

# Journal Pre-proof<sup>1</sup>

Phosphodiesterase-4 enzyme as a therapeutic target in neurological disorders<sup>2</sup>

Abid Bhat, Bipul Ray, Arehally Marappa Mahalakshmi, Sunanda Tuladhar, DN Nandakumar, Malathi Srinivasan, Musthafa Mohamed Essa, Saravana Babu Chidambaram, Gilles J. Guillemin, Meena Kishore Sakharkar<sup>3</sup>



PII: S1043-6618(20)31386-4<sup>4</sup>

DOI: <https://doi.org/10.1016/j.phrs.2020.105078>

Reference: YPHRS 105078

To appear in: *Pharmacological Research*

Received Date: 18 March 2020

Revised Date: 9 July 2020

Accepted Date: 10 July 2020

Please cite this article as: Bhat A, Ray B, Mahalakshmi AM, Tuladhar S, Nandakumar D, Srinivasan M, Essa MM, Chidambaram SB, Guillemin GJ, Sakharkar MK, Phosphodiesterase-4 enzyme as a therapeutic target in neurological disorders, *Pharmacological Research* (2020), doi: <https://doi.org/10.1016/j.phrs.2020.105078><sup>5</sup>

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.<sup>6</sup>

© 2020 Published by Elsevier.<sup>7</sup>

**Phosphodiesterase-4 enzyme as a therapeutic target in neurological disorders** <sup>1</sup>

Abid Bhat<sup>1</sup>, Bipul Ray<sup>1</sup>, Arehally Marappa Mahalakshmi<sup>1</sup>, Sunanda Tuladhar<sup>1</sup>, Nandakumar DN<sup>3</sup>,  
Malathi Srinivasan<sup>4</sup>, Musthafa Mohamed Essa<sup>5,6\*</sup>, Saravana Babu Chidambaram<sup>1,2\*</sup>, Gilles J.  
Guillemin<sup>7\*</sup>, Meena Kishore Sakharkar<sup>8</sup>

<sup>1</sup>Dept. of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education & Research, Mysuru, India <sup>2</sup>

<sup>2</sup>Centre for Experimental Pharmacology and Toxicology, Central Animal Facility, JSS Academy of Higher Education & Research, Mysuru, India

<sup>3</sup>Department of Neurochemistry, National Institute of Mental Health and Neurosciences (NIMHANS), Bengaluru, 560029, India.

<sup>4</sup>Department of Lipid Science, CSIR - Central Food Technological Research Institute (CFTRI), CFTRI Campus, Mysuru – 570020, India

<sup>5</sup>Ageing and Dementia Research Group, Sultan Qaboos University, Muscat, Oman

<sup>6</sup>Department of Food Science and Nutrition, CAMS, Sultan Qaboos University, Muscat, Oman

<sup>7</sup>Neuroinflammation group, Faculty of Medicine and Health Sciences, Macquarie University, NSW, 2109, Australia

<sup>8</sup>College of Pharmacy and Nutrition, University of Saskatchewan, 107, Wiggins Road, Saskatoon, SK, Canada S7N 5C9

**\*For correspondence – author's addresses** <sup>3</sup>

**Dr. Saravana Babu Chidambaram**, MPharm, PhD, FIST, FICS

Associate Professor, Department of Pharmacology

JSS College of Pharmacy & Coordinator, CPT, JSSAHER,

Mysuru, Karnataka 570015, India

Email: babupublications@gmail.com

Mob: +91-9042222277 / +91-9940434129

**Dr. Musthafa Mohamed Essa**, PhD <sup>4</sup>

Associate Professor

Food Science and Nutrition

Sultan Qaboos University, Oman

Tel: +(968) 2414 3604 Fax: +(968) 2441 3418

Email: drmdessa@squ.edu.om

**Prof Gilles J. Guillemin**

Head of the Neuroinflammation group

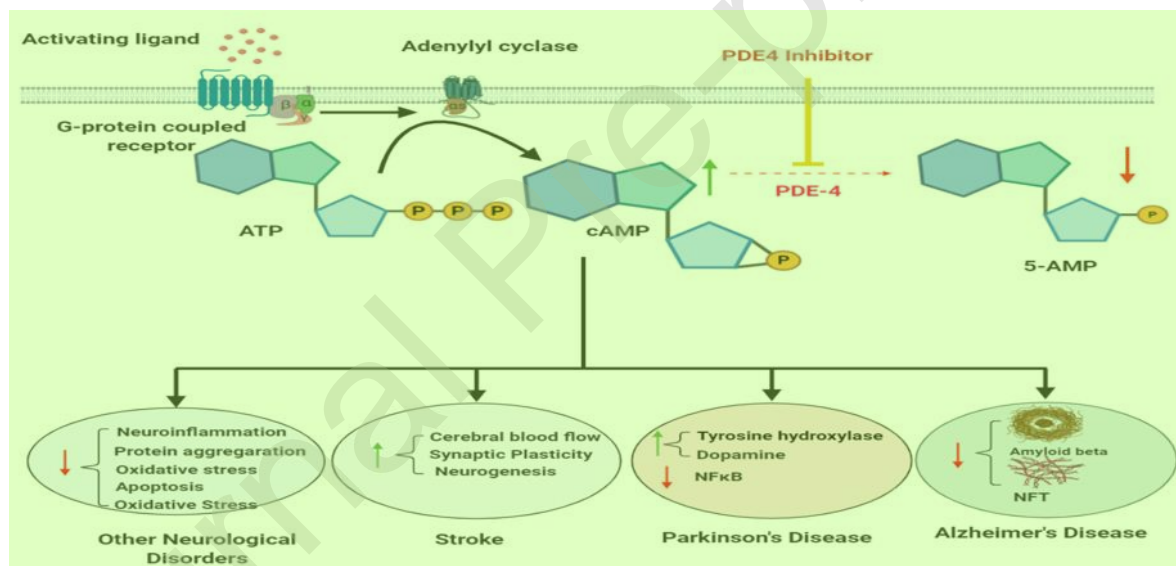
Faculty of Medicine and Health Sciences

Macquarie University|NSW, 2109 Australia

Email: gilles.guillemin@mq.edu.au

Tel: 61 (02) 9850 2727

#### Graphical abstract



#### Abstract

Phosphodiesterases (PDE) are a diverse family of enzymes (11 isoforms so far identified) responsible for the degradation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) which are involved in several cellular and biochemical functions. Phosphodiesterase 4 (PDE4) is the major isoform within this group and is highly expressed in the mammalian brain. An inverse association between PDE4 and cAMP levels is the key mechanism

in various pathophysiological conditions like airway inflammatory diseases – chronic obstruction pulmonary disease (COPD), asthma, psoriasis, rheumatoid arthritis, and neurological disorders etc.<sup>1</sup> In 2011, roflumilast, a PDE4 inhibitor (PDE4I) was approved for the treatment of COPD. Subsequently, other PDE4 inhibitors (PDE4Is) like apremilast and crisaborole were approved by the Food and Drug Administration (FDA) for psoriasis, atopic dermatitis etc. Due to the adverse effects like unbearable nausea and vomiting, dose intolerance and diarrhoea, PDE4 inhibitors have very less clinical compliance. Efforts are being made to develop allosteric modulation with high specificity to PDE4 isoforms having better efficacy and lesser adverse effects. Interestingly, repositioning PDE4Is towards neurological disorders including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS) and sleep disorders, is gaining attention. This review is an attempt to summarize the data on the effects of PDE4 overexpression in neurological disorders and the use of PDE4Is and newer allosteric modulators as therapeutic options. We have also compiled a list of on-going clinical trials on PDE4 inhibitors in neurological disorders.

## Abbreviations<sup>2</sup>

**AD:** Alzheimer's disease; **AIDS:** Acquired immunodeficiency syndrome; **AMP:** Adenosine monophosphate; **AMPK:** 5' AMP-activated protein kinase; **AMPA:**  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; **APP:** Amyloid precursor protein ; **ATP:** Adenosine triphosphate; **AB:** Amyloid- $\beta$ ; **BDNF:** Brain-derived neurotrophic factor; **CA1:** cornu ammonis-1; **cAMP:** Cyclic adenosine monophosphate; **CAR:** Conditioned avoidance response; **CBP:** CREB-binding protein; **cGMP:** cyclic guanosine monophosphate; **CNS:** Central nervous system; **COPD:** Chronic obstruction pulmonary disease; **CREB:** cAMP-response element binding protein; **DA:** Dopamine; **DISC1:** Disrupted in schizophrenia 1; **DNA:** Deoxyribonucleic acid; **EAE:** Experimental autoimmune encephalomyelitis; **EGF:** Epidermal growth factor; **ERK:** Extracellular signal-regulated kinase; **fALS:** Familial Amyotrophic Lateral Sclerosis; **FDA:** Food and Drug Administration; **fMRI:** Functional magnetic resonance imaging; **GABA:** Gamma aminobutyric acid; **GBM:** Glioblastoma; **GluR1:** Glutamate receptor1; **H<sub>2</sub>O<sub>2</sub>:** Hydrogen peroxide; **HD:** Huntington's disease; **Htt:** Huntingtin; **IBs:** Inclusion bodies; **IFN- $\gamma$ :** Interferon gamma; **IL-1  $\beta$ :** Interleukin-1beta; **IL-12:** Interleukin-12; **IL-23:** Interleukin-23; **IL-6:** Interleukin-6; **JNK:** c-Jun N-terminal kinase; **LPS:** Lipopolysaccharides; **LTP:** Long term plasticity; **MAPK:** Mitogen-activated protein kinase; **MDM2:** Mouse double minute 2 homolog; **MPP<sup>+</sup>:** 1-methyl-4-

phenylpyridinium; **MPTP**: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; **MS**: Multiple sclerosis; **NA**: Noradrenaline; **NFT**: Neurofibrillary tangles; **NF- $\kappa$ B**: Nuclear factor kappa-light-chain-enhancer of activated B cells; **NGF**: Nerve growth factor; **NMDAR**: *N*-methyl-D-aspartate-type glutamate receptors; **NO**: Nitric oxide; **NOS**: Nitric oxide synthase; **NT-3**: Neurotrophin-3; **NT-4**: Neurotrophin-4; **PC12**: Pheochromocytoma cells; **PD**: Parkinson's disease; **PDE**: Phosphodiesterase; **PDE4**: Phosphodiesterase 4; **PDE4I**: Phosphodiesterase 4 inhibitor; **PKA**: Protein kinase A; **PKG**: Protein kinase G; **PSD-95**: Postsynaptic density protein 95; **RSK2**: Ribosomal S6 kinase 2; **SAH**: Subarachnoid hemorrhage; **sALS**: Sporadic amyotrophic lateral sclerosis; **sGC**: Soluble guanylyl cyclase; **SH-SY5Y**: Human neuroblastoma; **SNpc**: substantia nigra pars compacta; **SSRI**: Selective serotonin reuptake inhibitor; **TBI**: Traumatic brain injury; **TH**: Tyrosine hydroxylase; **TNF $\alpha$** : Tumor necrosis factor alpha; **UCR**: Upstream conserved region; **VD**: Vascular dementia

**Keywords:** Phosphodiesterase, cAMP, cGMP, neurological disorders, PDE4 inhibitors, central nervous system

## 1. Introduction

Phosphodiesterases (PDEs) are a super-family of enzymes reported to be involved in inflammatory diseases like chronic obstructive pulmonary disease (COPD) and asthma (1). A total of 11 PDE enzymes have been identified and are present in almost all the cells in mammals. PDE enzymes hydrolyse cyclic nucleotides – cAMP and cGMP and thus plays important roles in cellular functions (1,2). PDE4 enzyme is predominant amongst the 11 PDE super-family enzymes and has four isoforms - PDE4A, PDE4B, PDE4C and PDE4D. PDEs interact with myomegalin (a structural protein), which is highly expressed in skeletal and cardiac muscles and play crucial role in cardiac contractility (3). Colocalization of myomegalin and PDE4D in Golgi-centrosomal structure is involved in cytoskeletal assembly and disassembly and organellar movements indicating that PDE4 plays crucial role in cellular functions (4). The deleterious effects of increased expression of PDE4 in different pathological conditions was confirmed in experimental models (5–7). Clinically, overexpression of PDE4 is recorded in cancer (8), retinal degeneration (9), acrodysostosis (10), Alzheimer's disease (AD) (11), stroke (12) and in several neurological

disorders (13,14). PDE4 enzymes hydrolyse cAMP and PDE4Is increase cAMP levels by preventing its breakdown. The ability of PDE4Is to increase cAMP in different pathological conditions has attracted clinical attention (15,16). US-FDA has approved PDE4Is for the treatment of various diseases, for example, PDE4B inhibitor (Roflumilast and Apremilast), for COPD (17) and psoriasis treatment, respectively. These inhibitors suppress  $\text{TNF}\alpha$ , IL-17, IL-23 expression and up-regulate the anti-inflammatory gene IL-10 (18). Interestingly, PDE4 enzyme is highly expressed in the brain and is deemed to be a potential target in the treatment of neurological disorders. Several PDE4Is are in Phase I and II clinical trials for neurological disorders like Parkinson's disease (PD), Huntington's disease (HD), AD, depression, and multiple sclerosis (MS). However, these inhibitors have dose limiting side effects such as nausea, vomiting and gastric acidity that has resulted in low clinical compliance (19).

Other PDE subfamily inhibitors like PDE5 and PDE7 inhibitors have shown neuroprotective ability in preclinical investigations but have serious side effects like non-arteritic anterior ischemic optic neuropathy (20) and hearing loss and hence are not a preferred option clinically (21). Furthermore, these PDE5 and PDE7 inhibitors exert undesirable effects on the cardiovascular functions including tachycardia and reduced peripheral blood pressure (22). No clear information on the safety and efficacy of PDE8, 10 and 11 inhibitors are available as of today and this needs to be investigated. Recently, PDE4 allosteric modulators -GSK256066, GEBR-7b, D159687, D159797 and TAK-648 were developed and have shown improved blood brain barrier penetrability, high potency and low side effects (23,24). These allosteric modulators are found to have improved efficacy in inflammatory diseases (25), diabetes (26), autoimmune disorders (27) and neurological disorders (28). The current review summarises data on the structure and localization and the role of PDE4 and PDE4Is in key molecular signalling pathways in brain. The therapeutic potential of PDE4 inhibitors in various neurological disorders and the data on the ongoing clinical trials on PDE4Is for treating neurological disorders is also presented.

## 1. Structure of PDE4

PDE enzymes possess conserved catalytic domain (25-49%) with almost 300 amino acids that suggests comparable three dimensional configurations of these enzyme domains (PDB code 1TB7) (29). PDE4 is highly specific for cAMP and has a low  $K_m$  of 1–3  $\mu\text{M}$  (30). PDE4 has four isoforms 4A, 4B, 4C, and 4D that have upstream conserved region (UCR) which regulates intracellular



signalling (29). Based on the UCRs they possess, PDE4s are divided into subgroups such as long, short, and super short isoforms (**Fig. 1**). Long isoforms have both UCR1 and UCR2, short isoforms have UCR2, while super-short isoforms have a truncated UCR2 (30,31) leading to variance in the regulation as UCR1 phosphorylates protein kinase A (PKA) dependent enzyme activation. Phosphorylation of short isoforms in catalytic domain by phosphoinositide-3-kinase and extracellular signal-regulated kinase (ERK) results in inhibition of PDE4 (32,33). The catalytic domain of PDE4B folds into a novel compact structure composed of 17- $\alpha$  helices and three sub-domains (34). Subdomain 1 is also known as N-terminal domain and comprises of seven  $\alpha$ -helices (H1–H7). Subdomain 2 contains four  $\alpha$ -helices (H8–H11) while subdomain 3 is made up of five  $\alpha$ -helices (H12–H16) with an extended loop that forms a  $\beta$  hairpin between H12 and H13 (35). The three sub-domains join to form a compact pouch which has 12 (out of 17) residues fully conserved in all PDE families. This is the catalytic pocket and has a volume of about  $450\text{\AA}^3$  and can accommodate a molecule of about  $250\text{\AA}^3$  in size (such as cAMP). In the crystal structure of PDE4B, residues of the catalytic pocket and Helix 17 (residue 496–508) interact through crystallographic symmetry. Also, the random loop of residues 422–434 in PDE4D2 corresponds to helix H17 in PDE4B (**Fig.2**). The conformational modification of the C-terminal residues in the PDE4 family along with the sequence multiplicity in the 11 PDE families suggests that the C-terminus is involved in the regulation of PDE hydrolytic activity. There are two conserved  $\text{Zn}^{2+}$  binding consensus motifs in the amino terminal segment of the catalytic site of the PDEs, and both sides are reportedly involved in the catalysis. Of these, site A has five conserved amino acids with a sequence HNXzHG/AX2, while site E and site B have a sequence HDZXHX<sub>24-26</sub>E, sharing more homology with the known  $\text{Zn}^{2+}$  binding site of thermolysin (36). Divalent metals like  $\text{Mg}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Zn}^{2+}$  are needed for the enzymatic activity of PDE4. The number of zinc atoms required for binding is under debate. Reports suggest that one  $\text{Zn}^{2+}$  ion per PDE4A monomer, two  $\text{Zn}^{2+}$  ions for PDE7 (*Vibrio fischeri*) and PDE4A, and three  $\text{Zn}^{2+}$  ions for PDE5 are required for activation. The enzymatic site of PDE4 comprises two metal ions separated by  $3.9\text{\AA}$ , suggesting its simultaneous interaction with the two metal binding sites. The first metal atom is located at the bottom of the catalytic pocket in association with the two histidine (H164, H200 in PDE4D2) and two aspartic acid residues (Asp201 and Asp318) (37). As the metal binding residues belong to the three PDE4 sub-domains, the metal ions stabilize the protein structure. It has been reported that in the PDE4–adenosine monophosphate (AMP) structure, the metal ion forms two coordinates: one

with His164, His200, Asp201, Asp318, and two phosphate oxygen atoms of AMP; and the second with Asp318, two phosphate oxygen atoms of AMP, and three bound water molecules. Therefore, it has a catalytic role (38). The strong irregular dispersion at the wavelength of the zinc absorption range is reflective of the fact that the first metal ion that activates the enzyme is zinc (36). In PDE4D2, the second metal ion interacts with D318, binds to water and the phosphate group of the substrate. However, due to the weak binding at this site, the second divalent metal ion is not known; although, magnesium (Mg) or manganese (Mn) are suggested by biochemical studies (39).

## 2. Expression and localization of PDE4 <sup>2</sup>

Expression of PDE4 is ubiquitous (40), although it varies both at regional and cellular levels (37). PDE4 overexpression promotes the progression of neurological disorders like AD, PD, MS, ALS, glioma, stroke, depression etc. PDE4 are mainly expressed in the brain, smooth muscles, cardiovascular tissues and are nearly absent in platelets (**Table. 1**). Intriguingly, expression of PDE4A, B, D was found to be high in rat brain, particularly in the anterior brain, cortex and olfactory bulb, whereas PDE4C was found to be high in the olfactory bulb (41).

## 3. PDE4 and PDE4Is in key molecular signalling pathways in brain <sup>4</sup>

### 3.1. *cAMP–PKA, CREB and BDNF signal*

PDE4 hydrolyses cAMP which play a significant role in long term potentiation (LTP) and synaptic plasticity (42). cAMP activates protein kinase A which controls the transcription of brain derived neurotropic factors (BDNF) and thus it has a pivotal role in cognitive functions (43). Mounting evidence confirms that PDE inhibitors enhance cAMP/cGMP signalling and in turn cAMP Response Element-Binding Protein (CREB) phosphorylation and the downstream effectors (44–46). Mutations in CREB causes cognitive dysfunction, mood disorders and alterations in neural regeneration (47). Rodents with a mutated form of CREB binding protein (CBP), a coactivator for major transcription factors, exhibit developmental aberrations like patients with mental retardation. These mouse models also suffer from memory dysfunction (48).

Rolipram is reported to improve long-term memory and CREB signalling instigated by CBP mutations (49). It also promotes neurogenesis in the hippocampal region and enhances the neuronal survival via cAMP/CREB signalling (50,51). Rolipram administered with imipramine



significantly increased the level of CREB and BDNF in the cortex and hippocampal regions of rats (52). Activation of cAMP/CREB/BDNF signalling by rolipram is shown to exert antidepressant-like effects (53). On the other hand, receptor for activated C kinase 1 (RACK1), called as guanine nucleotide-binding protein subunit beta-2-like 1 (GNB2L1) is tightly linked to cAMP/PKA pathway (54). RACK1 interacts (inhibits) with PDE4D5 and stimulates cAMP/PKA pathway (55). Activation of cAMP/PKA pathway in the hippocampal neurons dissociates RACK1 from NMDA-receptors allowing channels activity (56) which corroborates with its involvement in memory functions.

### 3.2. NO/cGMP/PKG pathway<sup>2</sup>

Nitric oxide (NO) signalling is extensively involved in synaptic plasticity and cognitive functions (57). NO is a soluble gas formed by the transformation of L-arginine to L-citrulline in the presence of  $\text{Ca}^{2+}$  regulated enzyme nitric oxide synthase (NOS). NO plays pivotal role at pre- and post-synapses. NO plays major effects in the hippocampus, cerebellum and lateral nucleus of the amygdala which are critical for memory formation (58,59). One immediate downstream effector of NO is soluble guanylyl cyclase (sGC) (60), which is responsible for cGMP synthesis and activation of cGMP dependent protein kinase (PKG). PKG, in turn, mobilises presynaptic vesicles and facilitate the release of neurotransmitters. It also activates postsynaptic PKG signalling leading to the activation of transcription factors that are important for LTP and memory formation (59,61). Pharmacological activation of NO/cGMP/PKG signalling is shown to enhance memory. Phosphodiesterase enzymes impede the signal transduction by affecting the second messengers like cAMP and cGMP (42). Stimulation of *N*-methyl-D-aspartate-type glutamate (NMDA) receptors activates NO synthase and increases cGMP levels (65). Inhibiting the breakdown of cGMP with a specific PDE2 inhibitor (Bay 60-7550) is shown to improve memory consolidation in MK801-induced memory deficits in mice (66). Preclinical studies have shown that PDE5 inhibitors increase NO/cGMP/PKG signalling in turn synaptic plasticity and cognitive performance in rodents (67,68). Zaprinast, a PDE5 inhibitor blocks cGMP specific phosphodiesterase and activates cGMP and cGMP-dependent ion channels, thereby potentiates calcium entry into presynaptic terminals and neurotransmitter release (69). Similarly, PDE5 inhibition with KJH-1002 was shown to improve cognitive function via activation of CREB signal in scopolamine intoxicated rats (70). Inhibition of PDE4 with rolipram elicited anxiolytic effects

through activation of cAMP/CREB/BDNF signalling (53). These data indicate the PDE enzymes influence NO/cGMP/PKG pathway and elicits beneficial effects in brain disorders.

### 3.3. MAPK-ERK signal

The role of the mitogen-activated protein kinase (MAPK) pathway in synaptic plasticity and memory is well-established (71,72). Activation of ERK signalling enhances LTP (73,74). Furthermore, both cyclic AMP/protein kinase A (PKA) and MAPK/ ERK are found to interact with each other (75). PDE4 is a common link between cAMP/CREB/BDNF and ERK signalling pathways in mediating memory. Inhibition of PDE4 enzymes by epidermal growth factor (EGF) via ERK activation increases cAMP levels (76). Crosstalk between cAMP and ERK signalling in hippocampal cornu ammonis-1(CA1) region is shown to be involved in synaptic plasticity (77,78). MAPK pathway activates CREB via p90 ribosomal S6 kinase 2 (RSK2) (79). Administration of MAPK and PKA inhibitors is shown to inhibit CREB phosphorylation. This suggests that MAPK-PKA pathways are involved in CREB function (80). Inhibition of MAPK/ ERK signalling is found to produce cognitive deficits and reduce hippocampal synaptic plasticity (72). ERK signalling affects PDE4 activity in neuronal cells (81). PDE4 inhibition by rolipram reverses ERK inhibition induced memory deficits in rats (74). Activation of NMDA receptors is a pre-requisite for LTP induction which is primarily mediated through MAPK (82). AP5, a NMDA receptors antagonist blocks the effects of rolipram on LTP (83). This shows that rolipram potentially trigger NMDA receptors mediated MAPK/ERK pathway and causes phosphorylation of CREB (84). Rolipram is reported to block MAPK signalling cascade by PKA initiated inhibition of Raf-1 activity (85). Furthermore, cAMP activates ERK via stimulation of B-Raf (86). Thus, inhibitors of PDEs have potential benefits in modulating the MAPK-ERK-CREB cascade primarily by cAMP and by altering changing NMDA receptor functions.

## 4. PDE4 inhibitors and neuroplasticity

Neuroplasticity is the capacity of neuronal cells to rewire or reorganize the structure and functions in normal or diseased state (87). Newer connections are established by normal cells which takes functional role of damaged neurons (88). Clinical and animal studies confirm that plasticity of brain is the main mechanism by which functional connectivity is restored after an injury (89). Multiple targets are shown to improve neuroplasticity, cAMP is one of them (90). Dysregulation of cAMP-PKA-CREB and cAMP-ERK1/2-CREB signalling cascades is linked to disruption of

neuroplasticity (91). cAMP/ PKA/CREB signalling regulates the transcription of BDNF which plays a vital role in the maintenance of hippocampal LTP (92,93). Upregulation of BDNF also accelerates neurogenesis and synaptic plasticity (94).

PDE4 hydrolyses cAMP and all the three isoforms (PDEA, B, D) are shown to inhibit neuroplasticity. PDE4 isoforms are highly expressed in cortex, hippocampus and amygdala (95). PDE4A5 is shown to reduce LTP and dendritic spine number and morphology in CA1 region of hippocampus due to the degradation of cAMP (96). Neuronal cell proliferation in dentate gyrus region of hippocampus is observed in PDE4A knockout mice (97). Inhibition of PDE4 is considered to be an ideal strategy in repairing the deficits in synaptic plasticity (98,99). PDE4 inhibition is shown to improve hippocampal LTP and dendritic spines density by activation of cAMP/PKA/CREB/BDNF signal (100,101). Rolipram is shown enhance the neural progenitor cell production in dentate gyrus region (102). Inhibition of proinflammatory cytokines release by PDE4I is linked to enhanced neuroplasticity. Mutation in PDE4 reduce the levels of interleukin-1-beta (IL-1 $\beta$ ), TNF- $\alpha$ , nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) and increase the expression of pCREB and BDNF in the hippocampal region of amyloid $\beta$ -42 injected mice (103). Interestingly, FFPM, a PDE4 inhibitor, exerts dual action viz activation of PKA, BDNF and CREB phosphorylation and simultaneously reduced NF- $\kappa$ B, p65, inducible nitric oxide synthase (iNOS), TNF- $\alpha$  and IL-1 $\beta$  levels in hippocampus of APP/PS1 transgenic mice as well as in lipopolysaccharides (LPS) injected mice (99,104). Roflumilast prevents primary blast-induced deficits in synaptic plasticity and increases the expression of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA)-glutamate receptor1(GluR1) (total and pGluR1-Ser831), and pStargazin-Ser239/240, and postsynaptic density protein 95 (PSD-95) that play a key role in LTP (101). PDE4 inhibition with Rolipram facilitates synaptic plasticity by activating cAMP/PKA/MAPK/CREB pathway and LTP in hippocampus of MK801, a non-competitive NMDA receptor antagonist of NMDA, intoxicated animals (105).

## 5. PDE4 as a therapeutic target in neurological disorders

Phosphodiesterase have a pivotal role in neuronal functions and survival (42,106). PDEs hydrolyze cAMP and cGMP and decrease the levels of these secondary messengers are corroborated for cognitive decline, and progression of AD, PD, psychosis, and depression. Thus inhibition of PDE4 appear as potential target in the treatment of neurological disorders (44,107).

## 6.1 Alzheimer's disease <sup>1</sup>

Alzheimer's disease is a fatal neurodegenerative disease with progressive cognitive decline (108). AD is distinguished by an increase in the accumulation of amyloid- $\beta$  peptide ( $A\beta$ ) neurofibrillary tangles (NFT), senile plaques which are made of  $A\beta$  peptide derived from amyloid precursor protein (APP) (108).  $\gamma$ -secretase enzymes cleave APP and produces insoluble  $\beta$ -amyloid protein which accumulates and forms plaques in the brain (109). NFT are aggregates of hyperphosphorylated tau proteins. Accumulation of  $A\beta$ , NFT and senile plaques inhibits LTP, disrupts the neuronal transport and ultimately leads to neuronal death (110). In addition, intracellular accumulation of  $A\beta$  decreases dendritic spine density and morphological alterations (111,112). Interestingly, cAMP and cGMP are shown to impact  $A\beta$  production by governing the conversion of the immature N-glycosylated APP, confined in the endoplasmic reticulum and Golgi apparatus, into the matured N- O-glycosylated protein (113). An increase in PDE4A and PDE4B expression are observed in the early stages of AD (114). Age related increase in cAMP dependent PKA plays a significant role in phosphorylation of tau in monkeys (115). Decreased levels of cAMP/ PKA/CREB have been found in the AD post-mortem brains (116–118). Inhibition of PDE4 enzymes improves long term potentiation, synaptic plasticity and corrects the memory deficits in double-transgenic mice having a high load of  $A\beta_{42}$  levels (119). FCPR03 is a novel PDE4 inhibitor inhibits pro-inflammatory cytokines like TNF- $\alpha$ , IL-1 $\beta$  and IL-6 and improves the levels of cAMP in BV-2 microglial cells exposed to lipopolysaccharide exposed and in mice. This anti-inflammatory activity is mediated by the cAMP/PKA/CREB signalling pathway and NF- $\kappa$ B inhibition (99). PDE4 inhibition with rolipram enhances hippocampal LTP, and improve memory in contextual fear conditioning test in transgenic mice by stabilizing the synaptic circuitry and via cAMP/PKA/CREB signalling (119). Dendritic spine loss and dystrophic neurites in hippocampal regions of APP/PS1 transgenic mice which classically mimics postmortem changes in AD brains. Rolipram treatment increased dendritic spines in the hippocampal region of APP/PS1 mouse model of AD (120). It also produced a dose dependent increase in hippocampal CREB levels and cognitive function in  $A\beta$  injected animals (121). Notably, it promotes proteasome activity and tau clearance improves cognition via cAMP-PKA activation in double-transgenic rTg4510 mice (122). GEBR-7b, a novel PDE4D selective inhibitor improves the consolidation process and increase the levels of cAMP in the hippocampal region of AD rats (123). Selective blocking of

PDE4D by D159687 and D159797, two allosteric PDE4D inhibitors, has been shown to improve the retrieval process in female Cynomolgus Monkeys (124). These data indicate the PDE4 is linked directly or indirectly in the regulation of key AD proteins like A $\beta$ , tau and NFT, cAMP/PKA/CREB signal and LTP that are involved in memory. In addition, PDE4 is also noted to play crucial role on dendritic spine morphology and numbers. Thus, PDE4 inhibitors might be beneficial in AD treatment.

## 6.2. Parkinson's disease

Parkinson's disease is a progressive neurodegenerative disease characterized by decreased levels of dopamine and accumulation of  $\alpha$ -synuclein aggregates in the substantia nigra and striatal region of brain (125–129). Sufficient evidence confirm the disruption of cyclic nucleotide signalling contributes to striatal dysfunction (130–132). Downregulation of CREB and neurotrophic factors like BDNF, nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) in substantia nigra (130) is well documented. An increase in the levels of cAMP has been reported to reduce the neuroinflammation by downregulating the expression of NF- $\kappa$ B and iNOS production (133). Pre-clinical and clinical studies have confirmed that PDE4 inhibitors appear to be promising drugs in the treatment of PD (130,134–136). FCPR16, a PDE4 inhibitor, prevents the dopaminergic loss by inhibiting reactive oxygen species production and preventing any change in the mitochondrial membrane potential in human neuroblastoma cells. This effect is reported to be mediated by cAMP/PKA/CREB and Epac/Akt signalling pathways (137). FCPR16 has also been found to trigger autophagy in 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>) intoxicated human neuroblastoma cells (SH-SY5Y cells) via 5' AMP-activated protein kinase (AMPK) pathway (138). XT-44 (1-n-butyl-3-n-propylxanthine), a PDE4 inhibitor increase the reuptake of DA and improve the intracellular dopamine levels in rat mesencephalic neurons. This protective effect was found to be mediated via cAMP/PKA/CREB pathway (139). Rolipram increases tyrosine hydroxylase (TH) phosphorylation and dopamine levels by increasing dopamine synthesis in the striatum without altering the dopamine release. Again, this action is found to be mediated via cAMP/PKA signalling (140). In addition, rolipram increased the striatal dopamine levels by preventing its metabolism and loss of tyrosine hydroxylase in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intoxicated C57BL/6 mice. Importantly, rolipram treatment improves the level of dopamine and alleviates the severity of Parkinsonism in a double-blind trial (141). On

a different note, ibudilast, PDE4 inhibitor, is reported to protect the astrocytes by inhibiting the release of cytochrome c, caspase-3 activation, and nuclear condensation in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) reperfusion rat model. The increase in cGMP levels through PDE inhibition is linked to the observed protection (142). These data indicate the crucial involvement PDE in pathology including  $\alpha$ -syn accumulation, TH and dopamine turn over. Hence, PDE4 inhibition can be a potential option the management of PD (**Fig.3**).<sup>1</sup>

### 6.3. Multiple sclerosis<sup>2</sup>

Multiple sclerosis (MS) is an autoimmune disease characterized by chronic neuroinflammation and demyelination which destroys oligodendrocytes, axons, and neurons. Inflammatory lesions found in white and grey matter are caused by over-activation of autoreactive T-cells and activated macrophages and microglia which form focal demyelinating plaques (143). Pro-inflammatory cytokines such as Interferon gamma (IFN- $\gamma$ ), TNF- $\alpha$ , Interleukin-6 (IL-6), Interleukin-12 (IL-12), and Interleukin-23 (IL-23) have been detected in post-mortem brains of MS patients (144) and in experimental autoimmune encephalomyelitis (EAE) model of MS (144,145). Pro-inflammatory mediators damage myelin and oligodendrocyte and disrupt the blood brain barrier (146). cAMP is recognized as a significant player in controlling the production of pro-inflammatory cytokines (147). Decreased levels of cAMP in the cerebrospinal fluid are linked to demyelination in MS patients (148). cAMP analogues are also reported to reduce inflammation and apoptosis. Previous studies have confirmed that cAMP analogue, Dibutyryl cyclic AMP, performs myelin repair in MS through recruitment of endogenous neural stem cells and their differentiation (149). Increasing cAMP levels through PDE4 inhibition suppresses the immune response and increases remyelination (150,151). PDE4 inhibition suppresses monocytes, macrophages, and cytokine secretion and proliferation of type 1 T helper cells specific to the myelin basic protein (152). Rolipram decreases TNF- $\alpha$  levels in J774 mouse macrophage cell line by increasing the expression of MAPK phosphatase-1(153) and by inhibiting NF- $\kappa$ B complexes in LPS intoxicated human chorionic cells PDE4 inhibition with rolipram reduced inflammation, demyelination and delayed onset of clinical signs in EAE rodent model of MS (154,155). Notably, rolipram prevents the development of clinical signs, demyelination and inflammation in marmosets (small monkeys) injected with human white matter, a gold standard model of EAE (156). Ibudilast is found to delay the onset of disease and inflammatory infiltration in EAE induced Dark August rats by increasing<sup>3</sup>



cAMP levels (157). BBB022, a PDE4 inhibitor, in combination with rolipram decreases the severity of EAE and stabilizes the blood brain barrier in EAE induced MS (158). Clinical trials are underway investigating the efficacy of PDE4 inhibitors in MS (23). Preclinical and clinical evidence clearly indicate that PDE participates in autoimmune function, cytokine release and demyelination. Further, few molecules under clinical trials indicate potential of PDE4 inhibition in MS treatment.

#### 6.4. Schizophrenia <sup>2</sup>

Schizophrenia is a serious psychiatric illness characterized by delusions, hallucinations, disorganized speech or behaviour, and impaired cognitive ability. The precise cause of schizophrenia is still not clear. However, the imbalance in neurochemicals such as dopamine, serotonin, and glutamate in brain are observed in schizophrenia patients (159). Recent studies have provided evidence on the possible role of PDE4 in schizophrenia (159–161). It has been reported that disrupted in PDE4B gene cause schizophrenia in two patients (162). Overexpression of PDE4B gene is shown to increase the risk of schizophrenia in the Scottish population (163). PDE4B associates with disrupted in schizophrenia 1 (DISC1), a genetic factor responsible for schizophrenia and related mental disorders (162). PDE4 with DISC1 and NDE1 or NDEL1 complexing suggests additional role of PDE4 in modulating cAMP levels via PKA and synaptic transmission. This reveals that PDE4 is a prime target in mental illness in schizophrenia research (164). Clapcote et al., (2007) found that production of two strains of DISC1-mutant mice with impaired affinity to PDE4B exhibit either schizophrenia or depression (165). Animal studies have given ample evidences on the possible role of PDE4 in schizophrenia (165). Rolipram is shown to antagonize the effects of phencyclidine and amphetamine-induced hyperactivity and to reduce methamphetamine-induced hyper-locomotion (166). It also reverses amphetamine-disrupted auditory sensory processing and prepulse inhibition (167) and NMDAR antagonist-induced deficits in latent inhibition (168). Roflumilast improves verbal learning and modulates the frontal brain area in schizophrenic patients. The improvement in memory, measured with functional magnetic resonance imaging (fMRI), is linked to the increase in cAMP levels and LTP (44,161). Thus, PDE4 inhibitors like rolipram and roflumilast possess antipsychotic-like properties and are interesting molecules for further research in schizophrenia.

### 6.5. Depression<sup>1</sup>

Depression, the most common mental illness that severely limits psychosocial functioning and diminishes life quality (169). Enormous evidence suggests that there is a dysfunction of cAMP signalling in depressive patients (170–172).  $\beta 1$  and  $\beta 2$ -adrenergic receptors produce anti-depressant like effects (173,174) and are shown to increase the formation of cAMP through adenylyl cyclase stimulation in brain (175).  $\beta 1$  and  $\beta 2$  receptors form complexes with PDEs, but their binding varies in terms of PDE4D splice variant recruited at the receptor.  $\beta 1$ -adrenergic receptor preferentially associates with PDE4D8 whilst  $\beta 2/\beta$ -arrestin complex has higher affinity to PDE4D5 (176). Zhang et al., (2005) demonstrated that PDE4 inhibition produced an “additive” anti-depressant effect when administered along with dobutamine,  $\beta 1$  agonist in rats. Similarly, a synergistic was observed when administered clenbuterol,  $\beta 2$ -agonist, in rats. Though many studies reveal that  $\beta 2$  mediated cAMP signalling and antidepressant effects are more sensitive to PDE4 inhibition, still  $\beta 1$  mediated noradrenergic anti-depressant effect has equal importance due to the greater sensitivity for the endogenous norepinephrine (177). Although, the interaction between  $\beta$ -adrenergic stimulation and PDE4 inhibition show prominent opportunity in depression research, involvement of cardiovascular risks hinders clinical investigation. On the other hand, administration of selective serotonin reuptake inhibitor along with rolipram, produced showed significant behavioural improvement in major depressive disorder patients (178), which indicate the PDE association with serotonergic system in brain.

Upregulation of PDE4 was observed the depressive rat cortex region (179–181). Surprisingly, decreased cAMP-PKA-CREB and cAMP-ERK1/2-CREB signalling and loss of dendritic spines are also observed in experimental depressive rodents (180). On a separate report, Fujita et al (2017) provide evidence on the decrease in cAMP levels in living depressive patients (178). Inhibition of PDE4 increases the level of cAMP, pCREB and has been reported to promote hippocampal neurogenesis which is corroborated to antidepressant-like effect (53,182). Clinical trials are underway to unravel the efficacy of PDE4 inhibitors in depression.

### 6.6. Huntington's disease<sup>4</sup>

Huntington's disease is a neurological disorder caused by an increase in polyglutamine (polyQ)<sup>5</sup> repeated inside the Huntingtin (Htt) protein. This mutant Huntingtin (Htt) protein with extended polyQ upon expression generates inclusion bodies (IBs), causes increased cellular toxicity and

results in development of motor disabilities (183,184). Subcellular actions like production of oxidative stress, mitochondrial dysfunction, activation of inflammatory responses and transcriptional dysregulation play a part in the development of HD (185). Dysfunction of cAMP/CREB signalling in HD is a result of overexpression of PDE4 (186). Interestingly, rolipram is shown to increase the phosphorylation of CREB and BDNF and the survival rate, and also improves the clinical signs in rodent model of HD (187). It has also been established that PDE4 inhibitors reduce cortical and striatal neuronal degeneration in transgenic mouse model of HD (188). Since, PDE4 inhibitors improve the cAMP and BDNF, and can regulate the neuronal functions, they have been considered as potential drugs to treat HD. GlaxoSmithKline is performing a clinical trial to determine the efficacy of rolipram in HD which is in Phase 1 (clinicaltrial.gov).

### 6.7. Stroke<sup>2</sup>

Stroke is a neurodegenerative disease caused by sudden disruption of blood flow to a specific region of the brain or the entire brain. Stroke causes weakness, lack of sensation, paralysis, slurred speech, aphasia, blurred vision (189). Disruption in the blood supply results in a series of metabolic and molecular alterations which leads to neuronal damage (189,190). Approximately 80% of strokes are ischemic while 20% are haemorrhagic in nature (189). An ischemic stroke occurs when blood to brain is blocked, while a haemorrhagic stroke occurs when an artery ruptures in brain (191). Gretarsdottir et al. (2003) reported a decrease in stroke risk with PDE4 inhibition (12). Several studies have elucidated the role of the PDE4 pathway in the pathogenesis of stroke (12,29). Increased expression of PDE4 in stroke plays a devastating role in mediating the neuronal cell death (192). Decrease in cAMP leads to activation of immune responses, oedema and hyperexcitability (12,29). Inhibition of cAMP-PDE4 reduces the infarct size in hippocampal region of rats and gerbil (193,194). Rolipram restores endothelial function in stroke by regulating vascular repair, inflammation and reduces the expression of tissue plasminogen which is connected to increased cAMP concentration via Epac pathway (195). Rolipram increases hippocampal neurogenesis and decreases ischemic injury in neuronal cultures (196). PDE4 inhibition prevents blood brain barrier disruption, thrombosis, and reduces the secretion of proinflammatory cytokines IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , and inhibits neuronal apoptosis in rat model of cerebral stroke (197). Successive reports also conclude that the increased survival index, angiogenesis, and cognitive

performance are connected to improvement in cAMP/CREB levels with PDE4 inhibition (196).<sup>1</sup> More research works are being focused towards the reposition of PDE 4 inhibitors for its use in cerebral stroke (Fig. 4).

### 6.8. *Amyotrophic lateral sclerosis*<sup>2</sup>

Amyotrophic lateral sclerosis is a neurodegenerative disease that causes loss of motor neurons in the brain and spinal cord leading to atrophy of voluntary muscles (198). ALS affects the motor neurons of the cerebral cortex, brain stem and spinal cord. About 90% of ALS cases are considered sporadic amyotrophic lateral sclerosis (sALS) while 10% are familial amyotrophic lateral sclerosis (fALS) (18). Yet the pathophysiology of ALS is not elucidated clearly. Nonetheless, aggregation of intracellular toxins, oxidative stress, neuroinflammation, excitotoxicity and mitochondrial dysfunction are reported to be involved (198,199). TAR DNA binding protein (TDP-43) have been identified to be involved in the progression of ALS. Mutations in TDP-43 have been found in patients with ALS (200). Dysregulation of the kynurenine pathway and production of neuroactive metabolites is involved in the development of ALS (201). Neuroinflammation, oxidative stress and NMDA excitotoxicity caused by the quinolinic acid produced during tryptophan metabolism aggravates the progression of ALS (202). Ibudilast used for COPD is repurposed for ALS (203). Ibudilast markedly enhances the clearance of TDP-43 by promoting autolysosomes via mTORC1 inhibition in motor neurons (204) and also inhibits neuroinflammation and microglial activation by increasing the cAMP levels in neuron and microglia co-cultures (133). Ibudilast is also found to reduce oxidative stress and increase the level of neurotrophic factors such as glia-derived neurotrophic factor, NGF, NT-43 which supports the development and growth of neurons (133). Preclinical studies have shown that Ibudilast protects hippocampal neurons, oligodendrocytes and astrocytes from excitotoxicity and apoptosis. These effects were mediated by upregulation of cGMP/PKG signaling (142,205,206). Clinical trials (NCT02238626) are going on Ibudilast to confirm its efficacy in ALS. In 2016, the European Medicines Agency recommended the use of ibudilast for ALS (**Fig. 5**).<sup>3</sup>

### 6.9. *Glioma*<sup>4</sup>

PDEs are implicated in the development of various tumours. The role of cAMP on brain tumours was established in 1977 when cAMP was discovered (207). Particularly, the role of cAMP on the development of glioma is well studied in both *in vitro* and *in vivo* models (208–211). Warrington<sup>5</sup>

et al (2015) found that overexpression of PDE4 increases glioma formation in *Nf1* mice.<sup>1</sup> Overexpression of PDE4 is observed in medulloblastoma, glioblastoma (GBM), oligodendroglioma, ependymoma, and meningioma (212) and the decreased adenylyl cyclase and cAMP levels shown to inhibit apoptosis and enhance tumour growth (213). cAMP regulates various physiological processes via cAMP/PKA or Epac1/Rap1-mediated pathways (214,215). Rolipram is shown to exert cytotoxicity in A172 and U87MG human glioblastoma cell lines via cAMP-dependent PKA and Epac1/Rap1-pathway (216,217). It induces apoptosis in U87MG cells through cAMP-dependent inhibition of AKT phosphorylation (218). Rolipram prevents the development of tumour resistance and produces tumour regression in U87 glioblastoma and Daoy medulloblastoma cells and xenograft model mice (219). It increases the life span in mice bearing glioblastoma when administered along with temozolomide (212). Rolipram increases the antitumor efficacy of bevacizumab in glioblastoma stem-like cells (GCSCs) by inhibiting ERK/AKT signalling and inhibiting MDM2 (mouse double minute 2 homolog) mediated p53 degradation. This in turn activates apoptosis and produces tumour cells death (220). Rolipram suppresses tumor progression, and apoptosis in xenograft brain tumour model by increasing the levels of cAMP/CREB (221) and by inhibiting p27 (Cip1) and p21 (Kip1) cell cycle inhibitors (232, 238). Rolipram is under investigation for its efficacy in patients with GBM.

#### 6.10. Traumatic brain injury<sup>2</sup>

Traumatic brain injury (TBI) imposes profound clinical problems such as disability, cognitive deficits (223). TBI alters glucose metabolism, increases the production of free radicals and mitochondrial dysfunction (224). TBI also increases the expression of PDE4 which results in dysfunction of the cAMP-PKA pathway (29). PDE4B2 levels increase by 10-fold within one hour after TBI. PDE4D2 is also highly expressed after TBI (225). A decrease in cAMP levels is observed in the cortex and hippocampus within 15 minutes after TBI (29,226). A decrease in the levels of cAMP triggers the release of proinflammatory mediators and affects LTP in the hippocampus. This leads to cognitive decline in an experimental model of TBI (223). Rolipram has been found to reduce the expression of inflammatory mediators like TNF- $\alpha$  and IL-1 $\beta$ . These are upregulated in microglial cells in EOC2 microglia cells and in rodent model of TBI (227,228). Rolipram also improves cognitive function in cerebral ischemic rats and is reported to prevent neuronal loss in the cortex and CA1 region of the hippocampus by increasing the expression of

cAMP-PKA/CREB signalling cascade in TBI animals (229,230). It also reduces the deposition of the beta-amyloid precursor protein in traumatic axonal injury (230). Since rolipram modulates PDE4 isoforms and can effectively manage TBI symptoms, we suggest that PDE4 is a potential target in the management of TBI.

### 6.11. Vascular dementia<sup>2</sup>

Vascular dementia (VD) is an age-related neurodegenerative condition, responsible for at least 20% of dementia cases (231). Pathophysiologically, it is characterized by reduced cerebral perfusion and causes mood disorders and attention and cognitive deficits (232). Multiple risk factors including arterial hypotension, cerebrovascular diseases, cerebral haemorrhage and infarcts causes VD (231). Furthermore, excitotoxicity, neuroinflammation, and alterations in the cyclic nucleotides levels are the pathogenetic mechanisms involved in the progression of VD (233,234). Inhibition of PDE4 results in the increase in cAMP level and is reported to play a critical role in regulating cerebral blood flow, synaptic plasticity, and learning and memory (46,235). Increased levels of cAMP are reported to dilate pial vessels and potentially inhibit platelet aggregation, which is correlated to improved brain blood flow in feline cerebral ischemia (236). Rolipram is shown to increase cortical blood flow measured via autoradiography in rats (237). Similarly, administration of roflumilast increases the survival rate, memory and reduces white matter injury in aged rats with chronic cerebral hypoperfusion, which corroborates to the increased cAMP content (238). Betulinic acid, a naturally occurring PDE4 inhibitor is shown to improve the cerebral blood flow measured by using the laser-Doppler flow meter in rats (260). Liang et al. (2020) reported that  $\alpha$ -mangostin, a natural PDE4 inhibitor, improves memory functions in beagle dogs that are subjected to unilateral common carotid artery occlusion. Interestingly, treatment with  $\alpha$ -mangostin does not cause emesis behaviour, as it selectively inhibits PDE4B (239). Convincingly, PDE4 inhibition improves blood flow to the brain by modulating blood vessels function indirectly in VD.

## 6. Clinical trials on PDE4 inhibitors for neurological disorders<sup>4</sup>

Clinical trials conducted so far have strengthened the therapeutic potential of PDE4 inhibitors in the treatment of various neurological diseases like AD, PD, MS, HD, stroke, and depression. PDE4 are highly expressed in the brain and modulate a wide range of physiological processes by



modulating the intracellular secondary messengers cAMP and cGMP. In general, most of PDE4 inhibitors have better blood-brain barrier penetration property and hence, they have been focused more to reposition in the treatment of brain related diseases (15). Rolipram, a cAMP specific PDE4 inhibitor, has been widely investigated in comparison to its counterparts (168). In 1992, a clinical trial with rolipram showed potent antidepressant activity (240). However, it produced dose dependent side effects like emesis, headache and increased gastric secretion (14). Inhibition of PDE4D in the postrema and nucleus of the solitary tract is emetogenic causative factor of rolipram (241). Another clinical trial was initiated by the National Institute of Health in 2006 to re-evaluate the antidepressant activity of rolipram. It was found that rolipram inhibits both PDE4B and PDE4D (which are prominently expressed in the brain) and has a potent antidepressant activity with less side effects (178). PDE4 inhibitors under clinical trials for some neurological disorders are listed in **Table 2**. The data is collected from ClinicalTrials.gov.

## 7. PDE4-isoform specific allosteric modulators and their advantages<sup>2</sup>

Inhibition of cAMP specific PDE4 enzymes is a promising therapeutic option for airway diseases treatment (19). Albeit, the first generation PDE4 inhibitors such as roflumilast, rolipram, apremilast, RS25344, PMNPQ, etc. have shown prominent therapeutic effect in preclinical and clinical trials (242–245), the clinical acceptance is limited because of their side effects like nausea and emesis, and gastric hypersecretion (246–248). These non-specific PDE4 inhibitors tightly bind at the high affinity site (HPDE4) of the enzyme and lead to increase in the levels of cAMP, which is responsible for the side effects (249). Cilomilast, a second generation PDE4 inhibitor binds at the low affinity site (LPDE4) but still shows emesis (250). Thus, the first and second generation PDE4 inhibitors are the non-specific inhibitors of all four subtypes (PDE4A, B, C, D) and increase cAMP concentrations beyond the normal physiological need (251).

Studies in knockout mice have shown that PDE4D isoform is responsible for emesis and PDE4D-deficient mice show an amplified sympathetic drive with decrease in sleeping time under xylazine/ketamine-induced anaesthesia, a behavioural symptom that corresponds to emesis. Further, PDE4D could potentially modulates the  $\alpha_2$ -adrenoceptor, which causes emesis and other side effects (252) and this warrants the need of development of specific PDE4 isoform inhibitors in alleviating the above reported side effects (253). X-ray crystallography techniques have helped in identifying the binding modes and the design of potent PDE4 isoform inhibitors (34). UCR2

specific allosteric modulators take advantage of an asymmetric PDE4 conformer by blocking only one active site, without affecting the second active site. UCR2-directed PDE4D allosteric modulators partially inhibit the cAMP hydrolysis and reduce emesis (251). D159687, a negative allosteric modulator of PDE4D, enhances learning and memory in TBI rats (254). Furthermore, it was also reported that D159687 does not induce emesis as predicted by the anaesthesia duration test in mice (181). D159797 is a UCR2-directed allosteric modulator of PDE4D and shows better performance in novel object recognition test with a 300 fold less emetic potential as compared to rolipram in female Cynomolgus monkeys (255). Allosteric modulation provides a big ray of hope in the clinical utility of PDE4 inhibitors in neurological disorders by improving synaptic plasticity (254), increasing the spine density and upregulating the expression of neurotrophic factors such as CREB, BDNF and VGF (256). Substantial research is underway to minimize the adverse effects of PGE4Is and to obtain a therapeutic option that has better benefit-to-risk ratio. Approaches towards the development of PDE4 isoform specific inhibitors with high binding affinity at the catalytic site are expected to have reduced emesis, due to the reduced affinity at HPDE4 site 4, whilst maintaining the potential therapeutic properties (257).

## 8. Conclusion<sup>2</sup>

Dysregulation of cAMP signalling in brain is said to have strong connection with various neurological disorders, of which some have been found to be related to the increased expression of PDE4 enzyme. Inhibition of PDE4 is shown to improve cAMP levels and offer neuroprotective effects in AD, PD, HD, depression, MS, epilepsy, schizophrenia, and other neurological disorders. Many of the non-specific PDE4 inhibitors show neuroprotective effects in preclinical models of neurological disorders, but unfortunately majority of them have failed in clinical trials. The first and second generation PDE4 inhibitors are non-specific between other members of PDE4 families and they produce a surge in cAMP levels beyond the physiological needs and this results in side effect. Allosteric modulation on PDE4 isoform with specific inhibitors, particularly PDE4B and PDE4D inhibitors have shown to possess required therapeutic properties and reduced side effect. None-the-less, there is a need for extensive investigation on long-term toxicities, pharmacokinetics, and data on biodistribution profiles for PDE4I. Also, the role of PDE4 subtype specific inhibitors' in neurological disorders largely and their ability to cross the blood brain

barrier needs investigation. Towards this end, the use of PDE4 subtype specific transgenic knockout rodent models will provide clear information on the benefit-to-risk ratio. In conclusion, PDE4 inhibition could be a worthwhile therapeutic option in the treatment of neurological disorders and the development of new subtype specific inhibitors and their efficacy and toxicity at preclinical and clinical levels needs to be investigated.

## Conflict of interest<sup>2</sup>

Authors declare no conflict of interest<sup>3</sup>

## Acknowledgments<sup>4</sup>

Mr Abid Bhat acknowledges the Indian Council of Medical Research, Govt of India, New Delhi, for financial assistance as Senior Research Fellowship. Prof Guillemin is supported by the NHRMC, ARC and Macquarie University. This manuscript is professionally edited by Red Fern Communication, Australia.

## References<sup>6</sup>

- [1] V. B.Smith, D. Spina,C.P. Page. Phosphodiesterase inhibitors. Br J Pharmacol. (2006): S252–7. [https://doi.org/ 10.1038/sj.bjp.0706495](https://doi.org/10.1038/sj.bjp.0706495)
- [2] C. Lugnier, Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. Pharmacol Ther. 109 (3) (2006):366–98. <https://doi.org/10.1016/j.pharmthera.2005.07.003>
- [3] G.M. Uys, A. Ramburan, B. Loos, C.J. Kinnear, L.J. Korkie, J. Mouton, et al. Myomegalin is a novel A-kinase anchoring protein involved in the phosphorylation of cardiac myosin binding protein C. BMC Cell Biol. (2011);12-18. [https://doi.org/ 10.1186/1471-2121-12-18](https://doi.org/10.1186/1471-2121-12-18)
- [4] I. Verde, G. Pahlke, M. Salanova, G. Zhang, S. Wang, D. Coletti, et al. Myomegalin is a novel protein of the golgi/centrosome that interacts with a cyclic nucleotide phosphodiesterase. J Biol Chem. 276(14) (2001);11189–98. [https://doi.org/ 10.1074/jbc.M006546200](https://doi.org/10.1074/jbc.M006546200)

- [5] H. Li, J. Zuo, W. Tang, Phosphodiesterase-4 Inhibitors for the Treatment of Inflammatory Diseases. *Front Pharmacol.* (9) (2018): 1048. <https://doi.org/10.3389/fphar.2018.01048> <sup>1</sup>
- [6] S. Vatter, G. Pahlke, J.W. Deitmer, G. Eisenbrand, Differential phosphodiesterase expression and cytosolic Ca<sup>2+</sup> in human CNS tumour cells and in non-malignant and malignant cells of rat origin. *J Neurochem.* 93 (2) (2005)321–9. <https://doi.org/10.1111/j.1471-4159.2005.03028.x> <sup>2</sup>
- [7] F. Yang, R.K. Sumbria, D. Xue, C. Yu, D. He, S. Liu, et al. Effects of PDE4 pathway inhibition in rat experimental stroke. *J Pharm Pharm Sci.* 17(3) (2014):362–70. <https://doi.org/10.18433/j3s02v> <sup>3</sup>
- [8] S.S. Pullamsetti, G.A. Banat, A. Schmall, M. Szibor, D. Pomagruk, J. Hänze, et al. Phosphodiesterase-4 promotes proliferation and angiogenesis of lung cancer by crosstalk with HIF. *Oncogene.* 32(9) (2013):1121–34. <https://doi.org/10.1038/onc.2012.136> <sup>4</sup>
- [9] M. Biel, S. Michalakis, Function and dysfunction of CNG channels: insights from channelopathies and mouse models. *Mol Neurobiol.* 35 (3) (2007):266–77. <https://doi.org/10.1007/s12035-007-0025-y> <sup>5</sup>
- [10] H. Lee, J.M. Graham, D.L. Rimoïn, Lachman RS, Krejci P, Thompson SW, et al. Exome sequencing identifies PDE4D mutations in acrodysostosis. *Am J Hum Genet.* 90(4) (2012):746–51. <https://doi.org/10.1016/j.ajhg.2012.03.003> <sup>6</sup>
- [11] Y.F. Li, Y.F. Cheng, Y. Huang, M. Conti, S.P. Wilson, J.M. O'Donnell, et al. Phosphodiesterase-4D knock-out and RNA interference-mediated knock-down enhance memory and increase hippocampal neurogenesis via increased cAMP signaling. *J Neurosci.* 31(1) (2011):172–83. <https://doi.org/10.1523/JNEUROSCI.5236-10.2011> <sup>7</sup>
- [12] S. Gretarsdottir, G. Thorleifsson, S.T. Reynisdottir, A. Manolescu, S. Jonsdottir, T. Jonsdottir, et al. The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. *Nat Genet.* 35(2) (2003):131–8. <https://doi.org/10.1038/ng1245> <sup>8</sup>

- [13] M.F. Azevedo, F.R. Faucz, E. Bimpaki, A. Horvath, I. Levy, R.B. de Alexandre, et al. Clinical and molecular genetics of the phosphodiesterases (PDEs). *Endocr Rev.*35(2) (2014);195–233. <https://doi.org/10.1210/er.2013-1053> <sup>1</sup>
- [14] R. Zebda, A.S. Paller, Phosphodiesterase 4 inhibitors. *J Am Acad Dermatol.* 78(3) (2018); :43–52. <https://doi.org/10.1016/j.jaad.2017.11.056> <sup>2</sup>
- [15] J. Prickaerts, P.R.A. Heckman, A. Blokland. Investigational phosphodiesterase inhibitors in phase I and phase II clinical trials for Alzheimer’s disease. *Expert Opin Investig Drugs.* 26(9) (2017):1033–48. <https://doi.org/10.1080/13543784.2017.1364360> <sup>3</sup>
- [16] A. Blokland, P. Heckman, T. Vanmierlo, R. Schreiber, D. Paes, J. Prickaerts, Phosphodiesterase Type 4 Inhibition in CNS Diseases. *Trends Pharmacol Sci.* 2019 Dec;40(12):971–85. <https://doi.org/10.1016/j.tips.2019.10.006> <sup>4</sup>
- [17] J. Baye. Roflumilast (Daliresp). *P T.* 37(3) (2012):149–61. <sup>5</sup>
- [18] L. Fala, Otezla (Apremilast), an Oral PDE-4 Inhibitor, Receives FDA Approval for the Treatment of Patients with Active Psoriatic Arthritis and Plaque Psoriasis. *Am Health Drug Benefits* 8 (2015):105–10. <sup>6</sup>
- [19] A.M. Vignola. PDE4 inhibitors in COPD—a more selective approach to treatment. *Respiratory Medicine.* 98(6) (2004):495–503. <https://doi.org/10.1016/j.rmed.2003.12.012> <sup>7</sup>
- [20] R. Akash, D. Hrishikesh, P. Amith, S. Sabah. Case report: association of combined nonarteritic anterior ischemic optic neuropathy (NAION) and obstruction of cilioretinal artery with overdose of Viagra. *J Ocul Pharmacol Ther.* 21(4) (2005); 315–7. <https://doi.org/10.1089/jop.2005.21.315> <sup>8</sup>
- [21] G. McGwin. Phosphodiesterase Type 5 Inhibitor Use and Hearing Impairment. *Arch Otolaryngol Head Neck Surg.* 136(5) (2010); 488–92. <https://doi.org/10.1001/archoto.2010.51> <sup>9</sup>
- [22] C. Kruuse, L.L. Thomsen, T.B. Jacobsen, J. Olesen. The Phosphodiesterase 5 Inhibitor Sildenafil Has No Effect on Cerebral Blood Flow or Blood Velocity, but Nevertheless <sup>10</sup>

- Induces Headache in Healthy Subjects: *Journal of Cerebral Blood Flow & Metabolism* 22(9) (2002);1124-31. <https://doi.org/10.1097/00004647-200209000-00010> <sup>1</sup>
- [23] N. Kumar, A.M. Goldminz, N. Kim, A.B. Gottlieb. Phosphodiesterase 4-targeted treatments for autoimmune diseases. *BMC Medicine*. 11(1) (2013):96. <https://doi.org/10.1186/1741-7015-11-96> <sup>2</sup>
- [24] C.J. Tralau-Stewart, R.A. Williamson, A.T. Nials, M. Gascoigne, J. Dawson, G.J. Hart, et al. GSK256066, an exceptionally high-affinity and selective inhibitor of phosphodiesterase 4 suitable for administration by inhalation: in vitro, kinetic, and in vivo characterization. *J Pharmacol Exp Ther*. 337(1) (2011):145–54. <https://doi.org/10.1124/jpet.110.173690> <sup>3</sup>
- [25] S. Yu, A.D. Pearson, R.K. Lim, D.T. Rodgers, S. Li, H.B. Parker, et al. Targeted Delivery of an Anti-inflammatory PDE4 Inhibitor to Immune Cells via an Antibody–drug Conjugate. *Mol Ther*. 24(12) (2016):2078–89. <https://doi.org/10.1038/mt.2016.175> <sup>4</sup>
- [26] N. Plock, S. Vollert, M. Mayer, G. Hanauer, G. Lahu. Pharmacokinetic/Pharmacodynamic Modeling of the PDE4 Inhibitor TAK-648 in Type 2 Diabetes: Early Translational Approaches for Human Dose Prediction. *Clin Transl Sci*. 10(3) (2017):185–93. <https://doi.org/10.1111/cts.12436> <sup>5</sup>
- [27] G. Schett, V.S. Sloan, R.M. Stevens, P. Schafer, Apremilast: a novel PDE4 inhibitor in the treatment of autoimmune and inflammatory diseases. *Ther Adv Musculoskelet Dis*. 2(5) (2010):271–8. <https://doi.org/10.1177/1759720X10381432> <sup>6</sup>
- [28] E.P. Knott, M. Assi, S.N.R. Rao, M. Ghosh, D.D. Pearse, Phosphodiesterase Inhibitors as a Therapeutic Approach to Neuroprotection and Repair. *Int J Mol Sci*. 18 (4) (2017). <https://doi.org/10.3390/ijms18040696> <sup>7</sup>
- [29] D.J. Titus, A.A. Oliva, N.M. Wilson, C.M. Atkins, Phosphodiesterase Inhibitors as Therapeutics for Traumatic Brain Injury. *Curr Pharm Des*. 21(3) (2014):332–42. <https://doi.org/10.2174/1381612820666140826113731> <sup>8</sup>



- [30] M.D. Houslay, P. Schafer, K.Y.J. Zhang, Keynote review: phosphodiesterase-4 as a therapeutic target. *Drug Discov Today*. 10(22) (2005):1503–19. [https://doi.org/10.1016/S1359-6446\(05\)03622-6](https://doi.org/10.1016/S1359-6446(05)03622-6) <sup>1</sup>
- [31] M.D. Houslay, Underpinning compartmentalised cAMP signalling through targeted cAMP breakdown. *Trends Biochem Sci*. 35(2) (2010):91–100. <https://doi.org/10.1016/j.tibs.2009.09.007> <sup>2</sup>
- [32] E.V. Hill, C.L. Sheppard, Y.F. Cheung, I. Gall, E. Krause, M.D. Houslay, Oxidative stress employs phosphatidyl inositol 3-kinase and ERK signalling pathways to activate cAMP phosphodiesterase-4D3 (PDE4D3) through multi-site phosphorylation at Ser239 and Ser579. *Cell Signal*. 18(11) (2006):2056–69. <https://doi.org/10.1016/j.cellsig.2006.07.018> <sup>3</sup>
- [33] H.T. Zhang, Cyclic AMP-specific phosphodiesterase-4 as a target for the development of antidepressant drugs. *Curr Pharm Des*. 15(14) (2009):1688–98. <https://doi.org/10.2174/138161209788168092> <sup>4</sup>
- [34] R.X. Xu, A.M. Hassell, D. Vanderwall, M.H. Lambert, W.D. Holmes, M.A. Luther, et al. Atomic structure of PDE4: insights into phosphodiesterase mechanism and specificity. *Science*. 288(5472) (2000):1822–5. <https://doi.org/10.1126/science.288.5472.1822> <sup>5</sup>
- [35] H. Wang, M.S. Peng, Y. Chen, J. Geng, H. Robinson, M.D. Houslay, et al. Structures of the four subfamilies of phosphodiesterase-4 provide insight into the selectivity of their inhibitors. *Biochem J*. 408 (2007):193–201. <https://doi.org/10.1042/BJ20070970> <sup>6</sup>
- [36] S.H. Francis, J.L. Colbran, L.M. McAllister-Lucas, J.D. Corbin, Zinc interactions and conserved motifs of the cGMP-binding cGMP-specific phosphodiesterase suggest that it is a zinc hydrolase. *J Biol Chem*. 269 (36) (1994):22477–80. <sup>7</sup>
- [37] L.M. McAllister-Lucas, T.L. Haik, J.L. Colbran, W.K. Sonnenburg, D. Seger, I.V. Turko, et al. An Essential Aspartic Acid at Each of Two Allosteric cGMP-binding Sites of a cGMP-specific Phosphodiesterase. *J Biol Chem*. 270(51) (1995):30671–9. <https://doi.org/10.1074/jbc.270.51.30671> <sup>8</sup>

- [38] Q. Huai, J. Colicelli, H. Ke, The crystal structure of AMP-bound PDE4 suggests a mechanism for phosphodiesterase catalysis. *Biochemistry*. 42(45) (2003):13220–6. <https://doi.org/10.1021/bi034653e> <sup>1</sup>
- [39] R. Alvarez, C. Sette, D. Yang, R.M. Eglen, R. Wilhelm, E.R. Shelton, et al. Activation and selective inhibition of a cyclic AMP-specific phosphodiesterase, PDE-4D3. *Mol Pharmacol*. 48(4) (1995):616–22. <sup>2</sup>
- [40] P. Engels, M. Sullivan, T. Müller, H. Lübbert. Molecular cloning and functional expression in yeast of a human cAMP-specific phosphodiesterase subtype (PDE IV-C). *FEBS Lett*. 358(3) (1995):305–10. [https://doi.org/10.1016/0014-5793\(94\)01460-i](https://doi.org/10.1016/0014-5793(94)01460-i) <sup>3</sup>
- [41] S. Pérez-Torres, X. Miró, J.M. Palacios, R. Cortés, P. Puigdoménech, G. Mengod, Phosphodiesterase type 4 isozymes expression in human brain examined by in situ hybridization histochemistry and [3H]rolipram binding autoradiography. Comparison with monkey and rat brain. *J Chem Neuroanat*. 20(3–4) (2000):349–74. [https://doi.org/10.1016/s0891-0618\(00\)00097-1](https://doi.org/10.1016/s0891-0618(00)00097-1) <sup>4</sup>
- [42] F.S. Menniti, W.S. Faraci, C.J. Schmidt, Phosphodiesterases in the CNS: targets for drug development. *Nature Reviews Drug Discovery*. 5(8) (2006):660–70. <https://doi.org/10.1038/nrd2058> <sup>5</sup>
- [43] B.E. Lonze, D.D. Ginty. Function and regulation of CREB family transcription factors in the nervous system. *Neuron*. 35(4) (2002): 605–23. <https://doi.org/> <sup>6</sup>
- [44] S.S.L. Jabaris, H. Sumathy, R. Girish, S. Narayanan, M. Sugumar, C. S. Babu, et al. Phosphodiesterase-4 inhibitors ameliorates cognitive deficits in deoxycorticosterone acetate induced hypertensive rats via cAMP/CREB signaling system. *Brain Res*. 1622 (2015):279–91. [https://doi.org/10.1016/s0896-6273\(02\)00828-0](https://doi.org/10.1016/s0896-6273(02)00828-0) <sup>7</sup>
- [45] O.A.H. Reneerkens, K. Rutten, H.W.M. Steinbusch, A. Blokland, J. Prickaerts. Selective phosphodiesterase inhibitors: a promising target for cognition enhancement. *Psychopharmacology* 202(1) (2009):419–43. <https://doi.org/10.1007/s00213-008-1273-x> <sup>8</sup>

- [46] H. Wang, J. Xu, P. Lazarovici, R. Quirion, W. Zheng, cAMP Response Element-Binding Protein (CREB): A Possible Signaling Molecule Link in the Pathophysiology of Schizophrenia. *Front Mol Neurosci.* 11 (2018). <https://doi.org/10.3389/fnmol.2018.00255> <sup>1</sup>
- [47] B. Monti, C. Berteotti, A. Contestabile, Subchronic rolipram delivery activates hippocampal CREB and arc, enhances retention and slows down extinction of conditioned fear. *Neuropsychopharmacology.* 31(2) (2006):278–86. <https://doi.org/10.1038/sj.npp.1300813> <sup>2</sup>
- [48] Y. Oike, A. Hata, T. Mamiya, T. Kaname, Y. Noda, M. Suzuki, et al. Truncated CBP protein leads to classical Rubinstein-Taybi syndrome phenotypes in mice: implications for a dominant-negative mechanism. *Hum Mol Genet.* 8(3) (1999):387–96. <https://doi.org/10.1093/hmg/8.3.387> <sup>3</sup>
- [49] K. Rutten, J. Prickaerts, G. Schaezle, H. Rosenbrock, A. Blokland. Sub-chronic rolipram treatment leads to a persistent improvement in long-term object memory in rats. *Neurobiol Learn Mem.* 90(3) (2008):569–75. <https://doi.org/10.1016/j.nlm.2008.04.016> <sup>4</sup>
- [50] T. Fujioka, A. Fujioka, R.S. Duman, Activation of cAMP signaling facilitates the morphological maturation of newborn neurons in adult hippocampus. *J Neurosci.* 24(2) (2004):319–28. <https://doi.org/10.1523/JNEUROSCI.1065.03.2004> <sup>5</sup>
- [51] Y.F. Li, Y. Huang, S.L. Amsdell, L. Xiao, J.M. O'Donnell, H.T. Zhang. Antidepressant- and anxiolytic-like effects of the phosphodiesterase-4 inhibitor rolipram on behavior depend on cyclic AMP response element binding protein-mediated neurogenesis in the hippocampus. *Neuropsychopharmacology.* 34(11) (2009):2404–19. <https://doi.org/10.1038/npp.2009.66> <sup>6</sup>
- [52] T. Itoh, Abe K, Tokumura M, Horiuchi M, Inoue O, Ibi N. Different regulation of adenylyl cyclase and rolipram-sensitive phosphodiesterase activity on the frontal cortex and hippocampus in learned helplessness rats. *Brain Res.* 991(1–2) (2003):142–9. <https://doi.org/10.1016/j.brainres.2003.08.007> <sup>7</sup>
- [53] Y.F. Li, Y. Huang, S.L. Amsdell, L. Xiao, J.M. O'Donnell, H.T. Zhang, Antidepressant- and anxiolytic-like effects of the phosphodiesterase-4 (PDE4) inhibitor rolipram on behavior depend on cyclic AMP-response element binding protein (CREB)-mediated neurogenesis <sup>8</sup>

in the hippocampus. *Neuropsychopharmacology* 34(11) (2009) <https://doi.org/10.1038/npp.2009.66> <sup>1</sup>

[54] R.J. Bird, G.S. Baillie, S.J. Yarwood. Interaction with receptor for activated C-kinase 1 (RACK1) sensitizes the phosphodiesterase PDE4D5 towards hydrolysis of cAMP and activation by protein kinase C. *Biochem J.* 432(1) (2010); 207–16. <https://doi.org/10.1042/BJ20101010>. <sup>2</sup>

[55] S.J. Yarwood, M.R. Steele, G. Scotland, M.D. Houslay, G.B. Bolger. The RACK1 signaling scaffold protein selectively interacts with the cAMP-specific phosphodiesterase PDE4D5 isoform. *J Biol Chem.* 274(21) (1999); 4909–17. <https://doi.org/10.1074/jbc.274.21.14909> <sup>3</sup>

[56] R. Yaka, C. Thornton, A.J. Vagts, K. Phamluong, A. Bonci, D. Ron. NMDA receptor function is regulated by the inhibitory scaffolding protein, RACK1. *Proc Natl Acad Sci USA.* 99(8) (2002);5710–5. <https://doi.org/10.1073/pnas.062046299> <sup>4</sup>

[57] K.T. Ota, V.J. Pierre, J.E. Ploski, K. Queen, G.E. Schafe, The NO-cGMP-PKG signaling pathway regulates synaptic plasticity and fear memory consolidation in the lateral amygdala via activation of ERK/MAP kinase. *Learn Mem.* 15(10) (2008):792–805. <https://doi.org/10.1101/lm.1114808> <sup>5</sup>

[58] W.L. Chien, K.C Liang, C.M. Teng, S.C. Kuo, F.Y. Lee, W.M. Fu, Enhancement of long-term potentiation by a potent nitric oxide-guanylyl cyclase activator, 3-(5-hydroxymethyl-2-furyl)-1-benzyl-indazole. *Mol Pharmacol.* 63(6)(2003):1322–8. <https://doi.org/10.1523/JNEUROSCI.21-01-00143.2001> <sup>6</sup>

[59] R.D Hawkins, H. Son, O. Arancio, Nitric oxide as a retrograde messenger during long-term potentiation in hippocampus. *Prog Brain Res.* 118 (1998):155–72. [https://doi.org/10.1016/s0079-6123\(08\)63206-9](https://doi.org/10.1016/s0079-6123(08)63206-9) <sup>7</sup>

[60] O. Arancio, I. Antonova, S. Gambaryan, S.M. Lohmann, J.S. Wood, D.S. Lawrence, et al. Presynaptic role of cGMP-dependent protein kinase during long-lasting potentiation. *J Neurosci.* 21(1) (2001):143–9. <https://doi.org/10.1523/JNEUROSCI.21-01-00143.2001>. <sup>8</sup>

- [61] W.L. Chien, K.C Liang, C.M. Teng, S.C. Kuo, F.Y. Lee, W.M. Fu, Enhancement of long-term potentiation by a potent nitric oxide-guanylyl cyclase activator, 3-(5-hydroxymethyl-2-furyl)-1-benzyl-indazole. *Mol Pharmacol.* 63(6)(2003):1322–8. <https://doi.org/10.1523/JNEUROSCI.21-01-00143.2001> <sup>1</sup>
- [62] R. Bernabeu, N. Schroder, J. Quevedo, M. Cammarota, I. Izquierdo, J.H. Medina, Further evidence for the involvement of a hippocampal cGMP/cGMP-dependent protein kinase cascade in memory consolidation. *Neuroreport.* 8(9–10) (1997):2221–4. <https://doi.org/10.1097/00001756-199707070-00026> <sup>2</sup>
- [63] P.F. Chapman, C.M. Atkins, M.T. Allen, J.E. Haley, J.E. Steinmetz, Inhibition of nitric oxide synthesis impairs two different forms of learning. *Neuroreport.* 3(7) (1992):567–70. <https://doi.org/10.1097/00001756-199207000-00005> <sup>3</sup>
- [64] G.E. Schafe, E.P. Bauer, S. Rosis, C.R. Farb, S.M. Rodrigues, J.E. LeDoux. Memory consolidation of Pavlovian fear conditioning requires nitric oxide signaling in the lateral amygdala. *Eur J Neurosci.* 22(1) (2005):201–11. <https://doi.org/10.1111/j.1460-9568.2005.04209.x> <sup>4</sup>
- [65] J. Garthwaite, Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci.* 1991 Feb;14(2):60–7. [https://doi.org/10.1016/0166-2236\(91\)90022-m](https://doi.org/10.1016/0166-2236(91)90022-m) <sup>5</sup>
- [66] F.G. Boess, M. Hendrix, F.J. van der Staay, C. Erb, R. Schreiber, W. van Staveren, et al. Inhibition of phosphodiesterase 2 increases neuronal cGMP, synaptic plasticity and memory performance. *Neuropharmacology.* 47(7) (2004):1081–92. <https://doi.org/10.1016/j.neuropharm.2004.07.040> <sup>6</sup>
- [67] A. Blokland, R. Schreiber, J. Prickaerts, Improving memory: a role for phosphodiesterases. *Curr Pharm Des.* 12(20) (2006):2511–23. <https://doi.org/10.2174/138161206777698855> <sup>7</sup>
- [68] D. Puzzo, S. Sapienza, O. Arancio, A. Palmeri, Role of phosphodiesterase 5 in synaptic plasticity and memory. *Neuropsychiatr Dis Treat.* 4(2) (2008):371–87. <https://doi.org/10.2147/ndt.s2447>

- [69] J.B. Kuzmiski, B.A. MacVicar, Cyclic Nucleotide-Gated Channels Contribute to the Cholinergic Plateau Potential in Hippocampal CA1 Pyramidal Neurons. *J Neurosci.* 21(22) (2001):8707–14. <https://doi.org/10.1523/JNEUROSCI.21-22-08707.2001> <sup>1</sup>
- [70] L. Zhang, J.H. Seo, H. Li, G. Nam, H.O. Yang, The phosphodiesterase 5 inhibitor, KJH-1002, reverses a mouse model of amnesia by activating a cGMP/cAMP response element binding protein pathway and decreasing oxidative damage. *Br J Pharmacol.* 175(16) (2018):3347–60. <https://doi.org/10.1111/bph.14377> <sup>2</sup>
- [71] J.P. Adams, J.D. Sweatt, Molecular psychology: roles for the ERK MAP kinase cascade in memory. *Annu Rev Pharmacol Toxicol.* 42 (2002):135–63. <https://doi.org/10.1146/annurev.pharmtox.42.082701.145401> <sup>3</sup>
- [72] S.K. Sharma, C.M. Sherff, J. Shobe, M.W. Bagnall, M.A. Sutton, T.J. Carew. Differential role of mitogen-activated protein kinase in three distinct phases of memory for sensitization in Aplysia. *J Neurosci.* 23(9) (2003):3899–907. <https://doi.org/10.1523/JNEUROSCI.23-09-03899.2003> <sup>4</sup>
- [73] A.L. Purcell, S.K. Sharma, M.W. Bagnall, M.A. Sutton, T.J. Carew, Activation of a tyrosine kinase-MAPK cascade enhances the induction of long-term synaptic facilitation and long-term memory in Aplysia. *Neuron.* 37(3) (2003):473–84. [https://doi.org/10.1016/s0896-6273\(03\)00030-8](https://doi.org/10.1016/s0896-6273(03)00030-8) <sup>5</sup>
- [74] H.T. Zhang, Y. Zhao, Y. Huang, N.R. Dorairaj, L.J. Chandler, J.M. O'Donnell, Inhibition of the Phosphodiesterase 4 (PDE4) Enzyme Reverses Memory Deficits Produced by Infusion of the MEK Inhibitor U0126 into the CA1 Subregion of the Rat Hippocampus. *Neuropsychopharmacol.* 29(8) (2004):1432–9. <https://doi.org/10.1038/sj.npp.1300440> <sup>6</sup>
- [75] W. Qiu, S. Zhuang, F.C. von Lintig, G.R. Boss, R.B. Pilz. Cell type-specific regulation of B-Raf kinase by cAMP and 14-3-3 proteins. *J Biol Chem.* 275(41) (2000):31921–9. <https://doi.org/10.1074/jbc.M003327200> <sup>7</sup>



- [76] R. Hoffmann, G.S. Baillie, S.J. MacKenzie, S.J. Yarwood, M.D. Houslay. The MAP kinase ERK2 inhibits the cyclic AMP-specific phosphodiesterase HSPDE4D3 by phosphorylating it at Ser579. *EMBO J.* 18(4) (1999):893–903. <https://doi.org/10.1093/emboj/18.4.893>
- [77] S.L. Patterson, C. Pittenger, A. Morozov, K.C. Martin, H. Scanlin, C. Drake, et al. Some forms of cAMP-mediated long-lasting potentiation are associated with release of BDNF and nuclear translocation of phospho-MAP kinase. *Neuron.* 32(1) (2001):123–40. [https://doi.org/10.1016/s0896-6273\(01\)00443-3](https://doi.org/10.1016/s0896-6273(01)00443-3)
- [78] P.J.S. Stork, J.M. Schmitt. Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. *Trends Cell Biol.* 12(6) (2002):258–66. [https://doi.org/10.1016/s0962-8924\(02\)02294-8](https://doi.org/10.1016/s0962-8924(02)02294-8)
- [79] R. Anjum, J. Blenis, The RSK family of kinases: emerging roles in cellular signalling. *Nat Rev Mol Cell Biol.* 9(10) (2008):747–58. <https://doi.org/10.1038/nrm2509>
- [80] Y. Gao, K. Deng, J. Hou, J.B. Bryson, A. Barco, E. Nikulina, et al. Activated CREB is sufficient to overcome inhibitors in myelin and promote spinal axon regeneration in vivo. *Neuron.* 44(4) (2004):609–21. <https://doi.org/10.1016/j.neuron.2004.10.030>
- [81] R.S. Song, B. Massenburg, W. Wenderski, V. Jayaraman, L. Thompson, S.R. Neves. ERK regulation of phosphodiesterase 4 enhances dopamine-stimulated AMPA receptor membrane insertion. *Proc Natl Acad Sci U S A.* 110 (38) (2013) :15437–42. <https://doi.org/10.1073/pnas.1311783110>.
- [82] E. Miyamoto, Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. *J Pharmacol Sci.* 100(5) (2006):433–42. <https://doi.org/10.1254/jphs.cpj06007x>
- [83] S. Navakkode, S. Sajikumar, J.U. Frey, Mitogen-activated protein kinase-mediated reinforcement of hippocampal early long-term depression by the type IV-specific phosphodiesterase inhibitor rolipram and its effect on synaptic tagging. *J Neurosci.* 25(46) (2005):10664–70. <https://doi.org/10.1523/JNEUROSCI.2443-05.2005>

- [84] E. Valera, F.J. Sánchez-Martín, A.V. Ferrer-Montiel, A. Messeguer, J.M. Merino. NMDA-induced neuroprotection in hippocampal neurons is mediated through the protein kinase A and CREB (cAMP-response element-binding protein) pathway. *Neurochem Int.* 53(5) (2008):148–54. <https://doi.org/10.1016/j.neuint.2008.07.007> <sup>1</sup>
- [85] K. Matousovic, J.P. Grande, C.C. Chini, E.N. Chini, T.P. Dousa. Inhibitors of cyclic nucleotide phosphodiesterase isozymes type-III and type-IV suppress mitogenesis of rat mesangial cells. *J Clin Invest.* 96(1) (1995):401–10. <https://doi.org/10.1172/JCI118049> <sup>2</sup>
- [86] Y. Obara, A. Yamauchi, S. Takehara, W. Nemoto, M. Takahashi, P.J.S. Stork, et al. ERK5 activity is required for nerve growth factor-induced neurite outgrowth and stabilization of tyrosine hydroxylase in PC12 cells. *J Biol Chem.* 284(35) (2009):23564–73. <https://doi.org/10.1074/jbc.M109.027821> <sup>3</sup>
- [87] S.L. Small, G. Buccino, A. Solodkin. Brain repair after stroke--a novel neurological model. *Nat Rev Neurol.* 9(12) (2013):698–707. <https://doi.org/10.1038/nrneurol.2013.222> <sup>4</sup>
- [88] D.M. Hermann, M. Chopp, Promoting brain remodelling and plasticity for stroke recovery: therapeutic promise and potential pitfalls of clinical translation. *Lancet Neurol.* 11(4) (2012):369–80. [https://doi.org/10.1016/S1474-4422\(12\)70039-X](https://doi.org/10.1016/S1474-4422(12)70039-X) <sup>5</sup>
- [89] C. Alia, C. Spalletti, S. Lai, A. Panarese, G. Lamola, F. Bertolucci, et al. Neuroplastic Changes Following Brain Ischemia and their Contribution to Stroke Recovery: Novel Approaches in Neurorehabilitation. *Front Cell Neurosci.* (2017):11. <https://doi.org/10.3389/fncel.2017.00076> <sup>6</sup>
- [90] P.R.A. Heckman, A. Blokland, J. Ramaekers, J. Prickaerts, PDE and cognitive processing: beyond the memory domain. *Neurobiol Learn Mem.* 119 (2015):108–22. <https://doi.org/10.1016/j.nlm.2014.10.011> <sup>7</sup>
- [91] C. Pittenger, R.S. Duman, Stress, depression, and neuroplasticity: a convergence of mechanisms. *Neuropsychopharmacology.* 33(1) (2008):88–109. <https://doi.org/10.1038/sj.npp.1301574> <sup>8</sup>

- [92] V. Di Lazzaro, G. Pellegrino, G. Di Pino, M. Corbetta, F. Ranieri, N. Brunelli, et al. Val66Met BDNF Gene Polymorphism Influences Human Motor Cortex Plasticity in Acute Stroke. *Brain Stimul* 8(1) (2015): 92–6. <https://doi.org/10.1016/j.brs.2014.08.006> <sup>1</sup>
- [93] D. Kotłęga, B. Peda, A. Zembroń-Łacny, M. Gołąb-Janowska, P. Nowacki, The role of brain-derived neurotrophic factor and its single nucleotide polymorphisms in stroke patients. *Neurol Neurochir Pol.* 51(3) (2017):240–6. <https://doi.org/10.1016/j.pjnns.2017.02.008> <sup>2</sup>
- [94] C.S. Mang, K.L. Campbell, C.J.D. Ross, L.A. Boyd. Promoting neuroplasticity for motor rehabilitation after stroke: considering the effects of aerobic exercise and genetic variation on brain-derived neurotrophic factor. *Phys Ther.* 93(12) (2013):1707–16. <https://doi.org/10.2522/ptj.20130053> <sup>3</sup>
- [95] E. Reyes-Irisarri, Pérez-Torres S, Miró X, Martínez E, Puigdomènech P, Palacios JM, et al. Differential distribution of PDE4B splice variant mRNAs in rat brain and the effects of systemic administration of LPS in their expression. *Synapse.* 2008 Jan;62(1):74–9. <https://doi.org/10.1002/syn.20459> <sup>4</sup>
- [96] R. Havekes, A.J. Park, J.C. Tudor, V.G. Luczak, R.T. Hansen, S.L. Ferri, et al. Sleep deprivation causes memory deficits by negatively impacting neuronal connectivity in hippocampal area CA1. *eLife* (2016);5:e13424. <https://doi.org/10.7554/eLife.13424> <https://doi.org/> <sup>5</sup>
- [97] H.T. Zhang, Y. Huang, A. Masood, L.R. Stolinski, Y. Li, L. Zhang, et al. Anxiogenic-like behavioral phenotype of mice deficient in phosphodiesterase 4B (PDE4B). *Neuropsychopharmacology.* 33(7) (2008):1611–23. <https://doi.org/10.1038/sj.npp.1301537> <sup>6</sup>
- [98] C. Jørgensen, S. Yasmeen, H.K. Iversen, C. Kruuse, Phosphodiesterase4D (PDE4D)--A risk factor for atrial fibrillation and stroke? *J Neurol Sci.* 359(1–2) (2015):266–74. <https://doi.org/10.1016/j.jns.2015.11.010> <sup>7</sup>
- [99] Z.Q. Zou, J.J. Chen, H.F. Feng, Y.F. Cheng, H.T. Wang, Z.Z. Zhou, et al. Novel Phosphodiesterase 4 Inhibitor FCPR03 Alleviates Lipopolysaccharide-Induced <sup>8</sup>

Neuroinflammation by Regulation of the cAMP/PKA/CREB Signaling Pathway and NF- $\kappa$ B Inhibition. *J Pharmacol Exp Ther.* 362(1) (2017):67–77. <https://doi.org/10.1124/jpet.116.239608> <sup>1</sup>

[100] R. Havekes, A.J. Park, R.E. Tolentino, V.M. Bruinenberg, J.C. Tudor, Y. Lee, et al. <sup>2</sup>  
Compartmentalized PDE4A5 Signaling Impairs Hippocampal Synaptic Plasticity and Long-Term Memory. *J Neurosci.* 36(34) (2016):8936–46. <https://doi.org/10.1523/JNEUROSCI.0248-16.2016>

[101] E.W. Vogel, F.N. Morales, D.F. Meaney, C.R. Bass, B. Morrison, <sup>3</sup>  
Phosphodiesterase-4 inhibition restored hippocampal long term potentiation after primary blast. *Exp Neurol.* 293 (2017):91–100. <https://doi.org/10.1016/j.expneurol.2017.03.025>

[102] Y.F. Li, Y.F. Cheng, Y. Huang, M. Conti, S.P. Wilson, J.M. O'Donnell, et al. <sup>4</sup>  
Phosphodiesterase-4D knockout and RNAi-mediated knockdown enhance memory and increase hippocampal neurogenesis via increased cAMP signaling. *J Neurosci.* 31(1) (2011):172–83. <https://doi.org/10.1523/JNEUROSCI.5236-10.2011>

[103] C. Zhang, Y. Cheng, H. Wang, C. Wang, S.P. Wilson, J. Xu, et al. <sup>5</sup>  
RNA interference-mediated knockdown of long-form phosphodiesterase-4D (PDE4D) enzyme reverses amyloid- $\beta$ 42-induced memory deficits in mice. *J Alzheimers Dis.* 38(2) (2014):269–80. <https://doi.org/10.3233/JAD-122236>

[104] H. Guo, Y. Cheng, C. Wang, J. Wu, Z. Zou, B. Niu, et al. <sup>6</sup>  
FFPM, a PDE4 inhibitor, reverses learning and memory deficits in APP/PS1 transgenic mice via cAMP/PKA/CREB signaling and anti-inflammatory effects. *Neuropharmacology.* 116 (2017):260–9. <https://doi.org/10.1016/j.neuropharm.2017.01.004>

[105] V. Wiescholleck, D. Manahan-Vaughan, <sup>7</sup>  
PDE4 inhibition enhances hippocampal synaptic plasticity in vivo and rescues MK801-induced impairment of long-term potentiation and object recognition memory in an animal model of psychosis. *Transl Psychiatry.* 2(3) (2012):e89. <https://doi.org/10.1038/tp.2012.17>

- [106] A.L.O. Hebb, H.A. Robertson, E.M. Denovan-Wright, Phosphodiesterase 10A inhibition is associated with locomotor and cognitive deficits and increased anxiety in mice. *Eur Neuropsychopharmacol.* 18(5) (2008):339–63. <https://doi.org/10.1016/j.euroneuro.2007.08.002> <sup>1</sup>
- [107] T. Kleppisch, Phosphodiesterases in the central nervous system. *Handb Exp Pharmacol.* (191) (2009):71–92. [https://doi.org/10.1007/978-3-540-68964-5\\_5](https://doi.org/10.1007/978-3-540-68964-5_5) <sup>2</sup>
- [108] P. Scheltens, K. Blennow, M.M.B. Breteler, B. de Strooper, G.B. Frisoni, S. Salloway, et al. Alzheimer's disease. *Lancet.* 388(10043) (2016):505–17. [https://doi.org/10.1016/S0140-6736\(15\)01124-1](https://doi.org/10.1016/S0140-6736(15)01124-1) <sup>3</sup>
- [109] A. Kumar, A. Singh, A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacol Rep.* 67(2) (2015):195–203. <https://doi.org/10.1016/j.pharep.2014.09.004> <sup>4</sup>
- [110] J.R.M. Coimbra, D.F.F. Marques, S.J. Baptista, C.M.F. Pereira, P.I. Moreira, T.C.P. Dinis, et al. Highlights in BACE1 Inhibitors for Alzheimer's Disease Treatment. *Front Chem.* 6 (2018):178. <https://doi.org/10.3389/fchem.2018.00178> <sup>5</sup>
- [111] S.B. Chidambaram, A.G. Rathipriya, S.R. Bolla, A. Bhat, B. Ray, A.M. Mahalakshmi, et al. Dendritic spines: Revisiting the physiological role. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 92 (2019):161–93. <https://doi.org/10.1016/j.pnpbp.2019.01.005> <sup>6</sup>
- [112] P. Merino-Serrais, R. Benavides-Piccione, L. Blazquez-Llorca, A. Kastanauskaite, A. Rábano, J. Avila, et al. The influence of phospho- $\tau$  on dendritic spines of cortical pyramidal neurons in patients with Alzheimer's disease. *Brain.* 136 (2013):1913–28. <https://doi.org/10.1093/brain/awt088> <sup>7</sup>
- [113] Y. Su, J. Ryder, B. Ni. Inhibition of Abeta production and APP maturation by a specific PKA inhibitor. *FEBS Lett.* 546(2–3) (2003):407–10. [https://doi.org/10.1016/s0014-5793\(03\)00645-8](https://doi.org/10.1016/s0014-5793(03)00645-8) <sup>8</sup>

- [114] S. Pérez-Torres, R. Cortés, M. Tolnay, A. Probst, J.M. Palacios, G. Mengod. Alterations on phosphodiesterase type 7 and 8 isozyme mRNA expression in Alzheimer's disease brains examined by in situ hybridization. *Exp Neurol.* 182(2) (2003):322–34. [https://doi.org/10.1016/s0014-4886\(03\)00042-6](https://doi.org/10.1016/s0014-4886(03)00042-6) <sup>1</sup>
- [115] B.C. Carlyle, A.C. Nairn, M. Wang, Y. Yang, L.E. Jin, A.A. Simen, et al. cAMP-PKA phosphorylation of tau confers risk for degeneration in aging association cortex. *Proc Natl Acad Sci U S A.* 111(13) (2014):5036–41. <https://doi.org/10.1073/pnas.1322360111> <sup>2</sup>
- [116] W.L. Bonkale, R.F. Cowburn, T.G. Ohm, N. Bogdanovic, J. Fastbom, A quantitative autoradiographic study of [3H]cAMP binding to cytosolic and particulate protein kinase A in post-mortem brain staged for Alzheimer's disease neurofibrillary changes and amyloid deposits. *Brain Res.* 818(2) (1999):383–96. [https://doi.org/10.1016/s0006-8993\(98\)01307-9](https://doi.org/10.1016/s0006-8993(98)01307-9) <sup>3</sup>
- [117] A. García-Jiménez, R.F. Cowburn, T.G. Ohm, N. Bogdanovic, B. Winblad, J. Fastbom. Quantitative autoradiography of [3H]forskolin binding sites in post-mortem brain staged for Alzheimer's disease neurofibrillary changes and amyloid deposits. *Brain Res.* 850(1–2) (1999):104–17. [https://doi.org/10.1016/s0006-8993\(99\)02111-3](https://doi.org/10.1016/s0006-8993(99)02111-3) <sup>4</sup>
- [118] Z. Liang, F. Liu, I. Grundke-Iqbal, K. Iqbal, C.X. Gong. Down-regulation of cAMP-dependent protein kinase by over-activated calpain in Alzheimer disease brain. *J Neurochem* 103(6) (2007):2462–70. <https://doi.org/10.1111/j.1471-4159.2007.04942.x> <sup>5</sup>
- [119] B. Gong, O.V. Vitolo, F. Trinchese, S. Liu, M. Shelanski, O. Arancio, Persistent improvement in synaptic and cognitive functions in an Alzheimer mouse model after rolipram treatment. *J Clin Invest.* 114(11) (2004):1624–34. <https://doi.org/10.1172/JCI22831> <sup>6</sup>
- [120] D.L. Smith, J. Pozueta, B. Gong, O. Arancio, M. Shelanski. Reversal of long-term dendritic spine alterations in Alzheimer disease models. *Proc Natl Acad Sci U S A* 106(39) (2009):16877–82. <https://doi.org/10.1073/pnas.0908706106> <sup>7</sup>



- [121] C. Wang, X.M. Yang, Y.Y. Zhuo, H. Zhou, H.B. Lin, Y.F. Cheng, et al. The phosphodiesterase-4 inhibitor rolipram reverses A $\beta$ -induced cognitive impairment and neuroinflammatory and apoptotic responses in rats. *Int J Neuropsychopharmacol.* 15(6) (2012):749–66. <https://doi.org/10.1017/S1461145711000836> <sup>1</sup>
- [122] N. Myeku, C.L. Clelland, S. Emrani, N.V. Kukushkin, W.H. Yu, A.L. Goldberg, et al. Tau-driven 26S proteasome impairment and cognitive dysfunction can be prevented early in disease by activating cAMP-PKA signaling. *Nat Med* 22(1) (2016):46–53. <https://doi.org/10.1038/nm.4011> <sup>2</sup>
- [123] O. Bruno, E. Fedele, J. Prickaerts, L. Parker, E. Canepa, C. Brullo, et al. GEBR-7b, a novel PDE4D selective inhibitor that improves memory in rodents at non-emetic doses. *Br J Pharmacol* 164(8) (2011):2054–63. <https://doi.org/10.1111/j.1476-5381.2011.01524.x> <sup>3</sup>
- [124] J.S. Sutcliffe, V. Beaumont, J.M. Watson, C.S. Chew, M. Beconi, D.M. Hutcheson, et al. Efficacy of Selective PDE4D Negative Allosteric Modulators in the Object Retrieval Task in Female Cynomolgus Monkeys (*Macaca fascicularis*). *PLOS ONE* (2014) ;9(7):e102449. <https://doi.org/10.1371/journal.pone.0102449> <sup>4</sup>
- [125] S.B. Chidambaram, A. Bhat, B. Ray, M. Sugumar, S.P. Muthukumar, T. Manivasagam, et al. Cocoa beans improve mitochondrial biogenesis via PPAR $\gamma$ /PGC1 $\alpha$  dependent signalling pathway in MPP<sup>+</sup> intoxicated human neuroblastoma cells (SH-SY5Y). *Nutr Neurosci.* (2018);1–10. <https://doi.org/> <sup>5</sup>
- [126] W. Poewe, K. Seppi, C.M. Tanner, G.M. Halliday, P. Brundin, J. Volkman, et al. Parkinson disease. *Nature Reviews Disease Primers* 3 (2017):17013. <https://doi.org/10.1080/1028415X.2018.1521088> <sup>6</sup>
- [127] S. Sathiy, V. Ranju, P. Kalaivani, R.J. Priya, H. Sumathy, A.G. Sunil, et al. Telmisartan attenuates MPTP induced dopaminergic degeneration and motor dysfunction through regulation of  $\alpha$ -synuclein and neurotrophic factors (BDNF and GDNF) expression in C57BL/6J mice. *Neuropharmacology.* 73 (2013):98–110. <https://doi.org/10.1016/j.neuropharm.2013.05.025> <sup>7</sup>

- [128] S. Sathiya, C.S. Babu. Telmisartan alleviates nitrosative stress in turn dopaminergic degeneration in mice mptp model of parkinsonism “biochemical and histopathological evidences. *IJPPS*. 1 (2015) 97–101. <sup>1</sup>
- [129] S. Sekar, S. Mani, B. Rajamani, T. Manivasagam, A.J. Thenmozhi, A. Bhat, et al. Telmisartan Ameliorates Astroglial and Dopaminergic Functions in a Mouse Model of Chronic Parkinsonism. *Neurotox Res*. (2018) <https://doi.org/10.1007/s12640-018-9921-3> <sup>2</sup>
- [130] P.R.A. Heckman, M.A. van Duinen, E.P.P. Bollen, A. Nishi, L.P. Wennogle, A. Blokland, et al. Phosphodiesterase Inhibition and Regulation of Dopaminergic Frontal and Striatal Functioning: Clinical Implications. *Int J Neuropsychopharmacol* 19(10) (2016). <https://doi.org/10.1093/ijnp/pyw030> <sup>3</sup>
- [131] N. Nishino, N. Kitamura, T. Hashimoto, C. Tanaka, Transmembrane signalling systems in the brain of patients with Parkinson’s disease. *Rev Neurosci*. 4(2) (1993):213–22. <https://doi.org/> <sup>4</sup>
- [132] S. Sharma, C.S. Moon, A. Khogali, A. Haidous, A. Chabenne, C. Ojo, et al. Biomarkers in Parkinson’s disease (recent update). *Neurochemistry International*. 63(3) (2013):201–29. <https://doi.org/10.1515/revneuro.1993.4.2.213> <sup>5</sup>
- [133] T. Mizuno, T. Kurotani, Y. Komatsu, J. Kawanokuchi, H. Kato, N. Mitsuma, et al. Neuroprotective role of phosphodiesterase inhibitor ibudilast on neuronal cell death induced by activated microglia. *Neuropharmacology*. 46(3) (2004):404–11. <https://doi.org/10.1016/j.neuropharm.2003.09.009> <sup>6</sup>
- [134] K. Kinoshita, Y. Muroi, T. Unno, T. Ishii, Rolipram improves facilitation of contextual fear extinction in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced mouse model of Parkinson’s disease. *Journal of Pharmacological Sciences* 134 (1) (2017):55–8. <https://doi.org/10.1016/j.jphs.2017.04.002> <sup>7</sup>
- [135] F. Niccolini, H. Wilson, G. Pagano, C. Coello, M.A. Mehta, G.E. Searle, et al. Loss of phosphodiesterase 4 in Parkinson disease: Relevance to cognitive deficits. *Neurology*. 89(6) (2017):586–93. <https://doi.org/10.1212/WNL.0000000000004201> <sup>8</sup>

- [136] J. Schwenkgrub, M. Zaremba, I. Joniec-Maciejak, A. Cudna, D. Mirowska-Guzel, I. Kurkowska-Jastrzębska, The phosphodiesterase inhibitor, ibudilast, attenuates neuroinflammation in the MPTP model of Parkinson's disease. *PLoS One* (2017):12(7). <https://doi.org/10.1371/journal.pone.0182019> <sup>1</sup>
- [137] J. Zhong, H. Yu, C. Huang, Q. Zhong, Y. Chen, J. Xie, et al. Inhibition of phosphodiesterase 4 by FCPR16 protects SH-SY5Y cells against MPP<sup>+</sup>-induced decline of mitochondrial membrane potential and oxidative stress. *Redox Biology* 16 (2018):47–58. <https://doi.org/10.1016/j.redox.2018.02.008> <sup>2</sup>
- [138] J. Zhong, J. Xie, J. Xiao, D. Li, B. Xu, X. Wang, et al. Inhibition of PDE4 by FCPR16 induces AMPK-dependent autophagy and confers neuroprotection in SH-SY5Y cells and neurons exposed to MPP<sup>+</sup>-induced oxidative insult. *Free Radic Biol Med.* 135 (2019):87–101. <https://doi.org/10.1016/j.freeradbiomed.2019.02.027> <sup>3</sup>
- [139] Hulley, J. Hartikka, S. Abdel'Al, P. Engels, H.R. Buerki, K.H. Wiederhold, et al. Inhibitors of type IV phosphodiesterases reduce the toxicity of MPTP in substantia nigra neurons in vivo. *Eur J Neurosci.* 7(12) (1995):2431–40. <https://doi.org/10.1111/j.1460-9568.1995.tb01041.x> <sup>4</sup>
- [140] A. Nishi, M. Kuroiwa, D.B. Miller, J.P. O'Callaghan, H.S. Bateup, T. Shuto, et al. Distinct roles of PDE4 and PDE10A in the regulation of cAMP/PKA signaling in the striatum. *J Neurosci.* 28(42) 2008: 10460–71. <https://doi.org/10.1523/JNEUROSCI.2518-08.2008> <sup>5</sup>
- [141] M. Casacchia, G. Meco, F. Castellana, L. Bedini, G. Cusimano, A. Agnoli, Therapeutic use of a selective cAMP phosphodiesterase inhibitor (Rolipram) in Parkinson's disease. *Pharmacol Res Commun.* 15(3) (1983):329–34. [https://doi.org/10.1016/s0031-6989\(83\)80017-4](https://doi.org/10.1016/s0031-6989(83)80017-4) <sup>6</sup>
- [142] K. Takuma, E. Lee, R. Enomoto, K. Mori, A. Baba, T. Matsuda. Ibudilast attenuates astrocyte apoptosis via cyclic GMP signalling pathway in an in vitro reperfusion model. *Br J Pharmacol* 133(6) (2001):841–8. <https://doi.org/10.1038/sj.bjp.0704146> <sup>7</sup>

- [143] M. Salou, A. Garcia, L. Michel, A. Gainche-Salmon, D. Loussouarn, B. Nicol, et al.<sup>1</sup>  
Expanded CD8 T-cell sharing between periphery and CNS in multiple sclerosis. *Ann Clin  
Transl Neurol.* 2(6) (2015):609–22. <https://doi.org/10.1002/acn3.199>
- [144] P. Deshpande, I.L. King, B.M. Segal, IL-12 driven upregulation of P-selectin ligand on<sup>2</sup>  
myelin-specific T cells is a critical step in an animal model of autoimmune demyelination.  
*J Neuroimmunol.* 173(1–2) (2006):35–44. <https://doi.org/10.1016/j.jneuroim.2005.11.016>
- [145] A.G. Trenova, G.S. Slavov, M.N. Draganova-Filipova, N.G. Mateva, M.G. Manova, L.D.<sup>3</sup>  
Miteva, et al. Circulating levels of interleukin-17A, tumor necrosis factor-alpha, interleukin-  
18, interleukin-10, and cognitive performance of patients with relapsing-remitting multiple  
sclerosis. *Neurol Res.* 40(3) (2018):153–9. <https://doi.org/10.1080/01616412.2017.1420522>
- [146] T.J. Kopper, J.C. Gensel. Myelin as an inflammatory mediator: myelin interactions with<sup>4</sup>  
complement, macrophages, and microglia in spinal cord injury. *J Neurosci Res.* 96(6)  
(2018):969–77. <https://doi.org/10.1002/jnr.24114>
- [147] T. Bopp, H. Jonuleit, E. Schmitt, Regulatory T cells--the renaissance of the suppressor T<sup>5</sup>  
cells. *Ann Med.* 39(5) (2007):322–34. <https://doi.org/10.1080/07853890701379700>
- [148] E. Maida, W. Kristoferitsch, Cyclic adenosine 3',5'monophosphate in cerebrospinal fluid of<sup>6</sup>  
multiple sclerosis patients. *J Neurol.* 225(2) (1981):145–51. <https://doi.org/10.1007/bf00313327>
- [149] S. Khezri, M. Javan, M. Goudarzvand, S. Semnanian, H. Baharvand. Dibutyl cyclic AMP<sup>7</sup>  
inhibits the progression of experimental autoimmune encephalomyelitis and potentiates  
recruitment of endogenous neural stem cells. *J Mol Neurosci.* 51(2) (2013):298–306.  
<https://doi.org/10.1007/s12031-013-9959-x>
- [150] E. Duarte-Silva, R. Araújo SM da, W.H. Oliveira, Lós DB de, França MER de, A.P. Bonfanti,<sup>8</sup>  
et al. Sildenafil ameliorates EAE by decreasing apoptosis in the spinal cord of C57BL/6  
mice. *Journal of Neuroimmunology.* 321 (2018):125–37. <https://doi.org/10.1016/j.jneuroim.2018.06.002>

- [151] E.M. Medina-Rodríguez, A. Bribián, A. Boyd, V. Palomo, J. Pastor, A. Lagares, et al.<sup>1</sup>  
Promoting in vivo remyelination with small molecules: a neuroreparative pharmacological treatment for Multiple Sclerosis. *Sci Rep.* 03;7 (2017):43545. <https://doi.org/10.1038/srep43545>
- [152] D. Ekholm, B. Hemmer, G. Gao, M. Vergelli, R. Martin, V. Manganiello, Differential<sup>2</sup>  
expression of cyclic nucleotide phosphodiesterase 3 and 4 activities in human T cell clones specific for myelin basic protein. *The Journal of Immunology.* 159(3) (1997):1520–9.
- [153] R. Korhonen, T. Hömmö, T. Keränen, M. Laavola, M. Hämäläinen, K. Vuolteenaho, et al.<sup>3</sup>  
Attenuation of TNF production and experimentally induced inflammation by PDE4 inhibitor rolipram is mediated by MAPK phosphatase-1. *Br J Pharmacol.* 169(7) (2013):1525–36. <https://doi.org/10.1111/bph.12189>
- [154] C. González-García, B. Bravo, A. Ballester, R. Gómez-Pérez, C. Eguiluz, M. Redondo, et al.<sup>4</sup>  
Comparative assessment of PDE 4 and 7 inhibitors as therapeutic agents in experimental autoimmune encephalomyelitis. *Br J Pharmacol.* 170(3) (2013):602–13. <https://doi.org/10.1111/bph.12308>
- [155] S. Jung, J. Zielasek, G. Köllner, T. Donhauser, K. Toyka, H.P. Hartung, Preventive but not<sup>5</sup>  
therapeutic application of Rolipram ameliorates experimental autoimmune encephalomyelitis in Lewis rats. *J Neuroimmunol.* 68(1–2) (1996):1–11. [https://doi.org/10.1016/0165-5728\(96\)00051-3](https://doi.org/10.1016/0165-5728(96)00051-3)
- [156] C.P. Genain, T. Roberts, R.L. Davis, M.H. Nguyen, A. Uccelli, D. Faulds, et al. Prevention<sup>6</sup>  
of autoimmune demyelination in non-human primates by a cAMP-specific phosphodiesterase inhibitor. *Proc Natl Acad Sci USA.* 92(8) (1995):3601–5. <https://doi.org/10.1073/pnas.92.8.3601>
- [157] Fujimoto T, Sakoda S, Fujimura H, Yanagihara T. Ibudilast, a phosphodiesterase inhibitor,<sup>7</sup>  
ameliorates experimental autoimmune encephalomyelitis in Dark August rats. *J Neuroimmunol.* 1999 Mar 1;95(1–2):35–42. [https://doi.org/10.1016/s0165-5728\(98\)00251-3](https://doi.org/10.1016/s0165-5728(98)00251-3)

- [158] V.A. Folcik, T. Smith, S. O'Bryant, J.A. Kawczak, B. Zhu, H. Sakurai, et al. Treatment with BBB022A or rolipram stabilizes the blood-brain barrier in experimental autoimmune encephalomyelitis: an additional mechanism for the therapeutic effect of type IV phosphodiesterase inhibitors. *J Neuroimmunol.* 1(97) (1999):119–28. [https://doi.org/10.1016/s0165-5728\(99\)00063-6](https://doi.org/10.1016/s0165-5728(99)00063-6) <sup>1</sup>
- [159] G.L. Snyder, K.E. Vanover, PDE Inhibitors for the Treatment of Schizophrenia. *Adv Neurobiol.* 17 (2017):385–409. [https://doi.org/10.1007/978-3-319-58811-7\\_14](https://doi.org/10.1007/978-3-319-58811-7_14) <sup>2</sup>
- [160] M.V. Duinen, O.A.H. Reneerkens, L. Lambrecht, A. Sambeth, B.P.F. Rutten, J.V. Os, et al. Treatment of Cognitive Impairment in Schizophrenia: Potential Value of Phosphodiesterase Inhibitors in Prefrontal Dysfunction. *Curr Pharm Des.* 21(26) (2015):3813–28. <https://doi.org/10.2174/1381612821666150605110941> <sup>3</sup>
- [161] J. Gilleen, Y. Farah, C. Davison, S. Kerins, L. Valdearenas, T. Uz, et al. An experimental medicine study of the phosphodiesterase-4 inhibitor, roflumilast, on working memory-related brain activity and episodic memory in schizophrenia patients. *Psychopharmacology.* (2018): <https://doi.org/10.1007/s00213-018-5134-y> <sup>4</sup>
- [162] J.K. Millar, B.S. Pickard, S. Mackie, R. James, S. Christie, S.R. Buchanan, et al. DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science.* 310(5751) (2005):1187–91. <https://doi.org/10.1126/science.1112915> <sup>5</sup>
- [163] B.S. Pickard, P.A. Thomson, A. Christoforou, K.L. Evans, S.W. Morris, D.J. Porteous, et al. The PDE4B gene confers sex-specific protection against schizophrenia. *Psychiatr Genet.* 17(3) (2007):129–33. <https://doi.org/10.1097/YPG.0b013e328014492b> <sup>6</sup>
- [164] Bradshaw NJ, Ogawa F, Antolin-Fontes B, Chubb JE, Carlyle BC, Christie S, et al. DISC1, PDE4B, and NDE1 at the centrosome and synapse. *Biochem Biophys Res Commun.* 2008 Dec 26;377(4):1091–6. <sup>7</sup>
- [165] S.J. Clapcote, T.V. Lipina, J.K. Millar, S. Mackie, S. Christie, F. Ogawa, et al. Behavioral phenotypes of Disc1 missense mutations in mice. *Neuron.* 54(3) (2007):387–402. <https://doi.org/10.1016/j.neuron.2007.04.015> <sup>8</sup>



- [166] J.A. Siuciak, D.S. Chapin, J.F. Harms, L.A. Lebel, S.A. McCarthy, L. Chambers, et al.<sup>1</sup>  
Inhibition of the striatum-enriched phosphodiesterase PDE10A: a novel approach to the  
treatment of psychosis. *Neuropharmacology*. 51(2) (2006):386–96. <https://doi.org/10.1016/j.neuropharm.2006.04.013>
- [167] T. Mori, J. Baba, Y. Ichimaru, T. Suzuki. Effects of rolipram, a selective inhibitor of<sup>2</sup>  
phosphodiesterase 4, on hyperlocomotion induced by several abused drugs in mice. *Jpn J  
Pharmacol*. 83(2) (2000):113–8. <https://doi.org/10.1254/jjp.83.113>
- [168] S.J. Kanes, J. Tokarczyk, S.J. Siegel, W. Bilker, T. Abel, M.P. Kelly. Rolipram: a specific<sup>3</sup>  
phosphodiesterase 4 inhibitor with potential antipsychotic activity. *Neuroscience*. 144(1)  
(2007):239–46. <https://doi.org/10.1016/j.neuroscience.2006.09.026>
- [169] G.S. Malhi, J.J. Mann. Depression. *The Lancet*. 392(10161) (2018):2299–312.<sup>4</sup>  
[https://doi.org/10.1016/S0140-6736\(18\)31948-2](https://doi.org/10.1016/S0140-6736(18)31948-2)
- [170] R.F. Cowburn, J.O. Marcusson, A. Eriksson, B. Wiehager, C. O'Neill. Adenylyl cyclase<sup>5</sup>  
activity and G-protein subunit levels in postmortem frontal cortex of suicide victims. *Brain  
Res*. 633(1–2) (1994):297–304. [https://doi.org/10.1016/0006-8993\(94\)91552-0](https://doi.org/10.1016/0006-8993(94)91552-0)
- [171] D. Dowlathshahi, G.M. MacQueen, J.F. Wang, L.T.s Young, Increased temporal cortex CREB<sup>6</sup>  
concentrations and antidepressant treatment in major depression. *Lancet*. 352(9142)  
(1998):1754–5. [https://doi.org/10.1016/S0140-6736\(05\)79827-5](https://doi.org/10.1016/S0140-6736(05)79827-5)
- [172] Y. Dwivedi, R.R. Conley, R.C. Roberts, C.A. Tamminga, G.N. Pandey. [(3)H]cAMP binding<sup>7</sup>  
sites and protein kinase a activity in the prefrontal cortex of suicide victims. *Am J  
Psychiatry*. 159(1) (2002):66–73. <https://doi.org/10.1176/appi.ajp.159.1.66>
- [173] O'Donnell JM, Frith S, Wilkins J. Involvement of beta-1 and beta-2 adrenergic receptors in<sup>8</sup>  
the antidepressant-like effects of centrally administered isoproterenol. *J Pharmacol Exp  
Ther*. ;271(1) (1994);46–54.
- [174] H.T. Zhang, S.A. Frith, J. Wilkins, J.M. O'Donnell. Comparison of the effects of<sup>9</sup>  
isoproterenol administered into the hippocampus, frontal cortex, or amygdala on behavior

of rats maintained by differential reinforcement of low response rate. *Psychopharmacology* (Berl). 159(1) (2001); 89–97. <https://doi.org/10.1007/s002130100889> <sup>1</sup>

[175] D. Morin, R. Sapena, J.P. Tillement, S. Urien. Evidence for different interactions between beta(1)- and beta(2)-adrenoceptor subtypes with adenylyl cyclase in the rat brain: a concentration-response study using forskolin. *Pharmacol Res.*41(4) (2000);435–43. <https://doi.org/10.1006/phrs.1999.0609> <sup>2</sup>

[176] W. Richter, P. Day, R. Agrawal, M.D. Bruss, S. Granier, Y.L. Wang, et al. Signaling from  $\beta$ 1- and  $\beta$ 2-adrenergic receptors is defined by differential interactions with PDE4. *EMBO J.* 27(2) (2008);384–93. <https://doi.org/10.1038/sj.emboj.7601968> <sup>3</sup>

[177] H.T. Zhang, Y. Huang, K. Mishler, S.C. Roerig, J.M. O'Donnell. Interaction between the antidepressant-like behavioral effects of beta adrenergic agonists and the cyclic AMP PDE inhibitor rolipram in rats. *Psychopharmacology* (Berl). 182(1) (2005);104–15. <https://doi.org/10.1007/s00213-005-0055-y>. <sup>4</sup>

[178] M. Fujita, E.M. Richards, M.J. Niciu, D.F. Ionescu, S.S. Zoghbi, J. Hong, et al. cAMP signaling in brain is decreased in unmedicated depressed patients and increased by treatment with a selective serotonin reuptake inhibitor. *Mol Psychiatry.* 22(5) (2017);754–9. <https://doi.org/10.1038/mp.2016.171> <sup>5</sup>

[179] A.M. Shalaby, S.M. Kamal, Effect of rolipram, a phosphodiesterase enzyme type-4 inhibitor, on  $\gamma$ -amino butyric acid content of the frontal cortex in mice exposed to chronic mild stress. *Journal of Pharmacology and Pharmacotherapeutics.* 3(2) (2012);132. <https://doi.org/10.4103/0976-500X.95509> <sup>6</sup>

[180] Z.Z. Wang, W.X. Yang, Y. Zhang, N. Zhao, Y.Z. Zhang, Y.Q. Liu, et al. Phosphodiesterase-4D Knock-down in the Prefrontal Cortex Alleviates Chronic Unpredictable Stress-Induced Depressive-Like Behaviors and Memory Deficits in Mice. *Scientific Reports* 5 (2015):11332. <https://doi.org/10.1038/srep11332> <sup>7</sup>

- [181] C. Zhang, Y. Xu, H.T. Zhang, M.E. Gurney, J.M. O'Donnell. Comparison of the Pharmacological Profiles of Selective PDE4B and PDE4D Inhibitors in the Central Nervous System. *Scientific Reports*. 7 (2017):40115. <https://doi.org/10.1038/srep40115> <sup>1</sup>
- [182] T. Sasaki, K. Kitagawa, E. Omura-Matsuoka, K. Todo, Y. Terasaki, S. Sugiura, et al. The phosphodiesterase inhibitor rolipram promotes survival of newborn hippocampal neurons after ischemia. *Stroke*. 38(5) (2007):1597–605. <https://doi.org/10.1161/STROKEAHA.106.476754> <sup>2</sup>
- [183] M.M. Essa, V. Singh, N. Guizani, T. Manivasagam, A.J. Thenmozhi, A. Bhat, et al. Phoenix dactylifera L. Fruits Date Fruit Ameliorate Oxidative Stress in 3-NP Intoxicated PC12 Cells. *International Journal of Nutrition, Pharmacology, Neurological Diseases*. 9(1) (2019):41. [https://doi.org/10.4103/ijnpnd.ijnpnd\\_51\\_18](https://doi.org/10.4103/ijnpnd.ijnpnd_51_18) <sup>3</sup>
- [184] H. Xu, J.J. An, B. Xu, Distinct cellular toxicity of two mutant huntingtin mRNA variants due to translation regulation. *PLoS One* 12 (5) (2017). <https://doi.org/10.1371/journal.pone.0177610> <sup>4</sup>
- [185] M.A. Hickey, M.F. Chesselet, Apoptosis in Huntington's disease. *Prog Neuropsychopharmacol Biol Psychiatry*. 27(2) (2003):255–65. [https://doi.org/10.1016/S0278-5846\(03\)00021-6](https://doi.org/10.1016/S0278-5846(03)00021-6) <sup>5</sup>
- [186] C. Zuccato, E. Cattaneo. Role of brain-derived neurotrophic factor in Huntington's disease. *Prog Neurobiol*. 81(5–6) (2007):294–330. <https://doi.org/10.1016/j.pneurobio.2007.01.003> <sup>6</sup>
- [187] Z. DeMarch, C. Giampà, S. Patassini, G. Bernardi, F.R. Fusco. Beneficial effects of rolipram in the R6/2 mouse model of Huntington's disease. *Neurobiol Dis*. 30(3) (2008):375–87. <https://doi.org/10.1016/j.nbd.2008.02.010> <sup>7</sup>
- [188] F.R. Fusco, E. Paldino, Role of Phosphodiesterases in Huntington's Disease. *Adv Neurobiol*. 17 (2017):285–304. [https://doi.org/10.1007/978-3-319-58811-7\\_11](https://doi.org/10.1007/978-3-319-58811-7_11) <sup>8</sup>
- [189] E. Auriel, Anatomy and Pathophysiology of Stroke. *Stroke*. 2 (2009):1–8. <https://doi.org/10.1159/000210267> <sup>9</sup>

- [190] C.S. Babu, M. Ramanathan, Post-ischemic administration of nimodipine following focal cerebral ischemic-reperfusion injury in rats alleviated excitotoxicity, neurobehavioural alterations and partially the bioenergetics. *Int J Dev Neurosci.* 29 (1) (2011):93–105. <https://doi.org/10.1016/j.ijdevneu.2010.08.001> <sup>1</sup>
- [191] J.J. Baztán, D.A. Pérez-Martínez, M. Fernández-Alonso, R. Aguado-Ortego, G. Bellando-Alvarez, A.M. de la Fuente-González. Prognostic factors of functional recovery in very elderly stroke patients. A one-year follow-up study. *Rev Neurol.* 44(10) (2007):577–83 <sup>2</sup>
- [192] H. Wang, U. Gaur, J. Xiao, B. Xu, J. Xu, W. Zheng. Targeting phosphodiesterase 4 as a potential therapeutic strategy for enhancing neuroplasticity following ischemic stroke. *Int J Biol Sci.* 14(12) (2018):1745–54. <https://doi.org/10.7150/ijbs.26230> <sup>3</sup>
- [193] F. Block, A. Tondar, W. Schmidt, M. Schwarz. Delayed treatment with rolipram protects against neuronal damage following global ischemia in rats. *Neuroreport.* 8(17) (1997):3829–32. <https://doi.org/10.1097/00001756-199712010-00033> <sup>4</sup>
- [194] H. Kato, T. Araki, Y. Itoyama, K. Kogure. Rolipram, a cyclic AMP-selective phosphodiesterase inhibitor, reduces neuronal damage following cerebral ischemia in the gerbil. *Eur J Pharmacol.* 272(1) (1995):107–10. [https://doi.org/10.1016/0014-2999\(94\)00694-3](https://doi.org/10.1016/0014-2999(94)00694-3). <sup>5</sup>
- [195] M. Bieber, M.K. Schuhmann, J. Volz, J.G. Kumar, J.V. Rao, N. Bernhard, et al. Description of a Novel Phosphodiesterase (PDE)-3 Inhibitor Protecting Mice From Ischemic Stroke Independent From Platelet Function. *Stroke.* 50 (2) (2019):478–86. <https://doi.org/10.1161/STROKEAHA.118.023664> <sup>6</sup>
- [196] E. Nikulina, J.L. Tidwell, H.N. Dai, B.S. Bregman, M.T. Filbin. The phosphodiesterase inhibitor rolipram delivered after a spinal cord lesion promotes axonal regeneration and functional recovery. *Proc Natl Acad Sci USA.* 101(23) (2004):8786–90. <https://doi.org/10.1073/pnas.0402595101> <sup>7</sup>
- [197] P. Kraft, T. Schwarz, E. Göb, N. Heydenreich, M. Brede, S.G. Meuth, et al. The phosphodiesterase-4 inhibitor rolipram protects from ischemic stroke in mice by reducing <sup>8</sup>

- blood-brain-barrier damage, inflammation and thrombosis. *Exp Neurol*. 247 (2013):80–90. <sup>1</sup>  
<https://doi.org/10.1016/j.expneurol.2013.03.026>
- [198] M.R. Turner, R. Bowser, L. Bruijn, L. Dupuis, A. Ludolph, M. McGrath, et al. Mechanisms, <sup>2</sup>  
models and biomarkers in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler*  
*Frontotemporal Degener.* 14 (2013):19–32. <https://doi.org/10.3109/21678421.2013.778554>
- [199] H.J. Chen, G. Anagnostou, A. Chai, J. Withers, A. Morris, Adhikaree J, et al. Characterization <sup>3</sup>  
of the properties of a novel mutation in VAPB in familial amyotrophic lateral sclerosis. *J*  
*Biol Chem*. 285(51) (2010):40266–81. <https://doi.org/10.1074/jbc.M110.161398>
- [200] J. Sreedharan, I.P. Blair, V.B. Tripathi, X. Hu, C. Vance, B. Rogelj, et al. TDP-43 mutations <sup>4</sup>  
in familial and sporadic amyotrophic lateral sclerosis. *Science*. 319(5870) (2008):1668–72.  
<https://doi.org/10.1126/science.1154584>
- [201] G.J. Guillemin, S.J. Kerr, G.A. Smythe, D.G. Smith, V. Kapoor, P.J. Armati, et al. Kynurenine <sup>5</sup>  
pathway metabolism in human astrocytes: a paradox for neuronal protection. *J Neurochem*.  
78(4) (2001):842–53. <https://doi.org/10.1046/j.1471-4159.2001.00498.x>
- [202] J.M. Lee, V. Tan, D. Lovejoy, N. Braidy, D.B. Rowe, B.J. Brew, et al. Involvement of <sup>6</sup>  
quinolinic acid in the neuropathogenesis of amyotrophic lateral sclerosis.  
*Neuropharmacology*. 112 (2017):346–64.  
<https://doi.org/10.1016/j.neuropharm.2016.05.011>
- [203] T. Filipi, Z. Hermanova, J. Tureckova, O. Vanatko, M. Anderova. Glial Cells—The Strategic <sup>7</sup>  
Targets in Amyotrophic Lateral Sclerosis Treatment. *J Clin Med*. 9(1) (2020):261.  
<https://doi.org/10.3390/jcm9010261>
- [204] Y. Chen, H. Wang, Z. Ying, Q. Gao. Ibudilast enhances the clearance of SOD1 and TDP-43 <sup>8</sup>  
aggregates through TFEB-mediated autophagy and lysosomal biogenesis: The new  
molecular mechanism of ibudilast and its implication for neuroprotective therapy. *Biochem*  
*Biophys Res Commun*. 526(1) (2020):231–8. <https://doi.org/10.1016/j.bbrc.2020.03.051>

- [205] Y. Tominaga, Y. Nakamura, K. Tsuji, T. Shibata, K. Kataoka. Ibudilast protects against neuronal damage induced by glutamate in cultured hippocampal neurons. *Clin Exp Pharmacol Physiol.* 23(6–7) (1996):519–23. <https://doi.org/10.1111/j.1440-1681.1996.tb02772.x> <sup>1</sup>
- [206] A. Yoshioka, Y. Yamaya, S. Saiki, M. Kanemoto, G. Hirose, D. Pleasure. Cyclic GMP/cyclic GMP-dependent protein kinase system prevents excitotoxicity in an immortalized oligodendroglial cell line. *J Neurochem.* 74(2) (2000):633–40. <https://doi.org/10.1046/j.1471-4159.2000.740633.x> <sup>2</sup>
- [207] M.A. Furman, K. Shulman. Cyclic AMP and adenylyl cyclase in brain tumors. *J Neurosurg.* 46(4) (1977):477–83. <https://doi.org/10.3171/jns.1977.46.4.0477> <sup>3</sup>
- [208] P.M. Daniel, G. Filiz, T. Mantamadiotis, Sensitivity of GBM cells to cAMP agonist-mediated apoptosis correlates with CD44 expression and agonist resistance with MAPK signaling. *Cell Death & Disease.* 7(12) (2016):e2494. <https://doi.org/10.1038/cddis.2016.393> <sup>4</sup>
- [209] S. Mukherjee, C. Tucker-Burden, E. Kaissi, A. Newsam, H. Duggireddy, M. Chau, et al. CDK5 Inhibition Resolves PKA/cAMP-Independent Activation of CREB1 Signaling in Glioma Stem Cells. *Cell Rep.* (2018) 8;23(6):1651–64. <https://doi.org/10.1016/j.celrep.2018.04.016> <sup>5</sup>
- [210] J. Oh, Y. Kim, L. Che, J.B. Kim, G.E. Chang, E. Cheong, et al. Regulation of cAMP and GSK3 signaling pathways contributes to the neuronal conversion of glioma. *PLoS ONE.* 12(11) (2017):e0178881. <https://doi.org/10.1371/journal.pone.0178881> <sup>6</sup>
- [211] X. Tan, S. Wang, L. Zhu, C. Wu, B. Yin, J. Zhao, et al. cAMP response element-binding protein promotes gliomagenesis by modulating the expression of oncogenic microRNA-23a. *PNAS.* 109(39) (2012):15805–10. <https://doi.org/10.1073/pnas.1207787109> <sup>7</sup>
- [212] P. Goldhoff, N.M. Warrington, D.D. Limbrick, A. Hope, B.M. Woerner, E. Jackson, et al. Targeted inhibition of cyclic AMP phosphodiesterase-4 promotes brain tumor regression. *Clin Cancer Res.* 14(23) (2008):7717–25. <https://doi.org/10.1158/1078-0432.CCR-08-0827> <sup>8</sup>



- [213] H. Dong, K.P. Claffey, S. Brocke, P.M. Epstein. Inhibition of breast cancer cell migration by activation of cAMP signaling. *Breast Cancer Res Treat.* 152(1) (2015):17–28. <https://doi.org/10.1007/s10549-015-3445-9> <sup>1</sup>
- [214] J.L. Bos. Epac: a new cAMP target and new avenues in cAMP research. *Nat Rev Mol Cell Biol.* 4(9) (2003);733–8. <sup>2</sup>
- [215] E.Y. Moon, A. Lerner, PDE4 inhibitors activate a mitochondrial apoptotic pathway in chronic lymphocytic leukemia cells that is regulated by protein phosphatase 2A. *Blood.* 101(10) (2003):4122–30. <https://doi.org/10.1182/blood-2002-10-3208> <sup>3</sup>
- [216] T.C. Chen, P. Wadsten, S. Su, N. Rawlinson, F.M. Hofman, C.K. Hill, et al. The type IV phosphodiesterase inhibitor rolipram induces expression of the cell cycle inhibitors p21(Cip1) and p27(Kip1), resulting in growth inhibition, increased differentiation, and subsequent apoptosis of malignant A-172 glioma cells. *Cancer Biol Ther.* 1(3) (2002):268–76. <https://doi.org/10.4161/cbt.80> <sup>4</sup>
- [217] E.Y. Moon, G.H. Lee, M.S. Lee, H.M. Kim, J.W. Lee, Phosphodiesterase inhibitors control A172 human glioblastoma cell death through cAMP-mediated activation of protein kinase A and Epac1/Rap1 pathways. *Life Sci.* 90(9–10) (2012): 373–80. <https://doi.org/10.1016/j.lfs.2011.12.010> <sup>5</sup>
- [218] S. Ramezani, M. Hadjighassem, N. Vosooghi, M. Parvaresh, F. Arbabi, N. Amini, et al. The Role of Protein Kinase B Signaling Pathway in Anti-cancer Effect of Rolipram on Glioblastoma Multiforme: An In Vitro Study. *Basic and Clinical Neuroscience.* 8(4) (2017):325–36. <https://doi.org/10.18869/nirp.bcn.8.4.325> <sup>6</sup>
- [219] P. Goldhoff, N. Warrington, D.D. Limbrick, A. Hope, B.M. Woerner, E. Jackson, et al. CCR-08-0827 Version 2 Targeted inhibition of cyclic AMP phosphodiesterase-4 promotes brain tumor regression. *Clin Cancer Res.* 14(23) (2008):7717–25. <https://doi.org/10.1158/1078-0432.CCR-08-0827> <sup>7</sup>

- [220] S. Ramezani, N. Vouseoghi, F.R. Kapourchali, M. Haddjighasem, P. Hayat, N. Amini, et al. Rolipram potentiates bevacizumab-induced cell death in human glioblastoma stem-like cells. *Life Sci.* 173 (2017):11–9. <https://doi.org/0.1016/j.lfs.2017.02.005> <sup>1</sup>
- [221] T.W. Kang, S.W. Choi, S.R. Yang, T.H. Shin, H.S. Kim, K.R. Yu, et al. Growth arrest and forced differentiation of human primary glioblastoma multiforme by a novel small molecule. *Sci Rep.* 4 (2014):5546. <https://doi.org/10.1038/srep05546> <sup>2</sup>
- [222] Yang L, Jackson E, Woerner BM, Perry A, Piwnica-Worms D, Rubin JB. Blocking CXCR4-mediated cyclic AMP suppression inhibits brain tumor growth in vivo. *Cancer Res.* 2007 Jan 15;67(2):651–8. <https://doi.org/10.1158/0008-5472.CAN-06-2762> <sup>3</sup>
- [223] D.J. Titus, A. Sakurai, Y. Kang, C. Furones, S. Jergova, R. Santos, et al. Phosphodiesterase inhibition rescues chronic cognitive deficits induced by traumatic brain injury. *J Neurosci.* 33(12) (2013):5216–26. <https://doi.org/10.1523/JNEUROSCI.5133-12.2013> <sup>4</sup>
- [224] M. Prins, T. Greco, D. Alexander, C.C. Giza. The pathophysiology of traumatic brain injury at a glance. *Dis Model Mech.* 6(6) (2013):1307–15. <https://doi.org/10.1242/dmm.011585> <sup>5</sup>
- [225] A.A. Oliva, Y. Kang, C. Furones, O.F. Alonso, O. Bruno, W.D. Dietrich, et al. Phosphodiesterase isoform-specific expression induced by traumatic brain injury. *J Neurochem.* 123(6) (2012):1019–29. <https://doi.org/10.1111/jnc.12049> <sup>6</sup>
- [226] A. Nagakura, M. Niimura, S. Takeo. Effects of a phosphodiesterase IV inhibitor rolipram on microsphere embolism-induced defects in memory function and cerebral cyclic AMP signal transduction system in rats. *Br J Pharmacol.* 135(7) (2002):1783–93. <https://doi.org/10.1038/sj.bjp.0704629> <sup>7</sup>
- [227] M. Ghosh, D. Garcia-Castillo, V. Aguirre, R. Golshani, C.M. Atkins, H.M. Bramlett, et al. Proinflammatory cytokine regulation of cyclic AMP-phosphodiesterase 4 signaling in microglia in vitro and following CNS injury. *Glia.* 60(12) (2012):1839–59. <https://doi.org/10.1002/glia.22401> <sup>8</sup>

- [228] N.M. Wilson, D.J. Titus, A.A. Oliva, C. Furones, C.M. Atkins. Traumatic Brain Injury Upregulates Phosphodiesterase Expression in the Hippocampus. *Frontiers in Systems Neuroscience*. (2016):10. [https://doi.org/ 10.3389/fnsys.2016.00005](https://doi.org/10.3389/fnsys.2016.00005) <sup>1</sup>
- [229] L.X. Li, Y.F. Cheng, H.B. Lin, C. Wang, J.P. Xu, H.T. Zhang, Prevention of cerebral ischemia-induced memory deficits by inhibition of phosphodiesterase-4 in rats. *Metab Brain Dis*. 26(1): (2011) 37–47. <https://doi.org/10.1007/s11011-011-9235-0> <sup>2</sup>
- [230] C.M. Atkins, A.A. Oliva, O.F. Alonso, D.D. Pearse, H.M. Bramlett, W.D. Dietrich. Modulation of the cAMP signaling pathway after traumatic brain injury. *Exp Neurol*. 208(1) (2007):145–58. <https://doi.org/10.1016/j.expneurol.2007.08.011> <sup>3</sup>
- [231] P.B. Gorelick, A. Scuteri, S.E. Black, C. Decarli, S.M. Greenberg, C. Iadecola, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the american heart association/american stroke association. *Stroke*. 42(9) (2011);2672–713. [https://doi.org/ 10.1161/STR.0b013e3182299496](https://doi.org/10.1161/STR.0b013e3182299496) <sup>4</sup>
- [232] J.T. O'Brien, A. Thomas. Vascular dementia. *Lancet*. 386(10004) (2015);1698–706. [https://doi.org/ 10.1016/S0140-6736\(15\)00463-8](https://doi.org/10.1016/S0140-6736(15)00463-8) <sup>5</sup>
- [233] S.M. de la Monte, Y.K. Sohn, D. Etienne, J. Kraft, J.R. Wands. Role of aberrant nitric oxide synthase-3 expression in cerebrovascular degeneration and vascular-mediated injury in Alzheimer's disease. *Ann N Y Acad Sci*. 903 (2000);61–71. [https://doi.org/ 10.1111/j.1749-6632.2000.tb06351.x](https://doi.org/10.1111/j.1749-6632.2000.tb06351.x). <sup>6</sup>
- [234] M. Kaundal, S. Zameer, A.K. Najmi, S. Parvez, M. Akhtar. Betulinic acid, a natural PDE inhibitor restores hippocampal cAMP/cGMP and BDNF, improve cerebral blood flow and recover memory deficits in permanent BCCAO induced vascular dementia in rats. *Eur J Pharmacol*. 832 (2018);56–66. [https://doi.org/ 10.1016/j.ejphar.2018.05.015](https://doi.org/10.1016/j.ejphar.2018.05.015) <sup>7</sup>
- [235] M.E. Gurney, E.C. D'Amato, A.B. Burgin. Phosphodiesterase-4 (PDE4) Molecular Pharmacology and Alzheimer's Disease. *Neurotherapeutics*. 12(1) (2015);49–56. [https://doi.org/ 10.1007/s13311-014-0309-7](https://doi.org/10.1007/s13311-014-0309-7) <sup>8</sup>

- [236] K. Tanaka, F. Gotoh, Y. Fukuuchi, T. Amano, D. Uematsu, J. Kawamura, et al. Effects of a selective inhibitor of cyclic AMP phosphodiesterase on the pial microcirculation in feline cerebral ischemia. *Stroke*. 20(5) (1989);668–73. [https://doi.org/ 10.1161/01.str.20.5.668](https://doi.org/10.1161/01.str.20.5.668) <sup>1</sup>
- [237] K. Rutten, E.L.V. Donkelaar, L. Ferrington, A. Blokland, E. Bollen, et al. Phosphodiesterase Inhibitors Enhance Object Memory Independent of Cerebral Blood Flow and Glucose Utilization in Rats. *Neuropsychopharmacology*. 34(8) (2009); 1914–25. [https://doi.org/ 10.1038/npp.2009.24](https://doi.org/10.1038/npp.2009.24) <sup>2</sup>
- [238] A. Santiago, L.M. Soares, M. Schepers, H. Milani, T. Vanmierlo, J. Prickaerts, et al. Roflumilast promotes memory recovery and attenuates white matter injury in aged rats subjected to chronic cerebral hypoperfusion. *Neuropharmacology*. 138 (2018); 360–70. [https://doi.org/ 10.1016/j.neuropharm.2018.06.019](https://doi.org/10.1016/j.neuropharm.2018.06.019) <sup>3</sup>
- [239] J. Liang, Y.Y. Huang, Q. Zhou, Y. Gao, Z. Li, D. Wu, et al. Discovery and Optimization of  $\alpha$ -Mangostin Derivatives as Novel PDE4 Inhibitors for the Treatment of Vascular Dementia. *Journal of Medicinal Chemistry* 63(6) (2020); 3370-3380. [https://doi.org/ 10.1021/acs.jmedchem.0c00060](https://doi.org/10.1021/acs.jmedchem.0c00060) <sup>4</sup>
- [240] W.W. Fleischhacker, H. Hinterhuber, H. Bauer, B. Pflug, P. Berner, C. Simhandl, et al. A multicenter double-blind study of three different doses of the new cAMP-phosphodiesterase inhibitor rolipram in patients with major depressive disorder. *Neuropsychobiology*. 26 (1–2) (1992);59–64. [https://doi.org/ 10.1159/000118897](https://doi.org/10.1159/000118897) <sup>5</sup>
- [241] A. Robichaud, C. Savoie, P.B. Stamatiou, N. Lachance, P. Jolicoeur, R. Rasori, et al. Assessing the emetic potential of PDE4 inhibitors in rats. *Br J Pharmacol*. 135 (1) (2002);113–8. [https://doi.org/ 10.1038/sj.bjp.0704457](https://doi.org/10.1038/sj.bjp.0704457) <sup>6</sup>
- [242] A. Kumar, N. Singh, Inhibitor of Phosphodiesterase-4 improves memory deficits, oxidative stress, neuroinflammation and neuropathological alterations in mouse models of dementia of Alzheimer's Type. *Biomed Pharmacother*. 88 (2017);698–707. [https://doi.org/ 10.1016/j.biopha.2017.01.059](https://doi.org/10.1016/j.biopha.2017.01.059) <sup>7</sup>

- [243] P.R.A. Heckman, J.V. Schweimer, T. Sharp, J. Prickaerts, A. Blokland. Phosphodiesterase 4<sup>1</sup> inhibition affects both the direct and indirect pathway: an electrophysiological study examining the tri-phasic response in the substantia nigra pars reticulata. *Brain Struct Funct.* 23(2) (2018);739–48. [https://doi.org/ 10.1007/s00429-017-1518-8](https://doi.org/10.1007/s00429-017-1518-8)
- [244] M.A.V. Duinen, A. Sambeth, P.R.A. Heckman, S. Smit, M. Tsai, G. Lahu, et al. Acute<sup>2</sup> administration of roflumilast enhances immediate recall of verbal word memory in healthy young adults. *Neuropharmacology.* 131 (2018); 31–8. [https://doi.org/ 10.1016/j.neuropharm.2017.12.019](https://doi.org/10.1016/j.neuropharm.2017.12.019).
- [245] E.H. Bennie, S.K. Chakravarti, C.M.B. Jarman, K. Khan, D. Master, G.H. Murray, et al. A<sup>3</sup> double-blind dose-finding study of rolipram in patients with major depressive disorder. *Human Psychopharmacology: Clinical and Experimental.* 3(4) (1988) :275–80. [https://doi.org/ 10.1159/000118897](https://doi.org/10.1159/000118897)
- [246] G.F. Hebenstreit, K. Fellerer, K. Fichte, G. Fischer, Geyer N, U. Meya, et al. Rolipram in<sup>4</sup> major depressive disorder: results of a double-blind comparative study with imipramine. *Pharmacopsychiatry.* 22(4) (1989):156–60. [https://doi.org/ 10.1055/s-2007-1014599](https://doi.org/10.1055/s-2007-1014599)
- [247] R. Horowski, M. Sastre-Y-Hernandez, Clinical effects of the neurotropic selective cAMP<sup>5</sup> phosphodiesterase inhibitor rolipram in depressed patients: Global evaluation of the preliminary reports. *Current Therapeutic Research.* (198)5;38(1):23–9.
- [248] E. Zeller, H.J. Stief, B. Pflug, M. Sastre-y-Hernández, Results of a phase II study of the<sup>6</sup> antidepressant effect of rolipram. *Pharmacopsychiatry.* 17(6) (1984):188–90. [https://doi.org/ 10.1055/s-2007-1017435](https://doi.org/10.1055/s-2007-1017435)
- [249] Y.H. Jeon, Y.S. Heo, C.M. Kim, Y.L. Hyun, T.G. Lee, S. Ro, et al. Phosphodiesterase:<sup>7</sup> overview of protein structures, potential therapeutic applications and recent progress in drug development. *Cell Mol Life Sci.* 62(11) (2005);1198–220. [https://doi.org/ 10.1007/s00018-005-4533-5](https://doi.org/10.1007/s00018-005-4533-5)
- [250] T.J. Torphy, M.S. Barnette, D.C. Underwood, D.E. Griswold, S.B. Christensen, R.D.<sup>8</sup> Murdoch, et al. Ariflo (SB 207499), a second generation phosphodiesterase 4 inhibitor for

the treatment of asthma and COPD: from concept to clinic. *Pulm Pharmacol Ther.* 12(2) (1999);131–5. <https://doi.org/10.1006/pupt.1999.0181>. <sup>1</sup>

[251] A.B. Burgin, O.T. Magnusson, J. Singh, P. Witte, B.L. Staker, J.M. Bjornsson, et al. Design of phosphodiesterase 4D (PDE4D) allosteric modulators for enhancing cognition with improved safety. *Nat Biotechnol.* 28(1) (2010);63–70. <https://doi.org/10.1038/nbt.1598> <sup>2</sup>

[252] A. Robichaud, P.B. Stamatou, S.L.C. Jin, N. Lachance, D. MacDonald, F. Laliberté, et al. Deletion of phosphodiesterase 4D in mice shortens alpha(2)-adrenoceptor-mediated anesthesia, a behavioral correlate of emesis. *J Clin Invest.* 110(7) (2002);1045–52. <https://doi.org/10.1172/JCI15506>. <sup>3</sup>

[253] L. Pagès, A. Gavalda, M.D. Lehner. PDE4 inhibitors: a review of current developments (2005 - 2009). *Expert Opin Ther Pat.* 19(11) (2009);1501–19. <https://doi.org/10.1517/13543770903313753>. <sup>4</sup>

[254] D.J. Titus, N.M. Wilson, O. Alcazar, D.A. Calixte, W.D. Dietrich, M.E. Gurney, et al. A negative allosteric modulator of PDE4D enhances learning after traumatic brain injury. *Neurobiol Learn Mem.* 148 (2018);38–49. <https://doi.org/10.1016/j.nlm.2017.12.008>. <sup>5</sup>

[255] J.S. Sutcliffe, V. Beaumont, J.M. Watson, C.S. Chew, M. Beconi, D.M. Hutcheson, et al. Efficacy of Selective PDE4D Negative Allosteric Modulators in the Object Retrieval Task in Female Cynomolgus Monkeys (*Macaca fascicularis*). *PLOS ONE.* 9(7) (2014); e102449. <https://doi.org/10.1371/journal.pone.0102449> <sup>6</sup>

[256] Y. Xu, M. Yang, H.T. Zhang, M. Gurney, J. O'Donnell. A selective phosphodiesterase 4D inhibitor BPN14770 reverses beta amyloid-induced memory impairment in humanized PDE4D mice. *The FASEB Journal.*;33 (2019);806.4-806.4. <sup>7</sup>

[257] A. Trifilieff, D. Wyss, C. Walker, L. Mazzoni, R. Hersperger. Pharmacological profile of a novel phosphodiesterase 4 inhibitor, 4-(8-benzo[1,2,5]oxadiazol-5-yl-[1,7]naphthyridin-6-yl)-benzoic acid (NVP-ABE171), a 1,7-naphthyridine derivative, with anti-inflammatory activities. *J Pharmacol Exp Ther.* 301(1) (2002); 241–8. <sup>8</sup>



- [258] G. Rena, F. Begg, A. Ross, C. MacKenzie, I. McPhee, L. Campbell, et al. Molecular cloning, genomic positioning, promoter identification, and characterization of the novel cyclic amp-specific phosphodiesterase PDE4A10. *Mol Pharmacol.* 59(5) (2001); 996–1011. <https://doi.org/10.1124/mol.59.5.996>. <sup>1</sup>
- [259] D.A. Wallace, L.A. Johnston, E. Huston, D. MacMaster, T.M. Houslay, Y.F. Cheung, et al. Identification and characterization of PDE4A11, a novel, widely expressed long isoform encoded by the human PDE4A cAMP phosphodiesterase gene. *Mol Pharmacol.* 67(6) (2005);1920–34. <https://doi.org/10.1124/mol.104.009423> <sup>2</sup>
- [260] P. Huang, Q. Sun, W. Zhuang, K. Peng, D. Wang, Y. Yao, et al. Epac1, PDE4, and PKC protein expression and their association with AKAP95, Cx43, and cyclinD2/E1 in breast cancer tissues. *Thorac Cancer* 8(5) (2017);495–500. <https://doi.org/10.1111/1759-7714.12475> <sup>3</sup>
- [261] K.F. Mackenzie, E.C. Topping, B.B. Gaweda, C. Deng, Y.F. Cheung, A.E. Olsen, et al. Human PDE4A8, a novel brain-expressed PDE4 cAMP-specific phosphodiesterase that has undergone rapid evolutionary change. *Biochem J.* 411(2) (2008); 361–9. <https://doi.org/10.1042/BJ20071251> <sup>4</sup>
- [262] B. Mahmood, M.M.B. Damm, T.S.R. Jensen, M.B. Backe, M.S. Dahllöf, S.S. Poulsen, et al. Phosphodiesterases in non-neoplastic appearing colonic mucosa from patients with colorectal neoplasia. *BMC Cancer* 16 (2016); 938. <https://doi.org/10.1186/s12885-016-2980-z> <sup>5</sup>
- [263] M. Sullivan, G. Rena, F. Begg, L. Gordon, A.S. Olsen, M.D. Houslay. Identification and characterization of the human homologue of the short PDE4A cAMP-specific phosphodiesterase RD1 (PDE4A1) by analysis of the human HSPDE4A gene locus located at chromosome 19p13.2. *Biochem J.* 333 (1998);693–703. <https://doi.org/10.1042/bj3330693>. <sup>6</sup>
- [264] Á. Agis-Torres, P. Recio, M.E. López-Oliva, M.P. Martínez, M.V. Barahona, S. Benedito, et al. Phosphodiesterase type 4 inhibition enhances nitric oxide- and hydrogen sulfide- <sup>7</sup>

mediated bladder neck inhibitory neurotransmission. *Scientific Reports* 8(1) (2018); 4711. <sup>1</sup>  
<https://doi.org/10.1038/s41598-018-22934-1>.

[265] D.V. Avila, D.F. Barker, J. Zhang, C.J. McClain, S. Barve, L. Gobejishvili. Dysregulation of <sup>2</sup>  
 hepatic cAMP levels via altered Pde4b expression plays a critical role in alcohol-induced  
 steatosis. *J Pathol.* 240(1) (2016);96–107. <https://doi.org/10.1002/path.4760>

[266] J.B. Cheng, J.W. Watson, C.J. Pazoles, J.D. Eskra, R.J. Griffiths, V.L. Cohan, et al. The <sup>3</sup>  
 phosphodiesterase type 4 (PDE4) inhibitor CP-80,633 elevates plasma cyclic AMP levels  
 and decreases tumor necrosis factor-alpha (TNFalpha) production in mice: effect of  
 adrenalectomy. *J Pharmacol Exp Ther.* 280(2) (1997); 621–6.

[267] E. Schinner, V. Wetzl, J. Schlossmann. Cyclic nucleotide signalling in kidney fibrosis. *Int J* <sup>4</sup>  
*Mol Sci.* 16(2) (2015); 2320–51. <https://doi.org/10.3390/ijms16022320>

[268] K. Komatsu, J.Y. Lee, M. Miyata, J.H. Lim, H. Jono, T. Koga, et al. Inhibition of PDE4B <sup>5</sup>  
 suppresses inflammation by increasing expression of the deubiquitinase CYLD. *Nature*  
*Communications* 4 (2013);1684. <https://doi.org/10.1038/ncomms2674>

[269] D.D. Pearse, Z.A. Hughes. PDE4B as a microglia target to reduce neuroinflammation. *Glia.* <sup>6</sup>  
 64(10) (2016);1698–709. <https://doi.org/10.1002/glia.22986>

[270] S.L.C. Jin, S. Goya, S. Nakae, D. Wang, M. Bruss, C. Hou, et al. Phosphodiesterase 4B is <sup>7</sup>  
 essential for T(H)2-cell function and development of airway hyperresponsiveness in allergic  
 asthma. *J Allergy Clin Immunol.* 126(6) (2010); 1252-1259.e12.  
<https://doi.org/10.1016/j.jaci.2010.08.014>

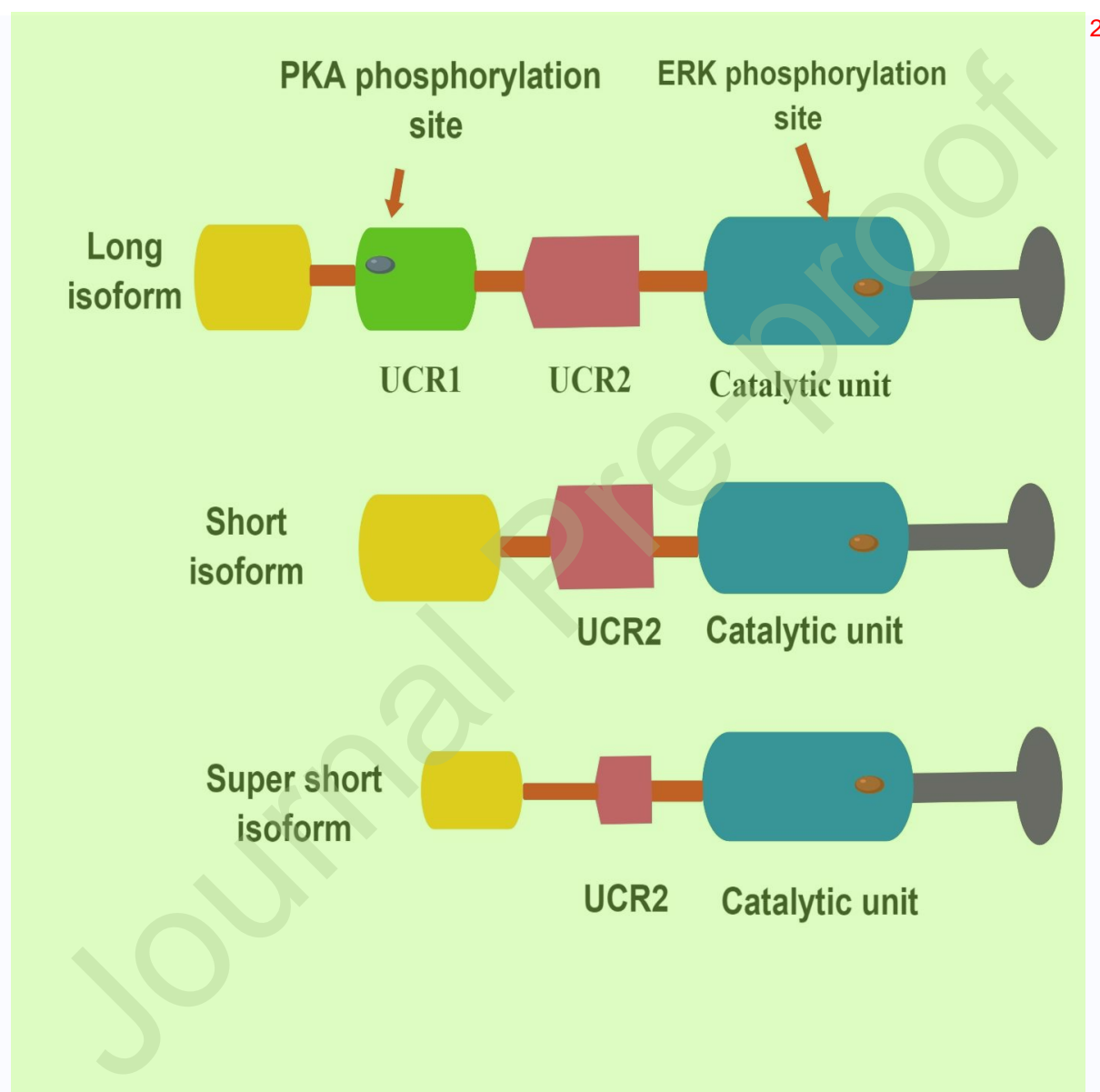
[271] E. Kashiwagi, M. Shiota, A. Yokomizo, M. Itsumi, J. Inokuchi, T. Uchiumi, et al. <sup>8</sup>  
 Downregulation of phosphodiesterase 4B (PDE4B) activates protein kinase A and  
 contributes to the progression of prostate cancer. *Prostate.* 72(7) (2012); 741–51.  
<https://doi.org/10.1002/pros.21478>

- [272] A.G. Vang, C. Basole, H. Dong, R.K. Nguyen, W. Housley, L. Guernsey, et al. Differential Expression and Function of PDE8 and PDE4 in Effector T cells: Implications for PDE8 as a Drug Target in Inflammation. *Front Pharmacol.* (2016);7. <https://doi.org/> <sup>1</sup>
- [273] R. Zhang, E. Maratos-Flier, J.S. Flier. Reduced Adiposity and High-Fat Diet-Induced Adipose Inflammation in Mice Deficient for Phosphodiesterase 4B. *Endocrinology* 150(7) (2009); 3076–82. <https://doi.org/10.3389/fphar.2016.00259> <sup>2</sup>
- [274] A. Balasubramaniam, S. Sheriff, L.A. Friend, J.H. James. Phosphodiesterase 4B knockout prevents skeletal muscle atrophy in rats with burn injury. *Am J Physiol Regul Integr Comp Physiol.* 315(2) (2018); 429–33. <https://doi.org/10.1152/ajpregu.00042.2018> <sup>3</sup>
- [275] Y.H. Choi, A. Suzuki, S. Hajarnis, Z. Ma, H.C. Chapin, M.J. Caplan, et al. Polycystin-2 and phosphodiesterase 4C are components of a ciliary A-kinase anchoring protein complex that is disrupted in cystic kidney diseases. *Proc Natl Acad Sci USA.* 108(26) (2011); 10679–84. <https://doi.org/10.1073/pnas.1016214108> <sup>4</sup>
- [276] D. Mika, W. Richter, R.E. Westenbroek, W.A. Catterall, M. Conti. PDE4B mediates local feedback regulation of  $\beta$ 1-adrenergic cAMP signaling in a sarcolemmal compartment of cardiac myocytes. *J Cell Sci.* 127(5) (2014);1033–42. <https://doi.org/10.1242/jcs.140251> <sup>5</sup>
- [277] Petersen TS, Stahlhut M, Andersen CY. Phosphodiesterases in the rat ovary: effect of cAMP in primordial follicles. *Reproduction.* 150(1) (2015);11–20. <https://doi.org/10.1530/REP-14-0436> <sup>6</sup>
- [278] S.M. Susuki, M. Miyata, B.C. Lee, H. Xu, H. Kai, C. Yan, et al. Cross-talk between PKA-C $\beta$  and p65 mediates synergistic induction of PDE4B by roflumilast and NTHi. *PNAS.* 112(14) (2015);E1800–9. <https://doi.org/10.1073/pnas.1418716112>. <sup>7</sup>
- [279] S.F. Hannah, F.R. Faucz, C.A. Stratakis. Alterations of Phosphodiesterases in Adrenocortical Tumors. *Front Endocrinol (Lausanne)* (2016);7;111. <https://doi.org/10.3389/fendo.2016.00111> <sup>8</sup>

- [280] D.J.P. Henderson, M.D. Houslay, C.H. Bangma, R. Hoffmann. Creating a potential diagnostic for prostate cancer risk stratification (InformMDx™) by translating novel scientific discoveries concerning cAMP degrading phosphodiesterase-4D7 (PDE4D7). *Clinical Science* 133(2) (2019);269–86. <https://doi.org/10.1042/CS20180519>. <sup>1</sup>
- [281] Y. Watanabe, T. Murata, K. Shimizu, H. Morita, M. Inui, T. Tagawa. Phosphodiesterase 4 regulates the migration of B16-F10 melanoma cells. *Exp Ther Med.* 4(2) (2012); 205–10. <https://doi.org/10.3892/etm.2012.587> <sup>2</sup>
- [282] R. Obernolte, J. Ratzliff, P.A. Baecker, D.V. Daniels, P. Zuppan, K. Jarnagin, et al. Multiple splice variants of phosphodiesterase PDE4C cloned from human lung and testis. *Biochim Biophys Acta.* 1353(3) (1997);287–97. [https://doi.org/10.1016/s0167-4781\(97\)00080-8](https://doi.org/10.1016/s0167-4781(97)00080-8) <sup>3</sup>
- [283] D. Peter, S.L.C. Jin, M. Conti, A. Hatzelmann, C. Zitt. Differential Expression and Function of Phosphodiesterase 4 (PDE4) Subtypes in Human Primary CD4+ T Cells: Predominant Role of PDE4D. *The Journal of Immunology* 178(8) (2007);4820–31. <https://doi.org/10.4049/jimmunol.178.8.4820>. <sup>4</sup>
- [284] E. Heimann, H.A. Jones, S. Resjö, V.C. Manganiello, L. Stenson, E. Degerman. Expression and Regulation of Cyclic Nucleotide Phosphodiesterases in Human and Rat Pancreatic Islets. *PLOS ONE* 5(12) (2010); e14191. <https://doi.org/10.1371/journal.pone.0014191> <sup>5</sup>
- [285] W. Richter, M. Xie, C. Scheitrum, J. Krall, M.A. Movsesian, M. Conti. Conserved expression and functions of PDE4 in rodent and human heart. *Basic Res Cardiol.* 106(2) (2011);249–62. <https://doi.org/10.1007/s00395-010-0138-8> <sup>6</sup>
- [286] D. Waddleton, W. Wu, Y. Feng, C. Thompson, M. Wu, Y.P. Zhou, et al. Phosphodiesterase 3 and 4 comprise the major cAMP metabolizing enzymes responsible for insulin secretion in INS-1 (832/13) cells and rat islets. *Biochem Pharmacol.* 76(7) (2008);884–93. <https://doi.org/10.1016/j.bcp.2008.07.025> <sup>7</sup>
- [287] H. Wang, N.K. Edens. mRNA expression and antilipolytic role of phosphodiesterase 4 in rat adipocytes in vitro. *J Lipid Res.* 48(5) (2007);1099–107. <https://doi.org/10.1194/jlr.M600519-JLR200> <sup>8</sup>

- [288] E.C. Lira, L.T. Parreiras-e-Silva, N.M. Zanon, C.M. da Costa Neto, I. do Carmo Kettelhut, L.C.C. Navegantes. The inhibition of phosphodiesterase 4 reduces skeletal muscle protein catabolism by suppressing autophagy/lysosomal and proteasomal pathways and atrophy-specific gene transcription. *The FASEB Journal*. 24 (2010); 801.11-801.11. <sup>1</sup>
- [289] Z. Huang, Z. Han, W. Cui, F. Zhang, H. He, T. Zeng, et al. Dynamic expression pattern of Pde4d and its relationship with CpG methylation in the promoter during mouse embryo development. *Biochem Biophys Res Commun*. 441(4) (2013); 982–7. <https://doi.org/10.1016/j.bbrc.2013.11.004> <sup>2</sup>
- [290] G. Levallet, J. Levallet, H. Bouraïma-Lelong, P.J. Bonnamy. Expression of the cAMP-Phosphodiesterase PDE4D Isoforms and Age-Related Changes in Follicle-Stimulating Hormone-Stimulated PDE4 Activities in Immature Rat Sertoli Cells. *Biol Reprod* 76(5) (2007);794–803. <https://doi.org/10.1095/biolreprod.106.055343> <sup>3</sup>
- [291] L.M. Grønning, G.S. Baillie, A. Cederberg, M.J. Lynch, M.D. Houslay, S. Enerbäck, et al. Reduced PDE4 expression and activity contributes to enhanced catecholamine-induced cAMP accumulation in adipocytes from FOXC2 transgenic mice. *FEBS Letters* 580 (17) (2006); 4126–30. <https://doi.org/10.1016/j.febslet.2006.06.058> <sup>4</sup>
- [292] D.C. Lin, L. Xu, L.W. Ding, A. Sharma, L.Z. Liu, H. Yang, et al. Genomic and functional characterizations of phosphodiesterase subtype 4D in human cancers. *PNAS*. 110(15) (2013); 6109–14. <https://doi.org/10.1073/pnas.1218206110> <sup>5</sup>
- [293] R.R. Mishra, N. Belder, S.A. Ansari, M. Kayhan, H. Bal, U. Raza, et al. Reactivation of cAMP Pathway by PDE4D Inhibition Represents a Novel Druggable Axis for Overcoming Tamoxifen Resistance in ER-positive Breast Cancer. *Clin Cancer Res*. 24(8) (2018);1987–2001. <https://doi.org/10.1158/1078-0432.CCR-17-2776> <sup>6</sup>
- [294] W. Richter, S.L.C. Jin, M. Conti. Splice variants of the cyclic nucleotide phosphodiesterase PDE4D are differentially expressed and regulated in rat tissue. *Biochem J*. 388(Pt 3) (2005);803–11. <https://doi.org/10.1042/BJ20050030> <sup>7</sup>

[295] P. Cedervall, A. Aulabaugh, K.F. Geoghegan, T.J. McLellan, J. Pandit. Engineered stabilization and structural analysis of the autoinhibited conformation of PDE4. PNAS. 112(12) (2015):E1414–22. <https://doi.org/10.1073/pnas.1419906112>

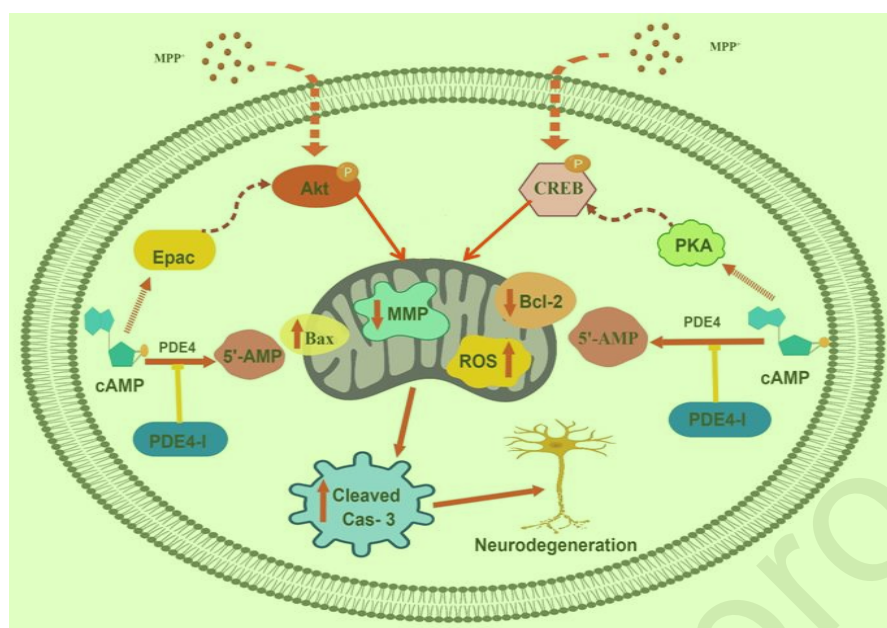


**Fig.1:** PDE4 isoforms and their post-translational regulation. PDE4 isoforms are classified based on the presence of their upstream conserved regions (UCR) and each isoform is differentially

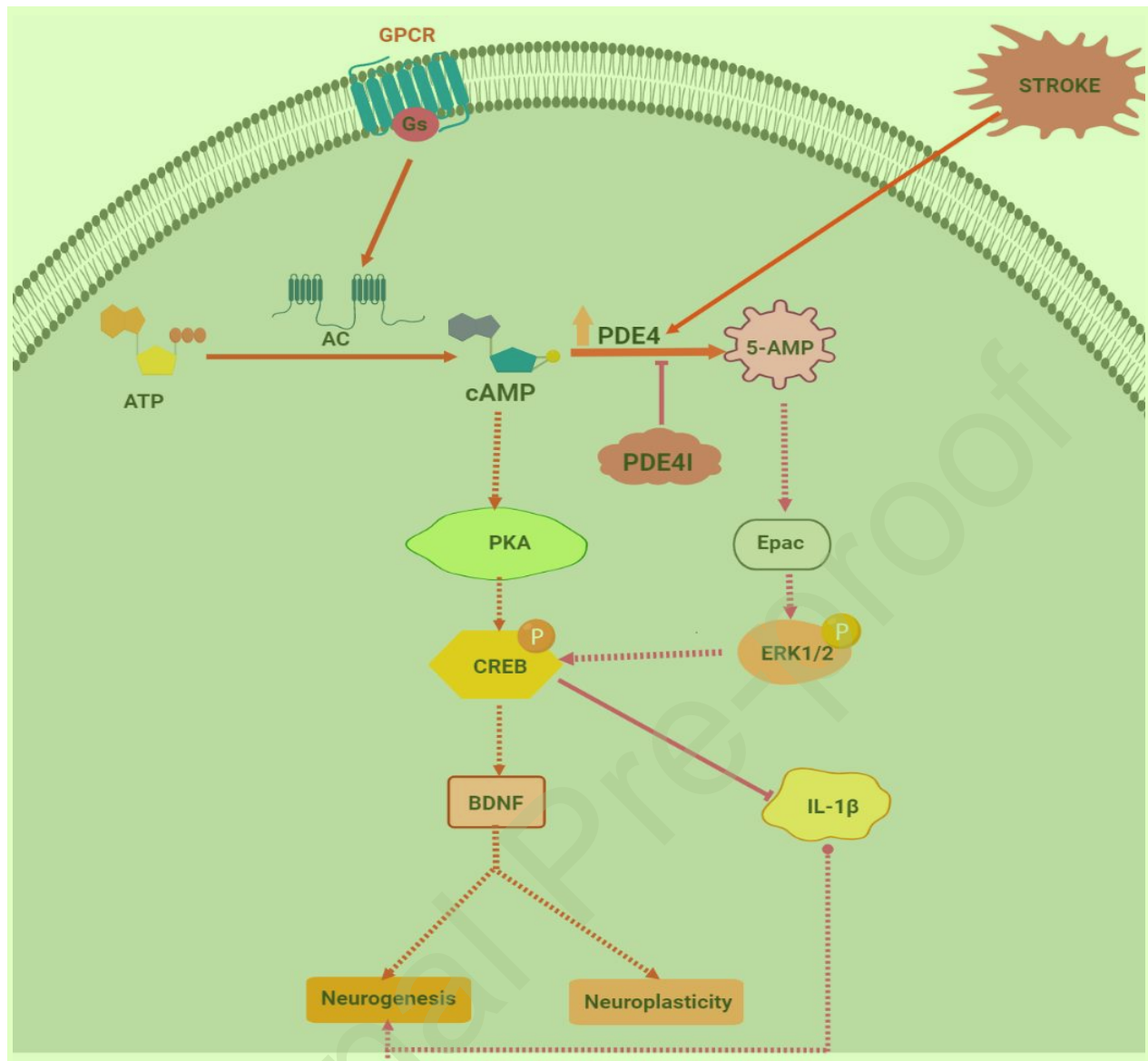




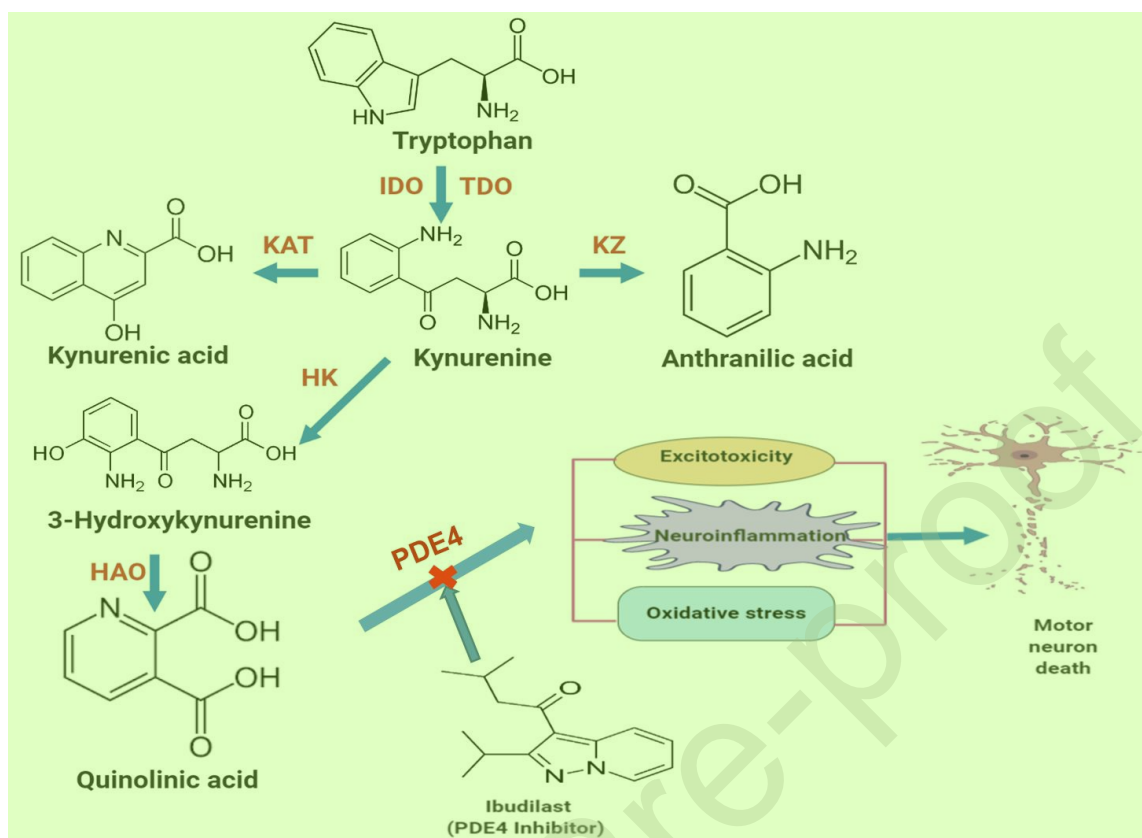




**Fig. 3:** 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>) triggers the accumulation of reactive oxygen species (ROS) and reduces mitochondrial membrane potential (MMP) along with an increase in expression of Bax and decrease in the level of Bcl-2. The levels of cleaved caspase 3 are also increased which leads to neurodegeneration. MPP<sup>+</sup> inhibits cAMP/PKA/CREB and Epac/Akt signalling pathway. PDE4 inhibitors act by increasing the level of cAMP which activates cAMP/PKA/CREB and Epac/Akt signalling pathways and prevents neurodegeneration.



**Fig. 4:** Activation of GPCR activates Gs $\alpha$  subunit and adenylyl cyclase (AC), which subsequently catalyses the conversion of ATP to cAMP. Increase in the level of local intracellular cAMP leads to activation of protein kinase A (PKA), exchange proteins activated by cAMP (Epac) which in turn activates cAMP-responsive element-binding protein (CREB). Under stroke conditions there is an over expression of PDE4 in brain which decreases the cAMP levels which inhibits PKA/CREB and Epac/ERK1/2 signalling. Activation of cAMP/PKA/CREB is critical for the production of neurotrophic factors like BDNF while as Epac/ERK1/2 activation inhibits proinflammatory factors. PDE4 inhibitors act by increasing the levels of cAMP/BDNF and thus play a crucial role in neurogenesis and neuroplasticity



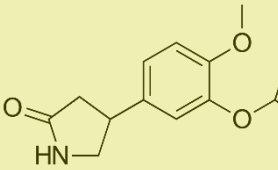
**Fig. 5:** Schematic representation of kynurenine pathway. TDO—tryptophan 2,3-dioxygenase, IDO—indoleamine 2,3-dioxygenase, KAT— kynurenine aminotransferase, KZ—kynureninase, HK—kynurenine 3-hydroxylase, HAO—3-hydroxyanthranilate-3,4-dioxygenase, PC— picolinic carboxylase, NC—nonenzymic cyclization. Increase in the production of Quinolinic acid by microglia increases the expression of PDE4 which leads to excitotoxicity, neuroinflammation and increase in the production of oxidative stress. PDE4 inhibitors act by blocking PDE4 expression and reducing excitotoxicity, neuroinflammation and oxidative stress, thereby inhibiting the neuronal loss in ALS.

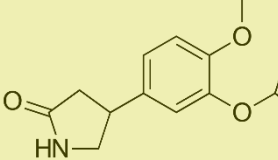
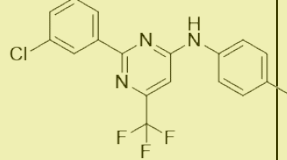
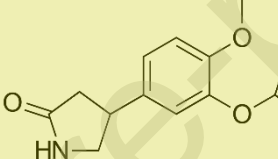
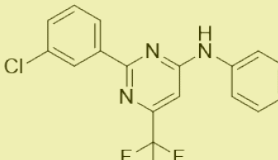
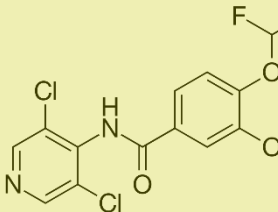
**Table 1:** Expression of PDE4 isoforms in different tissues

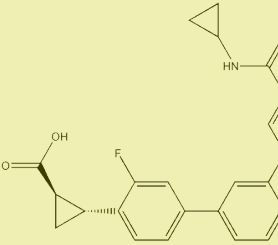
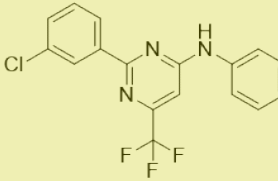
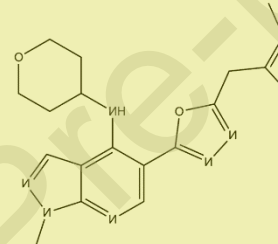
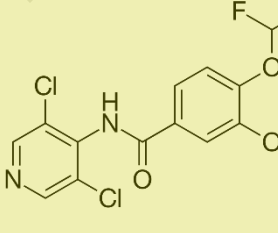
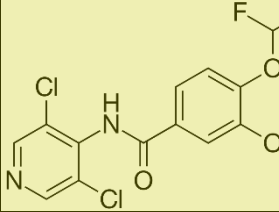
Isoform	Expression	Tissue	Reference
---------	------------	--------	-----------

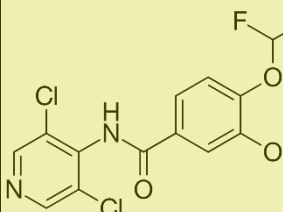
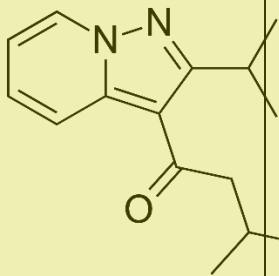
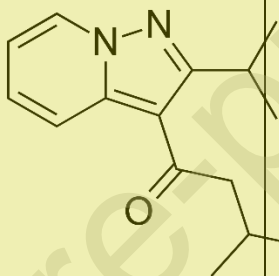
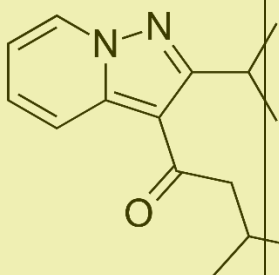
<b>PDE4A</b>	High	Adipose Tissue, Brain, Heart, Testis	(13,258,259)
	Moderate	Breast, Colon, Lung, Lymph Node, Skeletal Muscle, Thyroid	(8,260–263)
	Low / Very low / Absent	Adrenal, Kidney, Liver, Ovary, Prostate	(264–267)
<b>PDE4B</b>	High	Brain, Lungs	(261,268,269)
	Moderate	Adipose Tissue, Adrenal, Lymph Node, Prostate,	(270–273)
	Low / Very low / Absent	Breast, Colon, Heart, Kidney, Liver, Ovary, Skeletal Muscle, Testis, Thyroid	(265,268,272,274–278)
<b>PDE4C</b>	High	Adrenal, Colon, Prostate,	(279–281)
	Moderate	Lung, Testis	(282,283)
	Low / Very low / Absent	Adipose Tissue, Brain, Breast, Heart, Kidney, Liver, Lymph Node, Ovary, Skeletal Muscle, Thyroid	(13,40,41,272,275,284–287)
<b>PDE4D</b>	High	Skeletal Muscle	(4,288)
	Moderate	Ovary, Prostate, Thyroid	(10,289,290)
	Low / Very low / Absent	Adipose Tissue, Adrenal, Brain, Breast, Colon, Heart, Kidney, Liver, Lung, Lymph Node, Testis	(41,285,290–294)

**Table 2:** Clinical trials on PDE4 inhibitors for different neurological disorders <sup>1</sup>

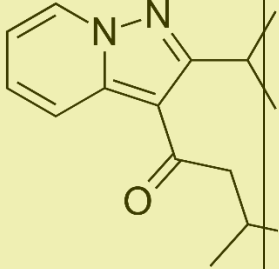
S. No.	NCT Number	Indication	Intervention	Chemical structure	Phase	Locations
1.	NCT00011375	Multiple Sclerosis	<ul style="list-style-type: none"> <li>Drug: Rolipram</li> </ul>		Phase 2	<ul style="list-style-type: none"> <li>National Institute of Neurological Disorders and Stroke (NINDS), Bethesda, Maryland,</li> </ul>

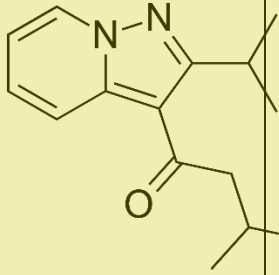
						United States
2.	NCT01602900	Huntington Disease	<ul style="list-style-type: none"> <li>• Drug: GSK356278</li> <li>• Drug: Rolipram</li> </ul>		Phase 1	<ul style="list-style-type: none"> <li>• GSK Investigational Site, London, United Kingdom</li> </ul>
3.	NCT03030105	Alzheimer Disease	<ul style="list-style-type: none"> <li>• Drug: BPN14770</li> </ul>		Phase 3	<ul style="list-style-type: none"> <li>• ICON Early Phase Services, LLC, Austin, Texas, United States</li> </ul>
4.	NCT00369798	Major Depressive Disorder	<ul style="list-style-type: none"> <li>• Drug: Rolipram</li> </ul>		Phase 1	<ul style="list-style-type: none"> <li>• National Institutes of Health Clinical Center, 9000 Rockville Pike, Bethesda, Maryland, United States</li> </ul>
5.	NCT03861000	Depression	<ul style="list-style-type: none"> <li>• Drug: [C-11]T-1650</li> <li>• Drug: BPN14770</li> </ul>		Phase 1	<ul style="list-style-type: none"> <li>• National Institutes of Health Clinical Center, Bethesda, Maryland, United States</li> </ul>
6.	NCT01433666	Dementia	<ul style="list-style-type: none"> <li>• Drug: Roflumilast</li> </ul>		Phase 2	<ul style="list-style-type: none"> <li>• Maastricht University, Faculty of Psychology and Neuroscience, Maastricht,</li> </ul>

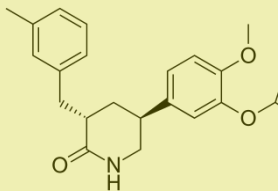
						Limburg, Netherlands
7.	NCT00362024	Alzheimer's Disease	<ul style="list-style-type: none"> <li>Drug: MK0952</li> </ul>		Phase 2	<ul style="list-style-type: none"> <li>National Institutes of Health Clinical Center, Bethesda, Maryland, United States</li> </ul>
8.	NCT02840279	Alzheimer's Disease	<ul style="list-style-type: none"> <li>Drug: BPN14770</li> </ul>		Phase 1	<ul style="list-style-type: none"> <li>Jasper Clinic, Kalamazoo, Michigan, United States</li> </ul>
9.	NCT01573819	Huntington Disease	<ul style="list-style-type: none"> <li>Drug: GSK356278</li> </ul>		Phase 1	<ul style="list-style-type: none"> <li>GSK Investigational Site, Zuidlaren, Netherlands</li> </ul>
10	NCT02835716	Alzheimer Disease	<ul style="list-style-type: none"> <li>Drug: Roflumilast</li> </ul> <ul style="list-style-type: none"> <li>Biological: Asteckinumab</li> </ul>		Phase 1	<ul style="list-style-type: none"> <li>Millennium Magnetic Technologies, LLC, Westport, Connecticut, United States</li> </ul>
11	NCT02079844	Schizophrenia	<ul style="list-style-type: none"> <li>Drug: Roflumilast</li> </ul>		Phase 1	<ul style="list-style-type: none"> <li>Denmark Hill, London, United Kingdom</li> </ul>

12	NCT02051335	Memory Impairment Alzheimer's Disease	<ul style="list-style-type: none"> <li>• Drug: Roflumilast</li> </ul>		Phase 1	<ul style="list-style-type: none"> <li>• London, United Kingdom</li> </ul>
13	NCT03782415	Glioblastoma Recurrent Glioblastoma	<ul style="list-style-type: none"> <li>• Drug: MN-166</li> </ul>		Phase 2	<ul style="list-style-type: none"> <li>• Dana Farber Cancer Institute, Boston, Massachusetts, United States</li> </ul>
14	NCT02714036	Amyotrophic Lateral Sclerosis	<ul style="list-style-type: none"> <li>• Drug: ibudilast</li> </ul>		Phase 2	<ul style="list-style-type: none"> <li>• Massachusetts General Hospital, Boston, Massachusetts, United States</li> <li>• South Shore Neurologic Associates, P.C., Patchogue, New York, United States</li> </ul>
15	NCT01389193	Migraine Headache	<ul style="list-style-type: none"> <li>• Drug: Ibudilast</li> </ul>		Phase 1	<ul style="list-style-type: none"> <li>• School of Medical sciences, University of Adelaide, Adelaide, Australia</li> </ul>



16	NCT01982942	Multiple Sclerosis, Primary Progressive	<ul style="list-style-type: none"> <li>• Drug: ibudilast</li> </ul>		<ul style="list-style-type: none"> <li>• University of Alabama at Birmingham, Birmingham, Alabama, United States</li> <li>• University of California Davis, Davis, California, United States</li> <li>• University of California Los Angeles, Los Angeles, California, United States</li> <li>• University of Colorado Denver, Denver, Colorado, United States</li> <li>• University of Miami Miller School of Medicine, Miami, Florida, United States</li> <li>• Emory University,</li> </ul>
----	-------------	---	---	--	--

						<p>Atlanta, Georgia, United States</p> <ul style="list-style-type: none"> <li>• Northwestern University, Evanston, Illinois, United States</li> <li>• University of Kansas Medical Center, Kansas City, Kansas, United States</li> <li>• Massachusetts General Hospital, Boston, Massachusetts, United States</li> <li>• Brigham and Women's Hospital, Boston, Massachusetts, United States</li> </ul>
17	NCT02238626	Amyotrophic Lateral Sclerosis	<ul style="list-style-type: none"> <li>• Drug: MN-166</li> <li>• Drug: riluzole</li> </ul>		Phase 2	<ul style="list-style-type: none"> <li>• Carolinas Healthcare System, Dept. of Neurology, Charlotte, North Carolina, United States</li> </ul>

18	NCT02013310	Age-Associated Memory Impairment (AAMI)	<ul style="list-style-type: none"><li>• Drug: HT-0712</li></ul>		Phase 2	<ul style="list-style-type: none"><li>• Sun City, Arizona, United States</li><li>• Long Beach, California, United States</li><li>• Santa Monica, California, United States</li></ul>
----	-------------	---	---	--	---------	--