Therapeutic application of histone deacetylase inhibitors for central nervous system disorders

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Abstract | Histone deacetylases (HDACs) — enzymes that affect the acetylation status of histones and other important cellular proteins — have been recognized as potentially useful therapeutic targets for a broad range of human disorders. Pharmacological manipulations using small-molecule HDAC inhibitors — which may restore transcriptional balance to neurons, modulate cytoskeletal function, affect immune responses and enhance protein degradation pathways — have been beneficial in various experimental models of brain diseases. Although mounting data predict a therapeutic benefit for HDAC-based therapy, drug discovery and development of clinical candidates face significant challenges. Here, we summarize the current state of development of HDAC therapeutics and their application for the treatment of human brain disorders such as Rubinstein–Taybi syndrome, Rett syndrome, Friedreich's ataxia, Huntington's disease and multiple sclerosis.

Chromatin remodelling
Defines effects of epigenetic modifiers on the dynamic state of silent versus active chromatin, which consists of differently packaged histones.

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doi:10.1038/nrd2681

The precise pathological mechanisms underlying many central nervous system (CNS) disorders remain unclear and are emerging slowly. It is evident that multiple genetic, environmental and other factors — including ageing — contribute to and modulate disease onset, progression and severity[1–3]. In part, the gaps in our understanding reflect the widespread challenges in the development of effective tools to study the enormous complexity of the brain[4,5]. More specifically, it is not yet clear whether *in vitro* and *in vivo* models that have been designed to recapitulate CNS diseases will ultimately be reflective of the pathophysiology of the corresponding human disease[6]. Consequently, therapeutic development efforts have been slow to validate molecular targets and drug candidates that have been identified in these models[7].

Remarkably, it seems that in some instances therapeutic benefits may be achieved for diverse CNS diseases by interfering with a common set of targets or pathways. The superfamily of histone deacetylases (HDACs) has been recognized as an important therapeutic area for a broad range of human disorders, particularly, at first, for cancer treatment. However, this Review focuses on another important application for HDAC-based therapeutics: as a potential treatment for human CNS disorders.

Protein acetylation in cells

The role of protein acetylation has emerged as an important post-translational modification that regulates multiple cellular functions, including chromatin remodelling and transcriptional regulation[8–10], microtubule dynamics and intracellular transport[11,12], metabolism, and ageing[13]. Modification of specific lysine residues by covalent bonding with an acetyl group modulates the biological functions of many proteins and protein complexes[14,15]. In cells, protein acetylation is dynamic and maintained by two classes of functionally antagonistic enzymes: the protein acetylases and the deacetylases[16].

Much research has focused on enzymes that modulate the acetylation of histones — major components of chromatin — owing to the important roles these proteins have in vital cellular functions and in disease[17,18]. Levels of histone acetylation depend on the activities of histone acetylases (HATs) and HDACs, which add or remove acetyl groups from protein substrates, respectively[19] (FIG. 1). Although transcriptional regulation is highly complex and dynamic, in general an increase in histone acetylation causes remodelling of chromatin from a tightly packed configuration to a loosely packed configuration, which subsequently leads to transcriptional activation. Conversely, a decrease in histone acetylation may cause chromatin structure to condense and result in

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Nuclear localization signal
An amino-acid consensus within a protein sequence that determines nuclear localization.

Nuclear export signal
An amino-acid consensus within a protein sequence that determines protein exit from the nucleus.

transcriptional silencing. So, upregulation of transcription can be achieved in cells either by stimulation of HAT or by inhibition of HDAC activities, and the opposite is true for transcriptional downregulation.

In addition to modification of histones, other (non-histone) cellular proteins are substrates for HDACs, and these proteins mediate diverse biological functions via transcriptional-dependent as well as independent mechanisms[20,21]. In fact, phylogenetic analysis of bacterial HDAC relatives suggests that evolutionary development of modern HDACs preceded the evolution of histone proteins, and raises the possibility that the primary activity of some HDACs is directed against non-histone substrates[22].

Intensive therapeutic development efforts have focused on targeting HDACs with small molecules. Initially, this interest was precipitated by the discovery of the anticancer potential of HDAC inhibitors[23,24]. Subsequently, potential therapeutic applications were broadened to include other human illnesses, including CNS diseases, based initially on promising *in vivo* applications to polyglutamine-repeat diseases (for reviews see REFS 9,25). Although cancer remains a primary target for HDAC-based therapy, significant efforts have been made to develop compounds for the treatment of brain disorders[26,27].

Structure–function analysis of human HDACs

The superfamily of HDACs consists of five main subtypes: classes I, IIa and IIb, and IV, and the structurally distinct class III[22,28] (FIG. 2a). The name HDAC is somewhat of a misnomer having a historic origin, as histones are not enzymatic substrates for some deacetylases in a given family, and are not exclusive substrates for other family members.

Class I and class II HDACs include the Zn^{2+}-dependent deacetylases, which share significant structural homology, especially within the highly conserved catalytic domains[29,30]. Class I HDACs contain the ubiquitously expressed HDAC1, HDAC2 and HDAC3, and the muscle-specific HDAC8. HDAC1 and HDAC2 are predominantly localized in the nucleus, whereas HDAC3 shuttles between the nucleus and cytoplasm (FIG. 3a). All three of these deacetylases contain a nuclear localization signal within their protein sequences, and HDAC3 additionally has a nuclear export signal[31]. The best-characterized enzymes HDAC1 and HDAC2 are components of three stable transcriptional complexes termed SIN3A, NuRD and CoREST. These complexes are recruited to gene promoters by DNA binding proteins, which suggests gene-specific rather then global transcriptional regulation[32–34].

Class IIa HDACs consist of four members — HDAC4, HDAC5, HDAC7 and HDAC9 — with distinct tissue-specific patterns of expression, predominantly in muscle and heart[35,36]. These proteins contain extended amino-terminal domains of ~600 amino acids, which mediate interactions with HDAC3, myocyte enhancer factor 2 (MEF2), repressor complex NCOR2/SMRT and 14-3-3 proteins[28] (FIG. 3a), followed by the Zn^{2+}-containing catalytic domain. MITR — an amino-terminal splice variant of HDAC9 lacking the catalytic domain — HDAC4 and HDAC5 interact with heterochromatin protein 1 (HP1). HP1 is an adaptor protein that recognizes histone methylated lysines and mediates transcriptional repression by recruiting histone methyltransferases[37]. Interestingly, some data indicate that HDAC4, HDAC5 and HDAC7 are unable to deacetylate histones themselves, but probably participate in gene-specific transcriptional regulation via an interaction with class I HDAC3 (REFS 34,38). However, new results have demonstrated intrinsic deacetylase activity of HDAC4 and other class IIa deacetylases, and have showed that these enzymes are particularly active on class IIa-specific substrates *in vitro*[39,40]. This suggests that vertebrate class IIa HDACs may have evolved to maintain low basal activities on acetyl-lysines and to efficiently process restricted sets of specific, still undefined, natural substrates[39]. HDAC4, HDAC5, HDAC7 and MITR shuttle between the nucleus and cytoplasm, whereas full-length HDAC9 is localized in the nucleus[41–44]. Retention in the cytoplasm is controlled by 14-3-3 anchor proteins, which bind exclusively to the phosphorylated from of HDACs and prevent entry into the nucleus, whereas nuclear re-entry occurs upon protein dephosphorylation[43,45–47].

Class IIb HDACs include HDAC6 and HDAC10. The structure of HDAC6 is unusual in that it contains two independently functioning catalytic domains and a carboxy-terminal Zn^{2+}-finger ubiquitin binding domain[42]. HDAC6 functions in the cytoplasm where it deacetylates α-tubulin and alters microtubule stability[11,12] (FIG. 3a). Its close structural homologue HDAC10 lacks the second functional catalytic domain[48]. HDAC10 has been found in a complex with HDAC3, although the exact functions of this deacetylase are not known.

The class IV enzyme HDAC11 is structurally different from the class I and class II deacetylases. HDAC11 is predominantly localized in the nucleus; however it co-precipitates with the primarily cytosolic HDAC6 (REF. 49). The function of this deacetylase is poorly understood, although region-specific and developmental expression patterns have been observed in the mouse brain[50].

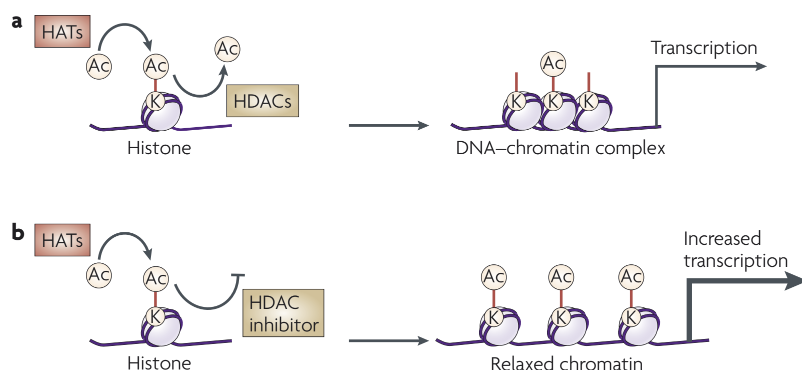

Figure 1 | **Effect of HDAC inhibitors on chromatin remodelling and transcription.** **a** | Levels of histone acetylation at specific lysine (K) residues are determined by concurrent reactions of acetylation (Ac) and deacetylation, which are mediated by histone acetylases (HATs) and histone deacetylases (HDACs). This histone acetylation is vital for establishing the conformational structure of DNA–chromatin complexes, and subsequently transcriptional gene expression. **b** | By blocking the deacetylation reaction, HDAC inhibitors change the equilibrium of histone acetylation levels, leading to increased acetylation, chromatin modification to relax conformation and transcription upregulation.

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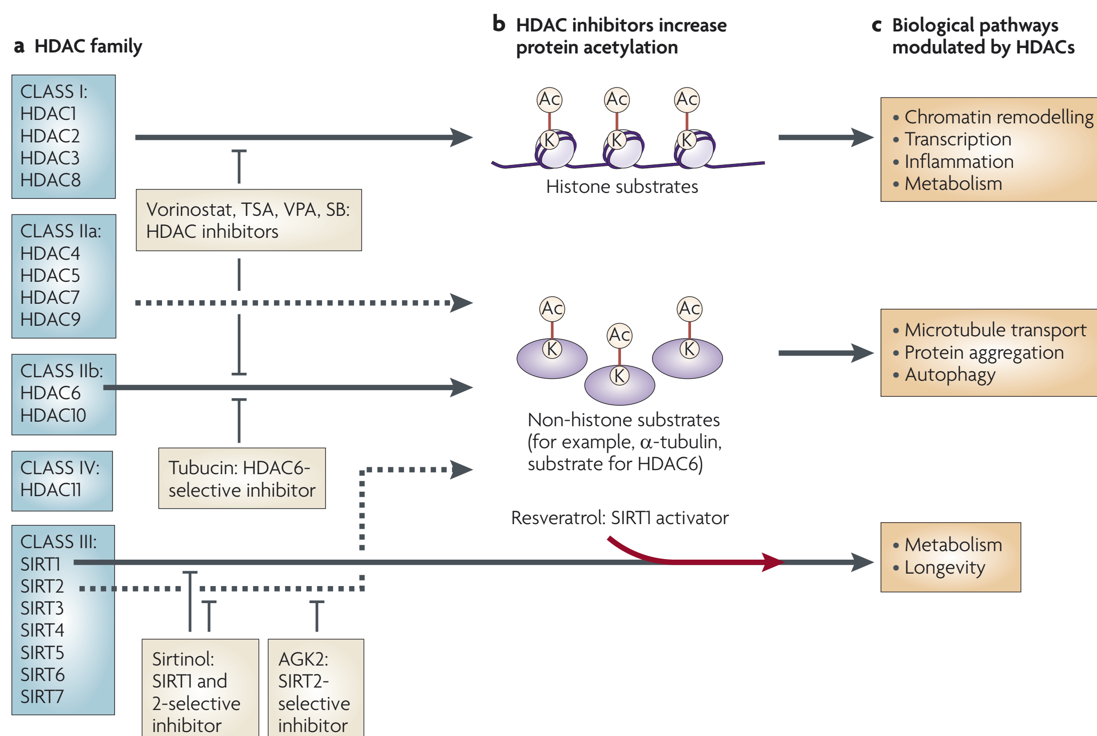

Figure 2 | **Therapeutic targeting CNS diseases with small-molecule inhibitors. a** | Histone deactylase (HDAC) family (classes I–IV) and HDACs implicated as disease modifiers are shown. **b** | Shown to be efficacious in animal disease models the HDAC inhibitors vorinostat (also known as SAHA), trichostatin A (TSA), valproic acid (VPA) and sodium butyrate (SB) upregulate histone and α-tubulin acetylation in cellular assays. The role of other specific HDAC isoforms in modulating disease pathology are yet to be determined with assistance of isoform-selective and class-selective inhibitors. Selective upregulation of α-tubulin acetylation in cellular assays is shown for the HDAC6-specific inhibitor tubucin as an example. **c** | Disease-modifying pathways including chromatin remodelling, transcription, inflammation, metabolism, ageing, degradation and microtubule transport, which underlie mechanisms implicated in HDAC inhibitor efficacy are shown.

By contrast, the class III deacetylases, or sirtuins, are structurally and functionally different from other HDACs. Named after the silent information regulator 2 (*Sir2*) gene — the first sirtuin identified in budding yeast — in humans, the class III HDACs include seven members[13,51]. Sirtuins are markedly different in their absolute dependence on NAD^+ to carry out catalytic reactions, which include both deacetylase and mono-ADP-transferase activities[52]. The predominant deacetylase activity has been shown for the class III HDACs SIRT1, SIRT2, SIRT3 and SIRT5 (REFS 51,53). The deacetylation reaction mediated by sirtuins is coupled to the cleavage of NAD^+, yielding nicotinamide and 2′-*O*-acetyl ADP-ribose, along with the deacetylated lysine residue within the protein substrate[54].

Structurally, human SIRT1 is the closest analogue of yeast Sir2, which regulates cellular metabolism and ageing[13]. SIRT2 has both nuclear and cytosolic localization, and interacts with numerous protein partners to execute multiple functions in cells. SIRT1 deacetylates a single lysine residue on several histones: K16 on histone 4 (H4), K14 on histone 3 (H3) and K26 on histone 1 (H1). Removal of acetyl groups from specific lysine residues may be determined by specific protein complexes, such as B-cell CLL/lymphoma 11A (zinc finger protein) (BCL11A) and p300/CBP-associated factor (pCAF)–myogenic differentiation 1 (MYOD1), of which SIRT1 is a component[13,55–57]. Non-histone substrates of SIRT1 include the transcription factors p53, TAF_I68, p300 and peroxisome proliferator-activated receptor-γ, coactivator 1α (PGC1α)[13] (FIG. 2b).

SIRT2 is a cytosolic protein that deacetylates α-tubulin and microtubules, a function that is redundant with that of HDAC6 (REFS 58,59). Subsequently, it has been shown that SIRT2 also deacetylates K16 on H4 (REF. 60) (FIG. 3b) and localizes to neuronal nuclei (G. Bates, personal communication). SIRT3, which shares the most structural similarity with SIRT2, is a mitochondrial protein (FIG. 3b). Acetyl-CoA synthetase (ACS2) has been identified as a SIRT3 substrate; however other data suggest a broad role for SIRT3 in regulating global mitochondrial lysine acetylation[61–63]. Mitochondrial subcellular localization has also been suggested for SIRT5 (REF. 64), but the function of this protein is not yet known. Finally, SIRT6 has recently been shown to be involved in the regulation of telomeric DNA during S phase where the putative enzymatic target is K9 on H3 (REF. 65).

S phase
The phase of the cell cycle when DNA is synthesized (replicated).

HDAC functions in the brain

A comprehensive gene-expression mapping of the 11 HDAC isoforms throughout the rat brain has been conducted using high-resolution *in situ* hybridization and

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imaging technology[66]. The signals obtained by *in situ* hybridization were compared with radiolabelled standards to obtain semi-quantitative analysis of individual HDAC isoforms and to define the relative levels of gene expression in more than 50 brain regions. In addition, an extensive atlas of expression of HDAC isoforms in the brain was assembled, which includes cell-type specific localization (Allen Institute for Brain Science; see Further information). Both of these resources have enhanced efforts to examine the roles of specific HDACs in the brain and to develop future modulators of HDAC activity.

The ubiquitous expression of class I HDACs in the CNS, as well as in peripheral tissues, suggests a broad role for these enzymes in controlling histone acetylation and transcription and argues against a neuron-specific function. By contrast, the class IIa HDACs show tissue-specific patterns of expression. Class IIa enzymes interact with the transcription factor MEF2 to control muscle differentiation[67,68]. Interestingly, however, class IIa HDACs and MEF2 are also highly expressed in the brain, which suggests that there is a specific role for these proteins in the CNS[69,70]. This role may be related to the activity of the calcium/calmodulin-dependent kinase (CaMK), which phosphorylates HDAC4, HDAC5 and HDAC7, and possibly MITR, resulting in the exit of these proteins from the nucleus[43,71] (FIG. 3a). In hippocampal neurons, nuclear translocation and export of HDAC4 and HDAC5 are dependent on synaptic activity and calcium influx, and are sensitive to CaMK inhibition[71,72]. Low potassium or excitotoxic glutamate levels in cultured neurons facilitate nuclear translocation of HDAC4, where it represses MEF2 and CREB-dependent transcription and causes apoptosis[73,74]. By contrast, elevated levels of MITR prevent neuronal apoptosis by transcriptional repression of c-Jun N-terminal kinase (JNK) through an interaction with HDAC1 (REF. 75).

The brain function of the class IIb enzyme HDAC6 reflects its role as a microtubule deacetylase. HDAC6 is expressed in most neurons but is most abundant in cerebellar Purkinje cells[59]. Expression of HDAC6 in post-mitotic cells suggests a novel function in the regulation of microtubule-dependent transport and cytoskeleton dynamics via modulation of acetylation levels of α-tubulin, a major component of microtubules[76]. Modulation of microtubule dynamics may provide a link to another HDAC6 function: regulation of protein degradation, which may be important to the maintenance of cellular homeostasis in the brain[77]. Recent results have implicated HDAC6 in facilitating both the UPS (ubiquitin proteasome system)-dependent and autophagy-mediated degradation pathways[78] (FIG. 3a). Notably, it has been shown that HDAC6 regulates the activity of the chaperone heat shock protein 90 (HSP90), which provides an additional mechanistic insight into the role of this deacetylase in protein degradation[79,80]. Expression of the other documented microtubule deacetylase, SIRT2, has been detected in the CNS, where SIRT2 has been localized to myelin sheaths in oligodendrocytes and implicated in the regulation of axonal myelination[81].

Purkinje cells
Large neurons with extensive dendritic arbor in the cerebellar cortex. Patients with spinocerebellar ataxia type 1 (SCA1) or SCA7 manifest cerebellar ataxia with degeneration of Purkinje cells, which is caused by polyglutamine extensions in the *SCA1* and *SCA7* genes.

Among the class III HDACs, SIRT1 has gained much attention as a mediator of metabolic changes and longevity in several model organisms. In a number of systems, SIRT1 activation, either genetically or pharmacologically, or via metabolic conditioning associated with calorie restriction, is neuroprotective[82,83]. In part, an increase in SIRT1 activity could enhance transcriptional expression of neuronal anti-stress, anti-apoptotic and anti-inflammatory genes[84]. SIRT1 is also expressed at high levels in the brain, spinal cord and dorsal root ganglia during embryonic development, which suggests an involvement of this deacetylase in neurogenesis[85]. Other sirtuin members are also implicated in the regulation of cellular metabolism and ageing, and targeting specific sirtuins such as SIRT2 provides neuroprotective benefit in neurodegeneration models[86,87].

HDAC inhibitor therapeutics for CNS disease

Recent progress in the field of chromatin remodelling and transcriptional regulation has dramatically changed our understanding of the ways in which genes are regulated. Epigenetic modifiers such as HDACs and HATs have been implicated as direct or indirect regulators of neuronal-specific, immune-specific and other tissue-specific gene-expression patterns in brain[25]. Furthermore, genetic evidence suggests crucial roles for HDACs and HATs in the maintenance of CNS homeostasis and in the mutations in genes encoding HATs or histone-binding proteins that underlie neurological disorders such as Rubinstein–Taybi syndrome and Rett syndrome, respectively (FIG. 4). We outline potential areas for therapeutic intervention below.

Rubinstein–Taybi syndrome. Mutations of CREB binding protein (CBP; also known as CREBBP) and p300, genes with HAT function, cause the mental retardation that is associated with Rubinstein–Taybi syndrome[88]. In a mouse model of Rubinstein–Taybi syndrome, mutant CBP heterozygotes develop a disorder that is characterized by growth retardation and skeletal abnormalities[89,90]. Several mouse models have been developed and these mice exhibit defects in chromatin acetylation and impairment of some forms of long-term memory, including the late phase of hippocampal long-term potentiation (L-LTP)[91]. The L-LTP deficits were ameliorated by either enhancing the expression of CREB-dependent genes or by inhibiting HDAC activity, which also partially restored long-term memory loss. Given the ubiquitous roles of CBP and p300 in all tissues, and the recent data suggesting an improvement in long-term memory and synaptic plasticity by the HDAC inhibitor trichostatin A (TSA) through its effect on CBP and CREB[92], it is reasonable to suggest that HDAC inhibitors may be a possible therapeutic option for Rubinstein–Taybi syndrome.

Psychiatric disorders. Chromatin remodelling may also play a central role in the cognitive impairment that is associated with psychiatric and neurodegenerative disorders[25]. So, targeting histone acetylation may provide benefit for the treatment of depression, schizophrenia, drug addiction and anxiety disorders[93]. HDACs,

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particularly HDAC5, seem to mediate antidepressant activity in animal studies[94]. Similarly, the HDAC inhibitor sodium butyrate exerted antidepressant-like effects in mice[95]. In a model showing temporally and spatially restricted induction of neuronal loss through induction of p25 expression, mice treated with HDAC inhibitors recovered learning and memory deficits[96]. Treated animals showed induced sprouting of dendrites, an increased number of synapses, and reinstated learning behaviour and access to long-term memories, which suggests that there could be a broad application for HDAC inhibitors in the treatment of cognitive disorders[96].

Rett syndrome. Rett syndrome is an X-linked dominant neurodevelopmental disorder of relatively high incidence caused by mutations in *MECP2*, which encodes the methyl-CpG-binding protein 2 (MECP2)[97]. This was the first disease to bring together epigenetics and neurobiology in human disease. MECP2 is a transcriptional repressor involved in chromatin remodelling and modulation of RNA splicing[98]. A complex relationship between MECP2 activity and gene imprinting exists such that a selective defect in postnatal neuronal maturation is observed in Rett syndrome[99], suggesting that further exploration of the therapeutic potentials of isoform-specific HDAC inhibitors for Rett syndrome may be warranted.

Friedreich's ataxia. Expansion of a triplet repeat region within an intron in the frataxin gene (*FXN*), which encodes a highly conserved mitochondrial protein, leads to transcriptional silencing in the neurodegenerative disease Friedreich's ataxia (for a review see REF. 100). Gene silencing of expanded frataxin alleles is accompanied by hypoacetylation of H3 and H4 and by trimethylation of H3 at K9, which is consistent with a heterochromatin-mediated repression mechanism[101] (FIG. 4). When tested in cultured cells obtained from patients with Friedreich's ataxia, benzamide-based HDAC inhibitors reversed frataxin-mediated silencing. In this cell model, HDAC inhibitors increased acetylation at specific lysine residues on H3 and H4 (H3K14, H4K5 and H4K12)[101]. In a recently published study, the novel benzamide HDAC inhibitor 106 corrected the frataxin deficiency in a mouse model of Friedreich's ataxia[102]. Thus, there is strong support for the potential therapeutic application of HDAC inhibitors for the treatment of Friedreich's ataxia.

Fragile X syndrome. A CGG-triplet repeat expansion in the 5′-UTR of the human *FMR1* gene is associated with the development of fragile X syndrome, the leading cause of inherited mental retardation. The mutation causes extensive local methylation within a CpG-rich 5′-flanking region, resulting in FMR1 transcriptional silencing and loss of its product, the FMRP protein (for reviews see REFS 103,104) (FIG. 4). Local hypermethylation and gene silencing is also associated with extensive changes to chromatin architecture, which can be ameliorated with TSA treatment in *Xenopus laevis* oocytes[105]. Furthermore, it has been shown in a cell-based model that transcriptional reactivation of *FMR1* expression can be achieved by inducing DNA demethylation with 5-azadeoxycytidine (5-azadC), or by treatment with the HDAC inhibitors 4-phenylbutyrate, sodium butyrate or TSA[106,107]. Although each individual treatment produced modest effects, the combination of 5-azadC with either HDAC inhibitor yielded a robust transcriptional reactivation[106]. These results provide an important example of the utility of HDAC inhibitors or 'drug cocktails' in combinatorial therapy and suggest a possible approach for the treatment of human CNS diseases[108]. In addition to class I and class II HDAC inhibitors, a potent reactivation of the *FMR1* gene was observed upon inhibition of the class III HDAC SIRT1 (REF. 109), which altered histone acetylation patterns. In addition to HDAC activity, a recent finding shows that SIRT1 positively regulates the H3K9 methyltransferase, which is implicated in heterochromatin formation and maintenance[110]. A reasonable interpretation of these results is that SIRT1 inhibition reverses heterochromatin-mediated silencing of the *FMR1* locus by increasing acetylation and decreasing methylation of histones. Therefore, pharmacological inhibition of SIRT1 could provide an alternative strategy to reactivate *FMR1* expression and to reverse the loss of a required neuronal protein, or could be used in combination with other treatments as mentioned above.

Motor-neuron disease. The inherited motor neuron disease spinal muscular atrophy (SMA) is caused by a mutation of the telomeric survival motor neuron 1 (*SMN1*) gene with retention of the centromeric *SMN2* gene[111], leading to insufficient levels of survival motor neuron (SMN) proteins. Owing to a silent mutation within an exonic splicing enhancer, SMN2 mainly produces alternatively spliced short transcripts with only a small degree of overlap with full length SMN1 (REF. 112). Even so, SMN2 may represent a promising target for SMA therapy using HDAC inhibitors[113]. During development in mouse tissues, histone acetylation levels decreased and HDAC2 levels increased at the region closest to the transcriptional start site, which correlated with decreased SMN transcript and protein levels. This suggests that the HDAC2 isoform in particular may serve as a therapeutic target for SMA[114]. In *ex vivo* studies using brain slices, vorinostat (also known as suberoylanilide hydroxamic acid; SAHA) was found to increase the expression of *SMN2* more potently in comparison with valproic acid, which is currently in clinical trials for treating SMA[115]. The benzamide HDAC inhibitor M344 also produced potent upregulation of SMN2 protein in fibroblasts derived from patients with SMA, suggesting apparent efficacy *in vitro*[112]. Finally, *in vivo* studies in a mouse model of SMA showed that TSA delivered orally or by intraperitoneal injections increased H3 and H4 acetylation and activated *SMN2* gene expression while also improving animal survival and protection of motor neurons when administered after disease onset[111].

HDAC inhibitors have also garnered interest as a potential therapeutic in amyotrophic lateral sclerosis (ALS) and spinal and bulbar muscular atrophy (SBMA).

Amyotrophic lateral sclerosis
(ALS). Is the most common form of motor-neuron disease.It is characterized by progressive selective degeneration of motor neurons and is mostly sporadic; however about 20% of familial ALS is caused by mutations in superoxide dismutase 1 (SOD1).

Spinal and bulbar muscular atrophy
(SBMA). Also known as Kennedy's disease, SBMA is an X-linked genetic disorder caused by a polyglutamine-repeat expansion within the androgen receptor gene.

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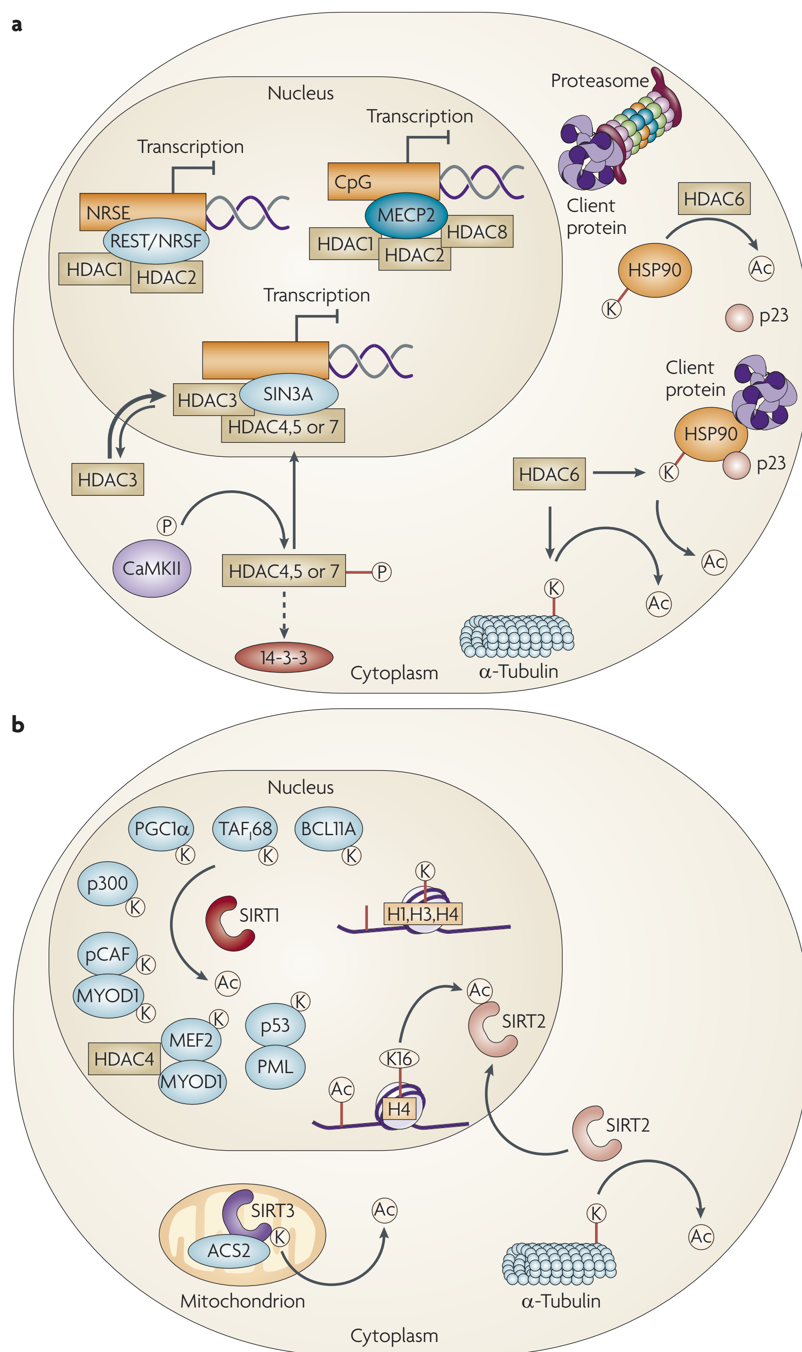

Figure 3 | **Schematic of representative HDAC effects in the nucleus and cytoplasm. a** | Histone deactylase (HDAC) complexes form in the nucleus to regulate transcription, typically through mechanisms leading to transcriptional repression, as depicted by the blocked lines at promoters. Some examples are depicted that are relevant to CNS disorders. In addition, HDAC6 is involved in cytoskeletal structure and proteasomal degradation, whereas HDAC4, HDAC5 or HDAC7 can shuttle between the nucleus and cytosol and are phosphorylated in the cytosol. **b** | Schematic depiction of several protein targets of sirtuin 1 (SIRT1) deacetylation in the nucleus, as well as proposed roles for SIRT2 in histone deacetylation and cytoskeletal structure, and SIRT3 in mitochondrial function. ACS2, acetyl-CoA synthetase; BCL11A, B-cell CLL/lymphoma 11A (zinc finger protein); CaMKII, calcium/calmodulin-dependent protein kinase 2; HSP90, heat-shock protein 90; MECP2, methyl-CpG-binding protein 2; MEF2, myocyte enhancing factor 2; MYOD1, myogenic differentiation 1; NRSE, neuron restrictive silencer element; pCAF, p300/CBP-associated factor (also known as KAT2B); PGC1α, peroxisome proliferator-activated receptor-γ, coactivator 1α; PML, promyelocytic leukaemia; REST/NRSF, RE1-silencing transcription factor.

Phenylbutyrate and sodium valproate promoted motor-neuron survival in mouse models of ALS, and compound efficacy was at least partially attributed to the amelioration of abnormal histone hypoacetylation and transcriptional dysregulation, which is implicated in ALS[116,117]. Similarly, in a mouse model of SBMA, caused by a polyglutamine-repeat expansion within the androgen receptor, a class of diseases discussed below, treatment with the HDAC inhibitor sodium butyrate partially ameliorated phenotypic symptoms and increased histone acetylation[118].

Polyglutamine-repeat diseases. Pleiotropic mechanisms of neurodegeneration are clearly associated with the nine dominant polyglutamine disorders, including Huntington's disease, spinocerebellar ataxias (SCAs) 1, 2, 3, 6, 7 and 17, SBMA, and dentatorubropallidoluysian atrophy (DRPLA)[119]. The precise mechanisms of neurodegeneration in these disorders are emerging, and perturbations to many aspects of neuronal homeostasis exist. These include abnormal histone acetylation and chromatin remodelling, and aberrant protein interactions. They also include mutant protein misfolding and aggregation, altered post-translational modifications, mitochondrial impairment, unbalanced metabolism, and altered sensitivity to oxidative stress (FIG. 4). A possible underlying defect in transcriptional regulation has been described for several of these diseases, including SCA1 and Huntington's disease[120–124] (for a review see REF. 125), leading to the premise that HDAC inhibition might be efficacious. Significantly, several of the polyglutamine-repeat diseases are caused by CAG-repeat expansions within genes encoding transcriptional regulatory proteins. For instance, an expanded repeat in the TATA-binding protein (TBP) leads to the neurodegenerative disease SCA17 (REF. 126), and mutation of the androgen receptor gene causes SBMA[127]. Likewise, SCA7 is caused by a mutation in ataxin 7, which has been identified as a subunit of a HAT co-activator complex[128], and ataxin 3 interacts with histones and HDAC3 and can repress transcription of target genes[129]. The neuroprotective effects of the HDAC inhibition has been well documented in both invertebrate and mouse models of Huntington's disease and other polyglutamine-repeat diseases[118,131–133].

A possible explanation for the observed neuroprotective effect has been attributed to a general HDAC-mediated chromatin remodelling and amelioration of transcriptional dysregulation in Huntington's disease (for a review see REF. 134). Alternatively, pathways may be altered in a manner that is specific for a given HDAC. For instance, pathway specificity may be inferred from studies showing that HDAC3, but not HDAC1, modulates polyglutamine-associated toxicity in *Caenorhabditis elegans* models, and that HDAC1and HDAC3 have different targets with opposing effects on polyglutamine toxicity[135]. Genetic modulation of HDACs in a *Drosophila melanogaster* model of Huntington's disease suggests that specific protection is mediated by knockdown of rpd3 (fly orthologue of HDAC1/2) and Sirt1 and Sirt2 orthologues[136]. Moreover, an early genetic screen in a fly model of SCA1 revealed

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genetic modifiers within the context of chromatin remodelling[137]. Furthermore, the observed efficacy of SIRT1 activation in a *C. elegans* model of Huntington's disease, mediated by FOXO1, is consistent with neuroprotective effects achieved through a specific pathway[138]. Transcriptional regulatory proteins such as CBP, nuclear factor-κb (NF-κB), p53, brain-derived neurotrophic factor (BDNF)[121,139,140] and more recently PGC1α[141], a key regulator of mitochondrial gene expression, are each implicated in some form of expanded polyglutamine-mediated neurodegeneration. These proteins are potentially amenable to modulation by HDAC inhibition[142] and conceivably with SIRT1 activators or inhibitors in Huntington's disease neurons. In keeping with a role for mitochondrial function, HDAC inhibition was found to improve mitochondrial-dependent Ca^{2+} handling in Huntington's disease striatal cells[143].However, the contribution of these pathways to disease progression and pathology, and the involvement of other HAT/HDAC-related effects such as non-histone-dependent protein activity and acetylation-dependent clearance mechanisms, remain to be elucidated.

Microtubule dynamics and protein aggregation

A defect in microtubule-based transport may contribute to the neuronal toxicity observed in Huntington's disease[144], and HDAC inhibition (for example, with TSA) may compensate for the transport deficit by increasing α-tubulin acetylation. TSA increases acetylation at K40 of α-tubulin in a HDAC6-dependent manner, which enhances recruitment of the molecular motors dynein and kinesin 1 to microtubules and leads to increased vesicle transport and subsequent release of BDNF. These results implicate HDAC6, and possibly SIRT2, modalities as therapeutic targets for diseases exhibiting impaired microtubule transport. The effects of HDAC6 and SIRT2 inhibitors on microtubule transport in the presence of aggregated tau (a microtubule-binding protein) in models of Alzheimer's disease and of tauopathy remain to be tested.

Recent results implicate HDAC6 as a regulatory protein of major cellular protein degradation pathways: the ubiquitin-proteasome system and autophagy[78] (FIG. 3). Protein degradation pathways are intimately linked to aggregation of misfolded mutant proteins, a hallmark of many neurological disorders, including Alzheimer's, Parkinson's, Huntington's and other diseases[145–147] (FIG. 4). Although it has become evident that HDAC6 activity can modulate neurodegenerative phenotypes in invertebrate systems[78] and protein aggregation[77], an evaluation of HDAC6 inhibitors in neurodegenerative models will benefit from the development of new inhibitors. It is noteworthy that the observed efficacy of the HDAC inhibitors vorinostat and sodium butyrate in the R6/2 mouse model of Huntington's disease seems to be independent from aggregation pathway(s), as treatment of animals with HDAC inhibitors does not affect the formation of visible neuronal polyglutamine aggregates[131,132]. Furthermore, *Hdac6*-knockout mice have hyperacetylated tubulin, are viable and develop normally, lacking any apparent neurological abnormalities[148]. We anticipate that HDAC6 will have a unique role in the clearance of misfolded proteins, which will be further elucidated in crosses of *Hdac6*-knockout strains and transgenic mouse models of neurodegenerative disease.

Finally, the efficacy of SIRT2 inhibitors has recently been investigated in models of Parkinson's disease[149], and beneficial effects correlate with increased acetylation of α-tubulin and increased formation of large α-synuclein inclusions. Nevertheless, the precise mechanism(s) by which SIRT2 inhibition exerts neuroprotective effects in dopaminergic neurons, both in cells in culture and in animal models, remains unclear.

Inflammation and apoptosis

Studies of the neuroprotective roles of HDAC inhibitors, which are known to broadly affect gene expression within the immune system, have been extended to other diseases that involve inflammation and neuronal apoptosis, such as multiple sclerosis[150–152] (FIG. 3). In an experimental autoimmune encephalomyelitis model of multiple sclerosis, the pan-HDAC inhibitor TSA reduces spinal-cord inflammation, demyelination, and neuronal and axonal loss through transcriptional modulation of gene expression[153]. It has also been shown that this compound upregulates genes that are associated with transcription of antioxidant, anti-excitotoxicity and pro-neuronal growth and differentiation. At the same time, TSA also inhibits caspase activation and downregulates gene targets of the pro-apoptotic E2F transcription factor pathway. So, TSA seems to ameliorate a transcriptional imbalance that may contribute to immune dysfunction and neurodegeneration in multiple sclerosis[153,154].

Additional recent studies have used a rat permanent middle cerebral artery occlusion model of stroke to study the effects of HDAC inhibitors on ischaemia-induced brain infarction, neuroinflammation, gene expression and neurological deficits. HDAC inhibitors exhibit anti-inflammatory and neuroprotective effects in a rat ischaemic model of stroke[155–158]. It has further been shown that post-stroke injections with the HDAC inhibitors valproic acid, sodium butyrate or TSA decrease brain infarct volume. Post-insult treatment with valproic acid or sodium butyrate also suppressed microglial activation, reduced the number of microglia and inhibited other inflammatory markers in the ischaemic brain[157]. Moreover, it seems that HDAC inhibitors also induce HSP70 and inhibit both activation of the pro-apoptotic phospho-AKT and p53 pathways, and induction of nitric oxide synthase and cyclooxygenase 2 in models of ischaemia[157,159]. Treatment with HDAC inhibitors also improved motor, sensory and reflex performance of tested animals. Similar results were observed in a mouse model of ischaemia treated with vorinostat[156]. These data suggest that HDAC inhibitors mediate multiple neuroprotective mechanisms of action in the ischaemic brain and that pharmacological manipulation with HDAC inhibitors may serve as a promising avenue to reduce post-ischaemic brain damage[9,154]. Importantly, the observed efficacy of valproic acid — an

R6/2 mouse model of Huntington's disease
First transgenic mouse model of Huntington's disease (HD), which is characterized by short life-span and robust neurological phenotype. The transgene encodes a polypeptide derived from the first exon 1 of the HD gene encoding the polyglutamine expansion. Expression of the transgene causes neurological phenotypes and extensive formation of neuronal inclusions and cytoplasmic aggregates.

α-Synuclein
Mutations in the α-synuclein gene product, which is of unknown function, have been identified in familial Parkinson's disease (PD). α-Synuclein protein readily forms insoluble aggregates, and is thought to have a key role in PD pathology.

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approved anticonvulsant and mood-stabilizing drug with a long history of clinical use — in rodent models of ischaemia suggests that testing of valproic acid in clinical trials for the treatment of patients with stroke is warranted[155].

Finally, in Huntington's disease, HDAC inhibition modulated gene expression associated with deleterious activation of the kynurenine pathway in microglia[160].

Metabolism and ageing

Since the discovery of the cholesterol-carrying apolipoprotein E (APOE) as a major risk factor for Alzheimer's disease, there has been a mounting interest in the role of this lipid as a possible pathogenic agent[161–163] in other neurodegenerative diseases. For instance, Niemann–Pick type C (NPC) disease is a neurodegenerative disease involving a lipid-storage disorder[164,165] (FIG. 4).

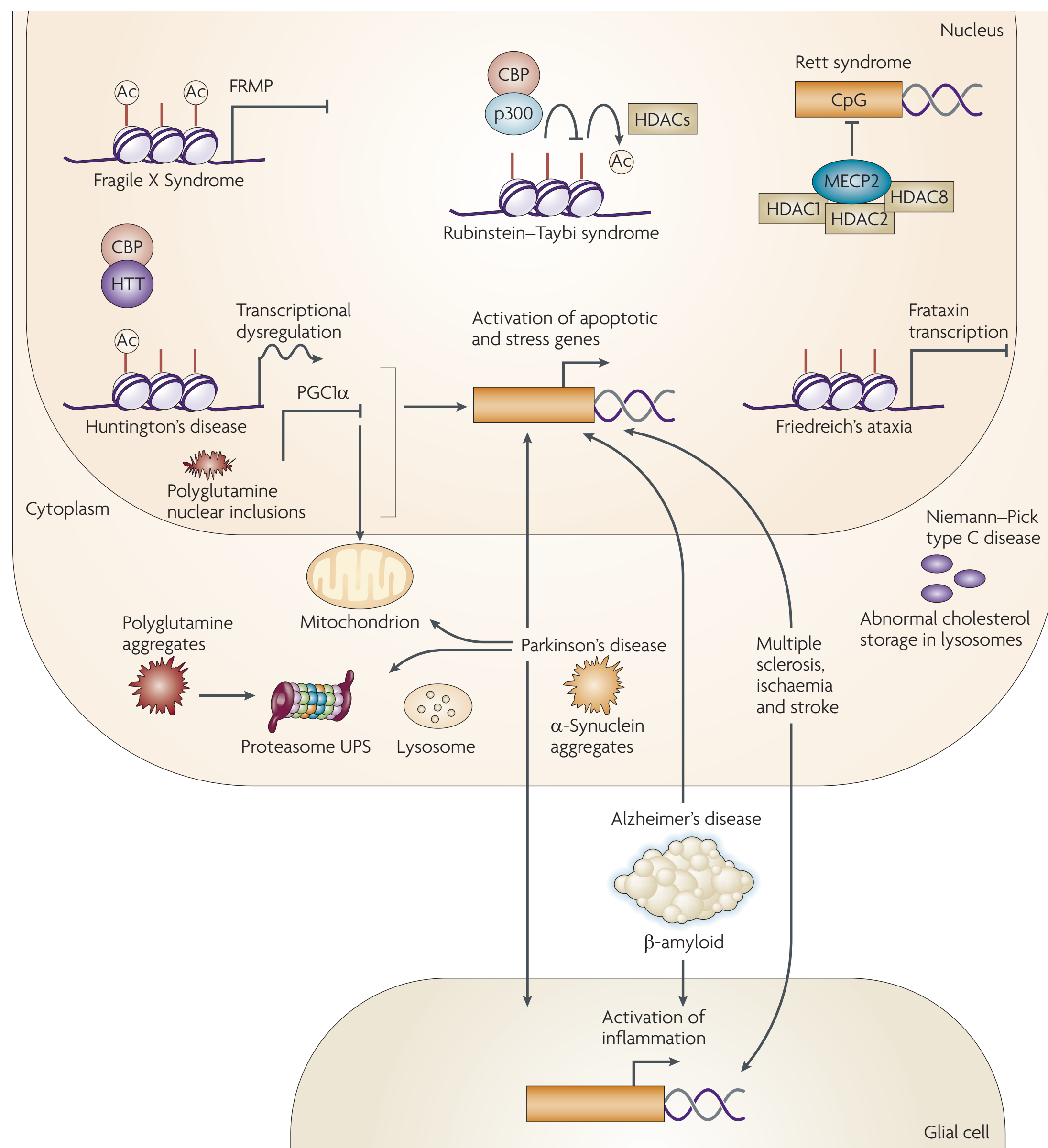

Figure 4 | **Role of HDACs and HATs in CNS disorders.** Several neurodegenerative diseases have been shown to involve disruptions in histone acetylase (HAT) and histone deactylase (HDAC) balance and are depicted schematically here. For instance, fragile X mental retardation 1 protein (FMRP) expression is reduced owing to extensive DNA methylation at its promoter, which can be alleviated through combined HDAC inhibition and demethylation. Decreased acetylation activity of CREB binding protein (CBP; also known as CREBBP), which is associated with Rubinstein–Taybi syndrome and polyglutamine-repeat diseases, appear to involve altered activity of key transcription factors such as the acetyltransferase activity of CBP. Because of known mutations or effects upon transcriptional regulation, HDAC inhibitors have been tested and shown to have protective effects in cell and animal model systems. Several of these disorders and possible sites of intervention are depicted schematically. For instance, frataxin expression is increased by treatment of model systems with HDAC inhibitors. HTT, huntingtin; MECP2, methyl-CpG-binding protein 2; PGC1α, peroxisome proliferator-activated receptor-γ, coactivator 1α; UPS, ubiquitin proteasome system.

Kynurenine pathway
Pathway leading to tryptophan degradation via a sequence of biochemical reactions and formation of bioactive intermediates such as kynurenic acid.

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A hallmark of this disease is impaired transport of cholesterol out of late endosomes/lysosomes and the accumulation of cholesterol in these organelles[166,167]. Although cholesterol accumulates in cell bodies of neurons from NPC1-deficient mice, the cholesterol content of axons is reduced[168,169]. The HDAC inhibitor valproic acid enhances neuronal differentiation and restores defective cholesterol metabolism in neural stem cells from NPC1-deficient mice[170]. Following treatment with valproic acid, expression of the essential neurotrophic genes neurotrophic tyrosine kinase, receptor, type 2 (*NTRK2*; also known as *TRKB*), *BDNF*, superoxide dismutase 2, mitochondrial (*SOD2*; also known as *Mn-SOD*) and neurogenic differentiation 1 (*NEUROD1*) was upregulated, leading to enhanced neural differentiation. By contrast, the elevated cholesterol levels in neural stem cells were reduced. These data suggest a therapeutic benefit from reduction of cholesterol levels for the treatment of NPC disease, and most recent data implicates the class III HDAC SIRT1 as a potential modulator of cellular cholesterol biosynthesis[171].

Disruptions in cholesterol biosynthetic pathways have been identified as a progressive and early marker of Huntington's disease pathogenesis in several mouse models of the disease. For instance, cholesterol precursors, lathosterol and lanosterol, and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity decline over time in transgenic mice that express either truncated or full-length mutant huntingtin (HTT) protein[172,173]. By contrast, cholesterol itself was found to accumulate in cultured neurons expressing mutant HTT and in the brains of mutant HTT-expressing animals, with evidence for impaired caveolin-1-mediated cellular trafficking[174]. The use of HDAC inhibitors may therefore be useful in elucidating the mechanisms involved with cholesterol homeostasis and could allow for both restored cholesterol biosynthesis and cholesterol transport.

A nutritional diet that is low in calories (calorie restriction) improves health and longevity in a wide range of model organisms, including rodents, in many cases by activating *SIRT1* gene expression[175–177]. SIRT1 has been identified as a key component linking cellular metabolism and ageing; therefore, enzyme agonists could potentially provide therapeutic benefits by targeting metabolic processes and extending longevity[179]. Activation of SIRT1, either pharmacologically or genetically, and conditional calorie restriction can both exert neuroprotective effects in various disease models[180,181]. For example, overexpression of the SIRT1 deacetylase and administration of resveratrol, shown to increase SIRT1 in several systems, markedly reduced NF-κB signalling stimulated by amyloid-β and had strong neuroprotective effects[182]. However, resveratrol exerts pleiotropic effects[180], inducing SIRT1-independent biological responses as well. The outcome of SIRT1 modulation is itself highly complex and under intense investigation in post-mitotic healthy and affected neurons in the context of CNS disease. For instance in *C. elegans* and cellular models of Huntington's disease, genetic activation of SIRT1 or resveratrol-dependent stimulation of SIRT2 are protective against polyglutamine-induced neurotoxicity[138]. By contrast, in a *C. elegans* model of oculopharyngeal muscular dystrophy, an increased dosage of SIRT1 exacerbated muscle pathology, whereas its null-mutant was protective. In addition, survival was decreased by resveratrol treatment, and promoted by the SIRT1/2 inhibitor sirtinol[183]. A neuroprotective effect of SIRT1 inhibition against lipid and protein oxidation damage further suggests complex cellular responses associated with targeting SIRT1 (REF. 184). Finally, inhibition of sirtuins using nicotinamide restored cognition in mouse models of Alzheimer's disease through a mechanism that seems to involve selective reduction of Thr231-phosphotau[185].

Oculopharyngeal muscular dystrophy
An autosomal dominant mutation causing extension of the naturally occurring 10 alanine sequence up to a maximum of 17 alanines, resulting in fibril formation of PABPN1, a nuclear protein, and the development of late-onset muscular dystrophy.

Pharmacological development

Historically, small-molecule HDAC inhibitors have been identified as active anti-proliferative agents in tissue culture, which inhibit tumour progression in rodent models[186–189]. However, the goal of reversing or blocking pathophysiological dysfunction or neuronal loss in CNS diseases, via modulation of HDAC-dependent pathways with small-molecule ligands, is fundamentally different from the objectives of anticancer therapy. To exploit the therapeutic potential of HDAC inhibitors in the CNS, stable and bioavailable compounds must penetrate the blood–brain barrier to reach affected brain tissues. Inhibitors need to be both potent and selective for specific intracellular molecular targets, and isoform-specificity or at least class-specificity is desired to minimize nonspecific side effects.

Potency and selectivity. Several highly potent *in vitro* and *in vivo* HDAC inhibitors have been designed and developed, and some lead compounds have demonstrated activity in the impressively low nanomolar range[187,190,191]. For example, deacetylase activities of purified HDAC1 and HDAC3 were potently inhibited by vorinostat (HDAC1 IC_{50} =119 nM; HDAC3 IC_{50} = 106nM) and TSA (HDAC1 IC_{50} =1.5 nM; HDAC3 IC_{50} = 0.6 nM)[190] (FIG. 5). Such potency of these small molecules has been attributed to the ability of the inhibitor functional groups to access the zinc cation in the HDAC active-site pocket. The structural characteristics of the hydroxamic acid inhibitors — consisting of a metal-binding domain, a linker domain and a hydrophobic capping group — have been examined in co-crystallization studies with HDAC enzymes[190]. Inhibitors, such as vorinostat and TSA, coordinate the zinc in a bidentate manner and simultaneously contacts residues that are likely to be involved in catalysis by its hydroxamate moiety[190]. This interaction efficiently inactivates the zinc-dependent deacetylase activity of HDAC enzymes[192–194]. However, the ability to effectively penetrate into the deep pocket of the active site was associated with other liabilities in structural design, which negatively affected the drug-like properties of the inhibitor[195].

In vitro specificity of previously identified HDAC inhibitors was determined using a panel of recombinant human HDAC enzymes in deacetylation assays with fluorophore-containing substrates optimized for

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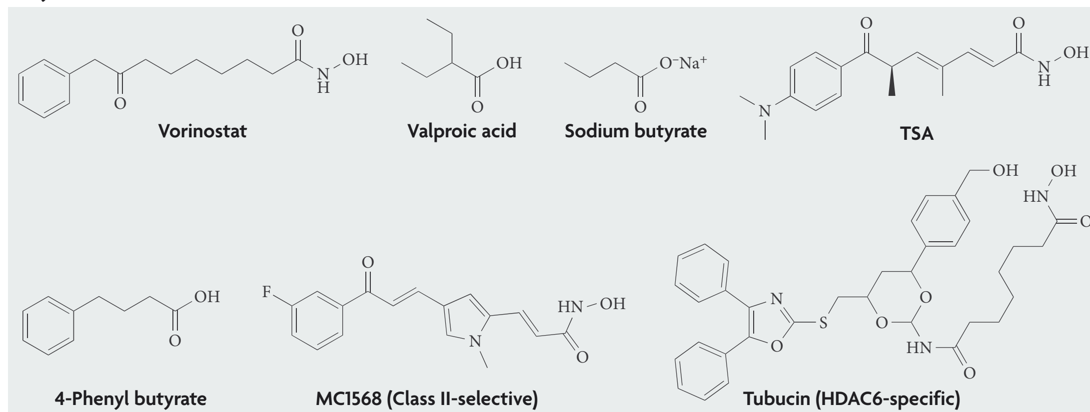
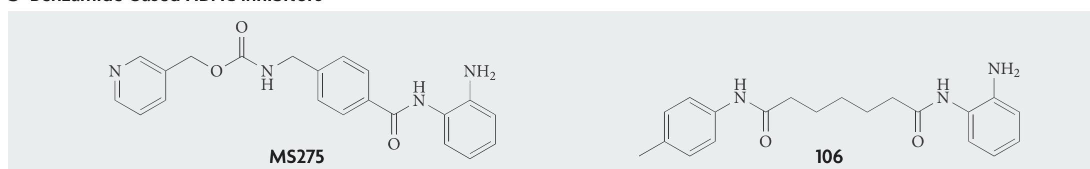
a Hydroxamate-based HDAC inhibitors



b Benzamide-based HDAC inhibitors



c Sirtuin ligands

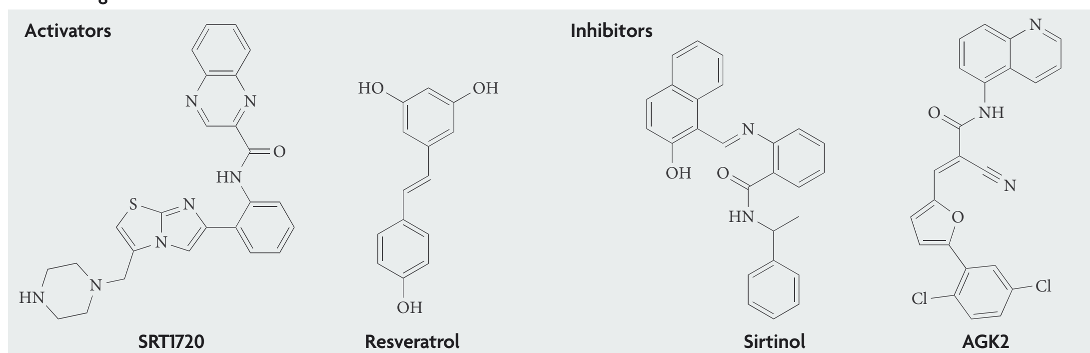


Figure 5 | **Structures of common and newly reported bioactive hydroxamate and non-hydroxamate HDAC inhibitors and sirtuin ligands.** Efficacies of hydroxamate-based vorinostat (also known as SAHA), trichostatin A (TSA), valproic acid, sodium butyrate, 4-phenyl butyrate, and benzamide-based MC275 and compound 106, were empirically demonstrated in disease animal models. MC1568 and tubucin are shown as examples of class-specific and isoform-selective HDAC inhibitors. Sirtuin 1 (SIRT1)-selective activators resveratrol and SIRT1720, SIRT1/2-selective inhibitor sirtinol, and SIRT2-selective inhibitor AGK2 are shown. Animal efficacy of the putative SIRT1 activator resveratrol was assessed and demonstrated in various disease models as described in the main text.

each isoform[196]. These experiments showed that inhibitors had varying selectivity for HDACs. The benzamide inhibitors MS275 and MGCD0103 exhibit *in vitro* selectivity for HDAC1 and HDAC2 isoforms; apicidin is selective for HDAC2 and HDAC3; R306465 is selective for HDAC1 and HDAC8; and valproic acid is selective for class I HDAC targets. TSA, LAQ824/LBG589, panobinostat, ITF2357, vorinostat and belinostat were found to be potent pan-HDAC inhibitors[186]. According to new findings, MS275 was found to be selective for HDAC1 (REF. 197). In previous reports, however, selectivity of the MS275 HDAC inhibitor was not observed[198].

Potencies (IC_{50} values) of common inhibitors against HDAC targets *in vitro* vary considerably, however, and these discrepancies are likely to be related to different sources of enzymes (native versus recombinant, and degree of purity), as well as the substrates used in the HDAC assays and reaction conditions. It is particularly important for class IIa enzymes, which have been previously thought to lack deacetylase activity. According to new findings, HDAC4 and other class IIa deacetylases have low but measurable activity, and these enzymes are particularly active on class IIa-specific substrates *in vitro*[39,40].

A recent focus of intensive investigation has been to develop class-specific and isoform-specific HDAC inhibitors, which has been difficult owing to the evolutionarily conserved architecture of the HDAC active site. Nevertheless, class I-selective HDAC inhibitors have been reported[39,199–201]. Class selectivity of HDAC inhibitors was evaluated in cellular assays in which compound treatment of intact cells was followed by measurements of

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levels of histone and α-tubulin acetylation. In these tests, pan-HDAC inhibitors upregulated both acetylation of histones and α-tubulin, whereas inhibitors selective for class I enzymes increased only histone acetylation[196,202,203] (FIG. 2c). However, the effects in cells of class I-selective inhibitors on HDAC class I–II complexes, and on class II HDAC-specific activities within these complexes, are yet to be examined.

Advances have also been made towards the development of class II isoform-selective HDAC inhibitors. One lead compound, MC1568, described as a class IIa-selective inhibitor, inhibits class IIa HDAC4 but not class I HDAC1 (REF. 203). It was later shown that MC1568 induced hyperacetylation of α-tubulin, a functional test for class IIb HDAC6 inhibition, and therefore was in fact class II selective. Tubacin was identified as a class IIb HDAC6-specific inhibitor, and notably appears to be domain selective[204]. Tubacin increases acetylation of α-tubulin, the known substrate of HDAC6, but not of histones, in cellular assays[204] (FIGS 2c,5).

Drug-like properties. Several formidable challenges that are associated with chemical development include nonspecific cytotoxicity, cellular impermeability and poor pharmacokinetic properties. The first identified pan-HDAC inhibitors, vorinostat and LAQ824, were hydroxamate-based[205] (FIG. 5). These inhibitors are currently in clinical development, but metabolite cytotoxicity of hydroxamic acid derivatives as well as low isoform selectivity may contribute to nonspecific cytotoxicity. In recent years, the search for non-hydroxamate HDAC inhibitors has intensified, leading to the discovery of HDAC inhibitors with a benzamide head group that exhibit significantly less cytotoxic properties (FIG. 5). A more advanced second generation of benzamide-based inhibitors, including CI994, MS275 and MGCD0103, are currently in clinical development[206].

Brain permeability. Brain permeability remains a major limitation for drug treatment of CNS disorders. Nevertheless, brain penetration has been demonstrated for the HDAC inhibitors vorinostat, sodium butyrate, phenyl butyrate, MS275 and valporic acid[131,132,155,156,207] (FIG. 5). It appears that structural properties of identified benzamides may allow greater brain penetration.

The benzamide inhibitor MS275 preferentially inhibits HDAC1 (IC_{50} ~0.3 μM) versus HDAC3 (IC_{50} ~8 μM); shows no inhibitory activity against HDAC8 (IC_{50} >100 μM); and increases histone acetylation in compound-treated cells[208]. Following administration by subcutaneous injection, MS275 effects are observed in the frontal cortex and the hippocampus, which suggests that MS275 has brain-region selectivity[209]. Using an increase in histone acetylation as a biomarker of HDAC inhibition activity, MS275 was shown to be 30-times to 100-times more potent then valproic acid[209]. In two recent publications, the utility of benzamide-based HDAC inhibitors for treating CNS disease has been explored further. One study shows that a novel HDAC inhibitor, 4-dimethylamino-*N*-[5-(2 mercaptoacetylamino) pentyl] benzamide, increases H3 acetylation and reduces the inflammatory response of microglia following traumatic brain injury in rats[210]. In another study (N^1-(2-aminophenyl)-N^7-*p*-tolylheptanediamide (compound 106) (FIG. 5), which lacks acute toxicity, normalizes frataxin levels and restores gene-expression profiles resulting from frataxin deficiency in a mouse model of Friedreich's ataxia[102].

Developing sirtuin activators and inhibitors. Different chemical classes of small molecules can activate human sirtuins: chalcones, flavones and stilbenes[211]. The most potent compound identified to date is resveratrol, a component of red wine, which stimulates SIRT1 activity tenfold by lowering the K_m for both NAD^+ and the peptide substrate. Activation of SIRT1 is detectable using some *in vitro* assays, including a fluorescent peptide-based assay in which the resveratrol effect seems to be highly selective for SIRT1 and does not change the activities of the SIRT2 or SIRT3 deacetylases[149,211,212]. However, as described above, resveratrol may exert pleiotropic effects on other cellular pathways as well[180,213]. The observed biological effects of resveratrol have established a link between SIRT1 activation and modulation of longevity and metabolism. The structure of resveratrol, which suggests that there may be low bioavailability in mammals, low solubility, and sensitivity to light and to oxidation, may also limit the utility of resveratrol as a drug[180]. However, improved SIRT1 agonists are beginning to emerge, facilitating both further investigation of SIRT1 as a validated target as well as opening up new therapeutic avenues[214] (FIG. 5). So far, no pan-sirtuin agonists or selective activators of SIRT2 or SIRT3 have been identified.

The first generation of sirtuin deacetylase inhibitors such as sirtinol (SIRT1 and SIRT2 inhibitor) (FIG. 5), identified in high-throughput screening, were characterized by low potency and selectivity[215]. Subsequently, potency and selectivity were greatly improved by rational drug design[216]. These redesigned sirtinol derivatives were found to be tenfold more potent, although they remain non-selective inhibitors of both SIRT1 and SIRT2. Further high-throughput screening has led to the discovery of indoles as potent and selective SIRT1 inhibitors[217]. The most potent compounds had IC_{50} values of 60–100 nM, representing a 500-fold improvement over previously reported sirtuin inhibitors. Interestingly, kinetic analyses suggest that these inhibitors bind after the release of nicotinamide from the enzyme and prevent the release of deacetylated peptide and 2′-*O*-acetyl-ADP-ribose, the products of enzyme-catalysed deacetylation. These newly discovered SIRT1 inhibitors seem to be cell-permeable, orally bioavailable and metabolically stable.

Efforts to design improved derivatives of the SIRT1 inhibitor suramin, a symmetric polyanionic naphthylurea, have led to the discovery of highly potent and selective inhibitors of SIRT1 and SIRT2 (REF. 218). Selective inhibitors of human SIRT1 and SIRT2 exhibit potency in the two-digit nanomolar range. Suramin has been shown to be a weak inhibitor of SIRT5 deacetylase as well. No SIRT3-selective inhibitors have yet been identified. The therapeutic application of the sirtuin ligands and

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inhibitors has just begun and the efficacy of animal trials will become crucial for evaluating therapeutic potentials of any new candidate molecule for a given CNS disease.

Perspectives on HDAC therapy

The concept of treating CNS diseases with HDAC inhibitors is viewed as a rational therapeutic approach by the scientific community and the pharmaceutical industry. These inhibitors will probably be acceptable to regulatory agencies and the general public as drugs with improved selectivity, decreased toxicity and validated targets emerge. Valproic acid has been used in humans as a drug for the treatment of schizophrenia and bipolar disorder for decades, and vorinostat has recently been approved for cancer treatment, while other HDAC inhibitors are currently in different phases of human clinical trials for CNS disorders such as Alzheimer's disease, Huntington's disease and ALS.

Given the tremendous therapeutic potential in cancer, it is likely that development of HDAC inhibitors, including entities to treat brain tumours, will continue for some time. However, after decades of translational and clinical research, drug delivery to the brain remains a major obstacle in CNS therapeutic development[219]. The brain is the most protected organ of the human body and is separated from peripheral tissues by the sophisticated defence system of the blood–brain barrier, which CNS drugs must penetrate. Further challenges in the development of effective CNS therapeutics are related to brain metabolism, which is unique from that of other tissues. Consequently, the pharmacokinetic and pharmacodynamic characteristics of drug candidates, along with their ADMET (adsorption, distribution, metabolism, excretion, toxicology) properties, are often different in brain tissues as compared to other organs. This observation magnifies a more general problem concerning drug safety and specificity: that a therapeutic agent may appear efficacious in the targeted disease tissue, yet causes adverse effects in other organs.

Thus, the therapeutic development of HDAC inhibitors and application for CNS diseases faces challenges, which are in part related to chemical design and synthesis of potent, brain-permeable, bioavalable small molecules with good pharmacological, pharmacodynamic and ADMET properties, but are also associated with the design of inhibitors that exhibit the necessary *in vivo* selectivity. It is presently unclear whether pan, class-specific or isoform-specific inhibitors will be more efficacious for treatment of each candidate disease. However, isoform-specific inhibitors would be anticipated to have fewer adverse effects, and thus be better tolerated during chronic or subchronic administration. To that end, rapid clearance of compounds in plasma and relative metabolic stability in the brain may become important pharmacokinetic properties to examine. The consideration is relevant for drugs that target deacetylases, which are responsible for regulating important and diverse biological functions in the brain and in peripheral tissues. Hence, adverse effects of silencing these modalities are likely. That formally contradicts the peculiar lack of neurological or other phenotypes observed in various HDAC-knockout mice. This is probably a consequence of compensatory effects of other HDAC enzymes. However, a lack of a knockout phenotype does not ensure the ability to safely target a specific HDAC modality with small molecules.

Despite expected difficulties in drug development, dramatic progress has been made in understanding the mechanisms of pathogenesis for a broad range of human brain disorders. Insights into molecular pathogenesis have led to the identification of HDAC targets as novel therapeutic interventions using small-molecule inhibitors. This suggests that HDAC inhibition could correct transcriptional defects and other acetylation-dependent impairments, and thus could be used as successful treatments for a number of neurodegenerative diseases including Rett syndrome, Rubinstein–Taybi syndrome, Friedreich's ataxia, Huntington's disease and many other disorders that affect brain function. The anti-inflammatory properties predicted for some of these compounds could render them useful for the treatment of multiple sclerosis and stroke. Broad therapeutic applications are envisioned for SIRT1 activators, while selective sirtuin inhibitors have just begun to emerge. Despite the acknowledged difficulties, intervention in human brain pathologies with HDAC inhibitors may represent a successful approach to treating a number of neurological disorders.

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Acknowledgements
We are grateful to M. Maxwell for critical review of this manuscript.

DATABASES
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