

Chapter 7

The Past, Present, and Future of Phosphodiesterase-4 Modulation for Age-Induced Memory Loss

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Abstract The purpose of this chapter is to highlight the state of progress for phosphodiesterase-4 (PDE4) modulation as a potential therapeutic for psychiatric illness, and to draw attention to particular hurdles and obstacles that must be overcome in future studies to develop PDE4-mediated therapeutics. Pathological and non-pathological related memory loss will be the focus of the chapter; however, we will at times also touch upon other psychiatric illnesses like anxiety and depression. First, we will provide a brief background of PDE4, and the rationale for its extensive study in cognition. Second, we will explore fundamental differences in individual PDE4 subtypes, and then begin to address differences between pathological and non-pathological aging. Alterations of cAMP/PDE4 signaling that occur within normal vs. pathological aging, and the potential for PDE4 modulation to combat these alterations within each context will be described. Finally, we will finish the chapter with obstacles that have hindered the field, and future studies and alternative viewpoints that need to be addressed. Overall, we hope this chapter will demonstrate the incredible complexity of PDE4 signaling in the brain, and will be useful in forming a strategy to develop future PDE4-mediated therapeutics for psychiatric illnesses.

Keywords Phosphodiesterase-4 (PDE4) • Aging • Memory • Cognition • cAMP signaling

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7.1 Introduction and Background of PDE4

Phosphodiesterases (PDEs) are a collective group of 11 enzyme families that hydrolyze the second messengers cAMP and/or cGMP. The PDE families are differentiated from one another through unique protein structure, tissue expression, cyclic nucleotide specificities, and distinct genes located on different chromosomes (Zhang et al. 2002; Conti et al. 2003; Houslay and Adams 2003; Shepherd et al. 2003; O'Donnell and Zhang 2004). Among the 11 PDE families, PDE4 has received much focus in regards to psychiatric illness due to its strong expression in the brain, in particular, the brain areas involved in psychiatric illness such as the prefrontal cortex, striatum, hippocampus, and amygdala.

PDE4 was first characterized as an enzyme capable of cAMP phosphodiesterase activity from rat liver plasma membranes (Marchmont and Houslay 1980); but, its involvement in cognition and behavior was first identified through mutations in the *Drosophila melanogaster* *dunce* PDE4D gene, which led to deficient cAMP-specific PDE activity and abnormal memory (Kauvar 1982; Qui et al. 1991). PDE4 was later discovered in both rodents (Davis et al. 1989; Swinnen et al. 1989) and humans (Bolger et al. 1993) to consist of a small family of enzymes encoded through four different genes, which are now referred to as PDE4-A, B, C, and D (Reeves et al. 1987; Wang et al. 1997; Houslay and Adams 2003; O'Donnell and Zhang 2004). These PDE4 subtypes are not expressed equally throughout the body. PDE4C has been shown to be largely deficient in the brain (Cherry and Davis 1999; Perez-Torres et al. 2000; Lakics et al. 2010). In regards to cognitive research, PDE4-A, B, and D are the predominant subtypes which are focused upon (Zhang 2009).

The canonical protein structure of PDE4 is divided into several domains along the N' to C' termini that each play unique functions in the role of the enzyme. The individual PDE4 splice variants have unique N' termini that are encoded through alternative promoters, and are involved with cellular localization and binding to unique binding partners (Beard et al. 1999, Beard et al. 2002; Jang et al. 2010). The PDE4 enzyme is contrasted from other PDEs in that it contains two upstream conserved regions (UCRs) termed UCR1 and UCR2 located immediately downstream of the N'-terminus region of the protein. These UCR regions play an important regulatory role, modulating both activity of the enzyme (Sette et al. 1996; Hoffmann et al. 1999; Houslay and Baillie 2003; Burgin et al. 2010) and dimerization with other PDE4 units (Richter and Conti 2002, 2004; Bolger et al. 2015). Within the C'-terminus lies the catalytic domain of the PDE4 enzyme which is responsible for the hydrolysis of cAMP to 5'-AMP (Saldou et al. 1998; Conti et al. 2003; Houslay and Adams 2003; Zhang 2009; Houslay 2010). From this canonical structure, there are then numerous PDE4 splice variants within each subtype that are produced through alternative promoters and splicing events. Twenty-five PDE4 splice variants have been identified to date, including 6 PDE4A splice variants, 5 PDE4B variants, 3 PDE4C variants, and 11 PDE4D variants (Bolger et al. 1996, Bolger et al. 1997, Bolger et al. 2003b; Huston et al. 1997; Sullivan et al. 1998; Rena et al. 2001; Miró et al. 2002; Shepherd et al. 2003; Wallace et al. 2005; Richter et al. 2005;

Chandrasekaran et al. 2008; Mackenzie et al. 2008; Zhang 2009). This alternative splicing results in the formation of different length PDE4s that are classified as long form, short form, super-short form, and dead-short form. Long-form PDEs are full length and contain no truncation, short forms lack a UCR1 region, super-short forms lack a UCR1 and have a truncated UCR2, and dead-short lack both UCR regions and also contain a truncated catalytic domain that render the PDE4 functionally inactive (O'Donnell and Zhang 2004; Zhang 2009; Richter et al. 2013). Interestingly, this alternative splicing has been shown to be increased in mammals and humans in parallel with increasing levels of cognitive capacity (Johnson et al. 2010). The following sections will describe the expression and broad behavioral roles of the individual PDE4 subtypes.

7.1.1 PDE4A

The PDE4A subtype has six splice variants; PDE4A1 is short (Sullivan et al. 1998), PDE4A7 is the dead-short form (Horton et al. 1995; Johnston et al. 2004), and PDE4A5 (PDE4A4 is the human orthologue) (McPhee et al. 1995; Huston et al. 2000; Beard et al. 2002), PDE4A8 (Bolger et al. 1996), PDE4A10 (Rena et al. 2001), and PDE4A11 (Wallace et al. 2005) are long forms (Bolger et al. 1997; Owens et al. 1997; Conti et al. 2003; O'Donnell and Zhang 2004; Cheung et al. 2007; Houslay et al. 2007; Chandrasekaran et al. 2008; Lynex et al. 2008; Zhang 2009). The broad PDE4A subtype has been found to be strongly expressed in the olfactory bulbs, deep layers of the cortex, amygdala, hypothalamus, hippocampus, piriform cortex, and cerebellum (Engels et al. 1995; Cherry and Davis 1999; Perez-Torres et al. 2000; MCPhee et al. 2001; D'Sa et al. 2005; Lakics et al. 2010; Johansson et al. 2012; Kelly et al. 2013).

PDE4A is one of the few PDE4 subtypes where differential splice-variant expression patterns have been looked at. MCPhee and colleagues found that the long PDE4A5/10 isoforms were present throughout most of the cortex, CA1, and CA2 in overlapping patterns. However, PDE4A10 was present in the islands of Calleja while PDE4A1 and PDE4A5 were not. In addition, PDE4A5 and PDE4A10 were present in the medial nucleus of the amygdala where PDE4A1 expression was absent. PDE4A1 had a different expression pattern than the longer isoforms, with very strong expression in the glomerular layer of the olfactory bulbs, CA3 of the hippocampus, the cerebellum, and low staining in the brain stem (McPhee et al. 2001). D'Sa and colleagues also looked at PDE4A expression, and observed *slightly* different patterns. Specifically, they saw that PDE4A1 was high in the medial septum, diagonal band, olfactory system, hippocampus and cerebellum; PDE4A5 was highly expressed in the olfactory nuclei, deep cortical layers, and the DG and CA1 of the hippocampus; PDE4A10 was highly expressed in the DG and CA1; and PDE4A8 expression was absent in the brain (D'Sa et al. 2005).

PDE4A1 has also been found to be unique, in that it is inserted into lipid bilayers through a TAPAS-1 (tryptophan anchoring phosphatidic acid selective-binding

domain 1); these bilayers are typically found on golgi that traffic out to the membrane (Baillie et al. 2002). PDE4A5 is unique from the remaining subtypes in that it can interact with SH3 domains on proteins through its N' Terminal region (O'Connell et al. 1996). This SH3 binding plays a significant role in the targeting of PDE4A5, which has been found to be expressed in perinuclear regions, and also within membrane ruffles (Beard et al. 2002). This expression pattern of PDE4A5 has been shown to be disrupted in apoptotic cells by cleavage of caspase 3 at its SH3 binding sites, and overexpression of PDE4A5 protects against apoptosis (Huston et al. 2000). Another unique trait of PDE4A5 is that it can bind to an immunophilin known as XAP2 which partially inhibits the enzyme up to 60%, and increases its sensitivity to the broad PDE4 inhibitor rolipram (Bolger et al. 2003b). Interestingly, PDE4A can also be activated by the P70S6 kinase in adipocytes during adipogenesis, which suggests it could play a role in obesity (MacKenzie et al. 1998). Lastly, PDE4A5 is the only member of the PDE4A family which can be phosphorylated by MAPK and subsequently attenuate its activation by PKA (MacKenzie et al. 2011). The PDE4A7 and PDE4A8 splice variants are exclusively found in the testis in rodents, however in humans PDE4A8 has undergone an evolutionary change which causes it to be expressed in the brain (Mackenzie et al. 2008).

The role of PDE4A in behavior is at a very preliminary level, however recent studies have shown that PDE4A knock out (KO) mice display increased anxiety-like behavior and enhancement in fear memory (Hansen et al. 2014). Other studies have also shown that PDE4A may play a role in memory deficits caused by sleep deprivation (Vecsey et al. 2009). To date, there are no known PDE4A mutations implicated in the etiology of any human disease, although PDE4A expression has been shown to be altered in the brains of autistic individuals (Fatemi et al. 2010). At this time, there are still no PDE4A selective pharmacological modulators.

7.1.2 PDE4B

PDE4B has five splice variants identified PDE4B1–5; PDE4B1, 3, and 4 are long isoforms, PDE4B2 is short, and PDE4B5 is super short (Bolger et al. 1997; Conti et al. 2003; O'Donnell and Zhang 2004; Cheung et al. 2007; Houslay et al. 2007; Chandrasekaran et al. 2008; Lynex et al. 2008; Zhang 2009).

Most of the PDE4 subtype expression patterns overlap a good bit and vary with different studies, but PDE4B is unique in the fact that it is consistently the highest PDE4 subtype expressed in the striatum, globus-pallidus, nucleus accumbens, hypothalamus, and amygdala (Engels et al. 1995; Cherry and Davis 1999; Perez-Torres et al. 2000; McPhee et al. 2001; D'Sa et al. 2005; Lakics et al. 2010; Johansson et al. 2012; Kelly et al. 2013). PDE4B is also unique in that mutations in PDE4B are strongly believed to play a role in schizophrenia through its interaction with disrupted in schizophrenia 1 protein (DISC1) (Millar et al. 2005; Fatemi et al. 2008; Numata et al. 2008, Numata et al. 2009; Kähler et al. 2010). Interestingly, it

was observed that this PDE4B mutation results in a decrease of PDE4B expression (Fatemi et al. 2008).

PDE4B KO mice studies have shown that PDE4B is the main PDE4 subtype responsible for the release of tumor necrosis factor- α (TNF- α) in response to lipopolysaccharide injections (LPS). In particular, these studies found that PDE4B KO reduced the amount of TNF- α released in response to LPS and PDE4A/PDE4D did not play a role (Jin and Conti 2002; Jin et al. 2005a). PDE4B was also specifically found to be altered in the brain after systemic LPS infusions, while PDE4A and PDE4D displayed no changes (Johansson et al. 2011). Thus, it appears that PDE4B may be one of the main PDE4 subtypes involved with regulation of microglia (Pearse and Hughes 2016), as PDE4B has also been shown to be involved with Amyloid beta induced microglia activation (Sebastiani et al. 2006). Additional studies have shown that PDE4B KO is anxiogenic and increases corticosterone levels, and also decreases depressive-like behavior in mice (Zhang et al. 2008).

Traditionally, it was not possible to target individual PDE4 subtypes pharmacologically due to the lack of highly selective inhibitors of specific PDE4 subtypes; however, recent studies have demonstrated feasibility for specific inhibition of PDE4B by targeting the Leu674 residue in PDE4B region control region 3 (CR3) (Fox et al. 2014; Hagen et al. 2014).

7.1.3 PDE4D

PDE4D has 11 identified splice variants termed PDE4D1–11; PDE4D1/2 are short forms, PDE4D6 is super short, and the remaining splice variants are all full length PDE4 proteins (Bolger et al. 1997; Conti et al. 2003; O'Donnell and Zhang 2004; Richter et al. 2005; Cheung et al. 2007; Houslay et al. 2007; Chandrasekaran et al. 2008; Lynex et al. 2008; Zhang 2009; Maurice et al. 2014).

PDE4D has a diffuse pattern of expression throughout the brain, with noticeable expression in the hippocampus, cortex, thalamus, area postrema, periaqueductal grey, brain stem, and cerebellum (Engels et al. 1995; Cherry and Davis 1999; Perez-Torres et al. 2000; McPhee et al. 2001; D'Sa et al. 2005; Lakics et al. 2010; Johansson et al. 2012; Kelly et al. 2013). Initial studies have demonstrated some differential expression of PDE4D splice variants in the brain, with PDE4D2 displaying distinct expression in the dorsal and median raphe nuclei, PDE4D1 in white matter cells, and PDE4D1 and 2 in the area postrema (Miró et al. 2002). PDE4D8 is predominantly expressed outside of the central nervous system, in particular in cardiac myocytes (De Arcangelis et al. 2009; Raymond et al. 2009; Mika and Conti 2015).

In addition, PDE4D also has specific binding partners such as myomegalin protein (Verde et al. 2001), beta arrestins (Baillie et al. 2003, Baillie et al. 2007; Bolger et al. 2003a; Lynch et al. 2005, Lynch et al. 2007; Li et al. 2009), RACK1 (Conti et al. 2003), SH3-domain regions (Beard et al. 1999) and the A-Kinase anchoring proteins (AKAPS), Erk, and exchange protein directly activated by cAMP (EPAC) (Dodge et al. 2001; Dodge-Kafka et al. 2005).

PDE4D has also been extensively studied using PDE4D KO mice designed by homologous recombination (Jin et al. 2005b) and through viral manipulation. PDE4D KO mice exhibit improved memory (Li et al. 2011), increased synaptic long-term potentiation (LTP) (Rutten et al. 2008), reduced levels of depressive-like behavior (Zhang et al. 2002), elevated neurogenesis (Li et al. 2011), and impaired growth and fertility (Jin et al. 1999). This is supported by studies showing that RNAi or inhibitors with relatively high selectivity for PDE4D also produce memory-enhancing effects (Burgin et al. 2010; Bruno et al. 2011; Li et al. 2011), reverse memory deficits induced by beta-amyloid peptide 1–42 (Zhang et al. 2014) and/or produce antidepressant activity (Wang et al. 2013). Furthermore, mice deficient in PDE4D have been shown to be less sensitive to the antidepressant effect of rolipam (Zhang et al. 2002). Together, these studies have strongly implicated PDE4D in memory as well as depressive-like behavior. Unfortunately, PDE4D KO also causes emetic-like behavior, the major side effect of broad PDE4 inhibitors such as rolipam (Robichaud et al. 2002a, b). Upon further investigation this data makes sense, as PDE4D has the highest expression of any of the PDE4 subtypes in the area postrema which is the emetic center of the brain (Cherry and Davis 1999; Miró et al. 2002; Mori et al. 2010).

PDE4D single nucleotide polymorphisms (SNP) have also been associated with stroke (He et al. 2013; Heyer et al. 2013; Liu et al. 2013a, b), however the results have been inconsistent (Shao et al. 2014). In a meta-analysis, Yan and colleagues found that PDE4D SNP83 is higher in Asian and Chinese populations, but not among Caucasians (Yan et al. 2014). PDE4D mutations have also been associated with skeletal dysplasia and intellectual disability (Lindstrand et al. 2014).

While it was previously very difficult to make a PDE4 subtype-specific inhibitor, x-ray structure of the PDE4 proteins (Wang et al. 2007a) revealed a phenylalanine-196 residue that was only present on PDE4D, which was able to be targeted by allosteric inhibitors (Burgin et al. 2010). These PDE4D allosteric inhibitors were able to reduce the emetic effects of PDE4D inhibition as measured through duration of anesthesia induced by ketamine/xylazine (Robichaud et al. 2002a, b) while maintaining the beneficial nootropic effects (Burgin et al. 2010). The memory enhancing effects of PDE4D disruption have also been supported by other recently developed PDE4D selective inhibitors (Bruno et al. 2011, 2014; Ricciarelli et al. 2017; Sierksma et al. 2013).

7.2 Differences Between Normal Aging and Pathological Aging

Contrary to public belief, normal aging does not necessitate a general decline of overall cognitive ability. Indeed, normal aging usually affects specific cognitive abilities such as spatial memory, working memory, reaction time, and long-term memory (Podtelezchnikov et al. 2011; Hansen and Zhang 2013). Not all individuals are affected in normal aging, and certain forms of cognition such as short-term

memory remain unchanged, while other forms of memory such as semantic memory can actually improve with age (Glisky 2007).

It was initially believed that with normal aging there is a natural loss of neurons in the brain (Brody 1955; Shefer 1973; Henderson et al. 1980); however, it is now believed there is no significant neuronal death that occurs with normal aging (Peters et al. 1998). The previous misconceptions were likely due to artifacts involved with the processing of aged brains (Haug 1985). Young brains shrink more during the fixation process, giving them the appearance of increased neuronal density (Peters et al. 1998). Once this confounding variable was accounted for through the use of unbiased stereological methods, the aged brain displays no differences in neuronal number (Giannaris and Rosene 2012).

One change normally observed in health aging is a decrease in brain volume (Skullerud 1985; Scahill et al. 2003; Driscoll et al. 2009) which may be due to decreases in neuronal size and somatic density (Terry et al. 1987). An alternative theory is that there is a loss of synapses in the aged brain (Geinisman 1999; Terry and Katzman 2001); however, others have observed no change of synapses in the aged brain (CA1 of the rat hippocampus), suggesting that memory deficits observed in senescence may be *functional* in nature (Geinisman et al. 2004). This decreased functional hypothesis is supported through observations that columns in the rhesus monkey brain display attenuated strength despite the fact that neuronal density and number are not changed (Cruz et al. 2004). These changes observed in normal aging are much different from what is seen in pathological aging.

Pathological aging or dementias such as Alzheimer's Disease (AD) are a significant diversion from the aging process, and have many differences from normal aging. Unlike normal aging which displays no significant changes in neuronal death, AD is characterized by massive atrophy in the brain which results in cognitive deficits much more severe than normal aging (Scheltens et al. 1995; Jack et al. 1997; Drachman 2006; Glisky 2007; Herrup 2010; Podtelezchnikov et al. 2011). The exact mechanisms and etiology for AD are still unknown, but in sporadic or non-familial cases (which account for the majority of AD cases) age is the main risk factor. Individuals over the age of 85 have a 50% chance of contracting this disease, and after contracting the disease there is an average survival of 5–10 years (Drachman 2006; Herrup 2010; Podtelezchnikov et al. 2011).

It has been suggested that AD could represent an accelerated aging process of the brain (Podtelezchnikov et al. 2011) and that the neurons could be undergoing massive functional transformation. Indeed, AD brains display significant changes in genes involved with lipid metabolism, the stress response, and inflammation. Interestingly, they also have changes in genes involved with cell adhesion, migration, and morphogenesis that bear a striking resemblance to patterns of gene expression also observed in epithelial mesenchymal transition (EMT) tissues (Podtelezchnikov et al. 2011). These findings reveal a similarity between AD brains and EMT that suggest a major transformation in brain tissue physiology and receptor signaling is occurring in AD. When the data obtained from the normal aged patients was extrapolated, it was predicted that in non-pathological aging, normal subjects would not reach cognitive decline comparable to AD subjects until

130–140 years of age (Podtelezchnikov et al. 2011). Other studies have confirmed that massive gene changes take place in AD. While some of these gene changes also occur in normal aging, the unique changes only in AD reinforce that pathological and normal aging processes are different (Miller et al. 2008). We will next focus on alterations that occur with cAMP and PDE4 with age, and discuss the correlations that this has with cognitive decline.

7.3 Alterations of cAMP/PDE4 Signaling in Normal Aging

There are many components to the cAMP pathway that could be altered with aging. The immediate downstream effector of cAMP is protein kinase A (PKA), which releases from autoinhibition upon cAMP binding, and then translocates to the nucleus where it phosphorylates cAMP response element binding protein (CREB). CREB is a 43 kDa transcription factor that binds to cAMP response element (CRE) promoter sites (Carlezon et al. 2005) located on specific CRE-mediated genes such as brain derived neurotrophic factor (BDNF) (Finkbeiner et al. 1997; Tao et al. 1998); however, thousands of other CREB targets have been identified (Conkright et al. 2003; Zhang et al. 2005). CREB has been highly implicated in learning and memory (Dash et al. 1990; Bourtchuladze et al. 1994) and long term potentiation (LTP), a putative mechanism for the formation of memory (Impey et al. 1996). Other downstream effectors for cAMP include exchange protein directly activated by cAMP (EPAC) (de Rooij et al. 1998; Kraemer et al. 2001; Bos 2006; Gloerich and Bos 2010); or hyperpolarization-activated cyclic nucleotide-gated (HCN) channels which are expressed throughout the brain (Moosmang et al. 1999) and can be activated by cAMP resulting in significant influences on neuronal activity strength and behavior (Ramos et al. 2006; Wang et al. 2007b; Arnsten et al. 2010). It is important to note that as a controller of cAMP, PDE4 and its subtypes therefore also regulate these downstream targets, and changes in PDE4 activity, expression, or even localization could all have significant effects downstream.

When addressing what effects normal aging has on cAMP levels there are many variables to take into consideration. First, it should not be expected that there would be a global increase or decrease of cAMP throughout the entire body or even within the brain itself; therefore, it's possible nuanced changes might be occurring within discrete brain regions or nuclei where one region displays increased cAMP and another displays decreased cAMP. It is also important to differentiate between baseline and stimulated levels of cAMP. Although baseline levels may remain unchanged, stimulated levels which would occur through application of a neurotransmitter such as dopamine or norepinephrine may reveal a different finding. Also, one needs to take into consideration the model organism, and the method with which the tissue was collected and cAMP measured. It has been suggested that cAMP and cGMP *in vivo* are notoriously difficult to study and can change quickly after death; because of this, some would argue that the only "accurate" way to measure cAMP from a living animal would be to perform microwave fixation (Stavinoha 1993;

Delaney and Geiger 1996; O'Callaghan and Sriram 2004; Murphy 2010). Microwave fixation is able to achieve near instantaneous denaturing of proteins (rendering them inactive) in the brain such as adenylyl cyclases (AC), proteases, phosphatases, and PDEs, all of which could contribute to confounding changes in cAMP levels after death in animals. Microwave fixation essentially allows the researcher to save a "snapshot" of the brain comparable to when the animal was alive, and avoids most of the confounds observed with various other methods of sacrificing (O'Callaghan and Sriram 2004).

With these variables in mind, there have been several studies which have sought to determine how cAMP changes with maturation and normal aging (Hansen and Zhang 2013). One of the first studies showed that in the cerebral cortex of aged rats there was a noticeable decrease in baseline cAMP from 26 to 2 pmol/mg at 2–6 months of age, respectively (Zimmerman and Berg 1974); however, levels were unchanged during the last 18 months of life. This is consistent with other studies that have shown decreased cAMP in aged rat cerebellum (Austin et al. 1978) and striatum (Sugawa and May 1994), senescent human lymphocytes (Birkenfeld and Ben-Zvi 1984), and the aged gerbil hippocampal CA1 region and cerebral cortex (Hara et al. 1992). Schmidt and Thornberry showed that in aged rats from 3–24 months of age, there was no decline of baseline cAMP levels in the hypothalamus, cortex, hippocampus, or brain stem; however, there was a 44% decrease of norepinephrine-stimulated cAMP levels in the cerebellum (Schmidt and Thornberry 1978). Others have also shown no change in baseline levels of cAMP with aging, yet observed attenuated dopamine activation of adenylyl cyclases (Puri and Volicer 1977; Sugawa and May 1994). Contradicting these results, elevations of cAMP have been observed in the striatum of aged rats (Sugawa and May 1993; Sugawa and May 1994). The data on how baseline levels of cAMP change with aging have yet to come to agreement; however, the effect of aging on activated levels of cAMP seems to be more consistent. It has been observed that adenylyl cyclase activity (Makman et al. 1980; O'Connor et al. 1981; Sugawa and May 1994) and expression (O'Connor et al. 1983; Araki et al. 1995; Ramos et al. 2003; Mons et al. 2004) decrease with age, suggesting that there may be a loss of hormonal sensitivity with aging (Boas et al. 1973). Baseline levels of adenylyl cyclase are actually increased in the aged caudate and cerebellum, while activated levels of AC are lower in all the brain regions tested (Boas et al. 1973). It is interesting that these two regions have altered baseline activity of adenylyl cyclase, as it was previously mentioned that both the striatum and cerebellum have been noted to have consistent changes in cAMP with aging. One possible explanation is that different brain regions age differently. While increased baseline adenylyl cyclase activity in the cerebellum could be a compensatory mechanism to account for a loss of cAMP (Austin et al. 1978; Schmidt and Thornberry 1978), increased baseline activity in the caudate could be the direct reason for elevated levels of cAMP observed in the aged striatum (Sugawa and May 1993; Sugawa and May 1994). Although these studies fail to converge on an overall unifying trend of how cAMP changes with aging in the brain, they at least suggest cAMP levels are altered, and these alterations may be specific to different brain regions.

Changes in cAMP observed with aging could be due to differences in neurotransmitter levels and altered PDE4 activity. Indeed, neurotransmitters such as dopamine, norepinephrine, acetylcholine, and glutamate have been observed to be decreased in the aged brain (Austin et al. 1978; Kaiser et al. 2005; Tomobe et al. 2007). Though there have been no consistent results on how PDE4 changes in the healthy aged brain, accumulating evidence suggests altered PDE4 activity and expression may contribute to changes in cAMP. PDE4 activity has been shown to be increased in the cortex, hypothalamus, and hippocampus of aged rats (Stancheva and Alova 1991); meanwhile decreases of PDE4 activity have been observed in the aged *Macaca mulatta* striatum and frontal cortex, and rat brain (Tohda et al. 1996). PDE4 expression was shown to be unchanged in the aged mouse hippocampus (Kelly et al. 2013), however it was decreased in the cerebral cortex which is in agreement with other studies (Tohda et al. 1996). Interestingly, PDE4A expression has also been shown to be decreased in the striatum (Kelly et al. 2013), which parallels the increase cAMP observed in this area (Sugawa and May 1993; Sugawa and May 1994). PDE4D is significantly decreased in the aged mouse cerebellum (Kelly et al. 2013), which has consistently shown age-related changes in cAMP levels (Austin et al. 1978; Schmidt and Thornberry 1978). One would expect decreased PDE4D expression in the cerebellum to result in increased cAMP levels. However, this decrease may be compensatory in nature to adapt to the falling levels of cAMP, which may be due to a loss of hormonal sensitivity or adenylyl cyclase activity. Other studies have not observed age-related changes of PDE4 in the brain (Puri and Volicer 1977; Tohda et al. 1996; Ramos et al. 2003). Overall, these findings highlight the incredible complexity of PDE4 signaling. Regulation of PDEs are elaborate, and the very molecule (cAMP) they hydrolyze also regulates their expression in a negative feedback loop (Liu et al. 2000; O'Donnell and Xu 2012). Interestingly, elevations of cAMP can increase PDE4 expression through a CREB dependent mechanism (D'Sa et al. 2002), thus downstream targets of cAMP also have further feedback regulation of its own activity.

Alterations of cAMP signaling should result in changes in downstream targets. Decreases of PKA activity have been seen in the aged fruit fly brain (Laviada et al. 1997) and hippocampus and frontal cortex of aged rats (Karege et al. 2001a, b). Decreases in PKA activity resulting from altered cAMP signaling should subsequently diminish phosphorylated activation of CREB (pCREB). Indeed, decreases of CREB signaling have been observed in the aged brain (Yamamoto-Sasaki et al. 1999; Hattiangady et al. 2005; Kudo et al. 2005; Porte et al. 2008; Xu et al. 2010). CREB is an important mediator of LTP and memory (Dash et al. 1990; Bourtchuladze et al. 1994; Yin et al. 1994; Impney et al. 1996; Johannessen et al. 2004; Carlezon et al. 2005; Brightwell et al. 2007) and enhancement of pCREB activation in the aged brain improves memory (Xu et al. 2010; Zhao et al. 2013). These results show that while cAMP signaling may be diminished in certain brain areas, restoration of downstream signaling may be able to rescue the behavioral deficits caused by this alteration in function. Together, this suggests that cognitive deficits which develop with normal aging are not permanent, and offers hope these deficits are functional in nature with the possibility of being treated.

7.4 Alterations of cAMP/PDE4 Signaling in Pathological Aging

Pathological aging such as AD also results in significant alterations of cAMP signaling. Alzheimer's patients display increased cAMP in their cerebrospinal fluid (CSF) (Martinez et al. 1999) and microvessels of the brain, particularly the hippocampus (Hernandez et al. 2001). BACE1 is the enzyme responsible for the proteolytic cleavage of amyloid- β protein that results in the hallmark neuritic plaques of AD. Interestingly, in addition to its contribution to the formation of amyloid plaques, BACE1 directly interacts with adenylyl cyclase via its transmembrane domain, and overexpression of BACE1 results in the reduction of cAMP signaling and downstream targets such as PKA and pCREB (Chen et al. 2012). Adenylyl cyclase activity and expression are also decreased in AD brains (Ohm et al. 1989, 1991; Cowburn et al. 1992; O'Neill et al. 1994; Schnecko et al. 1994; Yamamoto et al. 2000).

Altered adenylyl cyclase activity would most likely immediately affect PKA activation. Administration of amyloid- β to hippocampal neurons decreases PKA activity; when given the selective PDE4 inhibitor rolipram, this effect was able to be reversed (Vitolo et al. 2002). Interestingly, in both human AD and animal models there is a loss of LTP and spatial memory before neuronal death and morphological changes occur (Vitolo et al. 2002). These observations suggest biochemical changes precede anatomical changes, and might represent a potential site for therapeutic intervention. Increased PKA-regulatory subunit expression has also been observed in AD subjects (Blalock et al. 2003). Increased expression of the PKA-regulatory subunit could be predicted to decrease PKA activity and downstream signaling.

In addition to altered adenylyl cyclase activity, altered PDE4 signaling could directly result in cAMP alterations. McLachlan and colleagues observed decreased expression of PDE4 isoforms in the hippocampus of AD subjects, however, the PDE4D1 isoform doubled in expression (McLachlan et al. 2013). PDE4 expression also changes dynamically with the severity of the disease. During stage 1 through 2 of the Braak scale, AD brains display increased expression of PDE4A and PDE4B mRNA in the entorhinal cortex, and an increase of PDE4A mRNA in the frontal cortex (Pérez-Torres and Mengod 2003). Progression to Braak stages 3–4, results in decreased PDE4A expression in the frontal cortex and CA2 region of the hippocampus, and increased PDE4D expression in the putamen (Pérez-Torres and Mengod 2003). It is possible that in the early stages of AD or Braak stages 1–2, increased levels of PDE4 are a direct effect of amyloid- β plaques or oligomers, causing a decrease in cAMP levels and subsequent decrease in the cAMP/CREB pathway. However, in the later stages of the disease, the decrease in PDE4A expression seen in the frontal cortex and CA2 could be a compensatory mechanism due to chronically reduced levels of cAMP (O'Donnell and Xu 2012). Application of amyloid- β to rat microglial cells increases PDE4B expression and TNF- α production (Sebastiani et al. 2006). It is interesting to note that inflammation may be a major mediator of Alzheimer's disease (Rubio-Perez and Morillas-Ruiz 2012), and

that PDE4B is highly implicated in inflammation processes (Jin and Conti 2002; Jin et al. 2005a; Pearse and Hughes 2016). Inhibition of PDE4B and PDE4D may represent novel therapeutic targets for Alzheimer's disease (Gurney et al. 2015; Pearse and Hughes 2016).

Diminished cAMP signaling should manifest downstream through decreased phosphorylation of CREB. Phospho-CREB levels are reduced in the Alzheimer's Tg2576 mouse model, and exposure of rat primary hippocampal neurons to amyloid- β decreases CREB promoter activity (Pugazhenthil et al. 2011). Application of amyloid- β to hippocampal cultures also reduces glutamate-invoked pCREB levels (Vitolo et al. 2002), and rats infused with amyloid- β into the hippocampus display decreased pCREB and memory (Wang et al. 2012). Alzheimer's brains display an inverse correlation between amyloid- β levels and CREB (Pugazhenthil et al. 2011). Familial Alzheimer's mutations in amyloid precursor protein (APP) negatively regulate CRE-mediated transcription (Ikezu et al. 1996; Giambarella et al. 1997). Indeed, diminished pCREB has been observed in Alzheimer's brains (Yamamoto-Sasaki et al. 1999). However, other enzymes involved in the formation of amyloid- β such as APP and the presenilins do not always result in reduced pCREB and subsequent CRE mediated gene expression. Mutations in the presenilin gene which is observed in familial AD result in constitutive over-activation of CREB (Müller et al. 2011). The amount of amyloid- β present may also fundamentally affect how the CREB pathway is altered. For instance, moderate levels of intracellular amyloid- β increase CRE-mediated gene expression (Echeverria et al. 2005). However, when these levels are significantly raised beyond normal levels, or are aggregated into fibrils and plaques like in AD, there is a significant decrease in CRE-mediated gene expression (Echeverria et al. 2005; Arvanitis et al. 2007). This suggests the relationship between amyloid- β and CREB modulation may be more complicated than a simple linear relationship, and in fact may be more accurately represented by the "inverted U" model. Non-pathological levels of amyloid- β may increase CREB activity, while pathological levels decrease CREB activity. Both elevations and decreases in CREB-mediated transcription have been observed in AD brains, suggesting that CREB dysregulation as a whole is altered in AD pathology. CREB-mediated signaling is altered in the first stages of incipient AD, suggesting it could be one of the first pathways to change in the start and progression of the disease (Sato et al. 2009). Taking into consideration the aforementioned studies of how amyloid- β is able to both enhance and impair CREB, these observations should not be surprising. CREB has been shown to regulate thousands of gene transcription products (Johannessen et al. 2004; Carlezon et al. 2005). This dysregulation could represent both direct pathological manifestations of the disease and compensatory mechanisms aimed at combatting it.

7.5 Evidence for PDE4 Modulation as a Therapeutic Target in Pathological and Non-Pathological Aging

The literature suggests that cAMP signaling is altered in both normal aging and pathological aging; however, the question remains whether restoration of this signaling can improve cognitive function. Recent preclinical studies have shown that modulation of the cAMP pathway may attenuate cognitive deficits.

Amyloid- β decreases PKA and CREB activity in neuronal culture, and this effect is reversed through pretreatment with rolipram (Vitolo et al. 2002). Rolipram is also able to attenuate amyloid- β induced elevations of TNF- α and PDE4B in rat microglial cells (Sebastiani et al. 2006). This highlights the potential effects of PDE4 inhibition on inflammation, which is thought to play a main role in the disease (Frankola et al. 2011; Galimberti and Scarpini 2011; Clark et al. 2012; Montgomery and Bowers 2012; Rubio-Perez and Morillas-Ruiz 2012), and that targeting PDE4B may represent a novel therapeutic target for Alzheimer's disease (Pearse and Hughes 2016). In mouse models of AD, rolipram reverses deficits in LTP and memory, and pCREB levels in the hippocampus (Gong et al. 2004). These effects appear to be long lasting, as one course of chronic rolipram treatment was able to improve LTP and memory in AD mice 2 months after the end of their treatment (Gong et al. 2004). In other animal studies where amyloid- β was infused into the hippocampus, rolipram treatment was able to attenuate the effects of amyloid- β by improving memory and increasing pCREB levels (Cheng et al. 2010), and also reversed apoptotic responses (Wang et al. 2012). Reversal of apoptotic responses is particularly important in regards to pathological aging due to the large loss of neurons observed. PDE4 inhibition is also able to reverse morphological changes in AD models. Supporting this, Smith and colleagues found that treatment with rolipram reversed decreases in dendritic spine density in the hippocampus of transgenic AD-APP mice (Smith et al. 2009). The effects of rolipram have even been investigated against the deficits caused by iron loading in the brain, a process that occurs with aging and has been suggested to be responsible for age-induced cognitive impairments and neurodegeneration. Rolipram administration was able to reverse the effects of iron deposition on object recognition memory (de Lima et al. 2008). Rolipram and drugs that enhance cAMP signaling also rescue memory and LTP deficits in aged WT C57Bl/6J mice (Bach et al. 1999).

More recently there have been advances in the development of PDE4B and PDE4D subtype specific inhibitors (Burgin et al. 2010; Bruno et al. 2011, 2014; Azam and Tripuraneni 2014; Fox et al. 2014). The PDE4D specific inhibitor GEBR-7b enhances memory without the standard emetic side effects observed with rolipram (Miró et al. 2002; Mori et al. 2010; Bruno et al. 2011). GEBR-7b was also able to reverse the spatial memory deficits observed in AD APP^{swe}/PS1^{dE9} mice, although pCREB levels were unchanged (Sierksma et al. 2013). Knock down of the long form PDE4D splice variants also reversed the memory deficits caused by amyloid- β (Zhang et al. 2014). These findings are in agreement with earlier studies

suggesting PDE4D is the predominant subtype involved with memory processes (Burgin et al. 2010; Li et al. 2011).

Resveratrol is another compound that hints at the potential of PDE4 for aging related phenotypes; however it might work in a slightly different manner than rolipram. Resveratrol is a polyphenol compound typically found in red wine of which the nootropic neurotrophic effects have been known for some time (Tredici et al. 1999); however, it was recently discovered that resveratrol is a non-specific PDE inhibitor able to mimic the effects of caloric restriction, and reverse metabolic aging-like phenotypes (Park et al. 2012). Most recently, we found that resveratrol reversed A β 1-42-induced cognitive deficits via PDE4 inhibition and its subsequent activation of cAMP-CREB-BDNF signaling (Wang et al. 2016). The other pathway whereby resveratrol benefits aging is through a downstream cAMP effector known as EPAC, which is a guanine nucleotide exchange factor (GEF) for the small GTPases RAP1/2 (de Rooij et al. 1998; Kraemer et al. 2001; Bos 2006; Roscioni et al. 2008; Gloerich and Bos 2010) that exhibits implications for memory enhancement (Ouyang et al. 2008) and nerve growth (Murray and Shewan 2008; Murray et al. 2009). Reversal of age-induced metabolic phenotypes by resveratrol and EPAC was further found to be through the Sirtuin (SIRT) family (Park et al. 2012), which has been highly implicated in longevity and aging (Kim et al. 2007; Cristòfol et al. 2012). The effects of resveratrol were further explored in normally aged mice, and it was found that resveratrol was able to improve both memory and LTP following infusions into the cerebral ventricles (Zhao et al. 2013). Interestingly, the effect of resveratrol was not seen in SIRT1 KO mice. Further examination into the mechanisms of resveratrol action suggest downregulation of microRNAs 134 and 124, which are thought to regulate CREB expression (Zhao et al. 2013). These findings once again implicate CREB as one of the major potential downstream targets that might benefit from PDE4 inhibition and increased cAMP signaling.

The discovery of EPAC in downstream cAMP signaling has opened up new doors for therapeutic intervention. Interestingly, in the brains of AD patients, EPAC expression is altered (Mcphee et al. 2005). Typically, the two forms of EPAC (EPAC1 and EPAC2) have contrasting expression in the brain with EPAC2 more highly expressed in the CNS, and EPAC1 in the periphery; however, in AD brains EPAC1 expression is elevated, while EPAC2 expression is decreased (Mcphee et al. 2005). Other studies have also implicated EPAC in AD; activation of EPAC increased production of sAPP α (Maillet et al. 2003; Zaldua et al. 2007). This is a small neurotrophic molecule formed from the breakdown of APP in the non-amyloidogenic pathway. Thus, it is possible that activation of EPAC could shift the breakdown of APP away from the amyloidogenic pathway and the production of pathogenic levels of amyloid- β , towards the non-amyloidogenic pathway and production of therapeutic levels of the neurotrophic sAPP α .

7.6 Pitfalls and Side Effects of PDE4 Inhibition

Although targeting PDE4 for age-related memory loss seems promising, there are many side effects and pitfalls which need to be considered. The idea of using PDE4 as a target for psychiatric illness is not a novel one. Since PDE4 was first discovered, it was found shortly thereafter that the broad PDE4 inhibitor rolipram significantly improved memory (Randt et al. 1982; Egawa et al. 1997; Imanishi et al. 1997; Barad et al. 1998), and reversed depressive-like behavior in rodents (Wachtel 1983; Wachtel and Schneider 1986). Because of these initial findings, rolipram made it to clinical trials as a potential antidepressant and showed both promising (Zeller et al. 1984; Guiot-Goffioul et al. 1987; Laux et al. 1988) and inconsistent results (Bertolino et al. 1988). However, it was soon discovered that rolipram had a major flaw; several studies began confirming that rolipram induced severe nausea and emesis (Hebenstreit et al. 1989; Scott et al. 1991). The failure of rolipram in clinical trials leads to an important question, why *was* rolipram destined to fail? It has since been discovered that PDE4 is highly expressed in the area postrema (Cherry and Davis 1999) which is the emetic center of the brain (Mori et al. 2010). Inhibition of the PDE4 in this region “activates” the area postrema, and produces the emetic response typical of rolipram (Heaslip and Evans 1995; Robichaud et al. 2001; Richter et al. 2013). This emesis is most likely mediated by PDE4D, which has the highest expression levels in the area postrema (Cherry and Davis 1999; Miró et al. 2002; Mori et al. 2010). Subtype-specific inhibition would be one strategy to avoid the side effects caused by broad PDE4 inhibition. Recently subtype-specific inhibitors for PDE4B (Fox et al. 2014; Hagen et al. 2014) and PDE4D (Burgin et al. 2010; Bruno et al. 2011) have been developed that may avoid the strong emetic effects plaguing previous PDE4 inhibitors.

The development of broad PDE4 inhibitors for treating cognitive disorders in the future will be a difficult venture and is most likely not the most appropriate strategy. PDE4 is distributed throughout the body (Johansson et al. 2012), so treatment of a cognitive disorder with a broad PDE4 inhibitor would most likely result in many off-target effects. In addition, the multiple PDE4 subtypes and splice variants display differential expression in various brain regions and within individual neurons themselves. Within individual neurons distinct N' termini of the individual splice variants results in unique compartmentalization, which is important for the control of different transduction messages, and allows the neuron to retain specificity over different pathways (Catherine Jin et al. 1998; Houslay and Adams 2003; Martin and Cooper 2006; Baillie 2009; Houslay 2010; Oliveira et al. 2010; Blackman et al. 2011; Vincent et al. 2012). This suggests the PDE4 subtypes and splice variants have non-redundant functional roles, and further demonstrates that broad inhibition is not the most appropriate strategy.

In addition, it can't be assumed that elevations of cAMP/CREB signaling is beneficial in all brain regions. Although elevation of cAMP and pCREB enhances memory in the hippocampus, increasing cAMP in the amygdala could promote anxiety-like behavior or thoughts. In vitro studies demonstrate the anxiogenic

corticotrophin releasing factor (CRF) elevates cAMP signaling, whereas the anxiolytic compound neuropeptide Y (NPY) decreases cAMP signaling (Mulchahey et al. 1999; Sheriff et al. 2001). This parallels *in vivo* studies that show stress and anxiety are correlated with increases in pCREB (Adamec et al. 2011) and BDNF levels (Lakshminarasimhan and Chattarji 2012). Furthermore, overexpression of pCREB in the amygdala increases anxiety-like behavior (Wallace et al. 2004), and PDE4A and PDE4B KO mice display significant increases in anxiety-like behavior that correlate with an increase in cAMP signaling (Zhang et al. 2008; Hansen et al. 2014). Elevation of pCREB signaling in the nucleus accumbens can also have additional detrimental effects. Stress in rats produces an increase in CREB activation in the nucleus accumbens, and overexpression of CREB in this area leads to depressive-like anhedonia; dominant-negative infusion of CREB displays the opposite behavioral effect (Muschamp et al. 2011). Also, elevated cAMP/PKA activity in the prefrontal cortex impairs working memory (Taylor et al. 1999; Ramos et al. 2003), and aged rats have elevated levels of CREB and pCREB in the PFC as they age (Ramos et al. 2003; Vandesquille et al. 2013). It should come as no surprise that working memory and long-term memory (LTM) are affected differently by cAMP signaling as working memory “requires the continuous and dynamic updating of memory buffers, whereas long-term memory consolidation involves changes that are static and long lasting” (Arnsten et al. 2005).

One other pitfall is that the effects of altering cAMP levels on different pathological targets remain to be fully understood. While the majority of research shows amyloid- β decreases cAMP/CREB signaling, there are some studies showing the opposite (Echeverria et al. 2005; Satoh et al. 2009; Müller et al. 2011). CREB is one of the principal transcription factors affected in AD. A complete dysregulation of CREB signaling has been observed with some transcripts being up regulated and others down regulated (Blalock et al. 2003). Eliciting pathological changes from compensatory changes observed in this altered CREB signaling are a significant hurdle as cAMP/PDE4 signaling is notorious for being incredibly compensatory (O'Donnell and Xu 2012). Globally raising cAMP could have detrimental effects contributing to the pathology of AD. For instance, raising cAMP levels causes an increase in APP protein expression, and activation of both the amyloidogenic and non-amyloidogenic pathways (Canepa et al. 2013). Assuming the “amyloid hypothesis” of AD is correct, increasing APP could have detrimental effects on the amount of amyloid- β plaques produced and subsequent cognitive performance. Increased cAMP signaling also results in hyper-phosphorylation of tau protein, one of the hallmark pathologies of AD (Litersky and Johnson 1992; Jicha et al. 1999).

7.7 The Future of PDE4 for Therapeutic Intervention

Although much has been learned about PDE4 in cognition, there is still much to be discovered. First, the field needs to consistently characterize where individual PDE4 splice variants are expressed in the brain, and the roles these variants play in

behavior and cognition. This is one of the most complicated challenges that need to be overcome due to the large number of splice variants in the PDE4 family, unique expression of these splice variants in different brain regions, and localized expression of these variants within individual neurons which leads to microdomains and compartmentalization of signaling. Progress has been made with the development of PDE4B and PDE4D inhibitors (Burgin et al. 2010; Bruno et al. 2011; Fox et al. 2014); however, there are still no PDE4A specific inhibitors, nor are there drugs capable of targeting specific PDE4 splice variants. An optional approach to using drugs to identify the functional roles of PDE4 specific splice variants would be to use genetic approaches such as viral-mediated knockdown (Li et al. 2011; Wang et al. 2013; Zhang et al. 2014). Due to the incredible number of PDE4 splice variants, this will take time and patience. Characterization of unique splice variant compartmentalization is a fundamental issue that needs to be addressed in future research, as this may allow for targeted intervention of unique downstream targets for the individual splice variants that may not be able to be resolved through subtype inhibition.

Inhibition of PDE4 may also not be appropriate in all scenarios. One should recognize the incredibly delicate balance of cAMP signaling in the brain, and that global activation of cAMP through broad PDE4 inhibition might not be the best approach. In regards to working memory, it appears that decreases in cAMP levels in the PFC would actually improve memory. This also might hold true in the nucleus accumbens and amygdala, as overexpression of CREB in these regions has been shown to produce anhedonia (Muschamp et al. 2011) and anxiety, respectively (Wallace et al. 2004). There may need to be a paradigm shift in the way we think about PDE4 modulation as a therapeutic for cognitive disorders; in particular, researchers may need to begin thinking about PDE4 activation as a possible therapeutic strategy for the future. This makes sense in regards to anxiety or working memory. However, in regards to hippocampus-dependent memory PDE4 inhibition may still be the best approach.

Despite these drawbacks facing PDE4 as a therapeutic for aging and AD, PDE4 cognitive therapy remains promising. Many of the initial mechanisms that are disrupted in normal aging are also present in the early and later stages of AD. If animal models hold true, PDE4 inhibition may reverse morphological and functional behavioral changes that occur both in senescent memory and the initial pathological decline in AD. One of the main differences between AD and normal aging is the large number of neurons are killed as pathological aging progresses, whereas no significant neuronal loss occurs with normal aging. Thus, it seems that in normal aging the biological mechanisms may become stagnant, which shows great promise for PDE4 modulation as a therapeutic to “wake up” the cAMP signaling pathways. In regards to AD, the most promise for PDE4 modulation would be at the beginning of the disease before neuronal death begins to occur, and the signal transduction pathways are beginning to be changed. Once neuronal death begins to occur, the best hope for PDE4 therapies would be to stop further progression of the disease through increased neurogenesis or decreased apoptosis. It is unknown if PDE4 modulation would be able to restore lost cognitive ability derived from neuronal

loss; however, elevations of cAMP signaling increases neurogenesis and enhances memory (Li et al. 2011), indicating a role of PDE4 in cognitive deficits caused by neuronal loss. Overall, PDE4 modulation could represent a prophylactic therapeutic for AD and other CNS diseases with cognitive deficits. However, more research is needed into the field to characterize the consequences of altering cAMP levels in a disease as severe as AD.

Conflict of Interest The authors declare that they have no conflicts of interest.

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