

Alterations in Expression and Function of Phosphodiesterases in Huntington's Disease

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1. Introduction

Cyclic AMP (cAMP, cyclic 3', 5'-adenosine monophosphate) was first identified as a signalling molecule in 1958 (Rall & Sutherland, 1958), however it was not until 1962 that the enzyme responsible for hydrolysis of cAMP was identified and named phosphodiesterase (PDE; Butcher & Sutherland, 1962). Shortly afterwards, cyclic GMP (cGMP, cyclic 3', 5'-guanosine monophosphate) was identified as another important second messenger that was hydrolyzed by PDE (Ashman et al., 1963). PDEs inactivate cAMP or cGMP by hydrolyzing the 3' cyclic phosphate bond of the cyclic nucleotide in question (Bender & Beavo 2006). Through molecular cloning and sequencing, it is now known that mammalian PDEs are encoded by 21 distinct genes (Bender & Beavo, 2006). These 21 genes encode protein isoforms of which variants can exist through the use of multiple transcription start sites and alternative mRNA splicing (Bender & Beavo, 2006). The 21 identified isoforms have been grouped into 11 families based on similarities in amino acid sequence, structure and function.

1.1 Phosphodiesterase are key regulators of cyclic nucleotide signalling cascades

cAMP is formed from ATP by adenylyl cyclase (Fig. 1; Rall & Sutherland, 1958). Adenylyl cyclase is a membrane-bound enzyme that can be activated by the G α subunit, as well as the $\beta\gamma$ subunit of the G-protein family, by calcium, and by protein kinase C (Tang & Ziboh, 1991, Iyengar, 1993). Once formed, cAMP activates protein kinase A. Protein kinase A is a tetrameric protein composed of two catalytic subunits and two regulatory subunits (Johnson & Jameson, 2000; Johnson et al., 2001). Two cAMP molecules bind to each regulatory subunit, which results in the release of the active catalytic subunits. Protein kinase A is known to phosphorylate proteins involved in cell signalling, apoptosis, ion channel regulation, osmotic homeostasis, and protein trafficking (reviewed by Shabb, 2001). Protein kinase A can also enter the nucleus where it is known to phosphorylate cAMP-response element binding (CREB) protein (Delghandi et al., 2005). Phosphorylated CREB stimulates transcription of genes related to cell signalling and proliferation such as brain-derived

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neurotrophic factor (Delghandi et al., 2005). In addition to signalling through protein kinase A, cAMP can directly alter ion channel conductance (reviewed by Wang et al., 2007).

PDEs also regulate cGMP signalling cascades. The cGMP pathway is activated by nitric oxide, which is produced by nitric oxide synthase (Francis et al. 2010). Nitrous oxide activates guanylyl cyclase, which can be membrane-bound or cytosolic. Guanyl cyclase converts GTP to cGMP, which can go on to activate protein kinase G (Francis et al., 2010). The cGMP pathway, regulates smooth muscle relaxation (Walter, 1984), synaptic plasticity (Kleppisch & Feil, 2009), and regulation of platelet aggregation (Walter, 1984).

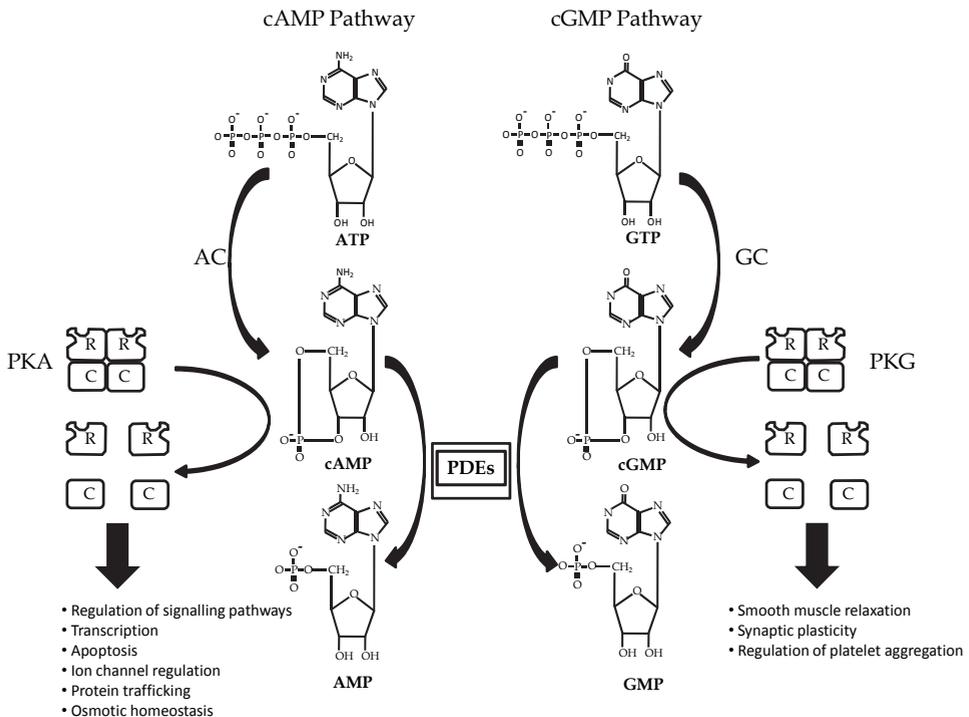


Fig. 1. PDEs regulate the cyclic nucleotide signalling pathways. The cAMP pathways is activated by adenylyl cyclase (AC) which converts ATP to cAMP. cAMP binds to the regulatory subunits (R) of protein kinase A (PKA), causing the release of the catalytic subunits (C). The cGMP pathway functions in a similar manner to the cAMP pathway. Guanylyl cyclase (GC) catalyzes the conversion of GTP to cGMP which activates protein kinase G (PKG). PDEs eliminate active cAMP and cGMP by hydrolyzing the molecules to their inactive AMP and GMP forms.

1.2 Phosphodiesterase isoforms are grouped into families based on similarities in catalytic and regulatory domains

All mammalian PDE isoforms share a conserved catalytic domain consisting of approximately 270 amino acids located in the C-terminal half of the protein (Degerman et al., 1997; Fig. 2). The catalytic domain is more similar within an individual PDE family

(>80% amino acid identity) than between different PDE families (~25-40% identity). Isoforms within PDE families 1, 2, 3, 4, 10 and 11 have dual specificity for both cAMP and cGMP, while PDEs within families 7 and 8 specifically hydrolyze cAMP and PDEs within families 5, 6 and 9 specially hydrolyze cGMP. The molecular basis for cAMP, cGMP, or cAMP/cGMP selectivity is believed to rely on a "glutamine switch" within the PDE catalytic domain which refers to an invariant glutamine that takes an orientation that favours binding of either cAMP or cGMP based on the presence of surrounding amino acid residues (Zhang et al., 2004).

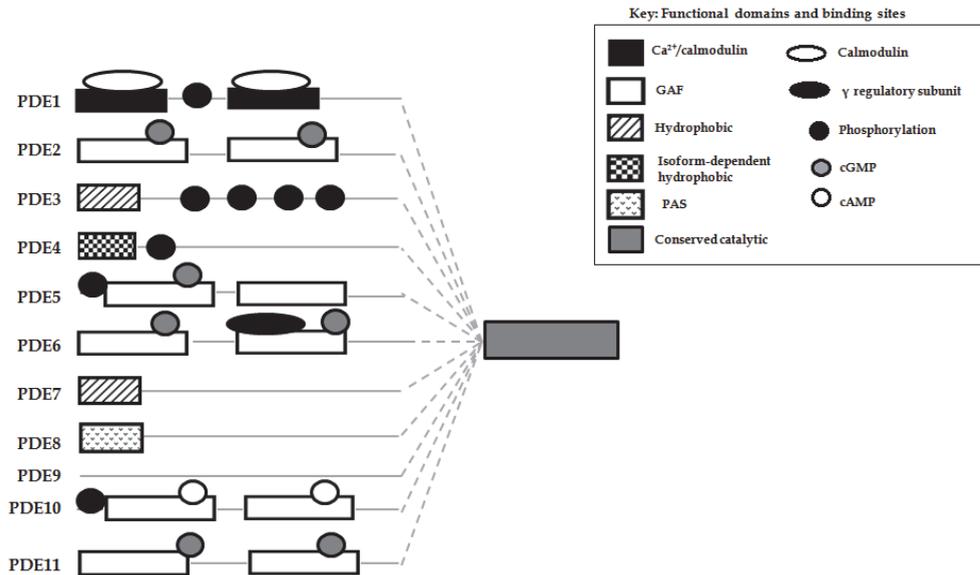


Fig. 2. Structural differences between the various PDE families. The different structural subunits that make up the individual PDE families help to dictate catalytic and regulatory specificity, as well as subcellular localization of the various PDEs.

The N-terminal portions of PDEs are widely divergent and contain functional domains that confer many of the regulatory and localization properties specific to the different PDE families (Degerman et al., 1997; Fig. 2). Isoforms of PDE families 2, 5, 6, and 7 contain two GAF domains (named after the proteins in which these domains are found: cGMP-specific phosphodiesterases, adenylyl cyclases and transcriptional activator of formate metabolism). Binding of cGMP, or cAMP in the case of PDE10, to the GAF domain stimulates enzymatic activity (Bender & Beavo, 2006). Isoforms of the PDE1 family share common dual Ca²⁺/Calmodulin binding sites, which, when bound by Ca²⁺/Calmodulin, stimulates enzymatic activity (Bender & Beavo, 2006). PDE3 isoforms contain hydrophobic domains near the N-terminus, which are believed to localize these enzymes to the plasma membrane (Degerman et al., 1997). PDE3 isoforms are also unique in that they are inhibited by cGMP, though the functional domain responsible for this has not been identified (Degerman et al., 1997). PDE6 isoforms contain an inhibitory subunit (γ), which must be removed to stimulate

catalytic activity (Bender & Beavo, 2006). PDE7 isoforms contain hydrophobic localization domains (Bender & Beavo, 2006). PDE8 isoforms contain PAS domains (named after the three proteins in which it occurs: period circadian protein, aryl hydrocarbon receptor nuclear translocator protein, single-minded protein), and REC, or receiver, domains, which are believed to function as environmental sensors (Bender & Beavo, 2006). Members of PDE families 1, 3, 4, 5, and 10 also contain phosphorylation sites which are known to play a role in activating or inhibiting enzymatic activity depending on the phosphorylation site in question (Bender & Beavo, 2006).

1.3 Subcellular localization of PDE isoforms plays an important role in compartmentation of cyclic nucleotide signalling

An important idea to come about in the last few years regarding cyclic nucleotide signalling is that of compartmentation of cAMP and cGMP (Bender & Beavo, 2006). Unique localization and protein-protein interaction domains allow PDEs isoforms to localize to specific areas of the cell which allows for compartmentation of cyclic nucleotides (Bender & Beavo, 2006). Because adenylyl cyclase and some proportion of guanylyl cyclase is membrane-bound, localization of PDEs to the membrane plays an important role in controlling cyclic nucleotide signalling. As previously discussed, PDE3A and PDE3B contain hydrophobic domains that can localize these proteins to the membrane. Hydrophobic domains are also found in PDE2A2, PDE2A3, and PDE4A1 (Bender & Beavo, 2006). Arrestin binding domains in PDE4 isoforms are also known to allow PDE4 isoforms to localize to arrestin / β -adrenergic receptor complexes where they can breakdown cyclic nucleotides and inhibit β -adrenergic receptor signalling (Baillie et al., 2003).

Subcellular localization of individual isoforms can also change through regulatory mechanisms. This is exemplified with PDE10A2 in medium spiny projection neurons (Fig. 3). When cAMP levels are low PDE10A2 is palmitoylated and becomes associated with vesicles or the plasma membrane (Charych et al., 2010). Once at the plasma membrane, PDE10A2 is trafficked to dendritic processes throughout the neuron where it may serve to regulate intracellular signalling cascades associated with dopaminergic and glutamatergic synapses (Charych et al., 2010). When levels of cAMP increase however PKA becomes activated which leads to phosphorylation of PDE10A2. Phosphorylation of PDE10A2 inhibits palmitoylation, which results in the cytosolic accumulation of PDE10A2 in the cell body where it can normalize cAMP levels through its catalytic activity (Charych et al., 2010). Consequently, subcellular localization of PDEs plays an important role in compartmentation of cyclic nucleotide signalling.

1.4 Conclusions

PDEs regulate cyclic nucleotide signalling through breakdown of cAMP and cGMP. Multiple PDE isoforms are expressed in mammals which differ in catalytic, regulatory and subcellular localization properties. Unique regulatory and localization properties allow for fine tuning of cyclic nucleotide levels through compartmentation of specific PDE isoforms. Properties of isoforms derived from each of the 21 PDE genes encoded in mammals are summarized in Table 1.

PDE isoforms	Preferred substrate	Regulatory properties	Subcellular localization
PDE1 A,B,C	cAMP/cGMP	Ca ²⁺ /Calmodulin-activated	Cytosolic
PDE2 A	cAMP/cGMP	GAF ⁺	A1: Cytosolic A2, A3: Membrane bound
PDE3 A,B	cAMP/cGMP	cGMP-inhibited	A: Membrane-bound or cytoplasmic ¹ B: Membrane-associated
PDE4 A,B,C,D	cAMP/cGMP	UCR may play as yet unknown role	A,B: Membrane-associated C: Cytosolic D: Membrane-bound or cytoplasmic ¹
PDE5 A	cGMP	GAF ⁺	Cytosolic
PDE6 A,B,C	cGMP	Inhibited by γ subunit; GAF ⁺	A,B: Membrane associated, but becomes cytosolic after association with δ subunit C: Cytosolic
PDE7 A,B	cAMP	Unknown	A1:Cytosolic A2: Membrane-bound
PDE8 A,B	cAMP	PAS and REC environmental sensors	Cytosolic
PDE9 A	cGMP	No known regulatory domains	A1:Nuclear A5: Cytosolic
PDE10 A	cAMP/cGMP	GAF ⁺	A1,A3: Cytosolic A2: Cytosolic when cAMP levels are high, membrane associated when cAMP levels low
PDE11 A	cAMP/cGMP	GAF ⁺	Cytosolic

¹ -Depends on splice variant and cell type.

Table 1. Properties of PDE isoforms. Adapted from Bender & Beavo (2006), Lugnier (2006), and Kleppisch & Feil (2009). Abbreviations: GAF, cGMP-activated PDEs, adenylyl cyclase, and transcriptional activator of formate metabolism; UCR, upstream conserved region; PAS, period circadian protein, aryl hydrocarbon receptor nuclear translocator protein, single-minded protein; REC, receiver.

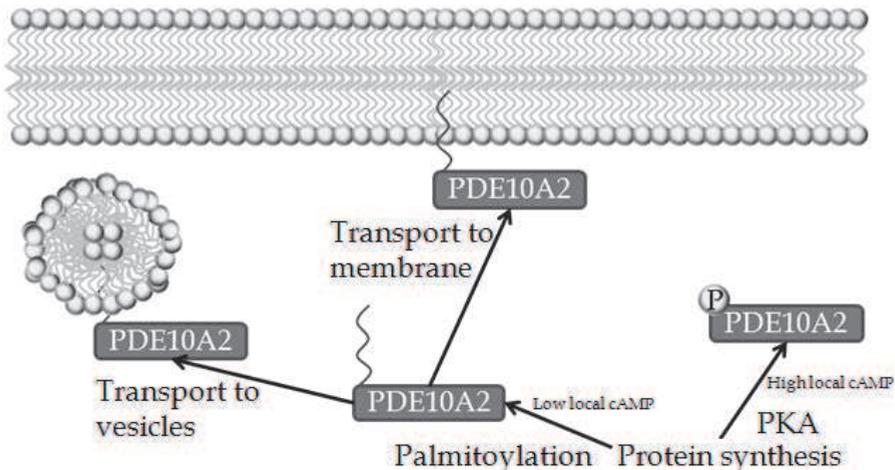


Fig. 3. Proposed model for the regulation of PDE10A2 localization in neurons in response to fluctuations in cAMP. PDE10A2 protein is synthesized in the cytoplasm. High levels of cAMP activate PKA to cause phosphorylation, and thus activation, of PDE10A2. During periods of low cAMP, PDE10A2 is palmitoylated and becomes associated with vesicles or the plasma membrane. Once at the plasma membrane, PDE10A2 is trafficked to dendritic processes throughout the neuron.

2. Phosphodiesterase isoforms have unique tissue distributions which can change during normal physiological processes

Of the 21 encoded PDE isoforms, only a small sub-set is expressed in any cell type. This cell-specific expression gives rise to unique distributions of PDE isoforms across tissues. Evidence suggests that expression of PDE isoforms changes during normal development and aging. Because different isoforms display distinct catalytic, regulatory, and subcellular localization properties, tissue-specific expression of PDE isoforms provides a mechanism to finely tune cyclic nucleotide levels within an organism.

2.1 Phosphodiesterase isoforms have unique tissue distributions

PDE isoforms have unique tissue distributions in the central nervous system (CNS) and non-nervous tissue. Tissue-specific expression of PDE isoforms was first noted in studies examining mRNA and protein expression of individual isoforms using northern blot (Fidock et al., 2002; Loughney et al., 1996), *in situ* hybridization (Prickaerts et al., 2002), western blot (Sadhu et al., 1999), and immunohistochemistry (Vandeput et al., 2007). Since then, heterogeneous tissue distribution of PDE isoform transcripts has been conclusively shown using quantitative reverse transcription (qRT) polymerase chain reaction (PCR) to quantify PDE isoform expression profiles in 12 distinct CNS and 12 distinct non-nervous tissues (Lakics et al., 2010). The PDE isoforms that are highly expressed in tissues are summarized in Table 2, while the relative distribution of highly expressed PDE isoforms across tissues is summarized in Table 3. It appears that individual tissues typically express between one and four PDE isoforms at high levels (Table 2), and individual PDE isoforms may be expressed at high levels in multiple tissues of both the CNS and non-nervous tissues (Table 3).

CNS		<i>Non-nervous tissue</i>	
Tissue	Highly Expressed PDE Isoforms	Tissue	Highly Expressed PDE isoforms
fCT	2A	THY	8B
pCT	2A	ADR	2A
tCT	2A	LIV	2A, 3B, 8A
HIP	2A	PAN	3A,5A, 8A
CAU	1B, 10A	STO	5A
SN	1C, 4B,	INT	2A, 4B, 5A, 9A
NAC	1B, 2A,	HEA	1C, 3A
CER	4A, 4B, 9A,10A	MUS	4B, 4D
THA	1C, 4B,	KID	1A, 4D, 9A
HPT	1C, 4B	BLA	5A
DRG	1C, 2A,5A, 9A	LUN	5A
SPI	4B	SPL	2A

Table 2. Highly expressed PDE isoforms within a given tissue based on quantitative reverse transcription PCR data reported by Lakics et al. (2010). PDE isoforms were considered highly expressed if mRNA levels were 60% or higher relative to the most highly expressed isoform within a given tissue. fCT, frontal cortex; pCT, parietal cortex; tCT, temporal cortex; HIP, hippocampus; CAU, caudate; SN, substantia nigra; NAC, nucleus accumbens; CER, cerebellum; THA, thalamus; HPT, hypothalamus; DRG, dorsal root ganglion; SPI, spinal cord; THY, thyroid; ADR, adrenal gland; LIV, liver; PAN, pancreas; STO, stomach; INT, intestine; HEA, heart; MUS, skeletal muscle; KID, kidney; BLA, bladder; LUN, lung; SPL, spleen.

Predominant isoform	Sites of high expression	
	CNS	<i>Non-nervous tissue</i>
1B	CAU	
2A	fCT, pCT, tCT, HIP, CAU, NAC	SPL
3A		HEA
4B	fCT, pCT, HIP, CAU, SN, NAC, THA, HPT, SPI	SPL
5A		BLA, LUN
7B	CAU	
8B		THY
9A	CAU, CER, DRG	KID, BLA, SPL
10A	CAU	
11A	DRG	THY, LIV, PAN, MUS

Table 3. Sites of high expression for predominant PDE isoforms based on quantitative reverse transcription PCR data from Lakics et al. (2010). Expression was considered high if mRNA levels were 60% or greater relative to other sites measured. Members of the PDE6 family are not shown because PDE6 isoforms are only expressed at appreciable levels in retina, which was not tested in this study. fCT, frontal cortex; pCT, parietal cortex; tCT, temporal cortex; HIP, hippocampus; CAU, caudate; SN, substantia nigra; NAC, nucleus accumbens; CER, cerebellum; THA, thalamus; HPT, hypothalamus; DRG, dorsal root ganglion; SPI, spinal cord; THY, thyroid; ADR, adrenal gland; LIV, liver; PAN, pancreas; STO, stomach; INT, intestine; HEA, heart; MUS, skeletal muscle; KID, kidney; BLA, bladder; LUN, lung; SPL, spleen.

2.2 Expression of phosphodiesterase isoforms can change during development and aging

Expression of PDE isoforms is dynamic and can change during different physiological processes such as development and aging. Prickaerts and colleagues (2002) showed that PDE5 mRNA is expressed in cerebellar Purkinje cells of rat brains only on and after postnatal (P) day 10, whereas PDE9A mRNA is present at 15 days gestation and several postnatal stages (P0, P5, P10, P21) until adulthood, thus providing an example of altered expression of PDE isoforms during development. Changes to PDE isoform expression during aging have also been documented, as PDE5 is significantly decreased in old compared to young adult rat brains, while expression of PDE 9 is higher in old compared to young rat brains (Prickaerts et al., 2002). Decreases in PDE4 expression have also been reported in the aging brain, as PDE4A mRNA levels are reduced in striatum of old compared to young mice (Hebb et al., 2004). Other PDEs including PDE1B and PDE10A do not appear to change with age (Hebb et al., 2004).

3. Expression of phosphodiesterase isoforms PDE1B, PDE4A, and PDE10A is decreased in Huntington's Disease

Huntington's Disease (HD) is caused by the inheritance of a mutant *huntingtin* gene containing an expanded CAG repeat region, which codes for an expanded polyglutamine (polyQ) region in the mutant huntingtin (mHtt) protein (reviewed by Zuccato et al., 2010). The CAG repeat length of mHtt is inversely correlated with the age of HD symptom onset. mHtt is cleaved by caspase enzymes (Graham et al., 2010). The resulting, truncated, amino-terminus of mHtt (N-mHtt) translocates to the nucleus. It is thought that the nuclear, soluble, N-mHtt interferes with transcription and thus effects gene expression, cell function, and survival (Hermel et al., 2004). The neurodegeneration observed during HD progression is tissue- and cell-specific, such that the medium spiny neurons of the striatum (caudate/putamen) are most severely affected (reviewed by Zuccato et al., 2010). Transcriptional dysregulation is a major component of HD pathogenesis. N-mHtt is thought to interfere with the assembly of the transcriptional machinery, either through the sequestration of certain transcription factors, or through the inappropriate binding and interactions with co-factors and transcription factors at the site of transcription initiation. Because transcription is dysregulated by N-mHtt during HD pathogenesis, several research groups have examined and identified the subset of genes whose expression is altered in the presence of N-mHtt to determine how altered gene expression might contribute to this disease. The identification of dysregulated genes in HD has been completed primarily with mouse models of HD and tissue from human patients suffering HD.

Several transgenic mouse models of HD exist, which can be broadly categorized as models over-expressing mHtt or knock-in models expressing mHtt within the mouse *huntingtin* locus at physiologically accurate levels (Heng et al., 2008). Of the over-expression mouse models of HD, the mouse N171-82Q, R6, and rat *huntingtin* cDNA models express N-mHtt containing between 82 (N171-82Q) and ~144 (R6/2) CAG repeats and maintain a full complement of wild-type, mouse Htt. HD symptom progression and neurodegeneration in these models is more rapid than in knock-in models of HD (Heng et al., 2008). Two distinct transgenic HD lines are derived from the R6 model: R6/1 and R6/2. R6/1 mice express N-

mHtt with ~113 CAG repeats and begin to exhibit HD motor symptoms at approximately 13 weeks of age. R6/2 mice expression N-mHtt with ~144 CAG repeats and begin to exhibit HD motor symptoms at approximately 8 weeks of age (Heng et al., 2008). Two other over-expression models, the mouse YAC128 and the HD cDNA models, express the full-length mHtt containing 128 CAG repeats. Disease progression is more rapid in these models than in the mouse knock-in models, but less rapid than in rodent models over-expressing N-mHtt (Heng et al., 2008). Also, the degree of neurodegeneration, as observed following euthanasia, is less severe in the full-length over-expression models than those models over-expressing N-mHtt (Heng et al., 2008). Knock-in mouse models of HD, including the Hdh/Q72 - 80 and Q111 - 150, express exon 1 of the human mutant *huntingtin* transgene in the mouse *huntingtin* locus. HD motor symptom onset, cognitive decline, and decreased socializing behaviours are delayed in these mouse models, relative to other rodent models of HD (Heng et al., 2008). Striatal cell loss, gross brain atrophy, and the size and number of neuronal intranuclear inclusions are also less prominent in mouse knock-in models of HD. Of the transgenic mouse models of HD, the R6 lines have been extensively studied because they display many behavioural and physiological changes associated with HD progression over a short period of time (Heng et al., 2008). R6/1 mice begin to exhibit motor symptoms related to the pathophysiology of HD between 15 and 18 weeks of age. R6/2 mice begin to exhibit HD-like symptoms between 8 and 9 weeks of age (Mangiarini et al., 1996). These symptoms include increased spontaneous locomotor activity, increased escape latency in the Morris water maze, spatial learning deficits, and progressive rotarod deficit (Cha et al., 1998).

3.1 Phosphodiesterase 1B mRNA levels decrease in the striatum of transgenic Huntington's Disease mice prior to symptom onset

PDE1B mRNA levels are lower in fully symptomatic, 12 week-old, R6/2 HD transgenic mice compared to age-matched wild-type mice, as demonstrated by microarray analysis (Luthi-Carter et al., 2004). Subsequent microarray analyses of PDE1B mRNA expression in symptomatic R6/1 mice, N171-82Q HD transgenic mice, a rat model of N-mHtt over-expression, and cDNA derived from the mRNA of symptomatic HD patients, all provide evidence for an N-mHtt-dependent decrease in PDE1B expression (Luthi-Carter et al., 2002; Desplats et al., 2006; Crocker et al., 2006; Nguyen et al., 2008). To determine when changes were first detected, and how CAG repeat length effected the rate or relative decline, PDE1B mRNA expression was measured in the striatum of the R6/1 and R6/2 HD transgenic mice, and wild-type mice, using *in situ* hybridization (Hebb et al., 2004; Fig. 4). An analysis of background-corrected optical density for PDE1B mRNA hybridization in coronal sections of mouse striatum revealed significant differences between genotypes and across ages. mRNA expression of PDE1B was reduced in R6/2 mice, relative to wild-type and R6/1 mice by 4 weeks of age. A significant decline in PDE1B was detectable by 10 week in R6/1 mice compared with wild-type. After the initial decline in transcript level observed in R6/1 and R6/2 mice, no further decline occurred. Therefore, PDE1B mRNA expression decreases in the presence of N-mHtt in the R6/1 and R6/2 transgenic mouse models of HD prior to motor symptom onset (Hebb et al., 2004). Decreased PDE1B expression may be a direct effect of expression of N-mHtt, or represent a compensatory mechanism during disease progression.

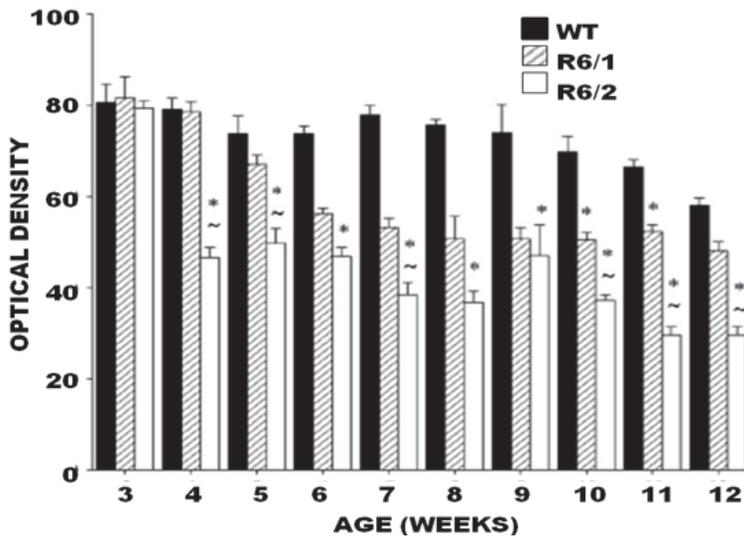


Fig. 4. PDE1B expression decreases in the striatum of R6/1 and R6/2 HD transgenic mice prior to symptom onset. This figure depicts the optical density of PDE1B mRNA *in situ* hybridization in the lateral striatum of wild-type (WT), R6/1, and R6/2 mice. In both R6/1 and R6/2 mice, there is an N-mHtt- and age-dependent decrease in PDE1B mRNA. Data represents means \pm S.E.M. for $n = 4$ of each genotype and of mice as indicated. * $P < 0.01$, significant difference from age-matched WT. $P < 0.01$, ~ significant difference from age-matched R6/1.

3.2 Phosphodiesterase 10A mRNA and protein levels decrease in the striatum of transgenic Huntington's Disease mice prior to symptom onset

PDE10A mRNA is expressed in the striatum, nucleus accumbens, and olfactory tubercle of R6 and wild-type mice. PDE10A mRNA distribution through the rostral-caudal axis of the mouse striatum is uniform (Fig. 5A). PDE10A mRNA expression is decreased in the striatum of R6/2 mice, relative to wild-type mice, by 4 weeks of age, as determined by *in situ* hybridization (Fig. 5A; Hebb et al., 2004). PDE10A mRNA levels continue to decline until reaching a new steady-state level, which is approximately 25% of that found in age-matched wild-type, by 9 weeks of age. Expression of PDE10A begins to decline between 6 and 7 weeks of age in R6/1 mice and continues to decline over the next 5 weeks, until reaching a new steady-state level of approximately 50% that of wild-type (Fig. 5B and C). Overall, three conclusions can be formed from *in situ* analysis of PDE10A expression in R6 mice. First, PDE10A mRNA levels do not normally change significantly within the striatum from 3 to 30 weeks of age. This conclusion suggests there is no effect of age on the expression of PDE10A. Second, PDE10A mRNA levels decline, and reach a final steady-state level, in an N-mHtt-dependent manner prior to symptom onset in both R6 lines. Third, the rate of PDE10A mRNA expression's decline is dependent upon the CAG repeat length of the mutant *huntingtin* transgene, as demonstrated by the more rapid rate of PDE10A mRNA in R6/2 mice, which express the *huntingtin* gene with a greater repeat length than R6/1 mice.

The N-mHtt-dependent decrease in PDE10A expression in the striatum of R6 mice was measured using western blot. PDE10A protein levels decrease in R6/2 mice, relative to wild-type, at 9 weeks of age and continue to decline until 15 weeks of age, when they achieve a new steady-state level (Fig. 5D; Hebb et al., 2004). In R6/1 mice a decrease in protein abundance is observed at 9 weeks of age, and the decrease continues until 18 weeks of age. The pattern of protein and mRNA decrease is similar in both R6 lines in that a significant decrease in levels is detected prior to or during motor symptom onset and the decline continues until a new steady state is achieved. In the case of PDE10A protein, the decrease is delayed, which is likely caused by a relatively long protein half-life.

As is observed in R6 transgenic mouse models of HD, PDE10A protein expression is decreased in human patients suffering from HD. PDE10A protein expression was analyzed in post-mortem human tissue from the caudate, nucleus accumbens, and putamen of grade 3 HD patients using western blot. When equal amounts of protein from healthy and HD patients were resolved by SDS-PAGE and probed with an anti-PDE10A antibody, little PDE10A could be detected in protein samples derived from HD patients (Fig. 6). These results demonstrate that PDE10A protein levels are decreased in HD, relative to age-matched healthy individuals (Hebb et al., 2004).

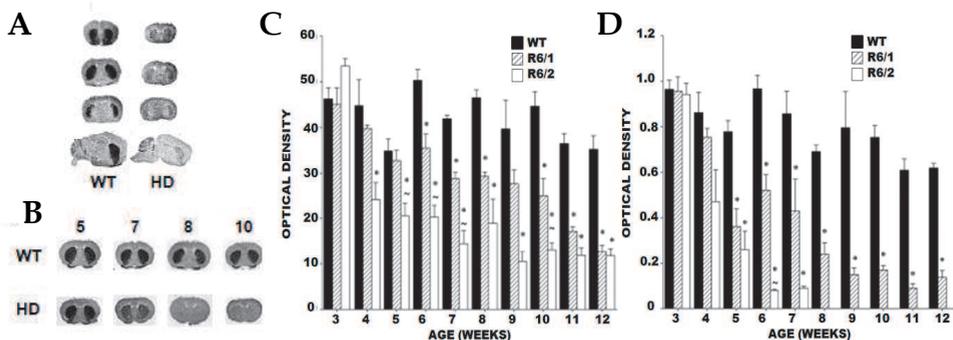


Fig. 5. PDE10A mRNA and protein levels decrease in the striatum of R6/1 and R6/2 HD transgenic mice prior to symptom onset. Panel A depicts PDE10A mRNA hybridization through the rostral-caudal axis of 10 week-old R6/2 HD and wild-type (WT) mice, with the bottom section shown in the sagittal plane. In panel B, the striatum-specific decline in PDE10A mRNA in R6/2 HD mice, relative to wild-type, is apparent by 7 weeks of age. Panel C depicts the optical density of PDE10A mRNA *in situ* hybridization in the lateral striatum of wild-type, R6/1, and R6/2 mice. In both R6/1 and R6/2 mice there is an N-mHtt- and age-dependent decrease in PDE10A mRNA. Panel D depicts the optical density of PDE10A protein from a western blot membrane for protein derived from striata of wild-type, R6/1, and R6/2 mice. Data represents means \pm S.E.M. for $n = 4$ of each genotype and of mice as indicated. * $P < 0.01$, significant difference from age-matched wild-type. $P < 0.01$, \sim significant difference from age-matched R6/1.

The decreased steady state levels of PDE10A2 in the R6 mouse striatum are caused by an altered rate of transcriptional initiation, rather than an alteration in mRNA stability (Hu et al., 2004; Gomez et al., 2006). A comparison of the human and mouse PDE10A2 promoters

reveals a high degree of conservation with respect to the presence and relative positions of several *cis*-regulatory elements. Altered expression of PDE10A2 may be a direct consequence of N-mHtt acting upon the transcriptional machinery present at the promoter of this gene (Hu et al., 2004).

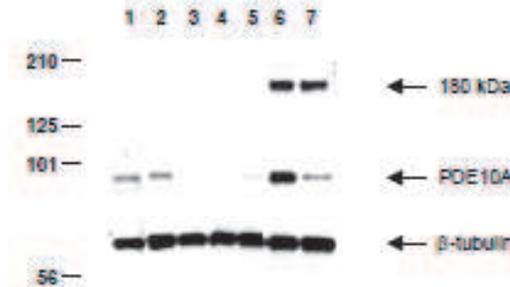


Fig. 6. PDE10A protein levels are lower in post-mortem samples from the caudate, nucleus accumbens, and putamen of patients with grade 3 HD, compared to age-matched controls. Lanes 1 and 2 represent 1 μ g of protein derived from the caudate and nucleus accumbens of 66 and 53 year-old non-HD males, respectively. Lanes 3 – 5 represent 1 μ g of protein derived from the caudate, nucleus accumbens, and putamen of grade 3 HD patients of 52, 67, and 48 year-old HD females (3 and 4) and a male (5). Lane 6 represents 1 μ g of protein from the striatum of wild-type mice, which was included as a positive control. Lane 7 represents 1 μ g of protein from the striatum of 12 wk-old R6/1 mice.

3.3 Phosphodiesterase 4A mRNA levels decrease in the striatum with age independently of mutant huntingtin

PDE4A expression is higher in the cortex than the striatum of wild-type and R6 mice. The optical density of cortical PDE4A mRNA has been measured by *in situ* hybridization. PDE4A mRNA abundance in the cortex declines as the animals age, and is significantly greater in wild-type mice relative to R6/2 at 3 weeks of age (Fig. 7). The decline in PDE4A mRNA expression is correlated with age, but not with the expression of N-mHtt, although it is possible that PDE4A mRNA begins to decline in R6/2 transgenic mice before it begins to decline in wild-type and R6/1 mice (Hebb et al., 2004).

3.4 Conclusions

PDE1B and 10A mRNA and protein levels are decreased in R6 mice relative to age-matched wild-type mice prior to motor symptom onset. PDE4A mRNA levels decline with increasing age. Decreased PDE1B and 10A expression in the R6 mouse models of HD is dependent upon expression of N-mHtt. Greater polyQ repeat length within the N-mHtt, such as in the fragment expressed in R6/2 mice relative to R6/1 mice, leads to earlier decreases in PDE1B and 10A expression. The lifespan of the R6 HD transgenic mouse models is summarized in figure 8. In the case of PDE10A2, decreased mRNA expression is the result of transcriptional interference by N-mHtt at the PDE10A2 promoter. Collectively, these data suggest expression of N-mHtt causes a decrease, at the level of transcription, in abundance of PDE1B, 10A, and possibly 4A, in the striatum (caudate/putamen). The functional consequence of decreased PDE expression in HD remains unclear.

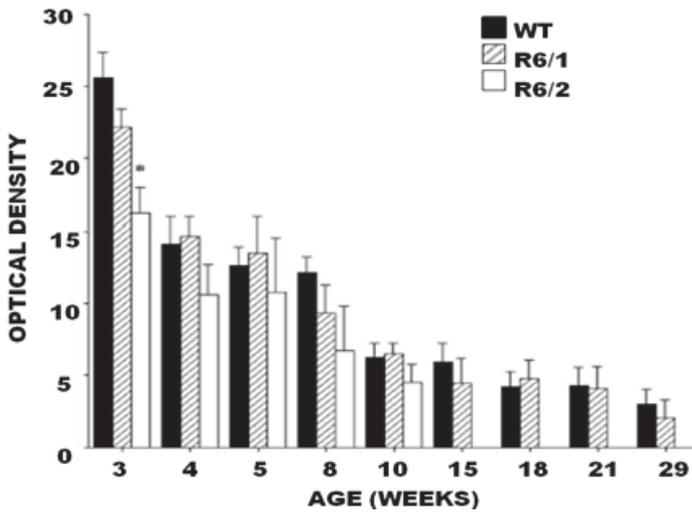


Fig. 7. PDE4A mRNA levels decrease with age. This figure depicts the optical density of PDE4A mRNA *in situ* hybridization in the cortex of WT, R6/1, and R6/2 HD transgenic mice. In all genotypes, there is a decrease in striatal PDE4A mRNA expression with increasing age. Data represents means \pm S.E.M. for $n = 4$ of each genotype and of mice as indicated. * $P < 0.001$, significant difference from age-matched WT.

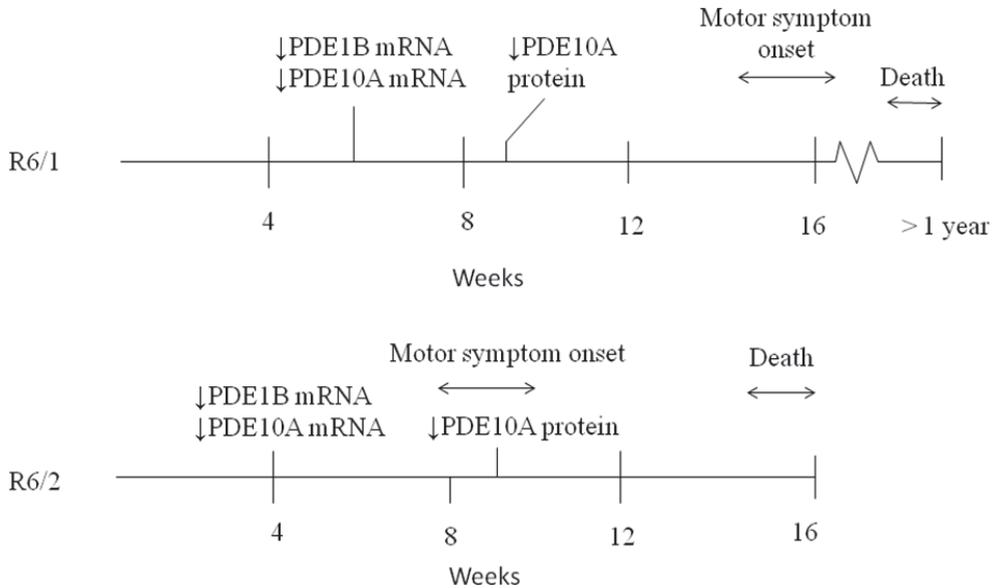


Fig. 8. Time-line of motor symptom onset, life span, and changes in PDE expression in R6/1 and R6/2 HD transgenic mice.

4. Impaired function of phosphodiesterase isoforms is associated with various pathological conditions of the central nervous system

HD progression is associated with distinct pathological changes in motor control and behaviour. Transgenic rodent models of HD that express mHtt, in part or whole, recapitulate many of the symptoms associated with HD and often experience cell-specific decreases in PDE1B and 10A mRNA expression (Table 4). However, the precise role of decreased PDE expression in these models is difficult to determine. Genetic knock-out of specific PDE isoforms, such as 1B, 4, 10A, and 11A, in mice causes phenotypic changes that often resemble the symptom profile of transgenic rodent models of HD (Kleppisch & Feil, 2009). Moreover, several mutations in specific PDE4, 6, 8, 10, and 11 isoforms are associated with disorders of the central nervous system, such as schizophrenia and major depressive disorder (Esposito et al., 2009). By comparing and contrasting the phenotype of HD to PDE knock-out models and other central nervous system disorders where PDE expression or activity are dysregulated, certain hypotheses can be made regarding the consequence of decreased PDE expression in HD.

4.1 Genetic knock-out of specific phosphodiesterases causes distinct behavioural phenotypes

Knock-out studies in which the expression of a specific PDE is eliminated by gene ablation or mutation reveal how changes in catalytic activity or expression of specific PDEs may contribute to disease pathophysiology. Mice lacking PDEs 1B, 1C, 4B, 4D, 6B, 9A, 10A, and 11A have been generated. These mice exhibit behaviours that resemble some behaviour associated with schizophrenia, major depressive disorder, hyperkinesias, and HD.

4.1.1 Phosphodiesterase 1B knock-out causes hyper-locomotion and spatial learning deficits in mice

PDE1B mRNA and protein are highly expressed in the striatum relative to other brain regions (Table 3). Expression of PDE1B decreases prior to symptom onset in R6 mouse models of HD. PDE1B knock-out mice were generated using homologous recombination to remove exons 2 – 13 of the mouse PDE1B gene (Reed et al., 2002). Knock-out of PDE1B is associated with increased locomotor activity, increased dopamine receptor-mediated phosphorylation of dopamine and cAMP-regulated neuronal phosphoprotein (DARPP-32), and performance deficits in spatial learning tasks. DARPP-32 is expressed in medium spiny projection neurons where it is phosphorylated and activated by protein kinase A following dopamine receptor-mediated cAMP production. Upon activation, DARPP-32 inhibits protein phosphatase 1, and thus facilitates phosphorylation and activation of pro-survival proteins. Double knock-out mice lacking PDE1B and DARPP-32 do not differ in phenotype from PDE1B null mice, suggesting that PDE1B activity upstream of DARPP-32 represents the major modulatory pathway for cyclic nucleotide messenger systems in the striatum (Ehrman et al., 2006). PDE1B knock-out mice show increased dopamine turnover and decreased serotonin levels in the striatum, and model depression-like behaviours such as decreased pleasure-seeking activity (Siuciak et al., 2007). These data indicate enhancement of cyclic nucleotide second messenger systems by PDE1B knock-out causes significant changes in locomotion and dopamine-mediated signal transduction within the striatum. Consequently, PDE1B null mice recapitulate the increased spontaneous locomotor activity and depression observed in HD mouse models and patients. Importantly during HD progression, DARPP-32 mRNA and protein levels

decrease in an N-mHtt dependent manner (Gomez et al., 2006). This indicates that treatments that alter PDE1B activity may be limited by defects in downstream DARPP-32 levels or activity.

4.1.2 Complete phosphodiesterase 4B, or conditional phosphodiesterase 4D, knock-out produces a schizophrenia-like phenotype in mice

The dual-specificity PDE4 isoforms, including PDE4A, B, and D, are expressed in the cerebral cortex and amygdala (Siuciak et al., 2007). In HD, PDE4A mRNA declines with age and may contribute to changes in mood and behaviour observed during disease progression (Hebb et al., 2004). PDE4B knock-out mice were generated by homologous recombination of exons 3 - 6, which ablated the catalytic subunit of the mouse PDE4B gene (Jin et al., 1999). Specific knock-out of PDE4B in mice reduces prepulse inhibition, which is considered a mouse behavioural model of schizophrenia. The prepulse inhibition test utilizes a series of paired stimuli to determine whether an animal is capable of filtering external stimuli. Mice with normal executive function have a reduced response to the second of two, paired, stimuli, relative to the first. Mice exhibiting schizophrenia-like symptoms have a heightened response to the second stimuli due to a deficit in the ability to filter external stimuli. Mice lacking PDE4B are defective in their response to prepulse inhibition, and have decreased baseline locomotor activity (Siuciak et al., 2007). In addition, PDE4B null mice display anxiogenic-like behaviour, as measured by decreased head-dips in the hole board test, reduced transitions into the light side of a light-dark chamber, and decreased exploration of an open field. PDE4B null mice do not display changes in memory or nociception (Zhang et al., 2002). PDE4B knock-out mice display impaired reversal learning in the Morris water maze, but no differences in spatial memory or fear conditioning, relative to wild-type littermates (Rutten et al., 2009). Taken together, these data illustrate that PDE4B expression in the cortex and amygdala contributes to control of locomotion and anxiety-like behaviours and that other PDEs do not compensate for loss of PDE4B function.

PDE4D expression is more abundant in the cerebral cortex and hippocampus than other brain regions. Loss of PDE4D is associated with behaviours that mimic the effects of antidepressants, although the precise role of PDE4D in MDD pathophysiology is unclear. Mice lacking PDE4D display increased mobility in the forced swim and tail-suspension tests, indicative of antidepressant-like behaviours in mice (Zhang et al., 2002). These data demonstrate PDE4 regulate susceptibility to psychoses and changes in mood. Depressive symptoms, such as anhedonia and decreased socializing behaviour are observed in mouse models of HD and may result from the decline in expression of certain PDE4 isoforms.

4.1.3 Phosphodiesterase 10A knock-out reduces spontaneous locomotor activity and increases social interactions in mice

PDE10A is highly expressed in the medium spiny projection neurons of the striatum. Protein and mRNA expression of PDE10A is decreased in the striatum (caudate/putamen) in human patients with, and mouse models of, HD. Knock-out of PDE10A in mice causes increased escape latency in the Morris water maze, impaired conditioned avoidance behaviour, reduced spontaneous locomotor activity, increased social interaction, and increased levels of striatal cAMP, relative to wild-type mice. PDE10A knock-out does not induce anxiety-, or depression-like behaviours, or produce altered nociception (Siuciak et al., 2006). Further, hyperlocomotion associated with amphetamine treatment is absent in PDE10A knock-out mice

(Siuciak et al., 2006). Siuciak and colleagues (2006) concluded that inhibition of PDE10A may represent a novel therapeutic approach to the treatment of schizophrenia. PDE10A knock-out mice recapitulate the reduced spontaneous locomotor activity characteristic of late-stage HD rigidity, and increased escape latency in the Morris water maze, but differ in that mouse models of HD display decreased, not increased, social interaction behaviours (Table 4).

Species	Model	Behavioural phenotype	Decreases in PDE expression	Reference
Mouse	N171-82Q	<ul style="list-style-type: none"> Increased spontaneous locomotor activity Progressive accelerated rotarod deficit beginning at 12 weeks 	<ul style="list-style-type: none"> PDE2A (6 weeks) 	Schilling et al. (1999) Yu et al. (2003) Runne et al. (2008)
Mouse	R6/1 and R6/2	<ul style="list-style-type: none"> Increased spontaneous locomotor activity Progressive accelerated rotarod deficit beginning at 5 and 12 weeks Increased escape latency in the MWM Spatial learning deficit 	<ul style="list-style-type: none"> PDE1B (4 weeks R6/2, 10 weeks R6/1) PDE4A (with aging) PDE10A (4 weeks R6/2, 6 weeks R6/1) 	Cha et al. (1998) Meade et al. (2002) Ribchester et al. (2004) Hebb et al. (2004)
Mouse	HD cDNA	<ul style="list-style-type: none"> Hyperactivity (12 weeks) Decreased baseline motor activity (24 weeks) 	<ul style="list-style-type: none"> PDE1B, 10A (14 weeks) 	Reddy et al. (1998) Thomas et al. (2008)
Mouse	YAC 128	<ul style="list-style-type: none"> Increased spontaneous locomotor activity (12 weeks) Decreased baseline motor activity (48 weeks) Increased escape latency in the MWM 	<ul style="list-style-type: none"> PDE1B, 10A (12 weeks) 	Benn et al. (2007) Mazarei et al. (2010)
Rat	<i>huntingtin</i> cDNA	<ul style="list-style-type: none"> Cognitive decline (40 weeks) Increased spontaneous locomotor activity 	<ul style="list-style-type: none"> PDE1B, PDE10A (12 weeks) 	Nguyen et al. (2008) Cao et al. (2006)
Mouse	Hdh/Q72 - 80	<ul style="list-style-type: none"> Increased aggression, decreased socializing behaviours Anhedonia 	<ul style="list-style-type: none"> None reported 	Kennedy et al. (2005)
Mouse	Hdh/Q111 - 150	<ul style="list-style-type: none"> Decreased baseline motor activity (24 weeks) Hyperactivity (4 weeks) 	<ul style="list-style-type: none"> None reported 	Wheeler et al. (2002)

Table 4. Behavioural phenotypes and decreases in PDE expression observed in rodent models of HD. Phenotypes for specific transgenic rodent models of HD are described with the approximate time at which behaviours become present where possible. Changes in PDE expression were determined via microarray and subsequently confirmed by quantitative polymerase chain reaction.

4.1.4 Phosphodiesterase 11A knock-out produces a schizophrenia-like phenotype in mice

PDE11A mRNA is expressed in the hippocampus CA1, subiculum, amygdalohippocampal area, and dorsal root ganglia, as demonstrated by *in situ* hybridization (Kelly et al., 2010). PDE11A knock-out mice were generated by creating a missense mutation in the catalytic subunit of the protein, which caused it to be non-functional. PDE11A knock-out mice exhibit hyperactivity in an open field test, deficits in social odour recognition and social avoidance behaviours, enlarged lateral ventricles, and increased CA1 activity. Overall, this knock-out mouse model displays symptoms that are thought to be like some symptoms seen in psychotic patients.

In contrast, humans homozygous for loss-of-function mutations in PDE11A were more likely to suffer major depressive disorder than those with normal levels of PDE11A expression (Wong et al., 2006). These studies highlight the essential differences between mouse models and human disorders. In both cases though, deficits in social behaviours were present, which suggests PDE11A function is required for normal socialization processes. Microarray analysis of gene expression in tissue derived from rodent models of HD demonstrate that PDE11A expression is not changed in HD (Cha et al., 1998). The phenotype of PDE11A knock-out mice does, however, resemble the hyperactivity and social avoidance behaviours observed in rodent models of HD.

In conclusion, altered expression of PDE1B, 4, 10A, or 11A appear to change behaviour in similar manners in rodent models of HD and PDE knock-out mice. The phenotypes associated with genetic knock-out of specific PDEs are summarized in table 5.

Species	Model	Mutant gene/ gene locus	Associated phenotype	References
Mouse	Knock-out	PDE1B	<ul style="list-style-type: none"> • Increased locomotor activity • Increased dopamine receptor-mediated phosphorylation of DARPP-32 • Spatial learning deficit • Reduced pleasure-seeking activity 	Reed et al. (2002)
Mouse	Knock-out	PDE4B	<ul style="list-style-type: none"> • Decreased baseline motor activity • Exaggerated locomotor response to amphetamine 	Siuciak et al. (2007)
Mouse	Knock-out	PDE4D	<ul style="list-style-type: none"> • Increased mobility in the forced swim and tail-suspension tests 	Zhang et al. (2002)
Mouse	Knock-out	PDE10A	<ul style="list-style-type: none"> • Increased escape latency in Morris water maze • Impaired conditioned avoidance learning • Reduced spontaneous locomotor activity 	Siuciak et al. (2006)
Human	SNPs	PDE11A	<ul style="list-style-type: none"> • Major Depressive Disorder 	Wong et al. (2006)
Mouse	Knock-out	PDE11A	<ul style="list-style-type: none"> • Hyperactivity • Deficits in social avoidance behaviours • Enlarged lateral ventricles 	Kelly et al. (2010)

Table 5. CNS phenotypes related to ablation and mutations of PDE genes in mice.

4.2 Mutations in phosphodiesterases and their interacting proteins are associated with schizophrenia

Schizophrenia is a neurological disorder described by a range of behavioural, attention, sensory, and executive function-based deficits (Ebix Inc. Animated Dissection of Anatomy for Medicine, [A.D.A.M.], 2010). Individuals with schizophrenia may experience psychoses, delusions, and hallucinations, collectively known as positive symptoms, as well as feelings of depression and social isolation, described as negative symptoms. This disorder affects approximately 24 million people worldwide (A.D.A.M., 2010). Schizophrenia is complex in that both the underlying cause and pathogenesis are highly variable when individuals suffering schizophrenia are compared. Several environmental factors, such as prenatal stress, infection, and substance abuse contribute, or predispose individuals, to developing schizophrenia (A.D.A.M., 2010). Genetic factors also play a role in the disorder's etiology. Specific mutations in PDEs, and the proteins they interact with, are an example of these genetic factors. The symptom profiles of schizophrenia and HD overlap in several respects. First, the behavioural changes associated with both disorders are highly variable. Second, schizophrenia and HD are both associated with symptoms of depression and social withdrawal. Third, individuals with schizophrenia may exhibit hyperactivity and individuals with HD present with choreic movements, which may be neurologically related to hyperactivity (Siuciak et al., 2007). In this section the role of PDEs and their interacting partners in the etiology of schizophrenia will be summarized. We will demonstrate the important role these enzymes play in the central nervous system and how a dysregulation of their activity can contribute to schizophrenic disorders.

Several authors have reported up-regulation of PDE5 protein in post-mortem tissue samples from patients with schizophrenia, particularly those with prominent negative symptoms (Akhondzadeh et al., 2011). PDE1C and PDE8B mRNA are up-regulated in post-mortem samples derived from the lateral cerebellum of patients with schizophrenia (Fatemi et al., 2009). The precise cause of this up-regulation is unknown, but the data demonstrate schizophrenia pathogenesis is associated with dysregulation of several PDE families and isoforms.

PDE10A has garnered significant attention as a potential therapeutic target for schizophrenia. As previously described, the PDE10A variant, PDE10A2, displays differential sub-cellular localization depending on local cAMP level. PDE10A2 localizes to the membrane and is transported along dendritic processes by palmitoylation at cysteine 11 (Charych et al., 2010). Protein kinase A is activated by high cAMP and phosphorylates PDE10A2 at threonine 16, which interferes with trafficking of PDE10A2 to the membrane. The authors postulate that differential dopamine signalling, as observed in schizophrenia, in the direct and indirect striatal output pathways, would change cAMP levels and thus localization and activation of PDE10A2. Their model of dopamine-dependent PDE10A2 localization and activity is summarized in figure 2.

Mutations in the disrupted-in-schizophrenia-1 protein are considered strong genetic risk factors for the development of schizophrenia. Specifically, mutation of glutamine 31 to leucine (Q31L) or leucine 100 to proline (L100P) in the N-terminal region of this protein are associated with depression-like and schizophrenia-like phenotypes in mutant mice, respectively (Lipina et al., 2011). Disrupted-in-schizophrenia-1 protein exists in a protein complex with glycogen synthase kinase-3 and PDE4B in the rat dorso-lateral prefrontal

cortex and hippocampus. This complex localizes to the synapse in primary mouse hippocampal cultured neurons (Lipina et al., 2011). In protein extracts derived from the hippocampus or dorso-lateral prefrontal cortex of L100P mice, disrupted-in-schizophrenia-1 protein -PDE4B binding was reduced by 75% and disrupted-in-schizophrenia-1 protein -glycogen synthase kinase-3 binding was reduced by 50%, relative to protein extracts derived from mice with wild-type disrupted-in-schizophrenia-1 protein. Similarly, disrupted-in-schizophrenia-1 protein -PDE4B binding was reduced by 50%, and disrupted-in-schizophrenia-1 protein -glycogen synthase kinase-3 binding by 75%, in Q31L mouse models of depression. The group hypothesized that disrupted-in-schizophrenia-1 protein acts as a scaffold to integrate and down-regulate the signalling pathways of PDE4B and glycogen synthase kinase-3. Sub-threshold, doses of the glycogen synthase kinase-3 inhibitor TDZD-8 and the PDE4 inhibitor rolipram effectively treat depression- and schizophrenia-like symptoms in both mutant mouse strains, as demonstrated by measuring pre-pulse inhibition deficit and mobility in the forced swim test. The authors conclude that disrupted-in-schizophrenia-1 protein mutations produce inappropriate interactions with PDE4B. The result of these inappropriate reactions was an inability to converge PDE4B and glycogen synthase kinase-3 signalling pathways contributing to schizophrenia-like phenotypes in mice. These data demonstrate the proper signalling of PDE4 isoforms is required for normal executive function, mood, and behaviour.

Mutations in the PDE4B gene itself have also been examined for associations with schizophrenia. The existence of PDE4B gene variants was examined in a population of 169 Caucasian patients taking antipsychotic medication. Two PDE4B variants associated with tardive dyskinesia and two additional variants associated with female-specific tardive dyskinesia were discovered (Souza et al., 2011). However, correction for multiple testing eliminated these variants as being truly genetically associated with the tardive dyskinesia observed in schizophrenia. In contrast, a similar study examined variations in the PDE4B gene in 837 individuals with schizophrenia and 1473 controls (Kahler et al., 2010). They found four variants in the PDE4B3 isoform nominally associated with schizophrenia in females, and four additional single nucleotide polymorphisms associated with positive symptom scores according to Positive And Negative Symptoms Scale (PANSS) testing of patients. Similar results were found in the PDE4B gene in a Japanese population, lending further support to the theory that certain PDE4B variants have a positive association with schizophrenia (Numata et al., 2009). Up-regulation of PDE4A and 4B mRNA has been observed in the frontal cortex of patients with schizophrenia (Fatemi et al., 2009). Overall, mutation of PDE4 isoforms, or changes in the level of expression of PDE4, is associated with changes in mood and behaviour related to schizophrenia. Therefore, decreased PDE4 expression during HD progression may contribute to changes in mood and behaviour as well.

4.3 Changes in phosphodiesterase 4 mRNA expression, but not allelic variability of phosphodiesterases, is implicated in major depressive disorder

Major depressive disorder is a neurological disorder characterized by emotional, attentional, sensory, and executive function-based deficits (A.D.A.M., 2011). Individuals with major depressive disorder may experience feelings of sadness, loss, anger, or frustration that persist for extended periods of time such that these feelings interfere with their normal ability to function and be productive. Major depressive disorder affects approximately 8 -

12% of all people at some point during their lives (A.D.A.M., 2011). Approximately 40% of patients suffering from HD exhibit symptoms of depression (A.D.A.M., 2011). The Hdh mouse models of HD exhibit anhedonia and decreased socializing behaviours, which are considered to be analogous to human depression (Kennedy et al., 2005). Genetic factors, or heritability, contribute 40 - 50% to the probability a person will suffer major depressive disorder (Numata et al., 2009). Changes in the mRNA expression or the activity of certain PDEs can contribute to major depressive disorder etiology.

PDE4B mRNA expression, single nucleotide polymorphisms, and haplotype variants were examined in a large Japanese population (655) suffering major depressive disorder (Numata et al., 2009). No significant correlation between allelic variation and major depressive disorder was found. PDE4B is most likely implicated in the pathophysiology of major depressive disorder because of the differential mRNA expression observed in animal models and human patients suffering from major depressive disorder. During HD progression, mRNA expression of PDE4A declines in a cell-specific manner in the cortex (Hebb et al., 2004). Depressive symptoms often observed in individuals suffering HD, and mouse models of HD, may therefore be explained by a decline in PDE4 expression.

Other authors have analyzed associations between allelic variation in PDE1A, 8A, 9A, and 11A and major depressive disorder (Wong et al., 2006; reviewed by Esposito et al., 2009). Nominally significant allelic associations between these PDEs and major depressive disorder have been found. However, independent analyses of these data, or attempts to replicate these findings in other populations, have failed to demonstrate significance. Of these, only one demonstrated a significant association between an inactivating mutation in PDE11A and individuals with adrenocortical hyperplasia and major depressive disorder (reviewed by Esposito et al., 2009).

4.4 Conclusions

Transgenic mouse models of HD display locomotor and cognitive deficits, including early-symptomatic increased spontaneous locomotor activity, late-symptomatic hypoactivity, and increased escape latency in the Morris water maze. These mouse models also display the depression-like phenotypes of anhedonia and decreased socializing behaviours (Heng et al., 2008). Similarly, genetic ablation of PDE1B, 4, 10A, or 11A is associated with specific locomotor and cognitive declines. Increased locomotor activity, spatial learning deficits, and reduced pleasure-seeking activity are observed in PDE1B knock-out mice (Reed et al., 2002). Increased escape latency and reduced spontaneous locomotor activity are observed in PDE10A knock-out mice (Siuciak et al., 2006). These data suggest that the consequence of decreased PDE expression, as observed in HD, may be a change in motor control and mood.

Human patients suffering from HD experience spontaneous choreic movements early in disease progression, rigidity late in disease progression, and symptoms of depression. Schizophrenia and major depressive disorder are two disorders of the central nervous system where PDE expression and/or catalytic activity are dysregulated. Therefore changes in PDE1B, 4, and 10A may play a contributing factor in the pathogenesis of HD and other central nervous system disorders. Expression and function of PDE4B and PDE11A in the amygdala, cortex, and hippocampus is critical to maintain normal cognitive function and social interaction. PDE4D also appears to be involved in social interaction and depression-

like behaviour. Expression of PDE8B is up-regulated in the cortex and hippocampus of Alzheimer's Disease patients compared to age-matched controls (Pérez-Torres et al., 2003). Although microarray analyses suggest no significant changes in the expression of PDE4B, 4D, 8A, or 11A mRNA, it is interesting to note that other central nervous system disorders are associated with cell-specific changes in PDE expression, which may contribute to their pathophysiology.

5. Pharmacological inhibition of phosphodiesterases in the central nervous system

Because multiple PDE isoforms are expressed in the central nervous system (Tables 2 and 3) and individual isoforms are tightly coupled to specific physiological functions, pharmacological inhibitors may be used to treat pathological conditions of the central nervous system without a high likelihood of causing non-specific side effects. PDEs represent a logical target for competitive inhibition because concentrations of their substrate (cAMP and cGMP) are low ($>1\mu\text{M}$ to $10\mu\text{M}$; Koyanagi et al., 1998). This means that competition with endogenous substrate could be achieved using low concentrations of PDE inhibitors. However, PDE isoforms share similar structure in the catalytic domain, which makes design and development of truly selective competitive inhibitors difficult. Within the active site of all PDE isoforms studied to date, 11 invariant residues have been identified which maintain a consistent arrangement between isoforms and are believed to be important for catalytic activity (Manallack et al., 2005). Nevertheless, multiple PDE competitive inhibitors have been developed which show some degree of isoform-specificity as demonstrated by a lower half-maximal inhibitory concentration (IC_{50}) for one isoform relative to other isoforms. For treatment of central nervous system disorders, competitive PDE inhibitors must also effectively cross the blood-brain-barrier. This section will review the pharmacological profile of PDE competitive inhibitors that show some degree of selectivity for individual PDE isoforms and have well known effects in the central nervous system.

5.1 Papaverine, TP-10 and MP-10 are selective competitive inhibitors of PDE10A

Papaverine is an opium alkaloid that was first isolated in 1848 from poppies, or *Papaver somniferum*, from which the name "papaverine" is derived (Hollman, 2005). Medical use of papaverine was first suggested in 1914 for treatment of hypertension and angina (Hollman, 2005). Papaverine was shown to competitively inhibit PDE10A following quantification of IC_{50} values in mice (Siuciak et al., 2006). The IC_{50} of papaverine for PDE10A is 36 nM, which is between 9 and 52-fold lower than the IC_{50} for the next most easily inhibited isoform, PDE4 (Siuciak et al., 2006).

TP-10 and MP-10 are two PDE10A competitive inhibitors that were developed by the pharmaceutical company Pfizer in 2008. The IC_{50} of TP-10 and MP-10 for PDE10A is approximately 0.3 nM and 0.18 nM respectively. This is between 3,333 and 10,000-fold lower than the IC_{50} for the 18 other PDE isoforms tested (Schmidt et al., 2008), which makes these compounds more selective for PDE10A than papaverine. TP-10 and MP-10 are more potent than papaverine as 3.2 mg/kg of TP-10 administered sub-cutaneously produced a 3- and 3.5-fold increase in extracellular cAMP and cGMP respectively in rat striatum (Schmidt et al., 2008). The dose of papaverine required to achieve a similar effect was 56 mg/kg (Siuciak et al., 2006).

5.2 Rolipram is a selective competitive inhibitor of PDE4 isoforms

Rolipram is a PDE4 competitive inhibitor originally developed as an antidepressant (Kehr et al., 1985). The IC_{50} of rolipram is approximately 500 nM for PDE4 in mice (Bader et al., 2006), which is approximately 24-fold lower than the IC_{50} for PDE10A (Bader et al., 2006). Within the PDE4 family, rolipram seems to inhibit PDE 4A most effectively as rolipram inhibited immunopurified PDE4A activity in U937 human histiocytic lymphoma cells with an IC_{50} of approximately 3 nM, compared to IC_{50} values of approximately 130 nM and 240 nM for PDE4B and PDE4D respectively (Bader et al., 2006). Inhibition of PDE4B and PDE4D is known to contribute to the antidepressant effects of rolipram, as the ability of rolipram to alleviate depression-like behaviours, is partially lost in PDE4B and PDE4D knock-out mice (Zhang et al., 2002; Siuciak et al., 2007).

5.3 Sildenafil is used for inhibition of PDE5A in the periphery, but also has effects in the central nervous system through inhibition of PDE5A and possibly PDE6

Sildenafil (trade name Viagra) was first developed by the pharmaceutical company Pfizer for treatment of angina, hypertension, and erectile dysfunction (Boolell et al., 1996). The IC_{50} of sildenafil for PDE5 is 3 nM as measured in human corpus cavernosum (Ballard et al., 1998). This is between 80- and 8500-fold lower than the IC_{50} for PDEs 1-4 (Ballard et al., 1998). Anti-angina, -hypertension, and -erectile dysfunction effects of sildenafil are mediated by inhibition of PDE5 which is enriched in smooth muscle of the lungs and corpus callosum (Boolell et al., 1996). PDE5 is also expressed in the brain and growing evidence suggests that orally delivered sildenafil has effects in the central nervous system, as inhibition of PDE5 in the brain is associated with improved object recognition memory in rats (Prickaerts et al., 2002) and altered event-related brain potentials in humans (Schultheiss et al., 2001). The IC_{50} of sildenafil for PDE6 is 9-fold greater than the IC_{50} for PDE5 (Ballard et al., 1998). Despite a higher IC_{50} for PDE6 than PDE5, inhibition of PDE6 in the retina is believed to contribute to visual disturbances reported in a minority of patients taking sildenafil (Marmor & Kessler, 1999). IC_{50} values of sildenafil for PDEs 7-11 have not yet been reported. Taken together, evidence suggests that sildenafil inhibits PDE5 and PDE6 in the central nervous system in addition to inhibiting PDE5 in the periphery.

6. Pharmacological inhibition of phosphodiesterase activity is useful in the treatment of several neurological disorders

Phosphodiesterase inhibitors are used for the treatment of embolism, thrombocytosis, inflammation, decreased cerebral blood flow, heart failure, asthma, chronic obstructive pulmonary disease, and erectile dysfunction. PDE inhibitors exhibit antidepressant, and nootropic (*i.e.* memory enhancing), properties, which has led to the development of central nervous system-specific PDE inhibitors for the treatment of several neurological disorders.

6.1 Phosphodiesterase inhibitors improve cognitive and sensorimotor deficits in schizophrenia

Inhibition of PDE4B, 5, and 10A, enzymes has been investigated as a potential therapeutic means of reducing psychoses. In particular, inhibitors of these enzymes improve attentional and sensorimotor deficits, as well as socializing deficits, in animal models of schizophrenia.

Several research groups have investigated the clinical efficacy of PDE inhibitors for the treatment of both positive and negative symptoms associated with schizophrenia. Two common rodent models of schizophrenia have been used to examine the effect of PDEs. Dopamine receptor-agonist treated mice and rats exhibit stereotypy and hyperactivity, which are behaviours thought to model the positive symptoms of schizophrenia in rodents. The other pharmacological rodent model of schizophrenia is the phencyclidine-treated mouse or rat. Phencyclidine acts as an N-methyl-D-aspartic acid (NMDA) receptor antagonist. Phencyclidine-treated rodents exhibit hyperactivity, prepulse inhibition, and anhedonia and are considered to be rodent models of the positive and negative symptoms of schizophrenia. The most well-known PDE inhibitor studied for use in schizophrenia is rolipram. Rolipram improves cognition, memory, and prepulse inhibition deficits in dopamine receptor agonist-treated mice. PDE4B activity and regulation are disrupted in the disrupted-in-schizophrenia-1 protein-L100P transgenic mouse model of schizophrenia (Lipina et al., 2011). Treatment of disrupted in schizophrenia-1 protein-L100P mice with the PDE4-specific inhibitor rolipram (0.1 mg/kg) corrects the deficit in prepulse inhibition and hyperactivity without producing overt side effects. Rolipram also reduces psychoses and improves attentional deficits in patients with chronic schizophrenia. However, rolipram has been discontinued as a treatment for schizophrenia because its use is associated with nausea, emesis, weight loss, and acute insomnia. These adverse effects are observed following treatment with all known PDE4 inhibitors. Acute insomnia, as it pertains to PDE4 inhibition, describes an inability to sleep consistently for less than 1 month during drug use (reviewed by Zhang et al., 2002). In addition to the adverse effects observed generally for PDE4 inhibitors, rolipram causes gastrointestinal pain and cardiac arrhythmia (Zhang et al., 2002).

PDE10A was first identified as a “druggable” target for the treatment of schizophrenia in 1999 (Itoh et al., 2011). The PDE10A-selective inhibitor papaverine has gained attention as a possible treatment for schizophrenia because of its neuroprotective actions. Papaverine induces NGF-dependent neurite outgrowth in PC12 neuroblastoma cells (Itoh et al., 2011). However, another PDE10A-selective inhibitor, MP-10, has no effect on neurite outgrowth in this model. Therefore, the effect of papaverine on neurite outgrowth may not be mediated by inhibition of PDE10A. Other PDE10A-selective inhibitors, such as the imidazol[1,5-a]pyridol[3,2-e]pyrazines, are effective at reducing stereotypy and hyperactivity in rats treated with phencyclidine or dopamine receptor agonists (Itoh et al., 2011). The highly selective PDE10A inhibitors MP-10 and TP-10 decrease hyperactivity, attenuate conditioned avoidance responses, recover prepulse inhibition deficits, and improve social odour recognition and novel object recognition in methamphetamine- or phencyclidine-treated rats (Kahler et al., 2010). Pfizer began a Phase I, placebo-controlled, randomized, double-blind, parallel assignment, safety/efficacy clinical trial for MP-10 in 2007. This trial demonstrated that MP-10 has a clearance of $4 \text{ mL min}^{-1} \text{ kg}^{-1}$, a half-life of 14 h, high oral bioavailability and low pharmacokinetic variability. Pfizer began a Phase II clinical trial for MP-10 with an anticipated end date of May 2008 and a primary end point of significant improvement for patients suffering schizophrenia on the Positive And Negative Symptoms Scale (PANSS). Unfortunately, this trial has been discontinued and the Pfizer website does not provide a clear statement regarding the reason for the trial being discontinued. Two Phase II clinical trials, utilizing the PDE4 inhibitor dipyrindamole are ongoing at the University of Maryland and Hospital Espirita de Porto Alegre (United States National Institutes of Health [NIH],

2011). Pfizer has also disclosed a patent for PQ-10, a papaverine-like PDE10A inhibitor. However, this compound appears to inhibit PDE10A and the cardiac-specific PDE3A isoforms, and can cause hypotension (Kahler et al., 2010).

The PDE5-selective inhibitor has been shown to improve socializing behaviours in animal models of schizophrenia. Sildenafil has been utilized as an adjunct therapy to risperidone for the treatment of patients suffering chronic schizophrenia (Akhondzedah et al., 2011). Forty patients were treated in a double-blind fashion with risperidone (6 mg/day), and sildenafil (75 mg/day) or placebo, for 8 weeks. Patients receiving sildenafil experienced a significant improvement compared with those given risperidone alone when symptoms were measured by positive and negative symptoms scale (PANSS). Importantly, no negative side effects were reported. The Massachusetts General Hospital recently completed a Phase IV clinical trial for the use of sildenafil on improving cognitive functioning, verbal memory, fluency, attention, spatial memory, motor speed, executive function, and reducing the incidence of psychoses and withdrawal symptoms in patients with schizophrenia. Their study utilized single daily doses of sildenafil (50 or 100 mg) for 12 days. Results have not yet been published (NIH, 2011).

6.2 Phosphodiesterase inhibition improves deficits in social interaction and mood associated with depression

Evidence from animal models and clinical trials demonstrating PDE inhibition could alleviate the negative symptoms of schizophrenia led investigators to explore the utility of these compounds in major depressive and bipolar disorders. Inhibition of certain PDEs can effectively improve depression-related symptoms in animal models. Moreover, several PDE inhibitors are currently being tested in clinical trials to confirm their efficacy in treating human depression. The animal model and clinical trial data concerning the antidepressant effects of PDE inhibitors add support to the utility of PDE inhibitors in treating the depressive symptoms observed among individuals with HD.

Isoforms of PDE4, particularly PDE4A and D, are expressed in the hippocampus and frontal cortex, which are areas classically considered to be the mediators of antidepressant drug effects. PDE4 inhibitors, such as rolipram, were first investigated for their antidepressant properties in animal models 27 years ago (Zhang et al., 2002). Treatment of rodents with the drug reserpine is used as a model of depression. Reserpine causes hypothermia in rodents, which is reversed by antidepressant drugs, such as tricyclic antidepressants. Rolipram is capable of reversing reserpine-induced hypothermia, and reducing immobility in the forced swim test. More recent animal models of depression include the learned helplessness model and the serotonin-depletion model. These animals are more likely to engage in pleasure-seeking activity (*i.e.* seek a mate) when treated with rolipram. Rolipram's antidepressant activities have been confirmed by several clinical trials, yet the drug has not been marketed because it causes emesis and gastrointestinal complications. Despite these complications, rolipram effectively demonstrates the utility of PDE4 inhibition for treatment of depression. As an antidepressant, rolipram is 30 times more potent than the tricyclic antidepressants imipramine or desipramine, and the effects of rolipram are potentiated by serotonin-selective reuptake inhibitors, suggesting serotonin-selective reuptake inhibitors and PDE inhibitors might be useful for the combinatorial treatment of depression (reviewed by Zhang et al., 2002). A new Phase II clinical trial is ongoing by the NIH to determine the

efficacy of low-dose rolipram for the treatment of major depressive disorder. The expected date of completion for this trial is December 2011. In this trial patients will receive rolipram, or placebo, for 3 years. During this time, symptoms of major depressive disorder will be monitored, brain PDE4 levels will be measured by PET scan, and the possible correlation of PDE4 level and major depressive disorder symptoms will be explored.

Inhibitors of PDE5 isoforms may represent equally promising means of treating depression. A double-blind, placebo-controlled clinical trial examined the usefulness of sildenafil for the treatment of mild-to-moderate, previously untreated, depression in men suffering erectile dysfunction (Kennedy et al., 2005). A total of 202 men were recruited for the trial, which lasted 6 weeks, and volunteers were treated with 50 mg sildenafil once daily, or placebo. Patients treated with sildenafil had significantly improved scores for the Beck Depression Inventory-II (BDI-II) and the erectile dysfunction domains, which are questionnaires to determine the depressive state and sexual satisfaction of a patient, respectively. Similar results were reported in an earlier, larger clinical trial, where the Self-Esteem And Relationship (SEAR) questionnaire was employed (Moncada et al., 2009). Inhibitors of PDE4 and 5 isoforms demonstrate robust antidepressant effects in animal models and clinical trials in men (Moncada et al., 2009).

PDE10A inhibition has also been investigated for antidepressant properties. Hypothermia is not reversed in reserpine-treated mice subsequently treated with papaverine or TP-10. Moreover, PDE10A knock-out mice do not differ from wild-type litter mates when they are tested in the forced swim test, which is considered a well-established test for depression in rodents (Siuciak et al., 2006).

6.3 Phosphodiesterase inhibition may be useful for cognitive enhancement in Alzheimer's Disease

Inhibitors of PDE isoforms have been investigated for their antidepressant and cognitive enhancement properties. Because of the ability of these compounds to enhance cognition and improve memory, they were tested in mouse models of Alzheimer's Disease. Treatment of transgenic Alzheimer's Disease mice (Tg2576), which over-express amyloid β precursor protein, with the PDE5 inhibitor sildenafil improves memory function, as measured in the Morris water maze, and significantly increases brain-derived neurotrophic factor levels in the hippocampus (Cuadrado-Tejedor et al., 2011). Brain-derived neurotrophic factor expression is decreased in human patients suffering from Alzheimer's Disease and major depressive disorder (Cuadrado-Tejedor et al., 2011). Brain-derived neurotrophic factor-mediated signal transduction is associated with increased protein kinase A activity, increased levels of phosphorylated CREB, and increased CREB-dependent gene expression (Zuccato et al., 2010). Brain-derived neurotrophic factor mRNA and protein levels are also decreased in HD. Decreased brain-derived neurotrophic factor is thought to contribute to decreased cell survival and gross atrophy during HD progression (Zuccato et al., 2010). Thus, the finding that sildenafil use might increase brain-derived neurotrophic factor levels suggests this drug might effectively delay neurodegeneration in disorders of the central nervous system such as HD.

Rolipram has also been investigated for Alzheimer's Disease treatment because of its nootropic properties. Initial evidence from pre-clinical models suggested inhibition of PDE4

isoforms would be logical in the context of Alzheimer's Disease because 1) they improved deficits in long-term memory in mouse models of Alzheimer's Disease, 2) they improved neurogenesis and pre-synaptic plasticity in mouse models of neurodegeneration, and 3) they evoked potent anti-inflammatory responses in mice challenged with lipopolysaccharide and other inflammatory agents (reviewed by Esposito et al., 2009). Moreover, expression of PDE4B is up-regulated in hippocampal neurons and microglia of mouse models of Alzheimer's Disease and autopsied Alzheimer's Disease human tissue (Cuadrado-Tejedor et al., 2011). Long-term potentiation, protein kinase A activity, and CREB phosphorylation are improved in hippocampal slice cultures derived from Alzheimer's Disease transgenic mice treated with rolipram (Cuadrado-Tejedor et al., 2011). Similarly, retention in the passive avoidance test is decreased, and an improvement in the Morris water maze test for memory is observed, in mice given amyloid β protein injections to the CA1 hippocampal region and treated with rolipram for 32 days (Cheng et al., 2010). Furthermore, coronal sections of hippocampus derived from mice treated with rolipram express phosphorylated CREB at significantly higher levels than mice that were not given rolipram. Despite these promising results, rolipram *per se* is not a useful drug for the treatment of Alzheimer's Disease because it causes emesis, insomnia, and cardiac arrhythmia. Current research in the area of PDE inhibitors for use in Alzheimer's Disease is focused on developing PDE4-selective inhibitors that do not cause emesis. Merck conducted a Phase II clinical trial for the use of their PDE4 inhibitor, MK-0952, on 55-year or older patients with mild-to-moderate Alzheimer's Disease in 2008. The trial has been completed and no significant improvements in patient cognitive function or memory were reported (NIH, 2011). One Phase II clinical trial, recently completed by Exonhit therapeutics, is attempting to establish the usefulness of the drug etazolate as a therapy for the treatment of Alzheimer's Disease. Etazolate is a PDE4 inhibitor and adenosine receptor antagonist. Data have not yet been published from this trial (NIH, 2011).

PDE1 inhibitors have shown nootropic properties in the treatment of Alzheimer's Disease, where PDE1 expression does not change. PDE1 inhibitors were first utilized clinically as vasodilators and anti-inflammatory agents. In 2003, the PDE1 inhibitor vinpocetine was investigated for its actions as a nootropic for therapeutic use in Alzheimer's Disease and dementia. Three clinical trials were conducted, all of which demonstrated significant cognitive improvement in patients treated with vinpocetine relative to those treated with placebo (reviewed by Szatmari & Whitehouse, 2003). However, the number of patients treated for a period greater than 6 months was too small for any conclusions to be drawn. The major side effect reported was agranulocytosis, a serious condition that reduces immune function. This potential adverse effect was considered too significant a risk to the treatment group. Consequently, no subsequent clinical investigations have been conducted.

6.4 Phosphodiesterase inhibition improves motor function in neurodegenerative disorders and traumatic central nervous system injury

PDE inhibition produces beneficial motor effects in animal models of neurodegenerative disorders such as Parkinson's Disease, and multiple sclerosis, as well as spinal cord injury, and ischemic-stroke. PDE7 inhibition, using the selective inhibitor S14, was shown to improve motor function in the lipopolysaccharide model of Parkinson's Disease, possibly due to protection of dopaminergic neurons in the substantia nigra (Picconi et al., 2011).

Long-term levodopa use by Parkinson's Disease patients causes involuntary jerky movements of the arms and/or head, which is described as dyskinesia. Dyskinesia also occurs in mice given 6-hydroxydopamine lesions to the substantia nigra and chronically treated with levodopa. Dyskinesia is reduced in these mice if they are administered striatal injections of zaprinast (PDE 5,6,9,11 inhibitor) or UK-343664 (PDE5 inhibitor) twice per day for 21 days (Picconi et al., 2011).

PDE5 inhibition by subcutaneous sildenafil treatment (10 mg/kg, once per day) for 8 days reduced the incidence and severity of abnormal movements in experimental autoimmune encephalomyelitis mice, a model of multiple sclerosis (Picconi et al., 2011). Sildenafil treatment in this study also improved neuropathology, as shown by reductions in 1) demyelination and axonal loss in spinal cord, 2) inflammatory cell infiltration, and 3) microglia activation (Picconi et al., 2011). PDE4 and PDE5 inhibition seem to play a beneficial role in motor recovery after traumatic injury of the central nervous system. Various selective PDE5 inhibitors have been shown to promote neurogenesis and improve motor recovery after cerebral ischemia (Picconi et al., 2011). Rolipram treatment also improves motor recovery following spinal cord injury in rats (Picconi et al., 2011).

6.5 Inhibitors of phosphodiesterases 2, 3, and 5, used in the treatment of peripheral disorders, have deleterious effects on the central nervous system

Several PDE inhibitors are used to treat disorders in the periphery. Although the main site of action for these inhibitors is peripheral tissue, several of these drugs effect central nervous system function either directly if they are blood-brain barrier penetrant, or indirectly via their effect on blood flow. The PDE2/3-selective inhibitor anagrelide is used to treat severe cases of essential thrombocytosis and is not considered blood-brain barrier penetrant (Sadhu et al., 1999). However, anagrelide is known to cause migraine headaches and dizziness. These effects are thought to stem from the vasodilation and decreased blood pressure often associated with chronic use of anagrelide.

Perhaps the most widely known and studied peripheral PDE inhibitors with actions in the central nervous system are PDE5-selective inhibitors, such as sildenafil. As mentioned above, sildenafil – and similar drugs – are commonly used to treat clinical erectile dysfunction. They also demonstrate nootropic, antidepressant, and motor control-enhancing properties in clinical trials for, and animal models of, depression, Alzheimer's Disease, and multiple sclerosis. However, sildenafil is also known to cause headaches in approximately 10% of all users, sudden hearing loss, and anterior optic neuropathy (NIH, 2011). Sildenafil-associated headaches are thought to be the result of altered blood flow. Sudden hearing loss and anterior optic neuropathy, however, are caused by PDE5 or 6 inhibition in the cochlear nerve and eye, respectively (NIH, 2011; see section 3.3). The Pfizer corporation recently completed a Phase III observational trial to determine the prevalence of anterior optic neuropathy in men using sildenafil for clinical erectile dysfunction. Their study found that the risk of developing anterior optic neuropathy for users of sildenafil was significant and posed a major risk to chronic users of sildenafil (odds ratio 5.73, prevalence of 0.35 per 1000 individuals). Pfizer is currently recruiting for a Phase IV clinical trial of sildenafil to explore the prevalence of anterior optic neuropathy in greater detail. The Eli Lilly company is conducting a parallel trial with another PDE5-selective inhibitor. Pfizer has also conducted clinical trials to determine the efficacy of sildenafil as a treatment of Miniere's Disease. In

their Phase II clinical trials, Pfizer found sildenafil did not significantly improve symptoms associated with Meniere's Disease, relative to placebo (NIH, 2011).

PDE inhibition has profound effects on physiology in the central nervous system and periphery. Preclinical data from animal models of schizophrenia, depression, Alzheimer's Disease, Parkinson's Disease, and multiple sclerosis suggest PDE 4, 5, and 10A inhibitors effectively improve deficits in socializing behaviour, cognitive function, and locomotor activity. Clinical data from schizophrenia, depression, and Alzheimer's Disease trials confirm the beneficial effects of PDE4 and 5 inhibitors on locomotor control, cognitive function, and mood. Unfortunately, PDE1, 4, 5, and 10A inhibitors are known to have certain adverse side effects, such as emesis in the case of rolipram, or anterior optic neuropathy in the case of sildenafil.

Given the efficacy of PDE inhibitors at improving locomotor and cognitive deficits, entertaining their potential utility in HD appears logical. Schizophrenia, depression, and Alzheimer's Disease share certain symptoms with HD, such as altered locomotor control, mood, or neurodegeneration. Therefore, the utility of PDE inhibitors has been investigated as a potential treatment of HD.

7. It is uncertain whether pharmacological inhibition of phosphodiesterases is beneficial for treatment of Huntington's Disease

Because inhibition of specific PDE isoforms has been shown to effectively reduce motor, cognitive, and emotional changes associated with several neurological disorders such as, Parkinson's Disease, schizophrenia, Alzheimer's Disease, and major depressive disorder, inhibition of PDEs may improve motor, cognitive and emotional changes that occur in HD. Despite data that PDE10A and PDE1B mRNA and protein levels are decreased early in HD and PDE4A mRNA levels decrease with aging, cAMP levels have been shown to be reduced in striatum of pre-symptomatic STHdh Q111/111 transgenic HD mice (Gines, 2003), and reduced cAMP levels have been observed in cerebral spinal fluid of HD patients (Cramer et al., 1984), and post-mortem caudate of HD patients (Gines, 2003). It has also been reported that phosphorylation of CREB, a transcription factor that is phosphorylated by cAMP-dependent protein kinase A, is decreased in models of HD. Consequently, it has been theorized that the decreased PDE10A, PDE1B, and PDE4A levels present in HD progression may represent compensatory changes that occur in response to even earlier decreases in cAMP levels (Kleiman et al., 2011). By this paradigm, inhibition of PDEs early in disease progression may represent a valid therapeutic approach to elevate cAMP levels and overcome changes to gene expression, which may contribute to HD pathogenesis. It is unknown at this point whether loss of PDEs is compensatory or pathogenic, however inhibition of PDE4 and PDE10A has been tested in animal models of HD.

7.1 PDE4 inhibition using rolipram shows beneficial effects in animal models of Huntington's Disease, however adverse effects associated with PDE4 inhibitors may limit their usefulness in Huntington's Disease

The first experiments to test whether pharmacological inhibition of PDEs was beneficial in HD used the PDE4 inhibitor rolipram. Rolipram treatment (1.5 mg/kg, intra-peritoneal injection once per day) for 2 and 8 weeks in rats that had received striatal lesions by direct

quinolinic acid injection to the striatum, a model used to recapitulate striatal degeneration that occurs in HD, resulted in decreased striatal cell loss as well as increased levels of phosphorylated CREB in the striatum (DeMarch et al., 2007). In a follow up study, Demarche and colleagues (2008) tested effects of rolipram treatment in the R6/2 transgenic mouse model of HD. Rolipram treatment (1.5 mg/kg, intra-peritoneal injection once per day) beginning at 4 weeks of age and continuing until euthanasia increased survival on a Kaplan-Meier curve by approximately 1.5 weeks, reduced gross brain atrophy, increased the number of surviving striatal neurons, reduced microglia activation, reduced the size and number of neuronal intranuclear inclusions, increased phosphorylated CREB levels in striatal and cortical neurons, and increased brain-derived neurotrophic factor levels in striatal cells (DeMarch et al., 2008). A later study from the same group extended previous findings by examining changes to the parvalbuminergic interneurons (Giampà et al., 2009). Parvalbuminergic interneurons display reduced CREB phosphorylation, and reductions in levels of a transcriptional co-activator CREB binding protein, which is believed to contribute to HD pathogenesis (Nucifora et al., 2001). In support of a beneficial effect of PDE4 inhibition in this study, rolipram treatment was found to increase the number of parvalbumin interneurons and normalize levels of CREB binding protein, a transcriptional co-activator that is thought to be inhibited by N-mHtt. Rolipram treatment also increased motor activity in an open field test, and increased the time spent on the rotarod. Taken together, these studies support a beneficial effect of rolipram treatment in rat chemical-lesion and transgenic mouse models of HD.

Although rolipram has shown beneficial effects for treatment of HD in animal models, clinical use of rolipram and other PDE4 inhibitors is not being pursued due to high incidence of adverse effects such as emesis, gastrointestinal problems, and insomnia (Giampà et al., 2010). Instead HD researchers have turned to other PDEs as a therapeutic target. The main candidate currently being investigated is PDE10A.

7.2 Inhibition of PDE10A produces conflicting results in mouse models of Huntington's Disease

PDE10A has been pursued as a pharmacological target for treatment of HD because PDE10A is selectively expressed in the caudate, which degenerates in HD (Hebb et al., 2004; Giampà et al., 2010; Lakics et al., 2010). To date, two studies have directly tested effects of PDE10A inhibitors in animal models of HD. In the first study, R6/1 and wild-type mice were treated with papaverine (20 mg/kg subcutaneous once daily, 30 minutes before behavioural testing) beginning at 8 weeks and continuing for 14 days. Wild-type mice displayed significantly increased anxiety-like behaviours using the light-dark test, but anxiety-like behaviour was absent in R6/1 mice. Reduced CREB protein levels in striatum of R6/1 and wild-type mice were also reported as shown by western blot and densitometric analysis. In the same study, effects of chronic papaverine treatment (20 mg/kg subcutaneous once daily for 42 days, given 30 mins before testing) in wild-type mice were also examined. Papaverine treatment led to distinct motor deficits, mild cognitive deficits, and anxiety-like behaviour as measured by rotarod, Morris Water Maze, and light-dark test respectively. In contrast, Giampa and colleagues (2010) showed that PDE10A inhibition using TP-10 (1.5 mg/kg intra-peritoneal injection, once daily) beginning at 4 weeks of age and continuing until euthanasia improved symptoms related to HD in R6/2 mice without

producing deficits in wild-type mice. TP-10 treatment was shown to improve motor deficits as shown by delayed development of hind paw claspings, increased time spent on rotarod, and increased distance travelled in an open field test. TP-10 treatment also decreased neurodegeneration, as shown by increased striatal and cortical neuron number, decreased number of neuronal intranuclear inclusions, and reduced microglia activation. Additionally, TP-10 treatment was shown to increase levels of phosphorylated CREB and brain-derived neurotrophic factor in the striatum and cortex of R6/2 mice. Differences between the results reported by Giampa et al. (2010) and Hebb et al. (2004) could be due to differences in pharmacological properties of papaverine and TP-10 or methodological differences such as behavioural tests used and age at which treatment was started. Consequently, effects of PDE10A inhibition in genetic mouse models of HD are not clear.

Other studies provide indirect evidence that PDE10A inhibition may be beneficial for treatment of HD. Threlfell and colleagues (2009) report that inhibition of PDE10A using striatal infusion of papaverine or TP-10, or systemic administration of TP-10, increases the probability that medium spiny projection neurons will depolarize in response to cortical input as shown by single-unit extracellular recordings performed in the dorsal striatum of anaesthetised rats. Loss of medium spiny neurons is believed to contribute to HD pathophysiology, so enhancement of medium spiny neurons responsiveness to cortical input represents a potentially beneficial effect of PDE10A inhibition in HD. Kleiman and colleagues (2011) observed changes in gene expression associated with chronic PDE10A inhibition that were predicted to provide neuroprotective effects in models of HD. Microarray analysis of RNA obtained from wild-type mice treated with TP-10 (25 mg/kg administered by oral gavage once per day for 18 days) showed down-regulation of mRNAs encoding histone deacetylase 4, follistatin, and claspin mRNAs in the striatum. Down-regulation of these mRNAs has been predicted to provide neuroprotection in HD (Hughes et al., 1999; Freudenreich and Lahiri, 2004; Thomas et al., 2008). In this study, no differences in gene expression were observed in PDE10A knock-out mice treated with TP-10, thus indicating that the effect of TP-10 on gene expression is selective for PDE10A. Kleiman and colleagues (2011) also showed that CREB-mediated transcription was significantly increased in striatum of wild-type mice treated with TP-10 (3.2 mg/kg subcutaneously for 1 week) using *in vivo* imaging of the bioluminescence produced from a CRE driven luciferase lentiviral vector. Taken together, these studies provide indirect evidence that inhibition of PDE10A may be beneficial in HD.

8. Conclusions

PDE10A and 1B mRNA and protein levels are decreased early in HD (Hebb et al., 2004). PDE4A mRNA levels decrease with aging (Hebb et al., 2004). Impaired function of PDE isoforms is associated with various pathological conditions of the central nervous system, so decreases in PDE10A and 1B may contribute to HD pathology. However, cAMP levels are reduced prior to symptom onset in rodent models of HD and it has been theorized that decreases in PDE10A and 1B may represent compensatory changes that occur in response to even earlier decreases in cAMP levels (Keliman et al., 2011). If this is true, then inhibition of PDEs early in disease progression represents a valid therapeutic approach to elevate cAMP levels and overcome changes in gene expression, which may contribute to HD pathogenesis. In support of this view, PDE4 inhibition via rolipram has shown beneficial results in mouse

models of HD (DeMarch et al., 2007; Giampa et al., 2009). Additionally, PDE10A inhibition by TP-10 treatment at 4 weeks of age reduces behavioural and cellular changes associated with HD progression in R6/2 mice (Giampa et al., 2010). Indirect evidence also supports a beneficial effect of PDE10A inhibition for treatment of HD, as TP-10 treatment increases the probability that medium spiny projection neurons fire in response to cortical input (Threfell et al., 2009) and both genetic ablation of PDE10A and TP-10 treatment result in gene expression changes that are predicted to be neuroprotective in HD (Kleiman et al., 2011). However, PDE10A2 mRNA expression is decreased at the level of transcription by N-mHtt in R6 mouse models of HD (Hu et al., 2004; Gomez et al., 2006). N-mHtt interacts with, and interferes with, the normal function of transcription factors and co-factors required for the appropriate expression of PDE10A2 (Hu et al., 2004). These data specifically argue against the hypothesis that decreased PDE expression represents a compensatory mechanism on the part of the cell during HD progression. PDE10A inhibition by papaverine produces marked cognitive and motor deficits in wild-type mice, while having no beneficial effect in R6/1 transgenic HD mice (Hebb et al., 2004). Moreover, PDE10A knock-out mice display cognitive deficits, including increased escape latency in the Morris water maze, and reduced spontaneous locomotor activity (Siuciak et al., 2006). Ablation of PDE1B is associated with increased spontaneous locomotor activity and reduced pleasure-seeking behaviour. The phenotype of PDE10A and 1B knock-out mice resembles those observed in several transgenic rodent models of HD. Taken together, these data indicate that decreased expression of PDEs 1B, 4A, and 10A may play a pathogenic role in HD.

HD progression is also associated with decreased expression of DARPP-32 and brain-derived neurotrophic factor. Evidence from PDE1B knock-out mice suggests that PDE1B is the major up-stream regulator of DARPP-32 activity in medium spiny projection neurons (Reed et al., 2002). Consequently, PDE1B inhibition in the context of HD, may have limited efficacy as DARPP-32 levels and activity are decreased. Decreased brain-derived neurotrophic factor is associated with decreased cell survival in Alzheimer's Disease and HD, and neuronal atrophy in major depressive disorder (Zuccato et al., 2010). Sildenafil may induce expression of brain-derived neurotrophic factor. However, PDE5 is not expressed at high levels in the caudate/putamen, so the effect of sildenafil may have little benefit in the treatment of HD neuronal cell loss.

To date, the majority of data regarding changes in PDE expression, or the efficacy of PDE inhibition in HD, have been collected in the R6 mouse model. The R6 transgenic mouse model of HD is limited in several respects. R6 mice over-express an N-terminal fragment of mHtt (Mangiarini et al., 1996). HD progression is accelerated by N-mHtt over-expression and certain aspects of HD pathophysiology, such as behavioural changes, may not be observed (Cha et al., 1998). Other, more physiologically accurate, transgenic mouse models of HD recapitulate the longitudinal progression of this disorder. For example, the Hdh/Q model, which is a knock-in mouse model expressing exon 1 of the human *huntingtin* gene containing 72 - 150 CAG repeats within the mouse *huntingtin* locus (Wheeler et al., 2002), display locomotor symptoms resembling those observed in human patients suffering HD, as well as decreased socializing behaviours, and anhedonia (Kennedy et al., 2005). Future studies that examine longitudinal changes in cAMP levels and PDE expression in the striatum of HD mice may elucidate whether decreased PDE1B, 4A, and 10A expression is compensatory or pathogenic. If PDE reductions are shown to be compensatory in HD

knock-in models, clinical trials could be conducted to determine whether PDE inhibitors could delay HD symptom onset without producing adverse side effects. PDE1 and 4 inhibitors cause agranulocytosis and emesis, respectively, and consequently may not be practical for use in treating HD. PDE10A inhibitors are not known to cause adverse side effects and may represent the most logical target in such clinical trials for the safe and effective treatment of HD.

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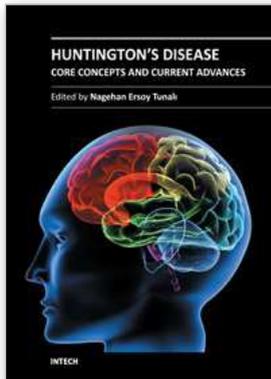
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Huntington's Disease is one of the well-studied neurodegenerative conditions, a quite devastating and currently incurable one. It is a brain disorder that causes certain types of neurons to become damaged, causing various parts of the brain to deteriorate and lose their function. This results in uncontrolled movements, loss of intellectual capabilities and behavioural disturbances. Since the identification of the causative mutation, there have been many significant developments in understanding the cellular and molecular perturbations. This book, "Huntington's Disease - Core Concepts and Current Advances", was prepared to serve as a source of up-to-date information on a wide range of issues involved in Huntington's Disease. It will help the clinicians, health care providers, researchers, graduate students and life science readers to increase their understanding of the clinical correlates, genetic aspects, neuropathological findings, cellular and molecular events and potential therapeutic interventions involved in HD. The book not only serves reviewed fundamental information on the disease but also presents original research in several disciplines, which collectively provide comprehensive description of the key issues in the area.

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