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Unique functional roles for class I and class II histone deacetylases in central nervous system development and function

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Abstract

Non-specific pharmacological inhibition of the histone deacetylase (HDAC) family of enzymes has largely beneficial effects in a variety of diverse contexts including cancer, cognitive function, and neurodegeneration. This review will discuss the role of individual HDAC isoforms in brain function during development and in the adult. Importantly class I and class II HDACs exhibit distinct cellular and subcellular expression patterns and utilize different signaling pathways to influence their substrates. Moreover, dissociable phenotypic outcomes emerge following manipulation of individual HDACs in the brain. To date, pharmacological inhibitors capable of targeting individual HDACs have proven difficult to develop, an obstacle that must be overcome to unlock the substantial clinical promise of manipulating endogenous HDAC isoforms in the central nervous system.

Introduction

Histone deacetylases (HDACs) are an evolutionarily ancient enzyme family that regulate activity of their substrates by removing acetyl groups from lysine residues (Gregoretti et al., 2004; Hildmann et al., 2007). *Histone* deacetylases are so named based on their ability to govern chromatin structural dynamics by deacetylating N-terminal lysine residues within the protruding tails of histone proteins, a modification associated with repression of gene transcription, however the acetylation/deacetylation reaction occurs in at least 80 proteins and HDACs can act on many substrates (Kouzarides, 2000; Glozak et al., 2005; Ocker, 2010). Alternatively, the histone acetyltransferases (HATs), by acetylation of histone tails, neutralize their positive charge thereby relaxing chromatin structure due to greater electrostatic repulsion from negatively charged DNA, a modification associated with transcriptional activation (Feng et al., 2007; Haberland et al., 2009a). In a provocative recent study, Wang et al. (2009) found that both HATs and HDACs are found at transcribed regions of active genes, and suggested that HDACs function to “reset” the conformational state of chromatin by removing acetylation at active genes (Wang et al., 2009).

Primarily through the use of non-specific, “pan-” HDAC inhibitors which inhibit many or all HDACs, HDACs have been implicated in diverse biological processes, including but not

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limited to, tissue specific developmental programming, apoptosis, synaptogenesis, cognition, cancer, and neurodegenerative disease (Bolger and Yao, 2005; Minucci and Pelicci, 2006; Hildmann et al., 2007; Barrett and Wood, 2008; Akhtar et al., 2009; Brunmeir et al., 2009; Chuang et al., 2009; Haberland et al., 2009a). Biological functions of individual HDACs have been difficult to determine due to the lack of specific pharmacological inhibitor compounds for particular HDACs. Moreover, constitutive knockout (KO) of many of the individual HDACs are lethal, underscoring the vital role of these enzymes in normal development, but rendering the use of constitutive KO models unsuitable for study in the adult. Conditional KO and small-interfering RNA (siRNA) strategies are now being employed to study the unique functional profiles of individual HDAC isoforms, with the obvious benefit that the animals are viable and knockdown can be made in a temporal- and anatomical region-specific manner.

Attention has recently been lavished upon the HDACs due to the efficacy of certain HDAC inhibitor compounds in treating some forms of cancer. To date two HDAC inhibitors have been approved for cutaneous T-cell lymphoma (vorinostat and depsipetide) and several are in phase II or III clinical trials for cervical and ovarian cancer (Minucci and Pelicci, 2006; Duvic and Vu, 2007; Kristensen et al., 2009). Beyond cancer, HDAC inhibitors are recognized for their ability to influence a wide spectrum of neurodevelopmental and neurophysiological processes. For example, HDAC inhibition is neuroprotective in *in vitro* and *in vivo* models of neurotoxicity and degeneration, and can improve cognitive function following traumatic brain injury or neurodegeneration in animal models (Ferrante et al., 2003; Petri et al., 2006; Avila et al., 2007; Dompierre et al., 2007; Fischer et al., 2007; Chuang et al., 2009; Dash et al., 2009; Ricobaraza et al., 2009; Bardai and D'Mello, 2011; Graff et al., 2012). Acute treatment with HDAC inhibitors enhances memory formation in vertebrates and invertebrates and boosts cellular mechanisms believed to underlie learning (e.g., long-term potentiation) (Levenson and Sweatt, 2006; Barrett and Wood, 2008; Federman et al., 2009; Graff and Tsai, 2011). Furthermore, there is evidence that drug addiction and affective disorder may be pathologies effectively targeted by HDAC manipulation (Renthal et al., 2007; Tsankova et al., 2007; Malvaez et al., 2009; Morris et al., 2010; Taniguchi et al., 2012). Clearly, the potential clinical applications of manipulating endogenous HDAC function transcend cancer and include neurodegenerative and psychiatric disorders.

The following review will discuss the diverse functional repertoire of this important enzyme family highlighting the distinct roles of class I versus class II HDACs in central nervous system development and adult brain function. The use of HDAC KO mice has begun to reveal distinct and specific roles of individual HDACs in development as well as in disease. Therefore in many applications broad HDAC inhibition will prove not as clinically beneficial as targeting individual HDAC isoforms.

Class I and Class II HDACs have distinct cellular and subcellular expression patterns and influence cellular function through diverse signaling pathways

Structural and functional differences as well as expression patterns provide a basis for grouping HDACs into at least 3 different classes in mammals (see Table 1, Figure 1). The class I HDACs (HDAC 1, 2, 3, and 8), are constitutively nuclear proteins, whereas the class II HDACs (HDAC 4, 5, 6, 7, 9, and 10; class II HDACs can be further divided into class IIa – HDAC4, 5, 7, 9 or class IIb – HDAC6, 10) are expressed in a more cell-specific manner and shuttle between the nucleus and cytoplasm (Figure 1) (Chawla et al., 2003; Broide et al., 2007; Haberland et al., 2009a). Class IV, currently consists of one member, HDAC11, with little known of its function. The sirtuins, sometimes referred to as the class III HDACs, possess deacetylase activity but are functionally unrelated to HDACs and rely upon NAD⁺ as opposed to zinc as a cofactor for enzymatic activity. Sirtuins have been discussed in

several recent reviews (Longo and Kennedy, 2006; Baur et al., 2012; Houtkooper et al., 2012) and will therefore not be further considered here.

In the adult rat and mouse all 11 HDACs are expressed in brain to some extent (Broide et al., 2007; Baltan et al., 2011). Class I HDACs are relatively highly expressed, with the exception of HDAC8 which is expressed at low levels in most areas of the brain. HDAC1 and 2 exhibit a unique spatial distribution in developing chick and mouse brain suggesting divergent functions of the two enzymes. As development progresses forebrain expression of HDAC2 becomes relatively greater than hindbrain expression, a pattern not seen for HDAC1 (Murko et al., 2010). Both HDAC1 and 2 are expressed in neural progenitors, however in the three month old mouse brain HDAC1 is found predominantly in glia and HDAC2 expression is expressed at low levels in glia but highly and ubiquitously expressed in neurons (MacDonald and Roskams, 2008; Murko et al., 2010; Baltan et al., 2011). HDAC3 is exceptional amongst class I HDACs in that it is found in cytoplasm and at the plasma membrane, as well as the nucleus in neurons throughout the brain (Takami and Nakayama, 2000; Longworth and Laimins, 2006). Two members of the class II HDACs, HDAC4 and 5, are highly expressed in brain (6, 7, 9, and 10 are expressed at lower levels) with highest expression in basal ganglia, hippocampus, and cerebellum. HDAC6 is expressed in raphe serotonergic neurons in adult mice, with weaker staining observed in hippocampus and other brain regions (Fukada et al., 2012). Given the variable tissue- and cell compartment- specific expression patterns for specific HDACs in brain and other tissues it is expected that robust functional differences exist between HDACs.

Generally speaking, although not without important exceptions, class I HDACs are found in the nucleus, appear more important for neuronal survival and proliferation, are expressed in all tissues, and are capable of acetylating histones as well as non-histone substrates. Alternatively, class II HDACs are expressed in a more tissue-restricted manner, exhibit nucleocytoplasmic shuttling, and thus are capable of influencing the acetylation status of both histones and cytoplasmic, non-histone substrates (Figure 1). In contrast with most class I HDACs, class IIa show weak enzymatic activity by themselves, but through recruitment of class I HDACs and interaction with the SMRT/NCOR co-repressor complex carry out deacetylase function (Fischle et al., 2002). HDACs lack intrinsic DNA-binding activity and must be recruited to target genes via their direct association with transcriptional activators and repressors. In many cases the mechanisms by which individual HDACs are attracted to and bind their specific target regions of the genome or their cytoplasmic substrates are not well understood. Furthermore, the bulk of experimental effort, and interpretation of experimental results in studies of HDAC function, has focused on the effects of HDACs on chromatin structure. These interpretations may be misleading in the sense that HDAC interactions with non-histone proteins are often ignored, both conceptually and empirically. Acetylation of non-histones can increase or decrease the DNA binding affinity of target proteins, increase or decrease transcriptional activation and protein stability, as well as promote or disrupt protein-protein interactions (Glozak et al., 2005; Ocker, 2010).

HDACs are critical for central nervous system development

Constitutive KO has been a powerful tool for understanding the functional relevance of individual HDACs in development. Constitutive deletion of most HDACs is lethal, either during embryonic development or very early postnatal with deleterious effects on distinct tissues or cellular processes recognized as the proximal cause of death (Haberland et al., 2009a). HDAC1 null mice exhibit widespread growth defects and do not survive beyond embryonic day 10.5 (E10.5) (Lagger et al., 2002). Constitutive HDAC2 KO mice die shortly after birth due to severe cardiovascular defects (Montgomery et al., 2007; Haberland et al., 2009a). Brain-specific deletion of both HDAC1 and 2 is also lethal before postnatal day 7

(Montgomery et al., 2009). The HDAC3 null mouse dies before E9.5 due to defects in gastrulation (Bhaskara et al., 2008; Montgomery et al., 2008). Constitutive HDAC8 KO mice survive but present with abnormal cranio-facial development (Haberland et al., 2009b). Individual constitutive KO of class II HDACs is similarly devastating, however HDAC5 and HDAC6 KO mice are exceptions and are viable, although HDAC5 KOs have abnormal adult cardiovascular function (Haberland et al., 2009a). Both HDAC5 and 9 are highly enriched in muscle, brain, and heart, and mutation of HDAC9, as well as its splice variant MEF2-interacting factor/histone deacetylase related protein (MITR/HDRP) disrupts the appropriate developmental timing of myocyte differentiation (Mejat et al., 2005; Haberland et al., 2009a). A double knockout of HDAC5 and HDAC9 is lethal due to defective heart development (Chang et al., 2004). HDAC6 KO mice have no discernible phenotype beyond hyperacetylated tubulin (Hubbert et al., 2002; Zhang et al., 2003). Interestingly, HDAC6 is alone amongst all HDACs in that its substrates are exclusively cytoplasmic proteins. Whether this relates to its relatively limited effect on gross phenotype is not clear. HDAC4 null mice die around postnatal week one due to skeleton malformation promoted by excessive bone formation (Vega et al., 2004). The HDAC7 null mouse shows embryonic lethality due to blood vessel rupture and a loss of the integrity of endothelial cell junctions, which is consistent with the high expression of HDAC7 in endothelial cells (Chang et al., 2006). These observations highlight the fundamental necessity of HDAC function for normal development as well as the fact that there is some degree of functional redundancy between HDACs, at least during embryonic and early postnatal development.

Early observations using pan-HDAC inhibitor compounds demonstrated the importance of HDAC function for tissue-specific embryonic and neonatal development. Pan-HDAC inhibitors promote the differentiation of embryonic stem cells, and intriguingly, are able to potentiate somatic cell reprogramming to pluripotency (Lee et al., 2004; Siebzehnrbuhl et al., 2007; Brunmeir et al., 2009; Kretsovali et al., 2012). In brain, broad HDAC inhibition induces differentiation of embryonic cortical neuronal progenitor cells to neurons, and promotes survival of neural progenitors in the hippocampus (Hsieh et al., 2004; Siebzehnrbuhl et al., 2007; Shaked et al., 2008; Kim et al., 2009). Inhibition using the HDAC1- and HDAC3-preferring compound MS-275 increases differentiation of neural precursor cells from the subventricular zone, decreases the number of oligodendrocytes, and increases the number of neurons (Siebzehnrbuhl et al., 2007). Collectively, results using HDAC inhibitors demonstrate that blockade of normal HDAC function at certain time periods during embryonic development shift the fate of neuronal progenitors from glial to neuronal and strongly implicate deacetylase enzymes in cell-fate decisions in the developing brain.

Both class I and class II HDACs are involved in the proper development of specific tissues, however class I appear to be more relevant for brain development. Montgomery et al. (2009) used the Cre-lox system under the control of the GFAP promoter to delete HDAC1, HDAC2 or both HDAC1 and 2 concurrently (HDAC1/2 DKO) in the brain beginning at E13.5 (Montgomery et al., 2009). They discovered that HDAC1/2 DKO severely disrupted cortical, hippocampal, and cerebellar organization, and the mice did not survive beyond postnatal day 7. Neuronal precursors in HDAC1/2 DKO mice failed to differentiate into neurons, and instead underwent apoptosis. By contrast mice with single deletions of HDAC1 or 2 were viable and exhibited no detectable developmental phenotype, demonstrating that HDAC1 and 2 have redundant functions during neural development. HDAC1 and HDAC2 also play redundant roles in the regulation of oligodendrocyte development by repressing the Wnt/ β -catenin pathway, suggesting that glial as well as neuronal differentiation require HDACs (Ye et al., 2009). Previous work has also suggested redundancy of HDAC1 and 2 function for cardiac development (Montgomery et al., 2007). However, dissociable roles for HDAC1 and 2 on cell proliferation have been documented. For example, HDAC1 is

essential for embryonic stem cell proliferation by repressing the expression of cell cycle inhibitors (e.g., cyclin associated kinases), and although HDAC2 and 3 are upregulated in embryonic stem cells with HDAC1 KO, these class I HDACs are unable to compensate for the loss of HDAC1 function (Lagger et al., 2002).

As suggested by the distinct neurodevelopmental expression patterns of HDAC1 and 2 described above, it is likely that at some point in early postnatal development the functional properties of HDAC1 and 2 undergo a switch, after which the roles of these enzymes in the adult brain are established. Our laboratory recently reported that HDAC1 and 2 function as a developmental “switch” that governs excitatory synapse maturation early in development (Akhtar et al., 2009). While broad HDAC inhibition using Trichostatin A (TSA) decreases spontaneous miniature excitatory post-synaptic currents (mEPSCs) in hippocampal cell culture, knockdown of HDAC1 or HDAC2 has specific effects that depend on the maturational state of neurons (Nelson et al., 2006; Akhtar et al., 2009). HDAC1/2 knockdown during early synaptic development, day 5 *in vitro* (5 DIV) increased mEPSC and synaptic vesicle mobilization however single KOs yielded no phenotype. Surprisingly, single-cell recordings made from HDAC1 or 2 deficient neurons at 16 DIV, a time point in which synapses in cultured hippocampal neurons share all of the functional and structural characteristics of mature synapses formed *in vivo* (Kavalali et al., 1999) showed that knockdown of HDAC2 reduced mEPSC frequency, while HDAC1 knockdown was without effect, thus unmasking an important developmental interaction between HDAC1 and 2 and synaptic function. Furthermore, over-expression of HDAC2 produced the opposite phenotype- an increase in mEPSC frequency in mature neurons, similar to what is observed following knockdown of both HDAC1 and 2 in immature neurons. Miniature inhibitory post synaptic currents (mIPSCs) displayed normal frequency and amplitude in HDAC1/2, HDAC1, and HDAC2 knockdown cultures. These results indicate that HDAC 1 and 2 promote synaptic network stability during early time points in development by specifically repressing excitatory synapse maturation, and that HDAC1 and 2 have functionally redundant effects on synaptic transmission in immature neurons although they become functionally divergent as neurons mature.

We have recently found that HDAC4 or HDAC5 knockdown in hippocampal cell cultures had no impact on synaptic transmission in hippocampal neuronal cultures (Kim et al., 2012). Therefore, class I and class II HDAC knockdown have dissociable effects on basal synaptic function as assessed in hippocampal neurons. Any roles in synaptic transmission for the class I HDAC, HDAC3, or the class II HDACs, HDAC6 and HDAC9, which are all expressed in brain, have not yet been elucidated. These studies will be important in order to clearly establish whether there are dissociable roles for class I versus class II HDACs in regulating synapse function. HDAC9 has been implicated in neuronal maturation. As cortical cells mature, HDAC9 is increasingly transported from the nucleus to the cytoplasm. This relocation is functionally relevant as an HDAC9 mutation that causes nuclear retention leads to defective cellular maturation and inappropriate expression of genes that are important for dendritic growth. Movement to the cytoplasm is regulated by neural activity, and impacts expression of the immediate early gene *c-fos* (Sugo et al., 2010).

Class I and Class II HDACs have distinct roles in learning and synaptic function

Chromatin dynamics are sensitive to experimental manipulations that foster associative learning and long-term memory formation. Specific histone modifications, including acetylation and methylation, likely influence memory formation by modifying promoters of transcription factors, neurotransmitter receptors, cytoskeletal proteins, and other cellular substrates (Barrett and Wood, 2008; Morris et al., 2010). Several laboratories have

demonstrated that pharmacological inhibition of HDACs improves cognitive function in various learning and memory paradigms. Intrahippocampal or systemic treatment with HDAC inhibitors enhances the acquisition and extinction of conditioned fear responses (Yeh et al., 2004; Lattal et al., 2007; Bredy and Barad, 2008). Similarly, in a less aversive episodic memory task, the novel object recognition task, treatment with the HDAC inhibitor sodium butyrate in wild-type mice enhances memory, and does so in a long-term fashion as sodium butyrate-treated animals continued to show evidence of memory long after untreated mice (Stefanko et al., 2009).

Recent observations strongly suggest that class I HDACs (particularly HDAC2), but not class II HDACs, act as an endogenous restraint on memory formation. Mice with neuron-specific over-expression of HDAC2 using the *tau* locus perform poorly in the Morris water maze spatial task and in a fear conditioning paradigm, relative to wild-type mice (Guan et al., 2009). HDAC1 over-expression, however, had no effect on fear conditioning or water maze learning. In addition, a brain-wide embryonic KO of HDAC2 surprisingly and robustly improved cognitive function in mice. In spite of 82% sequence homology between HDAC1 and HDAC2 (Brunmeir et al., 2009), and a demonstrated redundancy of function in embryonic brain (Montgomery et al., 2009), the effects of manipulating the two enzymes are clearly dissociable as regards memory formation in adults.

The above results strongly suggest that HDAC2 is important for the memory-boosting effects of broad HDAC inhibition in the brain, however post-training delivery of the class I-specific inhibitor MS-275, which preferentially inhibits HDAC1 and HDAC3 activity, into the hippocampus enhanced learning in an episodic memory task (Hawk et al., 2011). Further evidence for an impact of specific manipulation of HDAC1 on memory was recently provided by Bahari-Javan et al., however they found an enhancement of fear extinction learning when HDAC1 was over-expressed specifically in the hippocampus, and a decelerated rate of extinction in mice with inhibition of HDAC1 in hippocampus (Bahari-Javan et al., 2012). These data contradict previous work with pan-HDAC inhibitors and suggest that HDAC1 in hippocampus is required for normal extinction of fear responses. Moreover, HDAC1 manipulation had no effect on episodic or working memory. Although these results are not necessarily consistent with previous reports regarding extinction learning, they suggest the exciting possibility that individual HDACs are involved in different forms (e.g., associative versus episodic memory) or stages of memory formation (e.g., acquisition, extinction).

Another class I HDAC, HDAC3, may similarly act as a restraint on learning and memory potential. A focal deletion of HDAC3 in the CA1 region of the hippocampus enhanced long-term episodic memory for an object that had been displaced from a previously learned location, however had no effect on memory for the object itself (i.e., novel object recognition) consistent with the known importance for hippocampal function in spatial memory (McQuown et al., 2011). It will be important to determine if brain-wide HDAC3 KO yields a similar phenotype as HDAC2 KO in other behavioral tasks. This would be surprising as HDAC3 differs from HDAC2 in terms of subcellular expression patterns and protein-protein interactions. A KO of HDAC3 in the hippocampus increases the expression of HDAC4, which may complicate the interpretation of the effect of HDAC3 inhibition on memory formation (McQuown et al., 2011). Our laboratory has recently reported that a postnatal forebrain KO of HDAC4, but not HDAC5, forebrain KO, impairs learning and memory in mice (Kim et al., 2012). Furthermore, a recent study found that a truncated form of HDAC4 led to learning and memory and neurotransmission deficits by repressing genes important for synaptic plasticity and synapse structure (Sando et al., 2012). Thus, the impact of HDAC3 inhibition could be due to a subsequent increase in HDAC4 expression. Taken

together the data establish that manipulation of class I or class II HDACs can have opposing effects on adult learning behavior and memory formation.

Consistent with the results from behavioral studies demonstrating improved memory with HDAC inhibition, and perhaps pointing to a mechanistic cellular underpinning for the behavioral phenotypes that emerge following HDAC inhibition, are findings showing that HDAC inhibitors enhance long-term potentiation (LTP) at hippocampal synapses and also in the amygdala, two brain regions that are essential for associative learning (Levenson et al., 2004; Yeh et al., 2004; Vecsey et al., 2007; Barrett and Wood, 2008). LTP, an activity-dependent increase in synaptic strength, is widely regarded as an electrophysiological correlate of learning (Bliss and Collingridge, 1993; Kim and Linden, 2007). Perfusion of hippocampal slices with HDAC inhibitors sodium butyrate and TSA enhance LTP at the Schaffer-CA1 synapse (Levenson et al., 2004). Furthermore, using weak LTP-induction parameters (i.e., a single train of 100 Hz stimulation), HDAC inhibitor treatment transforms early-phase LTP, which is protein synthesis-independent, into sustained and robust late-phase LTP, which requires protein synthesis (Vecsey et al., 2007). As with performance in learning in memory tasks, HDAC2 appears to be the most relevant enzyme as over-expression of HDAC2 impairs, while KO enhances, LTP in the hippocampal CA1 subregion (Guan et al., 2009). HDAC1 over-expression, on the other hand, had no impact on LTP, and our laboratory has recently found that forebrain-specific KO of HDAC1 also does not affect LTP in the CA1 (Morris et al., 2012). Interestingly, a forebrain, neuron-specific postnatal KO of HDAC4 produces deficits in hippocampal CA1 LTP, while HDAC5 is without effect (Kim et al., 2012). This is consistent with the impact of HDAC4 or 5 KO on learning and memory performance in mice discussed above, and demonstrates a double dissociation between the effects of manipulating class I as opposed to class II HDACs on synaptic plasticity as well as learning.

The class I HDACs, HDAC2 and HDAC3 have been dubbed an “endogenous negative regulator of memory formation” or “a molecular brake pad” for long-term memory formation (Guan et al., 2009; McQuown and Wood, 2011), respectively. There are few precedent studies reporting global learning and memory enhancements following deletion of a gene and the consistent observation that HDAC inhibition or KO benefits cognitive function as well as enhances plastic events at synapses that likely support learning and memory is an exciting development in the study of the molecular neurobiology of memory. From the evolutionary biologist's perspective, there must be a physiological benefit to the presence of enzymes that restrain the learning potential of an organism. HDAC1 or 2 are clearly necessary during embryonic brain development but appear to be superfluous in adult brain given that mice that lack either enzyme are viable, and indeed thrive. One general function of the class I HDACs may be to maintain a “homeostatic” level of gene activation, potentially in a developmental-stage specific fashion, thus acting as a check on the deleterious effects of unfettered transcription at critical periods during development or possibly in adult brain as well. Unchecked transcription of HDACs gene targets could manifest as enhanced plasticity, as suggested by recent data (Akhtar et al., 2009; Guan et al., 2009). Although it seems unlikely, one possibility is that continued expression in adult brain of some members of the class I HDACs is essentially vestigial. In other words continued expression in adult brain, from an evolutionary fitness perspective, is inconsequential. It is perhaps laboratory sleight of hand to uncover a memory-boosting effect of inhibiting molecules in the adult brain that are essential for normal embryonic and neonatal development. More likely is that we are yet to discover the necessity of HDAC1 and HDAC2 for adult brain function. One recent study suggests that HDAC2 in particular is required for adult, but not embryonic, neurogenesis in the hippocampal dentate gyrus, although the functional relevance of hippocampal neurogenesis itself is not completely understood (Jawerka et al., 2010).

What are the upstream signaling pathways and downstream targets of Class I and Class II HDACs?

Clear distinctions can be drawn between class I and class II HDACs regarding the upstream signaling pathways that influence their activity, as well as the protein complexes in which class I or class II HDACs associate. This may be in part due to the differences in basal subcellular localization of the two classes of HDACs. Furthermore, class I and class II HDACs exhibit differential affinity and activity at acetylated substrates. While class I HDACs possess potent deacetylase activity (HDAC3 is an exception), class II HDACs are enzymatically inactive alone. Deacetylase activity of class IIa HDACs is likely due to association with other class I HDACs, particularly the SMRT/NCOR/HDAC3 complex, as class IIa HDACs preferentially interact with HDAC3 over HDAC1 or 2 (Huang et al., 2000; Fischle et al., 2002; Lahm et al., 2007).

Calcium-calmodulin kinases (CaMK) are bona fide upstream mediators of class IIa HDAC activity. CAMKI and II phosphorylate and induce nuclear export of HDAC4 and 5, although a kinase other than CaMK may be responsible for phosphorylating HDAC4 (McKinsey et al., 2000; Zhao et al., 2001). CaMKI and CaMK IV are capable of phosphorylating all four class II HDACs (Zhao et al., 2001; Parra and Verdin, 2010). Protein kinase D1 may also act as an important class II kinase, however thus far this has only been established in vascular smooth muscle cells, not yet in neurons (Xu et al., 2007). Phosphorylation of the N-terminus and association with 14-3-3 proteins leads to a conformational change that exposes a nuclear export signal, therefore the net balance between the phosphorylated and unphosphorylated state of class IIa HDACs determines subcellular localization, and downstream effects on transcription of class IIa HDAC targets (Verdin et al., 2003; Yang and Gregoire, 2005). The cyclic-AMP (cAMP) pathway may also be an important signaling cascade for the regulation of class II HDACs, as a recent study by Taniguchi et al. suggests (Taniguchi et al., 2012). cAMP induces transport of HDAC5 to the nucleus which is associated with dephosphorylation of HDAC5 at serine 279 (s279), a modification that is mediated by increased protein phosphatase 2A activity and important for behavioral responses to acute cocaine treatment.

Little is known about the upstream signaling cascades that affect class I HDAC activity. Similar to class II HDACs, phosphorylation enhances class I enzyme activity and promotes co-repressor complex interactions (Cai et al., 2001; Pflum et al., 2001; Smillie et al., 2004; Sun et al., 2007). HDAC1 and HDAC2 form several co-repressor complexes, which are distinct from those formed by HDAC3. Co-repressor complexes formed with Sin3, MECP2, NuRD, CoREST, and NODE are responsible for delivering HDACs 1 and 2 to their intranuclear targets, whereas HDAC3 associates with the SMRT/NCOR complex (Jones et al., 1998; Guenther et al., 2001; You et al., 2001; Brunmeir et al., 2009). Nitric oxide (NO) is a recently discovered upstream regulator of HDAC2 activity as it dissociates HDAC2 from CREB-regulated gene promoters, which leads to increased transcription of c-fos, VGF, nNos, and erg-1 (Nott et al., 2008; Nott and Riccio, 2009). NO-dependent modulation of HDAC2 is needed for dendritic growth *in vitro* (Nott et al., 2008; Nott and Riccio, 2009).

Less than 10% of all genes are regulated by HDACs, and HDAC inhibition leads to gene repression as well as activation depending on the specific gene target (Glaser et al., 2003; Kato et al., 2004; Nusinzon and Horvath, 2005; Peart et al., 2005). The ratio of up to downregulated genes appears to be approximately equal, strongly suggesting that HDACs are not global transcriptional repressors (Nusinzon and Horvath, 2005; Minucci and Pelicci, 2006). Upregulation of specific genes following HDAC inhibition, however, does not rule out secondary effects that may result in transcriptional repression (e.g. disinhibition of a repressor).

The N-terminal domain of class II HDACs interacts with tissue-specific transcription factors and co-repressors. This has been convincingly demonstrated for the interaction between class II HDACs and myocyte enhancer factor-2 (MEF2), a transcription factor that is critical for neuronal survival and apoptosis in response to extracellular signals, along with well known roles in regulating genes critical for muscle differentiation (Chawla et al., 2003; Shalizi and Bonni, 2005). A MEF2 binding site on the N-terminal domain of class II HDACs promotes their nuclear localization until the necessary cellular signal (e.g., CaMKs) releases class II HDACs from MEF2 (Lu et al., 2000; Gregoire and Yang, 2005; Shalizi and Bonni, 2005). Phosphorylation of conserved class IIa HDAC serine and threonine residues in the N-terminus interfere with the MEF2-HDAC association and unmask HDAC binding sites for 14-3-3 proteins which lead to nuclear export. This interaction between MEF2 and class IIa HDACs is established in developing muscle, and it appears to be relevant for brain function as well (Chawla et al., 2003; Shalizi and Bonni, 2005; Parra and Verdin, 2010). Nuclear import of HDAC5 in neurons results in reduced MEF2-dependent transcription in neurons, and subsequently an increase in apoptosis of depolarized neurons (Linseman et al., 2003). Along with binding to, and interfering with the transcriptional activation domain of MEF2, all four class IIa HDAC isoforms potentiate the sumoylation of MEF2 in HEK293, and HeLa cells, a modification that represses transcription (Gregoire and Yang, 2005).

There are different thresholds of activity for shuttling HDAC4 or 5 out of the nucleus. Spontaneous activity is sufficient to drive HDAC4 out of the nucleus, however, HDAC5 remains and requires calcium influx through L-type calcium channels or the NMDA receptor (Chawla et al., 2003). In myocytes calcium influx and electrical activity increase the expression of the HDAC9 splice variant MITR/HDRP, which lack a deacetylase domain (Mejat et al., 2005). As with other class IIa HDACs, MITR/HDRP is thought to repress the expression of myogenic genes by inhibiting MEF2-dependent transcriptional events (Mejat et al., 2005). An exciting possibility is that the level of recent activity in a synaptic circuit can differentially “tag” distinct HDAC isoforms which subsequently relieve repression of specific genes regulated by, for example, HDAC5. Elucidating the activity-dependent molecular pathways that trigger chromatin modifying enzymes will substantially advance our understanding of the contribution of HDAC isoforms to brain development, synaptic function, and complex behavior.

Class I HDACs regulate the expression of subsets of genes involved in mediating plastic cellular events (e.g., synapse development, activity-dependent changes in synaptic strength, cardiovascular hypertrophy) during development and adulthood. HDAC2 binds to several synaptic plasticity-related genes- *BDNF*, *Erg1*, *Creb1*, *CBP*, *nrn1*, NMDA receptor subunit genes, and *fos*, as well as genes important for synapse formation (Guan et al., 2009). The fact that HDAC2 binds at CREB and CBP gene promoters suggests that HDAC2 may communicate with a well-established CREB-CBP pathway to regulate activity-dependent gene expression and memory formation (Guan et al., 2009). Interestingly, CREB is required for the enhancement of LTP in hippocampal slices following non-specific HDAC inhibition (Vecsey et al., 2007). Additionally, the coREST complex, an important regulator of neuron-specific genes, for example genes encoding ion channels, synaptic vesicle proteins, and neurotransmitter receptors, preferentially associates with HDAC2 relative to HDAC1 (Schoenherr and Anderson, 1995). Following treatment with the HDAC inhibitor MS-275, which preferentially inhibits HDAC1 and to a lesser extent HDAC3, Covington III et al. (2009) found upregulation of various genes involved in dendritic remodeling (e.g., *SLIT*, *TGFa*, *JNK*, and *Rho*) as well as various transcription factors that have been implicated in memory formation (e.g., CREB, REST, coREST, STAT, *nrn1*).

Candidate genes for mediating learning and memory enhancements that result from HDAC inhibition include *Nr4a1* and *Nr4a2* which code for the immediate early transcription factors

Nurr77 and Nurr1, respectively. These genes have previously been implicated in memory, affective behavior, and addiction and increased expression of Nr4a1 and Nr4a2 mRNA is observed two hours after training in fear conditioning (von Hertzen and Giese, 2005; Colon-Cesario et al., 2006; Rojas et al., 2007; Vecsey et al., 2007). Treatment with TSA immediately after contextual fear conditioning increased the acetylation of H3 and H4 at the promoters of *Nr4a1* and *Nr4a2* and enhanced the expression of Nr4a1 and Nr4a2 mRNA relative to wild type and CREB mutant mice, an effect not observed following TSA treatment without prior fear conditioning training (Vecsey et al., 2007). Additionally, TSA treatment leads to recruitment of RNA polymerase and transcription factor IIB to the *Nr4a1* promoter *in vitro* (Fass et al., 2003). The memory enhancing effects of HDAC3 deletion require the HAT CREB binding protein (CBP), and subsequent expression of Nr4a2, a CREB dependent gene, as CBP mutants do not exhibit enhanced LTP following HDAC inhibition and siRNA for Nr4a2 prevents the improved memory effect (McQuown and Wood, 2011). It is not known if HDAC1 or 2 manipulation impacts Nr4a1 or Nr4a2 expression, and this could be a molecular pathway that specifically mediates HDAC3 involvement in certain forms of learning.

A better understanding of the protein complexes in which HDACs associate, along with the cross talk between epigenetic pathways, will be critical for mechanistically determining how HDACs influence gene expression, and ultimately synaptic function and behavior. For example HDAC1 and 2 are known to form part of a multiprotein corepressor complex with methyl-CpG binding protein 2 (MeCP2), a protein that binds to methylated CpG dinucleotides (Jones et al., 1998; Dannenberg et al., 2005; Monteggia and Kavalali, 2009). Although the functional relevance of this interaction is not yet known, interruptions to the normal arrangement of this complex are likely to be clinically relevant as the neurodevelopmental disorder Rett Syndrome is caused by mutations in *MECP2* (Amir et al., 1999). The MeCP2-HDAC1/2 complex may be important for stabilizing heterochromatin and TSA treatment can relieve transcriptional repression by MeCP2 (Jones et al., 1998; Nan et al., 1998). HDACs also interact with the DNA methyltransferase (DNMT) enzymes responsible for adding methyl groups to specific cytosine nucleotides of DNA, which is an important mechanism for gene silencing. DNMT1, necessary for maintenance methylation of DNA, recruits HDAC1 and 2 as well as the histone methyltransferase Suv39h1 (Espada et al., 2004). HDAC1 and 2 have also been shown to interact with DNMT3a and DNMT3b, both *de novo* DNMTs (Fuks et al., 2000; Fuks et al., 2001; Bai et al., 2005). An example of a functional outcome of DNMT-HDAC interaction has been demonstrated for neurite outgrowth. DNMT3b activity is important for neurite outgrowth, and this is mediated by DNMT3b interaction with HDAC2 (Bai et al., 2005).

Broad potential clinical applications for isoform-specific HDAC inhibition in the brain

Beneficial effects of HDAC inhibition have been demonstrated using *in vitro* as well as *in vivo* models of neurodegenerative disease. Importantly, broad HDAC inhibition is neuroprotective when cells are challenged by diverse insults including polyglutamine toxicity, cuprizone, glutamate, and ischemia (Ferrante et al., 2003; Hockly et al., 2003; Jeong et al., 2003; Ryu et al., 2003; Faraco et al., 2006; Leng and Chuang, 2006; Riveccio et al., 2009). However, contradictory evidence is available demonstrating that HDAC inhibition can induce apoptosis in cerebellar granule neuron cultures and cortical neurons undergo apoptosis following chronic HDAC inhibition (Salminen et al., 1998; Boutillier et al., 2003; Langley et al., 2008). One potential reason for the conflicting results with pan-HDAC inhibitors may be that distinct HDAC isoforms are pro-apoptotic or neuroprotective (Table 1). Separate studies have documented the pro-apoptotic potential of HDAC1, HDAC3, and HDAC5 in contrast to neuroprotection afforded by HDAC4, HDAC6,

HDAC7, and the alternatively spliced form of HDAC9, HDRP (Linseman et al., 2003; Bolger and Yao, 2005; Kim et al., 2010; Bardai and D'Mello, 2011). Thus, the impact of HDACs on neuronal viability is isoform-specific, and not related to class (i.e. class I vs. class II).

Overexpression of the class I HDAC, HDAC3 is highly toxic to neurons, likely through a cooperative interaction with another class I HDAC, HDAC1. Indeed inhibition of HDAC1 ameliorates cell death induced by HDAC3, and vice versa. While HDAC1 knockdown has previously been shown to enhance apoptosis in cultured cortical neurons, (Kim et al., 2008) recent data suggest a mechanism whereby HDAC1 behaves as a “molecular switch” between neuroprotection and neuronal apoptosis. The neurotoxic properties of both HDAC1 and HDAC3 are prevented by inhibition of the GSK3 β , a kinase implicated in a number of neurodegenerative diseases, or by activation of the IGF-1-Akt pathway, which inhibits GSK3 β , a likely upstream effector of HDAC3. On the other hand HDAC1 is neuroprotective as a result of interactions with HDRP, a truncated form of HDAC9 previously established as neuroprotective (Morrison et al., 2006; Bardai et al., 2012). HDRP interferes with the death-promoting HDAC1-HDAC3 interaction is capable of recruiting HDAC1 to the c-Jun promoter, deacetylation of which favors neuronal viability. Thus, an individual HDAC isoform can be both neurotoxic and neuroprotective depending upon cellular context and protein-protein interactions with distinct binding partners. HDAC1 appears to be a central player in establishing cell viability by partnering with HDAC3 to promote apoptosis, or with HDRP convey neuroprotection. Interestingly, and an exception to the strict nuclear localization of HDAC1, in humans with multiple sclerosis or in demyelination models in animals, HDAC1 is observed in axons. The axonal localization appears to be mediated by calcium and axonal HDAC1 is capable of binding to alpha-tubulin and the motor protein kinesin to inhibit mitochondrial transport, an effect that is independent of HDAC6 activity. Inhibition of HDAC1 following glutamate toxicity or TNF-alpha treatment reverses the mitochondrial transport deficit (Kim et al., 2010).

The class II HDAC, HDAC4 has also been shown to promote neuroprotection and headway is being made in determining the molecular pathways responsible (Majdzadeh et al., 2008; Chen and Cepko, 2009; Hageman et al., 2010). In cultured cerebellar granule neurons, overexpression of HDAC4 protects from low-potassium induced apoptosis, and this may require inhibition of cyclin-dependent kinase-1 as well as prevention of maladaptive cell-cycle progression (Majdzadeh et al., 2008). HDAC4 may be necessary for the survival of retinal neurons, as *in vivo* siRNA against HDAC4 in the developing mouse retina promoted apoptosis, and HDAC4 overexpression led to a significantly greater number of bipolar cells (Chen and Cepko, 2009). Moreover, in a mouse model of retinal degeneration, HDAC4 overexpression enhanced photoreceptor survival, an effect blocked by expression of a dominant negative form of the HIF1 α , a protein important for oxygen homeostasis (Chen and Cepko, 2009). Data implicating other class IIa HDACs in apoptotic pathways or neuroprotection are sparse, however in cerebellar granule neurons overexpression of HDAC5 led to enhanced cell death in neurons depolarized with potassium chloride (Linseman et al., 2003). By contrast HDAC7 appears neuroprotective for cerebellar granule neurons challenged by low potassium culture media by repressing c-Jun expression (Ma and D'Mello, 2011).

The class IIb HDAC, HDAC6 is an attractive target for cellular protection in the context of neurodegenerative disease (Du and Jiao, 2011). HDAC6, which is critical for the clearance of misfolded cytoplasmic proteins, is increased in cortical neurons in Alzheimer's patients, which is consistent with reduced acetylation of tubulin in cortical neurons in these patients (Hempfen and Brion, 1996; Ding et al., 2008). The affinity of HDAC6 for cytoskeletal elements, and its regulation of cytoskeletal dynamics, may play a role in impaired trafficking

in neurological disorders. In an animal model of Huntington's disease inhibition of HDAC6 reversed the observed transport deficits, in part by increasing vesicular trafficking of BDNF, a kinesin-1 cargo protein (Dompierre et al., 2007). Specific HDAC6 inhibition can also enhance mitochondrial trafficking in cultured hippocampal neurons (Chen et al., 2010). HDAC6 has been suggested to be neuroprotective as well (Pandey et al., 2007b; Du and Jiao, 2011). In a *Drosophila melanogaster* model of spinobulbar muscular atrophy, and in *Drosophila* with mutations that impair proteasome function, HDAC6 overexpression protected against neurodegeneration (Pandey et al., 2007a; Pandey et al., 2007b). Work by these authors suggests that HDAC6 is critical for autophagic degradation of noxious proteins (Pandey et al., 2007a).

As discussed, in normal mice inhibition of class I HDAC function benefits learning and memory, and several exciting recent studies have translated this phenomenon to rodent models of neurodegenerative disease (Fischer et al., 2007; Ricobaraza et al., 2009; Graff et al., 2012). Beneficial effects of central or peripheral treatments with HDAC inhibitors on retention of fear associations have been observed in mouse models of Alzheimer's disease (Fischer et al., 2007). Furthermore, HDAC inhibition can improve learning after traumatic brain injury in mice (Dash et al., 2009). Given the putative neuroprotective effects of HDAC inhibition it is not clear if benefits are a result of enhanced cellular survival or increasing the plastic potential of existing cells. A recent study by Graff et al. (2012) using an Alzheimer's model suggests the latter. Using a p25 over-expressing mouse to model neurodegeneration the authors show that HDAC2 is increased in degenerating neurons, and the increase is responsible for suppressing genes related to synaptic plasticity and memory formation. Short-hairpin mediated knockdown of HDAC2 reinstated gene expression and improved learning, without improving neuronal survival (Graff et al., 2012).

Class II HDACs are important for adaptive behavioral responses to chronic stress, a known precipitating factor in many cases of major depressive disorder (Kendler et al., 1999). Chronic social defeat stress, a putative mouse model of depressive-like behavior, down-regulates HDAC5 mRNA in the nucleus accumbens, while chronic treatment with the antidepressant imipramine increases HDAC5 expression in animals that have been subjected to the social defeat paradigm (Renthall et al., 2007). By contrast, over-expression of HDAC5 antagonizes imipramine's antidepressant effects (Tsankova et al., 2006). Chronic injection of HDAC inhibitors into the nucleus accumbens prevented the social avoidance phenotype typically seen in mice that have been socially defeated (Covington et al., 2009). Importantly, intra-accumbens HDAC inhibition had antidepressant action in the forced swim and sucrose preference tests, establishing that HDAC inhibition rescues several depressive-like symptoms induced by chronic stress (e.g., anhedonia, behavioral despair, reduced social interaction). Using gene microarrays the authors also demonstrated that chronic MS-275 infusion into nucleus accumbens reversed the pattern of gene expression observed following social defeat in a fashion similar to chronic treatment with fluoxetine, a somewhat perplexing observation considering that MS-275 does not inhibit class IIa HDACs, and may implicate class I HDACs in the behavioral responses. Recently it was reported that HDAC5 is decreased in peripheral white blood cells of depressed patients, along with HDAC2 (Hobara et al., 2010). It is not yet clear how this observation relates to the onset or maintenance of the depressive state. Initial behavioral studies in HDAC6 mutant mice suggest that this class IIb HDAC may also be important for mood regulation. Constitutive deletion of HDAC6 induces hyperactivity in a novel environment, but not in the home cage. Furthermore deletion of HDAC6 reduces anxiety, and has antidepressant effects (Fukada et al., 2012).

The transition from recreational drug use to addiction may involve enduring changes in gene expression patterns in brain regions critical for processing rewarding stimuli (Kumar et al.,

2005; Wallace et al., 2008). Consistent with this hypothesis is the observation that drug-induced alterations in chromatin structure occur in the nucleus accumbens, a forebrain region that is critical for reward processing, and these alterations are dissociable depending on the temporal nature of drug exposure. Similar to what is observed following stressors, rats given acute treatments with cocaine display increased acetylation of H4 in the promoter regions of the immediate early genes *fosB* and *cfos*, whereas chronic exposure preferentially induces hyperacetylation of H3 around the *fosB*, cyclin-dependent kinase 5 (*CDK5*), and *BDNF* gene promoters (Brami-Cherrier et al., 2005; Kumar et al., 2005; Levine et al., 2005). Interestingly, over-expression of HDAC4 in nucleus accumbens reverses the acetylation patterns as well as the behavioral adaptations induced by chronic cocaine administration (Kumar et al., 2005). Also, treatment with the non-specific HDAC inhibitors TSA and sodium butyrate enhanced the locomotor activating effects of cocaine, as well as preference for a cocaine-paired environment.

Another class II HDAC, HDAC5, has also been implicated in mediating neural alterations that result from chronic exposure to drugs of abuse. High levels of HDAC5 are expressed in the nucleus accumbens, and Nestler and coworkers have shown that genetic manipulations of HDAC5 in this brain region profoundly influence an animal's sensitivity to, and interactions with, cocaine (Renthal et al., 2007). HDAC5 phosphorylation is increased in mice with a history of cocaine administration as opposed to acute experience. Furthermore, over-expression of HDAC5 in the accumbens diminished conditioned place preference for a cocaine-paired environment, an effect that could be blocked by TSA treatment. HDAC5 KO mice exhibited the opposite phenotype, an enhanced sensitivity to cocaine, provided the animals were chronically given cocaine. HDAC5 KO mice displayed normal responses to acute cocaine, suggesting that HDAC5 may be specifically important for the behavioral transition from acute to chronic drug use. Through a cAMP-dependent mechanism, acute cocaine, unlike chronic experience, dephosphorylates HDAC5, which lessens the rewarding effects of cocaine exposure (Taniguchi et al., 2012). The dephosphorylation leads to nuclear accumulation of HDAC5 which colocalizes with MEF2, an effect that is blocked by the inhibition of protein phosphatase 2A.

A consistent finding is the effect of HDAC inhibition on the learning of fear associations. Fear conditioning creates *de novo* associative memories through the pairing of an initially innocuous stimulus (a “conditioned stimulus”) such as an auditory tone or a novel environment with a salient, biologically relevant stimulus (“unconditioned stimulus”), for example, a mild footshock. Posttraumatic stress disorder, anxiety disorders, and the development of phobias are characterized by an inability to extinguish fear responses. HDAC inhibition is a potentially relevant avenue for treating these disorders, as they accelerate the extinction of fear responses in animals (Lattal et al., 2007; Bredy and Barad, 2008). However, acute knockdown of HDAC1 in hippocampus has also been reported to impair the rate of fear extinction (Bahari-Javan et al., 2012), hence it remains unclear whether a blockade or potentiation of class I HDAC activity would be most appropriate in targeting disorders marked by maladaptive extinction processes.

Concluding Remarks

As recently suggested by Haberland et al. (2009) “one of the most perplexing aspects of HDAC biology is that pharmacological inhibition of HDAC activity provides a therapeutic benefit in such a wide variety of disease states”. The overwhelming benefits observed following HDAC inhibition in a number of diverse contexts begs the question - should everyone be taking HDAC inhibitors? In short - no, or at least not yet. The HDAC inhibitors currently available are not well tolerated when administered chronically, and patients present with side effects including fatigue, diarrhea, and cardiac side effects (Marsoni et al.,

2008), however HDAC inhibitors present with lower toxicity and fewer side effects than most other cancer drugs (Minucci and Pelicci, 2006). Deleterious cellular effects of HDAC inhibition have been noted, including apoptosis, dysfunctional activity of chaperone proteins (e.g., HSP90), interference with tubulin assembly, induction of reactive oxygen species, and inhibition of angiogenesis and adult neurogenesis (Glozak et al., 2005; Marsoni et al., 2008; Jawerka et al., 2010). In addition all HDAC inhibitors promote cell cycle arrest to some degree (an exception is HDAC6-selective inhibitors), however normal cells are more resistant than tumor cells to the apoptotic effects of HDAC inhibition, for reasons still unknown (Minucci and Pelicci, 2006; Ocker, 2010).

Isoform-specific HDAC inhibitors have proven to be a challenge to develop. The majority of HDAC inhibitors used currently inhibit multiple isoforms among class I, II, and IV HDACs. HDAC inhibitors are typically among the following structural classes: cyclic peptides, depsipeptides, hydroxymates, and short-chain fatty acids. Several HDAC inhibitors have been developed from naturally occurring sources (e.g., luteolin from celery). A handful of inhibitors have been developed that exhibit class I selectivity with nanomolar potency, although they target several isoforms within the class (e.g., MS-275 targets HDAC1, 2, and 3). Similarly, HDAC6-selective inhibitors have been developed (e.g., tubacin), however in many cases these compounds exhibit a high degree of lipophilicity and are difficult to synthesize, rendering them more useful as tools for research (Dallavalle et al., 2012). All HDAC inhibitors function by chelating the zinc ion at the HDAC active site, however, a high degree of structural homology at the enzyme active site of the HDACs presents a hurdle that must be overcome to achieve the significant clinical promise in manipulating individual HDAC isoforms (Balasubramanian et al., 2009; Dallavalle et al., 2012). Most inhibitor compounds consist of 3 domains, the zinc binding domain, a cap group which interacts with amino acids at the *N*-acetylated lysine binding channel, and a linker region that connects the zinc binding and cap domains. Given the homology between HDACs in the zinc-binding active site, it has been suggested that modifying the cap group may be the most promising strategy for ultimately developing isoform specific compounds (Suzuki, 2009; Dallavalle et al., 2012).

This review has highlighted how manipulation of individual HDACs can have diverse phenotypic outcomes, most notably for cognitive function and neurodegeneration. Distinct and opposing effects of class I versus class II HDAC inhibition argue against a sledgehammer pharmacological approach, although the benefits of broad HDAC inhibition undoubtedly outweigh the costs in some circumstances (e.g., cancer). Study of HDAC biology is still in relative infancy, however in the future domains in health-care and psychiatry will benefit from the development of better pharmacological tools to manipulate the activity of individual HDACs in the brain.

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	Protein domains	Amino acids	Subcellular localization	Reference(s)
Class I				
HDAC1		482	Nucleus	Lagger et al. , 2002
HDAC2		488	Nucleus	Haberland et al. , 2009
HDAC3		428	Nucleus/ Cytoplasm	Yang et al. , 1997 Longworth & Laimins, 2001
HDAC8		377	Nucleus	Hu et al. , 2000
Class IIa				
HDAC4		1084	Nucleus/ Cytoplasm	Grozinger et al. , 1999
HDAC5		1122	Nucleus/ Cytoplasm	Grozinger et al. , 1999
HDAC7		912	Nucleus/ Cytoplasm	Kao et al. , 2000
HDAC9		1011	Nucleus/ Cytoplasm	Zhou et al. , 2001
Class IIb				
HDAC6*		1215	Cytoplasm	Grozinger et al. , 1999
HDAC10		669	Cytoplasm	Kao et al. , 2002
Class IV				
HDAC11		347	Nucleus	Gao et al. , 2002

Figure 1.

Comparison of class I, II, and IV HDAC protein size and subcellular localization. All HDACs contain a highly conserved catalytic domain (represented by black rectangle). Class IIa enzymes are characterized by an N-terminal extension (solid black line) of approximately 600 residues not found in Class I HDACs. Open circles and rectangles represent serine phosphorylation sites. ZnF – zinc finger. *HDAC6 contains an identical duplication of 2 catalytic domains.

Table 1
Constitutive And Conditional Brain-Specific Knockout (Ko) Of Individual Histone Deacetylase (Hdac) Isoforms

	Constitutive KO cause of death	Conditional brain KO-behavioral phenotype	Effect on neuronal viability	Reference(s)
<i>Class I</i>				
HDAC1 ^(N)	General growth defects	Mild/no phenotype	Toxic/protective	Lagger et al., 2002; Morris et al., 2012; Kim et al., 2008; Bardai et al., 2012
HDAC2 ^(N)	Cardiovascular defects	Enhanced memory, LTP	NR [*]	Montgomery et al., 2007; Guan et al., 2009
HDAC3 ^(N/C)	Gastrulation defects	Enhanced episodic memory	Toxic	Montgomery et al., 2008; Bardai et al., 2011; Bardai et al., 2012; McQuown et al., 2011
HDAC8 ^(N)	Viable, cranial defects	NR	NR	Haberland et al., 2009
<i>Class IIa</i>				
HDAC4 ^(N/C)	Skeleton malformation	Impaired learning, LTP	Protective	Vega et al., 2004; Akhtar et al., 2012; Sando et al., 2012; Majdzadeh et al., 2008; Chen & Cepko, 2009
HDAC5 ^(N/C)	Viable	Enhanced cocaine sensitivity; anhedonia	Toxic	Haberland et al., 2009; Renthal et al., Linseman et al., 2003
HDAC7 ^(N/C)	Endothelial cell defects	NR	Protective	Chang et al., 2006; Ma et al., 2011
HDAC9 ^(N/C)	Viable	NR	NR [^]	Haberland et al., 2009; Morrison et al., 2006
<i>Class IIb</i>				
HDAC6 ^(C)	Viable	Hyperactive, anxiolytic	Toxic/protective	Hubbert et al., 2002; Fukada et al., 2012; Pandey et al., 2007; Dompierre et al., 2007; Riviello et al., 2009
HDAC10 ^(N/C)	NR	NR	NR	Kao et al., 2002
<i>Class IV</i>				
HDAC11 ^(N/C)	NR	NR	NR	Gao et al., 2002

In parentheses – subcellular localization; N = nucleus, C = cytoplasm; NR = not reported. LTP = long term potentiation,

^{*} = Brain-specific double KO of HDAC1 and 2 during embryonic development is lethal ~ one week postnatal and leads to apoptosis/poorly developed brain (Montgomery et al., 2009)

[^] = truncated form of HDAC9, HDRP has shown neuroprotective properties (Morrison et al., 2006).