changes in surface *GluR1* levels following *Tet3* KD. Together, these results suggest that *Tet3* and active DNA demethylation signaling respond to changes in global synaptic activity to re-establish a responsive cellular state. Moreover, transcriptome analysis of *Tet3*-KD neurons revealed differential expression of genes involved in the synapse and synaptic transmission, suggesting an essential role for *Tet3* in regulating gene expression in response to changes in global synaptic activity. *Bdnf*, already described as undergoing active demethylation in depolarized neurons (Ma et al., 2009) and implicated in synaptic transmission and synaptic scaling (Rutherford et al., 1998), was hypermethylated at the promoter IV region in *Tet3* KD neurons, with a consequent decrease in its expression. Interestingly, whereas *Tet1*-deficient neurons exhibited hypermethylation at *Arc* and *Npas4* promoters (Rudenko et al., 2013), Tet3-KD neurons did not. No changes in methylation were observed at the *Arc* or *Npas4* promoter regions, suggesting that activity-induced expression of immediate early genes *Arc* and *Npas4* is mediated by the oxidative function of TET1, but not of TET3. A physical interaction between TET3 and *Bdnf IV* promoter region was described by the authors in neurons, using chromatin immunoprecipitation (ChIP)-PCR analysis (Yu et al., 2015).

A more recent paper used CRISPR-Cas9 technology, termed 2-cell embryo-CRISPR-Cas9 injection (2CC), to induce *in vivo Tet3* loss-of-function and recorded AMPAR-mediated miniature **excitatory** post-

synaptic currents (mEPSCs) from layer 2/3 pyramidal neurons of the primary somatosensory cortex of P14 chimeric mice and from hippocampal CA1 neurons (Wang et al., 2017). The authors observed that *Tet3*-mutant neurons had a significantly higher mEPSC frequency and a similar mEPSC amplitude in layer 2/3 neurons whereas in the hippocampus both the frequency and amplitudes were significantly increased, suggesting an important role of endogenous *Tet3* in negatively regulating excitatory synaptic transmission in young mice. These findings corroborated Yu and colleagues *in vitro* studies reporting the role of TET3 in the downregulation of excitatory synaptic transmission. Bisulfite sequencing analyses revealed slightly increased CpG methylation at the *Bdnf* IV, IX and *Wfdc2* promoter regions, consistent with Yu and colleagues, but not on the *Npas4* promoter-exon 1 junction or the *Fgf1G* and *Ndst1* promoter regions. Additionally, loss of TET3 function significantly reduced both the frequency and amplitude of $GABA_A$R-mediated **inhibitory** synaptic transmission, as measured by miniature inhibitory post-synaptic currents (mIPSCs) in the cortical layers 2/3 pyramidal neurons and hippocampal CA1 region, suggesting a promoting role of endogenous *Tet3* in regulating inhibitory synaptic transmission as well (Wang et al., 2017).

In vivo **behavioral** studies correlated *Tet3* mRNA expression levels in the hippocampus with neuronal activity after Contextual Fear Conditioning (CFC) behavioral test. The authors observed that *Tet3* mRNA transcripts, but not *Tet1* and *Tet2*, were upregulated after 30 min and 3 h, but returned to baseline after 24 h (Kremer et al., 2018). Importantly, *Tet3* expression was not modified by cold swim stress suggesting that the changes were specific to memory formation in CFC and were not related to the stress response elicited by fear conditioning. When the NMDA (N-methyl-D-aspartate) receptors were activated in primary hippocampal neurons, *Tet3* mRNA levels were upregulated, suggesting that NMDA receptor signaling increases *Tet3* transcription. Expression levels of *mir-29b* were also altered, being downregulated, after NMDA receptors stimulation indicating another target of this glutamate receptor. Transcriptional analysis in hippocampus 30 min after training showed that synaptic plasticity and genes related with memory, such as *Notch1*, *Creb1*, *Crebbp* and *Gadd45b* are sensitive to TET3 upregulation (Kremer et al., 2018).

Li and collaborators described upregulation of *Tet3* transcript levels, but not *Tet1* as reported by others (Guo et al., 2011; Zhang et al., 2013), in primary cortical neurons after 7 h and 10 h of KCl-induced depolarization (Li et al., 2014). Consistently, *Tet3* was also upregulated in the infralimbic prefrontal cortex (ILPFC) after fear extinction training. Moreover, *Tet3* knockdown in the ILPFC resulted in normal fear memory acquisition but impairment in fear extinction memory (Li et al., 2014). Genome-wide analyses revealed that 16% of genes with 5hmC gain after fear extinction training were associated with synaptic signaling. One example was *Gephyrin* gene, which anchors GABA receptors to the postsynaptic membrane and is directly involved in fear extinction, showing a gain of 5hmC accompanied by a 5mC decrease within an intron, 24 h post-extinction training. An increase in *Gephyrin* mRNA transcripts was also observed transiently 2 h after extinction training, together with an increase in TET3 occupancy surrounding the *Gephyrin* gene, suggesting that DNA methylation can be dynamically regulated after learning. The effect of extinction learning on TET3 occupancy at the *Gephyrin* locus, as well as the dynamic changes in the accumulation of 5hmC and 5mC, G*ephyrin* mRNA and associated effects on the chromatin landscape were completely blocked in the presence of *Tet3* shRNA (Li et al., 2014). Together, these results suggest that *Tet3* activity within the ILPFC is necessary for the learning-dependent accumulation of 5hmC and related chromatin modifications, which underpins rapid behavioral adaptation.

Overall, these studies suggest that TET3 has an important role in fear extinction memory, probably through modulation of synaptic genes. However, it is still unclear if TET3 influences other cognitive behaviors, such as memory and learning, and what are the mechanisms underlying the neuronal activity, mediated by this enzyme.

3. Conclusions and future directions

Since all three TET enzymes are present in the mammalian brain and share the capacity of oxidizing 5mC into 5hmC, a putative intermediate in the DNA demethylation process, their functions could be assumed to be mostly redundant. However, recent publications describing different effects of each TET knockout or knockdown in the brain physiology and development, have stirred the debate. As we have comprehended from the above-mentioned works, loss or gain-of-function of each of these individual isoenzymes produced singular findings, suggesting non-redundant functions for TET enzymes in the brain. Indeed, TET1 was implicated in a wide range of specific behaviors, such as spatial and fear learning, short-term and object location memories. TET2 was shown as an unequivocal player in controlling short and long-term spatial learning, as well as memory processes. TET3 enzyme was identified as a key enzyme to regulate fear extinction memory. Altogether, these studies have demonstrated that TET deficiencies produce significant changes in neuronal function. This is probably due to the critical role of TET enzymes in regulation of the epigenetic state of key regulatory regions, such as promoters, of neuronal activity-associated genes and its consequence on the transcription levels and gene functions.

Further studies using double and triple TET KO models could help increase our knowledge on the relative contribution and potential cooperation of the different TET enzymes. Also, considering the dynamic nature of DNA modifications in the nervous system, a temporal perspective on TET mediated activity throughout life is mandatory. Importantly, so far many of the studies were performed using full TET KO. Therefore, some phenotypes may result from the developmental roles of TET enzymes rather than dysregulation of the function of mature neurons. In the future, the conditional ablation of TET proteins in specific cell types and considering the development stage is needed.

Despite recent advances, a full understanding of how epigenetic modifications regulate neuronal physiology, plasticity and cognitive functions is still a matter of debate and require further investigation. Particularly, it would be of utmost importance to generate conditional knockouts for each TET enzyme in specific areas of the brain or types of neurons, for example. Additionally, it would be interesting to knockout or knockdown TET enzymes in the brain of specific models of diseases affecting the CNS, in order to investigate the possible contribution of these epigenetic players in disease onset, progression or even possible therapeutics. One very important technical breakthrough is the possibility of performing epigenetic editing of the genome, using CRISPR/Cas system. This would be a promising tool to manipulate neurons *in vitro* or brain cells *in vivo*, trying to modulate brain cognitive processes related to depression, anxiety, amongst others, possibly leading to the production of new chemical compounds that could target epigenetic pathways, looking for novel treatments for brain dysfunction.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

We thank the reviewers for their constructive comments that significantly improved this review.

The authors also wish to thank Patrícia Monteiro and Jorge Diogo Silva (ICVS, University of Minho) for helpful comments after critical reading the manuscript. This work was supported by National Funds through Portuguese Foundation for Science and Technology (FCT) fellowships (PD/BD/106049/2015 to C.A., IF/01079/2014 to L.P. and IF/00047/2012 and CEECIND/00371/2017 to C.J.M); FCT project grant (PTDC/BIA-BCM/121276/2010) to C.J.M; EpiGeneSys Small Collaborative project to L.P.; BIAL Foundation Grant427/14 to L.P.; Northern Portugal Regional Operational Programme (NORTE 2020),

under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (FEDER; NORTE-01-0145-FEDER-000013); FEDER funds, through the Competitiveness Factors Operational Programme (COMPETE), and National Funds, through the FCT (POCI-01-0145-FEDER-007038).

References

Azpurua, J., Eaton, B.A., 2015. Neuronal epigenetics and the aging synapse. Front. Cell. Neurosci. 9 208-208.

Bird, A., 2002. DNA methylation patterns and epigenetic memory. Genes Dev. 16, 6–21.

Branco, M.R., Ficz, G., Reik, W., 2011. Uncovering the role of 5-hydroxymethylcytosine in the epigenome. Nat. Rev. Genet. 13, 7–13.

Broadbent, N.J., Squire, L.R., Clark, R.E., 2004. Spatial memory, recognition memory, and the hippocampus. Proc. Natl. Acad. Sci. U. S. A. 101, 14515–14520.

Cadena-del-Castillo, C., Valdes-Quezada, C., Carmona-Aldana, F., Arias, C., Bermudez-Rattoni, F., Recillas-Targa, F., 2014. Age-dependent increment of hydroxymethylation in the brain cortex in the triple-transgenic mouse model of Alzheimer's disease. J. Alzheimers Dis. 41, 845–854.

Campbell, R.R., Wood, M.A., 2019. How the epigenome integrates information and reshapes the synapse. Nat. Rev. Neurosci. 20, 133–147.

Clem, R.L., Huganir, R.L., 2010. Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. Science (New York, N.Y.) 330, 1108–1112.

Coutellier, L., Beraki, S., Ardestani, P.M., Saw, N.L., Shamloo, M., 2012. Npas4: a neuronal transcription factor with a key role in social and cognitive functions relevant to developmental disorders. PLoS One 7, e46604.

Dalton, G.L., Wang, Y.T., Floresco, S.B., Phillips, A.G., 2008. Disruption of AMPA receptor endocytosis impairs the extinction, but not acquisition of learned fear. Neuropsychopharmacology 33, 2416–2426.

Day, J.J., Sweatt, J.D., 2011. Epigenetic mechanisms in cognition. Neuron 70, 813–829.

Deaton, A.M., Bird, A., 2011. CpG islands and the regulation of transcription. Genes Dev. 25, 1010–1022.

Fasolino, M., Welsh, S.A., Zhou, Z., 2017. Chapter 4 – TET and 5hmC in neurodevelopment and the adult brain. In: Bredy, T.W. (Ed.), DNA Modifications in the Brain. Academic Press, San Diego, pp. 61–79.

Globisch, D., Munzel, M., Muller, M., Michalakis, S., Wagner, M., Koch, S., Bruckl, T., Biel, M., Carell, T., 2010. Tissue distribution of 5-hydroxymethylcytosine and search for active demethylation intermediates. PLoS One 5, 0015367.

Gontier, G., Iyer, M., Shea, J.M., Bieri, G., Wheatley, E.G., Ramalho-Santos, M., Villeda, S.A., 2018. Tet2 rescues age-related regenerative decline and enhances cognitive function in the adult mouse brain. Cell Rep. 22, 1974–1981.

Guo, J.U., Su, Y., Zhong, C., Ming, G.L., Song, H., 2011. Hydroxylation of 5-methylcytosine by TET1 promotes active DNA demethylation in the adult brain. Cell 145, 423–434.

Guo, J.U., Su, Y., Shin, J.H., Shin, J., Li, H., Xie, B., Zhong, C., Hu, S., Le, T., Fan, G., Zhu, H., Chang, Q., Gao, Y., Ming, G.L., Song, H., 2014. Distribution, recognition and regulation of non-CpG methylation in the adult mammalian brain. Nat. Neurosci. 17, 215–222.

Hahn, M.A., Qiu, R., Wu, X., Li, A.X., Zhang, H., Wang, J., Jui, J., Jin, S.G., Jiang, Y., Pfeifer, G.P., Lu, Q., 2013. Dynamics of 5-hydroxymethylcytosine and chromatin marks in Mammalian neurogenesis. Cell Rep. 3, 291–300.

Ito, S., D'Alessio, A.C., Taranova, O.V., Hong, K., Sowers, L.C., Zhang, Y., 2010. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature 466, 1129–1133.

Ito, S., Shen, L., Dai, Q., Wu, S.C., Collins, L.B., Swenberg, J.A., He, C., Zhang, Y., 2011. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science (New York, N.Y.) 333, 1300–1303.

Kaas, G.A., Zhong, C., Eason, D.E., Ross, D.L., Vachhani, R.V., Ming, G.L., King, J.R., Song, H., Sweatt, J.D., 2013. TET1 controls CNS 5-methylcytosine hydroxylation, active DNA demethylation, gene transcription, and memory formation. Neuron 79, 1086–1093.

Kawasaki, F., Beraldi, D., Hardisty, R.E., McInroy, G.R., van Delft, P., Balasubramanian, S., 2017. Genome-wide mapping of 5-hydroxymethyluracil in the eukaryote parasite Leishmania. Genome Biol. 18 23-23.

Kim, J.I., Lee, H.R., Sim, S.E., Baek, J., Yu, N.K., Choi, J.H., Ko, H.G., Lee, Y.S., Park, S.W., Kwak, C., Ahn, S.J., Choi, S.Y., Kim, H., Kim, K.H., Backx, P.H., Bradley, C.A., Kim, E., Jang, D.J., Lee, K., Kim, S.J., Zhuo, M., Collingridge, G.L., Kaang, B.K., 2011. PI3Kgamma is required for NMDA receptor-dependent long-term depression and behavioral flexibility. Nat. Neurosci. 14, 1447–1454.

Ko, M., Bandukwala, H.S., An, J., Lamperti, E.D., Thompson, E.C., Hastie, R., Tsangaratou, A., Rajewsky, K., Koralov, S.B., Rao, A., 2011. Ten-Eleven-Translocation 2 (TET2) negatively regulates homeostasis and differentiation of hematopoietic stem cells in mice. Proc. Natl. Acad. Sci. U. S. A. 108, 14566–14571.

Ko, M., An, J., Bandukwala, H.S., Chavez, L., Äijö, T., Pastor, W.A., Segal, M.F., Li, H., Koh, K.P., Lähdesmäki, H., Hogan, P.G., Aravind, L., Rao, A., 2013. Modulation of TET2 expression and 5-methylcytosine oxidation by the CXXC domain protein IDAX. Nature 497, 122–126.

Kohli, R.M., Zhang, Y., 2013. TET enzymes, TDG and the dynamics of DNA demethylation. Nature 502, 472–479.

Kremer, E.A., Gaur, N., Lee, M.A., Engmann, O., Bohacek, J., Mansuy, I.M., 2018. Interplay between TETs and microRNAs in the adult brain for memory formation. Sci. Rep. 8, 1678.

Kriaucionis, S., Heintz, N., 2009. The nuclear DNA base 5-hydroxymethylcytosine is present in Purkinje neurons and the brain. Science (New York, N.Y.) 324, 929–930.

Kumar, D., Aggarwal, M., Kaas, G.A., Lewis, J., Wang, J., Ross, D.L., Zhong, C., Kennedy, A., Song, H., Sweatt, J.D., 2015. Tet1 oxidase regulates neuronal gene transcription, active DNA hydroxy-methylation, object location memory, and threat recognition memory. Neuroepigenetics 4, 12–27.

Li, Z., Cai, X., Cai, C.L., Wang, J., Zhang, W., Petersen, B.E., Yang, F.C., Xu, M., 2011. Deletion of Tet2 in mice leads to dysregulated hematopoietic stem cells and subsequent development of myeloid malignancies. Blood 118, 4509–4518.

Li, X., Wei, W., Zhao, Q.Y., Widagdo, J., Baker-Andresen, D., Flavell, C.R., D'Alessio, A., Zhang, Y., Bredy, T.W., 2014. Neocortical Tet3-mediated accumulation of 5-hydroxymethylcytosine promotes rapid behavioral adaptation. Proc. Natl. Acad. Sci. U. S. A. 111, 7120–7125.

Li, T., Yang, D., Li, J., Tang, Y., Yang, J., Le, W., 2015. Critical role of Tet3 in neural progenitor cell maintenance and terminal differentiation. Mol. Neurobiol. 51, 142–154.

Li, X., Yue, X., Pastor, W.A., Lin, L., Georges, R., Chavez, L., Evans, S.M., Rao, A., 2016. Tet proteins influence the balance between neuroectodermal and mesodermal fate choice by inhibiting Wnt signaling. Proc. Natl. Acad. Sci. U. S. A. 113, E8267–E8276.

Li, X., Yao, B., Chen, L., Kang, Y., Li, Y., Cheng, Y., Li, L., Lin, L., Wang, Z., Wang, M., Pan, F., Dai, Q., Zhang, W., Wu, H., Shu, Q., Qin, Z., He, C., Xu, M., Jin, P., 2017. Ten-eleven translocation 2 interacts with forkhead box O3 and regulates adult neurogenesis. Nat. Commun. 8, 15903.

Liu, S.Q., Cull-Candy, S.G., 2000. Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. Nature 405, 454–458.

Liu, S., Wang, J., Su, Y., Guerrero, C., Zeng, Y., Mitra, D., Brooks, P.J., Fisher, D.E., Song, H., Wang, Y., 2013. Quantitative assessment of Tet-induced oxidation products of 5-methylcytosine in cellular and tissue DNA. Nucleic Acids Res. 41, 6421–6429.

Long, H.K., Blackledge, N.P., Klose, R.J., 2013. ZF-CxxC domain-containing proteins, CpG islands and the chromatin connection. Biochem. Soc. Trans. 41, 727–740.

Lv, X., Jiang, H., Liu, Y., Lei, X., Jiao, J., 2014. MicroRNA-15b promotes neurogenesis and inhibits neural progenitor proliferation by directly repressing TET3 during early neocortical development. EMBO Rep. 15, 1305–1314.

Ma, D.K., Jang, M.-H., Guo, J.U., Kitabatake, Y., Chang, M.-l., Pow-anpongkul, N., Flavell, R.A., Lu, B., Ming, G.-l., Song, H., 2009. Neuronal activity–induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Science (New York, N.Y.) 323, 1074–1077.

Mi, Y., Gao, X., Dai, J., Ma, Y., Xu, L., Jin, W., 2015. A novel function of TET2 in CNS: sustaining neuronal survival. Int. J. Mol. Sci. 16, 21846–21857.

Munzel, M., Globisch, D., Bruckl, T., Wagner, M., Welzmiller, V., Michalakis, S., Muller, M., Biel, M., Carell, T., 2010. Quantification of the sixth DNA base hydroxymethylcytosine in the brain. Angew. Chem. Int. Ed. Engl. 49, 5375–5377.

Pastor, W.A., Aravind, L., Rao, A., 2013. TETonic shift: biological roles of TET proteins in DNA demethylation and transcription. Nat. Rev. Mol. Cell Biol. 14, 341–356.

Pfaffeneder, T., Spada, F., Wagner, M., Brandmayr, C., Laube, S.K., Eisen, D., Truss, M., Steinbacher, J., Hackner, B., Kotljarova, O., Schuermann, D., Michalakis, S., Kosmatchev, O., Schiesser, S., Steigenberger, B., Raddaoui, N., Kashiwazaki, G., Muller, U., Spruijt, C.G., Vermeulen, M., Leonhardt, H., Schar, P., Muller, M., Carell, T., 2014. Tet oxidizes thymine to 5-hydroxymethyluracil in mouse embryonic stem cell DNA. Nat. Chem. Biol. 10, 574–581.

Rafalski, V.A., Brunet, A., 2011. Energy metabolism in adult neural stem cell fate. Prog. Neurobiol. 93, 182–203.

Ramamoorthi, K., Fropf, R., Belfort, G.M., Fitzmaurice, H.L., McKinney, R.M., Neve, R.L., Otto, T., Lin, Y., 2011. Npas4 regulates a transcriptional program in CA3 required for contextual memory formation. Science (New York, N.Y.) 334, 1669–1675.

Rudenko, A., Dawlaty, M.M., Seo, J., Cheng, A.W., Meng, J., Le, T., Faull, K.F., Jaenisch, R., Tsai, L.H., 2013. Tet1 is critical for neuronal activity-regulated gene expression and memory extinction. Neuron 79, 1109–1122.

Rutherford, L.C., Nelson, S.B., Turrigiano, G.G., 1998. BDNF has opposite effects on the quantal amplitude of pyramidal neuron and interneuron excitatory synapses. Neuron 21, 521–530.

Ryu, J., Futai, K., Feliu, M., Weinberg, R., Sheng, M., 2008. Constitutively active Rap2 transgenic mice display fewer dendritic spines, reduced extracellular signal-regulated kinase signaling, enhanced long-term depression, and impaired spatial learning and fear extinction. J. Neurosci. 28, 8178–8188.

Shin, J., Ming, G.-l., Song, H., 2014. DNA modifications in the mammalian brain. Philos. Trans. Biol. Sci. 369, 20130512.

Smith, Z.D., Meissner, A., 2013. DNA methylation: roles in mammalian development. Nat. Rev. Genetics 14, 204–220.

Song, C.X., Szulwach, K.E., Fu, Y., Dai, Q., Yi, C., Li, X., Li, Y., Chen, C.H., Zhang, W., Jian, X., Wang, J., Zhang, L., Looney, T.J., Zhang, B., Godley, L.A., Hicks, L.M., Lahn, B.T., Jin, P., He, C., 2011. Selective chemical labeling reveals the genome-wide distribution of 5-hydroxymethylcytosine. Nat. Biotechnol. 29, 68–72.

Sweatt, J.D., 2013. The emerging field of neuroepigenetics. Neuron 80, 624–632.

Szulwach, K.E., Li, X., Li, Y., Song, C.-X., Wu, H., Dai, Q., Irier, H., Upadhyay, A.K., Gearing, M., Levey, A.I., Vasanthakumar, A., Godley, L.A., Chang, Q., Cheng, X., He, C., Jin, P., 2011. 5-hmC-mediated epigenetic dynamics during postnatal neurodevelopment and aging. Nat. Neurosci. 14, 1607–1616.

Szwagierczak, A., Bultmann, S., Schmidt, C.S., Spada, F., Leonhardt, H., 2010. Sensitive enzymatic quantification of 5-hydroxymethylcytosine in genomic DNA. Nucleic Acids Res. 38, e181.

Tahiliani, M., Koh, K.P., Shen, Y., Pastor, W.A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L.M., Liu, D.R., Aravind, L., Rao, A., 2009. Conversion of 5-methylcytosine to 5-Hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science (New York, N.Y.) 324, 930–935.

Tsetsenis, T., Younts, T.J., Chiu, C.Q., Kaeser, P.S., Castillo, P.E., Sudhof, T.C., 2011. Rab3B protein is required for long-term depression of hippocampal inhibitory

synapses and for normal reversal learning. Proc. Natl. Acad Sci. U. S. A. 108, 14300–14305.
Wang, L., Li, M.Y., Qu, C., Miao, W.Y., Yin, Q., Liao, J., Cao, H.T., Huang, M., Wang, K., Zuo, E., Peng, G., Zhang, S.X., Chen, G., Li, Q., Tang, K., Yu, Q., Li, Z., Wong, C.C., Xu, G., Jing, N., Yu, X., Li, J., 2017. CRISPR-Cas9-mediated genome editing in one blastomere of two-cell embryos reveals a novel Tet3 function in regulating neocortical development. Cell Res. 27, 815–829.
Williams, K., Christensen, J., Pedersen, M.T., Johansen, J.V., Cloos, P.A.C., Rappsilber, J., Helin, K., 2011. TET1 and hydroxymethylcytosine in transcription and DNA methylation fidelity. Nature 473, 343.
Wu, H., D'Alessio, A.C., Ito, S., Xia, K., Wang, Z., Cui, K., Zhao, K., Sun, Y.E., Zhang, Y., 2011. Dual functions of Tet1 in transcriptional regulation in mouse embryonic stem cells. Nature 473, 389–393.
Xu, Y., Wu, F., Tan, L., Kong, L., Xiong, L., Deng, J., Barbera, A.J., Zheng, L., Zhang, H., Huang, S., Min, J., Nicholson, T., Chen, T., Xu, G., Shi, Y., Zhang, K., Shi, Y.G., 2011. Genome-wide regulation of 5hmC, 5mC, and gene expression by Tet1 hydroxylase in mouse embryonic stem cells. Mol. Cell 42, 451–464.
Xu, Y., Xu, C., Kato, A., Tempel, W., Abreu, J.G., Bian, C., Hu, Y., Hu, D., Zhao, B., Cerovina, T., Diao, J., Wu, F., He, H.H., Cui, Q., Clark, E., Ma, C., Barbara, A., Veenstra, G.J., Xu, G., Kaiser, U.B., Liu, X.S., Sugrue, S.P., He, X., Min, J., Kato, Y., Shi, Y.G., 2012. Tet3 CXXC domain and dioxygenase activity cooperatively regulate key genes for Xenopus eye and neural development. Cell 151, 1200–1213.
Yu, H., Su, Y., Shin, J., Zhong, C., Guo, J.U., Weng, Y.L., Gao, F., Geschwind, D.H., Coppola, G., Ming, G.L., Song, H., 2015. Tet3 regulates synaptic transmission and homeostatic plasticity via DNA oxidation and repair. Nat. Neurosci. 18, 836–843.
Zhang, H., Zhang, X., Clark, E., Mulcahey, M., Huang, S., Shi, Y.G., 2010. TET1 is a DNA-binding protein that modulates DNA methylation and gene transcription via hydroxylation of 5-methylcytosine. Cell Res. 20, 1390–1393.
Zhang, R.R., Cui, Q.Y., Murai, K., Lim, Y.C., Smith, Z.D., Jin, S., Ye, P., Rosa, L., Lee, Y.K., Wu, H.P., Liu, W., Xu, Z.M., Yang, L., Ding, Y.Q., Tang, F., Meissner, A., Ding, C., Shi, Y., Xu, G.L., 2013. Tet1 regulates adult hippocampal neurogenesis and cognition. Cell Stem Cell 13, 237–245.
Zhao, X., Dai, J., Ma, Y., Mi, Y., Cui, D., Ju, G., Macklin, W.B., Jin, W., 2014. Dynamics of ten-eleven translocation hydroxylase family proteins and 5-hydroxymethylcytosine in oligodendrocyte differentiation. Glia 62, 914–926.