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The prevalence of epigenetic mechanisms in the regulation of cognitive functions and behaviour

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A complex interplay between the pattern of DNA methylation and a large and growing number of post-translational modifications (PTMs) of histones contribute to the epigenetic regulation of gene transcription. This epigenetic regulation involves histone acetylation, phosphorylation and methylation, and is now known to be important for several forms and phases of long-term memory. Anomalies in the epigenome have also been demonstrated to be critical factors in a number of cognitive and behavioural disorders. The epigenetic mechanisms that contribute to these deficits include: first, the dysregulation of key components of the epigenetic machinery; second, alterations in the expression of genes important for cognition and behaviour by epigenetic mechanisms; third, instability at trinucleotide repeats; and fourth, the breakdown of major epigenetic processes like imprinting and X-chromosome inactivation. Thus, both pharmacological and environmental interventions that act on epigenetic mechanisms provide a promising tool for the treatment of a wide variety of cognitive and behavioural disorders.

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Introduction

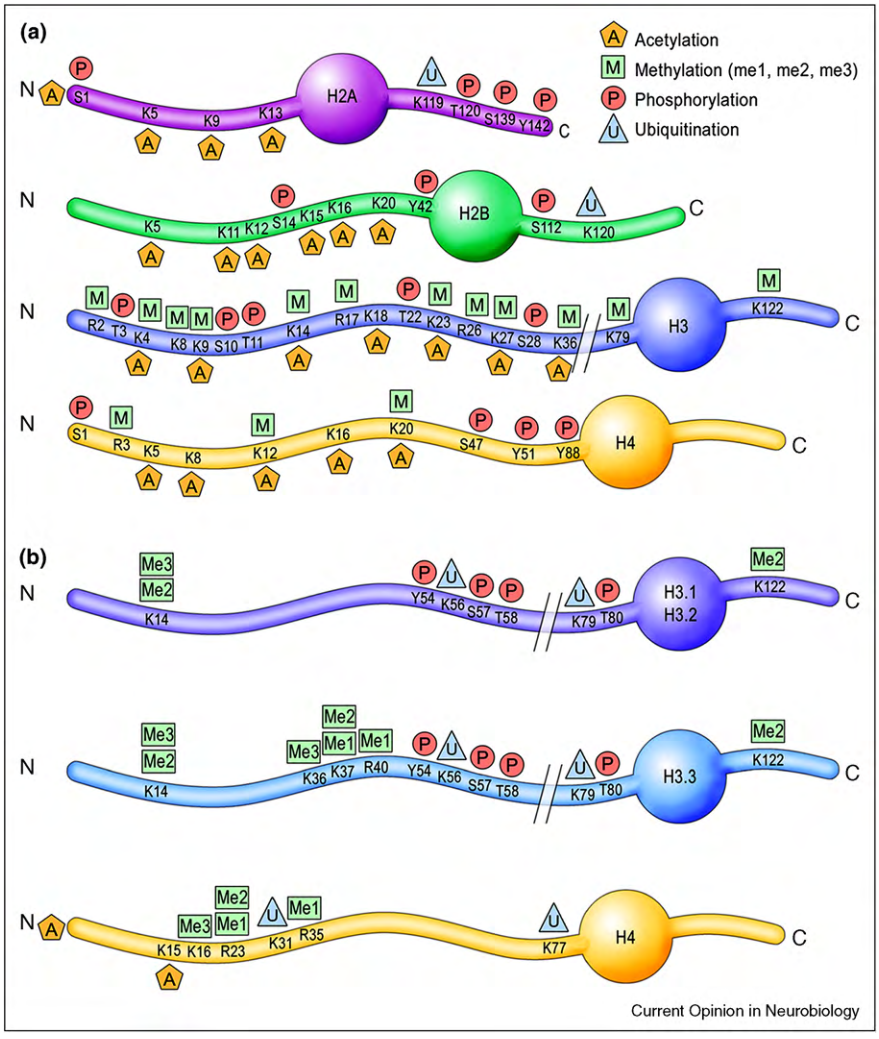
Epigenetics is classically defined as the ensemble of changes in gene functions that are transmitted through mitosis and meiosis, but do not result from a permanent alteration of the DNA sequence itself. It primarily implicates covalent modifications of histone proteins (regular and variants) and of the DNA, but includes also RNA interference, DNA looping and nucleosome repositioning [1]. These processes modulate gene transcription by altering local chromatin structure, and its access for the transcriptional machinery.

Multiple post-translational modifications (PTMs) exist on histone proteins (Figure 1). In the brain, the best studied are acetylation and phosphorylation, commonly associated with actively transcribed genes. Histone methylation is another prominent PTM linked to both transcriptional activation and silencing. Additional PTMs include ubiquitination, sumoylation, ADP-ribosylation, biotinylation, formylation, palmitoylation, glycosylation and proline isomerization. These are also likely linked to gene transcription although their role remains not well defined. For instance, ubiquitination is a PTM classically known for its cytosolic role in tagging proteins for degradation, but is also associated with transcriptional silencing and activation when present on H2A or H2B, respectively. In the adult mouse brain, histone ubiquitination was recently shown to account for up to 16% of all histone PTMs [2•], suggesting its functional importance. Histone sumoylation is also associated with transcriptional silencing, but in yeast [3], and H3 proline isomerization with transcriptional activation via negative coupling with H3K36 methylation [4]. Finally, N^6-formylation of H1 was demonstrated in mammalian cells, but histone biotinylation only *in vitro*, and their functional relevance is not yet known [5,6].

PTMs are present primarily on the protruding N-terminal tail of histones, but also affect the histone core and C-terminus [2•]. In addition to being complex and numerous, they also demonstrate extensive crosstalk. Histone PTMs are often regulated together (either positively or negatively), and act in synergy to favour transcriptional activation or repression [7]. For instance, phosphorylation of H3S10 and acetylation of H4K16 act cooperatively to promote transcriptional activation. Phosphorylated S10 favours the recruitment of the histone acetyltransferase (HAT) MOF, leading to H4K16 acetylation [8•]. The mammalian MLL3/4 Set1–H3K4 methyltransferase complex also promotes transcriptional activation by removing a repressive mark, H3K27 methylation, and adding an activating mark, H3K4 methylation [9•]. Furthermore, histone PTMs are engaged in epigenetic bidirectional 'conversations' with the DNA, and can participate in mutually enhancing feedback loops [10,11]. Histone methylation can shape the profile of DNA methylation; for instance H3K9 methylation promotes DNA methylation [12]. In turn, DNA methylation can recruit components of the histone-modifying machinery such as silencing complexes containing HDACs [11,13,14•].

DNA methylation is another epigenetic modification critical for transcriptional regulation. In mammals it occurs on cytosine in CpG dinucleotides, and critically regulates CpG dense regions (CpG islands) that often

Figure 1



Epigenetic modifications on histones that contribute to chromatin remodelling. **(a)** Schematic representation of the N-termini and C-termini of the core histones with their major PTMs. M represents mono-methylation, bi-methylation or tri-methylation. Updated from [75]. **(b)** Novel PTMs (65) identified on H3 variants (H3.1, H3.2 and H3.3) and H4 by ESI-LTQ Orbitrap mass spectrometry illustrating their large variety and variant specificity. Mono-methylation, bi-methylation or tri-methylation can be distinguished with this method of analysis (from [2•]). C, C-terminus; N, N′, N-terminal tails.

exist in promoters and intragenic regions. DNA methylation is established and maintained by complex mechanisms that recruit DNA methyltransferases (DNMTs), methyl CpG-binding proteins (MBPs) and small non-coding RNAs [1]. It controls cell-specific gene expression, X-chromosome inactivation and parental imprinting [15], and can be modulated by environmental stimuli such as nutrition, stress and postnatal care [16]. Recently, DNA methylation was shown to be highly dynamic and to cycle rapidly in human cells [17••,18••]. CpG methylation and demethylation can alternate within 100 min in the promoter region of several transcriptionally active genes, and increased methylation in this region coincides with the initiation of transcription.

Epigenetic mechanisms in learning and memory

Several forms and phases of long-term memory (LTM) are associated with specific histone PTMs [16]. H3K14 acetylation is increased in the CA1 subregion of the hippocampus one hour after contextual fear conditioning, whilst it is increased on H4 but not H3K14 after latent inhibition, another form of associative memory [19]. This specificity suggests that the involvement of epigenetic alterations in learning and memory is not because of changes in gene transcription in general, but may involve specific alterations in the epigenetic machinery likely to impact subsets of genes important for differing forms of memory formation. Thus, it may also be possible that

certain forms of LTM require epigenetic regulation, whilst others do not. Histone acetylation in LTM is thought to implicate CREB-binding protein (CBP), a transcriptional co-activator with endogenous HAT activity. A deficiency in CPB in the mouse brain impairs contextual and cued fear memory, and affects the acquisition of spatial memory and object recognition [20,21]. These impairments can be reversed by i.p. or i.c.v. injection of HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA), suggesting an imbalance in HAT/HDAC activity in these mice [20,21]. In control mice, such imbalance in favour of HAT induced by, for instance, HDAC inhibition with sodium butyrate, improves the recognition of familiar objects and prolongs object memory [22]. Further, intra-amygdalar infusion of sodium butyrate or TSA enhances fear potentiated startle memory [23], and TSA infused into the hippocampus ameliorates contextual, but not cued fear conditioning, an effect thought to recruit CBP via a CREB/CBP transcriptional complex [24]. Systemic HDAC inhibition by sodium butyrate can also reinstate the access to emotional memories that were previously lost in Ck-p25 transgenic mice displaying neurodegeneration, a finding that correlates with increased synapse number [25••]. In this mouse model, memory restoration associated with increased histone acetylation is also achieved by environmental enrichment [25••], indicating the influence of environmental factors on acetylation. Interestingly, sodium butyrate also improves short-term spatial and associative memory after traumatic brain injury, but only when combined with training on a water maze [26], revealing the potential of the combination of pharmacological and environmental factors on memory.

Although several HDACs exist in the brain, HDAC2 has been proposed to be one of the most important for the regulation of memory, and HDAC1 has no major role. When overexpressed in neurons *in vivo*, HDAC2 impairs emotional and spatial memory formation, and spatial working memory, and decreases synapse number and spine density. In contrast, a deficiency in HDAC2 facilitates emotional memory and spatial working memory and increases synapse number [27••]. These findings suggest that HDAC2 negatively controls memory and that inhibitors specifically targeting HDAC2 may have clinical value in the treatment of neurodegenerative diseases.

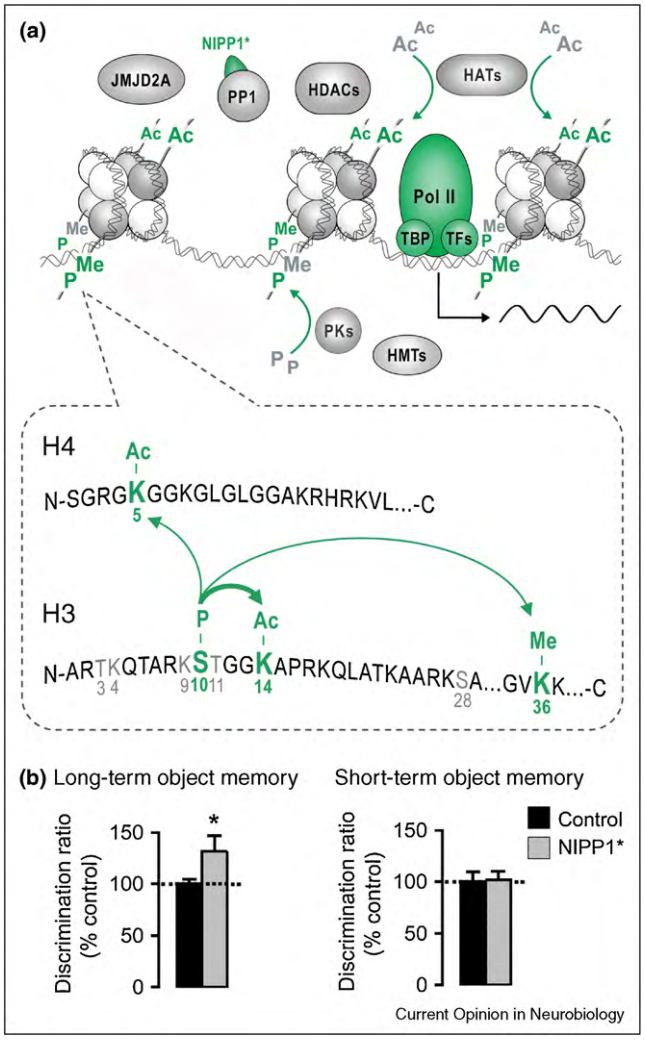
Further to acetylation, histone phosphorylation also occurs during LTM in the hippocampus, for instance following contextual fear conditioning and novel object recognition [28]. Phosphorylation of H3S10 is increased in the promoter of specific genes such as *CREB*, but is also decreased in other loci like the *NFκb* promoter [29•]. These epigenetic changes were associated with increased *CREB* and decreased *NFκb* expression. This bidirectional regulation likely results from the dual action of protein kinases and phosphatases. One of the kinases involved is Msk1, a member of the ERK/MAPK family. Msk1 knockout in mice eliminates H3 phosphorylation in the hippocampus and alters contextual fear memory [30]. The protein phosphatase PP1 was recently shown to also be involved and selectively dephosphorylates H3S10 by directly interacting with H3 (Figure 2a) [29•]. PP1 also regulates H3K14 and H4K5 acetylation, and H3K36 trimethylation, by forming complexes with HDAC1 and JMJD2A, suggesting that it has central role in the epigenetic machinery. This role is critical for LTM since the induction of these PTMs by selective inhibition of nuclear PP1 in adult hippocampal neurons improves LTM for objects and space without affecting short-term memory (Figure 2b) [29•].

Like histone PTMs, DNA methylation is also dynamically regulated in the adult mouse brain during LTM, despite the general view that it is static in post-mitotic cells. Initial observations showed that infusion of the DNMT inhibitor 5-aza-2-deoxycytidine (5-aza) in hippocampus subregion CA1 blocks the consolidation of contextual fear memory [31•]. This form of memory itself upregulates the expression of *de novo* DNMTs, *DNMT3a* and *b*, and increases DNA methylation at specific promoters, such as *PP1γ*, in the adult rat hippocampus [32••]. However, it also decreases DNA methylation in other loci such as the *reelin* promoter [32••], indicating that DNA methylation is bidirectionally regulated in the adult brain in association with memory formation. Moreover, conditional mutant mice lacking both DNMT1 and DNMT3a in forebrain excitatory neurons have impaired long-term synaptic plasticity, and deficits in learning and memory on the water maze [33]. These abnormalities are not seen when either DNMT1 or DNMT3a are knocked-out separately, suggesting that these DNMTs fulfil overlapping roles in establishing DNA methylation patterns required for learning and memory.

DNA methylation is also linked to histone PTMs since 5-aza favours H3 acetylation during memory consolidation [31•]. The mechanisms of this interplay involve complex interactions between epigenetic components such as HDACs and methylated DNA through, for instance, methyl CpG-binding protein 2 (MeCP2), or DNMTs and histone methyltransferases [11].

Further to memory consolidation, memory extinction is also subject to epigenetic regulation. Extinction of cued fear conditioning correlates with increased H4 acetylation around the *BDNF* P4 promoter and with *BDNF* upregulation in the prefrontal cortex [34]. It is improved by valproic acid, a drug clinically used as anticonvulsant and mood stabilizer, and known to act as an HDAC inhibitor [34]. Similarly, intra-hippocampal administration of sodium butyrate or TSA enhances the extinction of contextual fear [35]. Enhanced extinction by HDAC inhibition may have therapeutic use in the treatment of drug

Figure 2



Model of a PP1-dependent histone code for the control of gene transcription. **(a)** PP1 is a protein Ser/Thr phosphatase present in neuronal cells that can associate with chromatin. When it is inhibited by, for instance, the endogenous inhibitor NIPP1, histone phosphorylation on H3S10 is increased. PP1 inhibition also leads to the dissociation of PP1 from HDACs, which reduces HDAC activity, and increases histone acetylation on H3K14 and H4K5. These changes are also associated with an increase in tri-methylation of H3K36, and decreased JMJD2A activity occurring when interaction with PP1 is disrupted. These PTMs may loosen chromatin and increase the binding of RNA polymerase II (Pol II), TATA box binding protein (TBP) and other transcription factors (TFs). Bottom, histone PTMs, which depend on nuclear PP1 are in green, histone residues thought not to be regulated by nuclear PP1 are in grey. The thick arrow represents a well-established crosstalk between H3S10 phosphorylation and H3K14 acetylation in the context of memory formation [28,30]. Thin arrows illustrate potential crosstalk suggested by previous data [29•]. For clarity, the crosstalk between these residues and acetylated H2B has been omitted. **(b)** Long-term, but not short-term, object recognition memory is improved in transgenic mice overexpressing the endogenous inhibitor of PP1, NIPP1. A positive discrimination ratio (% control) represents enhanced memory formation. From [29•].

addiction. Indeed, sodium butyrate was recently demonstrated to facilitate the extinction of cocaine-induced conditioned place preference, and prevent the reinstatement of drug-induced behaviours in rodents [36].

Different mechanisms of epigenetic alteration in cognitive and behavioural pathologies

Neurodevelopmental and neurodegenerative diseases characterized by cognitive and behavioural deficits such as autism, mental retardation, schizophrenia and Alzheimer's disease (AD), are all linked to epigenetic anomalies but involve several different mechanisms (Table 1) [16].

Dysregulation of components of the epigenetic machinery

Loss-of-function mutations in key components of the epigenetic machinery are associated with several developmental and cognitive disorders. Mutations in the X-linked gene *mecp2* cause Rett syndrome (RS) [37], and mutations in *CBP* cause Rubinstein–Taybi syndrome [21], two neurodevelopmental conditions characterized by mental retardation. The modelling of these mutations in mice recapitulates most disease symptoms including increased anxiety, social withdrawal and cognitive deficits. MeCP2 deficiency also correlates with H3 hyperacetylation, perhaps as a result of altered HDAC recruitment by MeCP2 [38], whilst CBP deficiency decreases overall acetylation [21]. The behavioural and cognitive impairments resulting from the mutations can be corrected by choline nutrient supplementation during the critical period of brain development in the MeCP2 model, or by systemic injection of sodium butyrate or TSA, or intraventricular administration of SAHA in CBP models, confirming their epigenetic nature.

DNA hypermethylation resulting from elevated levels of the methyl donor *S*-adenosyl methionine (SAM), and/or increased DNMT1, is another epigenetic mechanism at play in mental diseases [39,40]. It has been well studied in schizophrenia following early observations that SAM induces psychotic episodes [41]. Clinically relevant doses of two antipsychotics, clozapine and sulpiride, increase demethylation in rodents, an effect facilitated by concomitant valproic acid administration, indicating the combined therapeutic actions of these drugs [42]. The use of DNMT inhibitors has also been suggested in the treatment of schizophrenia [43].

Epigenetic alterations can also result from the detrimental influence of environmental factors. Chronic stress or cocaine exposure can alter the epigenetic machinery, for example by decreasing HDAC5 activity in the nucleus accumbens, an important reward-associated brain region [44]. Chronic stress was also shown to decrease HDAC2 in rodents, mimicking post-mortem findings in depressed patients [45]. Infusion of [*N*-(2-aminophenyl)]-4-[*N*-(pyridine-3-ylmethoxy-carbonyl)aminomethyl]benzamide

Table 1

Summary of mechanisms of epigenetic alterations associated with cognitive and behavioural pathologies

Mechanisms of epigenetic alterations	Cognitive and behavioural pathologies	Examples of specific epigenetic alterations
Dysregulation of components of the epigenetic machinery	Rett syndrome	Mutation in *mecp2* [37] H3 hyperacetylation [38]
	Rubinstein–Taybi syndrome	Mutation in *CBP* [21] Decreased overall acetylation [21]
	Schizophrenia	Increased SAM [40] Increased *DNMT1* [39,40]
	Drug addiction	Decreased *HDAC5* in the nucleus accumbens [44]
	Depression	Decreased *HDAC2* in the nucleus accumbens [45]
Alterations of genes involved in cognitive functions and behaviour by epigenetic mechanisms	Alzheimer's disease	Increased *PS1* associated with decreased *PS1* methylation [46,47•]
	Schizophrenia	Decreased reelin and *GAD67* associated with increased methylation [48–50]
	Depression (with suicide)	Decreased *GABA-A α1* receptor subunit associated with increased methylation [51]
Instability of trinucleotide repeats	Fragile X syndrome	Extension of CGG repeats associated with heterochromatin [58,59]
	Huntington's disease	Decreased CBP HAT activity by polyQ and increased histone monoubiquitylation [65]
Alteration of imprinting	Angelman syndrome, Prader–Willi syndrome, autism spectrum disorder	Alterations of imprinted domains on chromosome 15q11-13 [66,68,69]

(MS-275) or SAHA directly into the nucleus accumbens rescues the depressive-like behaviours induced by chronic stress in mice, suggesting that HDAC inhibitors may also be beneficial for the treatment of depression [45].

Alterations of genes involved in cognitive functions and behaviour by epigenetic mechanisms

Aberrant gene transcription resulting from altered epigenetic regulation is associated with cognitive defects in several pathologies including AD, schizophrenia and depression. Increased expression of *presenilin 1* (*PS1*), a member of the γ-secretase complex, correlates with *PS1* promoter hypomethylation in post-mortem brain samples from AD patients, and with increased β-amyloid formation *in vitro* [46,47•]. Downregulation of reelin, a glycoprotein involved in neuronal migration during development and cognitive functions in adults, and of the glutamate decarboxylase that catalyzes GABA synthesis (GAD67), are associated with promoter hypermethylation in post-mortem schizophrenic samples [48–50]. Hypermethylation of the *GABA-A α1* receptor subunit promoter and gene downregulation are also observed in depressed patients who committed suicide but not in control individuals who died of other causes [51].

Further to DNA methylation, histone PTMs are also implicated since treatments with therapeutic benefit correlate with changes in PTMs. However, a causal link has not been firmly established. In a mouse model with schizophrenia-like symptoms, valproic acid blocks DNA hypermethylation at the reelin promoter, and reverses abnormal cognitive and social behaviours in this model [52]. Chronic electroconvulsive therapy, effective in many depressed patients, also induces hyperacetylation of H4 at the *BDNF* P2 promoter and of H3 at the P3 promoter [53]. Similarly, chronic treatment with the antidepressant imipramine increases H3 acetylation at P3 and P4 promoters and H3K4 dimethylation, an activational mark, at the P3 promoter in the hippocampus of mice exposed to chronic stress, pointing to BDNF as an important target of epigenetic regulation in depression [54,55]. Recently, increased methylation of *BDNF* at P4 has been demonstrated in the brain of people who died from suicide versus those that died from other causes, further suggesting a link between mental disease and abnormal methylation at *BDNF* promoters [56].

Instability of trinucleotide repeats

Expansion of DNA trinucleotide repeats is another important mechanism that affects specific genes or loci and underlies several neurological diseases. Abnormal extension of CGG or CAG repeats has been identified in the 5′-UTR of *fragile X mental retardation 1* (*fmr1*) gene in fragile X syndrome (FXS) patients, and in the *huntingtin* (*htt*) gene in Huntington's disease (HD), respectively [57]. These repeats are associated with heterochromatin. CGG repeats in *fmr1* correlate with DNA hypermethylation in the repeat and flanking DNA regions, with increased H3K9 methylation near the expanded repeat, and reduced euchromatin marks (acetylated H3 and H4) [58,59]. Gene silencing in FXS can be alleviated by class III-selective HDAC inhibitors (i.e. Sirt1 inhibitors), suggesting the potential of such inhibitors for gene reactivation in post-mitotic neurons [60•].

The mechanisms underlying the formation and the spreading of heterochromatin to adjacent euchromatic

regions are not fully understood but require that the number of repeats reaches a certain threshold. In patients with maximum repeat expansion (up to 200 CGGs), the *fmr1* promoter is fully methylated, whilst methylation is only mosaic in individuals carrying pre-expansion alleles (60–200 CGG repeats) [61,62]. Repeats are expanded by the misfolding of repeat sequences into secondary structures that form hairpins, slipped-strand structures, triplexes and quadruplexes. Expansion is increased by low levels of global DNA methylation (as during gametogenesis and embryogenesis), and by altered DNMT1 activity. It is postulated to also involve DNA repair factors thought to be required for epigenetic reprogramming [57]. Indeed, the level of DNA methylation in somatic tissues correlates with stability, both *in vivo* and *in vitro* [63,64].

In HD, CAG repeats in *htt* are extended beyond the normal range of 17–29 repeats and result in aberrant polyglutamine tract expansion in the huntingtin protein. PolyQ huntingtin is toxic, resistant to proteolytic cleavage and susceptible to aggregation. It ultimately leads to cell death by elevation of key cell death players. Transcriptional alteration in HD is also associated with epigenetic dysregulation through sequestration and decrease of CBP HAT activity by polyQ, and induction of histone mono-ubiquitylation [65].

Alteration of imprinting

Several brain disorders are caused by abnormal imprinting, a gene silencing mechanism based on DNA methylation resulting in monoallelic and parent-specific expression of selected alleles. Imprinted genes are thought to represent less than 1% of autosomal genes [66], and occur primarily in clusters in chromosomal domains. These clusters also often contain imprinting control regions (ICRs) [67], which are differentially methylated depending on their parental origin, and regulate the silencing of neighbouring genes in the cluster. Alteration in imprinted domains on chromosome 15q11-13 are associated with Angelman and Prader–Willi syndromes, two neurodevelopmental disorders characterized by mental retardation and learning disabilities [66]. Angelman syndrome often results from a loss of maternal chromosomal contribution but can also derive from maternal uniparental disomies or from mutations in imprinting centres. Prader–Willi syndrome is most often because of a loss of paternal contribution. Linkage analyses identified 15q11-13 as a locus also relevant for autism spectrum disorders (ASD) [68,69]. Maternally expressed imprinted genes in this region are primarily implicated, since maternal uniparental disomies are most commonly linked with ASD and account for over 85% risk for the development of the disease [66,70]. However, in post-mortem brain tissue, the expected parental gene dosage effect was demonstrated in only half of autistic patients with 15q11-13 duplication, suggesting that other epigenetic mechanisms of gene regulation contribute to the disorder. Indeed, they may underlie the variability in clinical expression of 15q11-13 duplication syndrome [71].

Conclusion

Because epigenetic changes are prominent in several behavioural and cognitive disorders, epigenetic drugs such as HDAC and DNMT inhibitors, already of proven clinical utility in the treatment of cancer, provide promising therapeutic means. Some of these drugs have previously been investigated in this respect [19,20,23]. However, there is need for caution given their lack of specificity, the widespread changes they may induce in the epigenome, and their possible impact during development and on the germline [62]. Nonetheless, their specificity may be improved, for instance by using engineered transcription factors such as zinc factor proteins which target specific promoters [72]. Further to pharmacological compounds, epigenetic anomalies may also be treated with dietary supplements such as methionine, folic acid, vitamin B6 and 12 or with environmental factors like enriched environmental complexity. Despite being systemic, diet-based treatment may still provide good specificity, since methionine was shown to alter the expression of only 300 genes in the hippocampus, but does not modify DNA methylation overall [73]. Whilst environmentally driven epigenetic variations may increase the susceptibility to certain diseases, they can also bring some phenotypic variability and ultimately enhance fitness [74•]. A better understanding of epigenetic regulation in the nervous system is therefore a primary requisite to fully appreciate the basic mechanisms of behaviour and their evolution.

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