Phosphodiesterase 4B: Master Regulator of Brain Signaling

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Abstract: Phosphodiesterases (PDEs) are the only superfamily of enzymes that have the ability to break down cyclic nucleotides and, as such, they have a pivotal role in neurological disease and brain development. PDEs have a modular structure that allows targeting of individual isoforms to discrete brain locations and it is often the location of a PDE that shapes its cellular function. Many of the eleven different families of PDEs have been associated with specific diseases. However, we evaluate the evidence, which suggests the activity from a sub-family of the PDE4 family, namely PDE4B, underpins a range of important functions in the brain that positions the PDE4B enzymes as a therapeutic target for a diverse collection of indications, such as, schizophrenia, neuroinflammation, and cognitive function.

Keywords: phosphodiesterase; cyclic-AMP; rolipram; PDE4B; neuroinflammation

1. Introduction

Cyclic nucleotides are ubiquitous signaling molecules that are recognized as archetypal second messengers. Since their discovery, there has been an unprecedented drive to understand signal transduction systems that utilize them and to characterize physiological systems under their control. It is well established that both cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) signaling systems underpin critical pathways necessary for brain development and function [1–4]. CAMP is generated by adenylyl cyclase (AC) following activation of G_s-protein coupled receptors (GPCR) [5]. Recent appreciation of the discrete cellular positioning of these receptors and AC has supported the concept of compartmentalized cAMP signaling [6,7] that explains the ability of one second messenger to evoke a range of outcomes [8]. The spatial and temporal control of cAMP within cells is maintained via the action of a highly localized super family of enzymes called 3′5′-cyclic nucleotide phosphodiesterases (PDEs) [9–11]. PDEs are the only known enzymes capable of degrading cyclic nucleotides positioning them as highly important intracellular signaling regulators [12,13]. The distinct localization of PDEs results in hydrolysis of discrete pools of cAMP, controlling the duration of signal transduction in cellular nano-domains [14–16]. There are 11 families of PDEs (PDE1-11) that are the product of differential splicing of 21 genes. The cAMP specific PDE4 family is divided into four sub-families (A, B, C, and D; reviewed in [17]). Within PDE4B sub-family, five members exist (PDE4B1-5) consisting of long, short, and super-short isoforms (Figure 1).

Analysis of mRNA levels has identified that, along with PDE4D, PDE4B is the most abundant sub-family within in human, monkey, and rat brain [18]. Investigations of the roles of PDE4 in the brain have been by encouraged by the discovery that the selective-PDE4 inhibitor, known as rolipram (4-[3-(cytopentyloxy)-4 methoxyphenyl]-2-pyrrolidinone) (IC_{50} for PDE4B approximately 130 nM), promotes anti-depressant effects [19,20]. Rolipram possesses a 100-fold higher selectivity for PDE4 over the other subfamilies [21]. These studies now span a wide variety of neurological and

central nervous system (CNS) disorders such as schizophrenia and traumatic brain injury [22–25]. Hence, investigations of the changes in PDE levels and activity in the brain during neurological development, aging, and disease are relevant to both basic mechanistic research and more translation studies [24,26–31]. This review discusses current opinions about the role of PDE4B in the brain and in brain and related diseases.
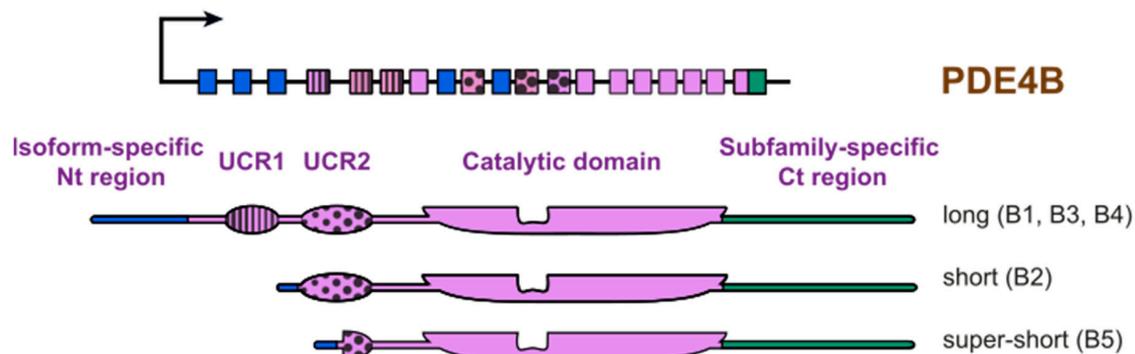

Figure 1. Scheme of PDE4B isoforms. The PDE4B subfamily generates five distinct isoforms. Each contains an isoform-specific N-terminal region, which is encoded for by specific exons denoted in blue. The family members are further specified through the presence of the upstream conserved regions 1 and 2 (UCR1 (stripes) and UCR2 (spots), respectively). All isoforms share an identical catalytic domain (purple) and C-terminal (Ct region) encoded by the green colored exon.

2. Structure and Activation of PDE4B Enzymes

Found on chromosome 1p31.3, the *PDE4B* gene gives rise to five protein isoforms in mammals, PDE4B1-5 [32]. Although individual isoforms contain highly conserved catalytic regions, they can be separated on a structural level by regulatory regions and unique N-terminal domains. Via the presence and size of the Upstream Conserved Region (UCR1) and UCR2 domains, isoforms can be categorized into long, short, super-short, or dead-short categories [33]. Within the PDE4B sub-family exist: three long isoforms, PDE4B1 (736 a.a), PDE4B3 (721 a.a), and PDE4B4 (659 a.a); a short isoform PDE4B2 (564 a.a); and a super-short isoform, PDE4B5 (484 a.a) (see Figure 1) [34–36]. Long forms contain both the UCR1 and UCR2 domains, short forms contain only the UCR2 domain, and super-short forms possess a truncated UCR2 region [33].

The UCR regions are responsible for modulating phosphorylation-dependent changes in enzymatic activity [33,37–39]. For example, UCR1 contains a conserved protein kinase A (PKA) site crucial for activation of all long isoforms. Long-form PDE4Bs also are known to contain a regulatory extracellular signal-regulated kinase (ERK) phosphorylation site at the end of the catalytic domain [38]. In contrast to the long PDE4B isoforms, which are inhibited by ERK phosphorylation, short-form PDE4B2 is activated by the same modification [38]. PDE4B's ability to bind to the kinase is a function of an ERK specificity motif (Phe693, Glu694, Phe695; FQF motif) located on an α-helix around 5–30 amino acids downstream of the targeted serine [39]. The catalytic domain of PDE4B enzymes also contain a more general kinase interacting motif (KIM) on a β-hairpin loop [39]. These two motifs allow for the docking and subsequent phosphorylation by ERK2.

It has been well established that the formation of dimeric long-form PDE4 complexes define the properties of the enzyme [40–42]. Dimerization is mediated by the UCR1 domain and, given the sequence conservation, PDE4s can form both homo- and heterodimers. The dimerization of PDE4B has been shown to be critical for the regulation of the enzyme. As mentioned, PDE4B is activated by PKA-dependent phosphorylation. The structural basis of the phospho-dependent activation and inhibition of PDE4B enzymes has been suggested by crystallographic studies, which identified the mechanism by which the UCR1 domain of one monomeric subunit of the long isoform PDE4B1 crosses over and allows for the regulation of the catalytic activity of the other monomeric subunit [37].

3. Role of PDE4 Isoforms in Cognitive Function and Memory

The prevalence of age-related diseases of the brain has grown exponentially in accordance with an increased life expectancy [43]. Cognitive dysfunction is a characteristic feature of age-related memory decline and although research regarding the loss of cognition and memory impairment has been considerable, the need for successful targeted treatment remains high. Memory formation is underpinned by gene expression controlled by the cAMP response element binding protein (CREB), a transcription factor activated by PKA phosphorylation [24]. The conversion to long-term memory depends on both transcription and translation [44]. In mice behavioral studies, the deficiency of CREB as well as the increase of CREB-binding protein (CBP) identified a key role for the transcription factor in the facilitating of long-term memory storage [45]. Long-term potentiation (LTP) is transcription-dependent synaptic plasticity, which has been extensively utilized as an investigatory model from long-term memory [46]. There are temporally different LTP stages that are broken down into three categories: short-term potentiation (STP), early-LTP and late-LTP [46]. These stages are dependent upon a transient increase in cAMP levels, PKA activation and CREB phosphorylation [47]. As PDEs modulate cAMP levels within cells, there has been increased interest PDE inhibition to improve cognitive function.

In the brain, PDE4B has been detected in the amygdala, thalamus, hypothalamus, white matter tracts, striatum, and, importantly, the hippocampus [18,43,48–50]. Hippocampal neurons possess unique properties that allow them to promote memory formation. Hippocampal LTP is the best-characterized cellular model for investigating learning and memory formation [51,52]. The first association of PDE4B with learning and memory was discovered in an investigation of long-term potentiation (LTP) in hippocampal neurons. Ahmed and Frey [51] identified PDE4B3 as the first cAMP-specific phosphodiesterase to be associated with the control of LTP stages in rat hippocampal slices. There is documented evidence that late-LTP is dependent upon a transient increase in cAMP levels, which results in the phosphorylation of transcription factors such as CREB. Two studies have independently shown that a mouse double AC knockout mutant failed to transition to late-LTP supporting the notion that cAMP modulation in neurons is important for late-LTP [51,53,54]. Interestingly, both the PDE4B3 transcript and PDE4B3 protein fluctuated during transition through the different stages of LTP in area cornu ammonis (CA1, the first region of the hippocampus) leading to the stage dependent transient modulation of cAMP [51,55]. Delayed cAMP elevation could be involved in the PKA dependent process of covalent modifications and as such creating a functional state of "plasticity-related proteins" (PRPs) [56]. PRPs may be directly involved in maintaining LTP processes. This mechanism remains unclear but has been named the 'synaptic tagging and capture hypothesis'. This proposes that a strong synaptic pathway leads to two pathways: local tag setting and the synthesis of PRPs. The PRPs are captured by tagged synapses allowing the sustenance of LTP maintaining it up to 8 h [57]. The delay in the elevation of cAMP levels may be crucial to the function of such tag molecules and PRPs driving LTP into its late stage. PDE4B may be characterized as a secondary PRP due to modulation of cAMP levels that promote the process through initial to late stage. As such PDE4B would function to modulate the synaptic plasticity stimuli within the cell and allow for the change in reaction later in the memory forming process [57].

In humans and rodents, the tendency to explore their environment is a commonly used characteristic to evaluate generalized cognitive abilities; however, an increase in exploration does not directly correlate with improved cognitive function [58,59]. Through the reduction of aversive stimuli, such as loud noises and bright light, leads to a shift in the motivation, which underlies environmental exploration therefore facilitating learning [60]. PDE4B^{Y358C} mutant mice, containing a catalytic domain mutation reducing its cAMP hydrolytic ability in a C57BL/6J mouse background, displayed decreased fear responses and increased exploration accompanied with cognitive enhancement during non-aversive tests [61]. During fear conditioning in the lateral amygdala, where PDE4B is highly expressed, inputs are formed through associations between the conditioned and unconditioned stimulus [62]. The decrease in fear responses in PDE4B^{Y358C} mice is consistent with the upregulation of β-arrestin and disrupted in schizophrenia 1 protein (DISC1) and decrease of the β-arrestin-PDE4B interaction, which is crucial

to the formation of associative fear [61,63]. The PDE4B[Y358C] mutant mice fear conditioning phenotype was replicated using a subchronic dose of rolipram (1 mg/kg twice daily), which inhibits all PDE4 activity and does not interfere with the interaction of the complex [24]. The same phenotype was replicated when rolipram was administered 24 h after fear conditioning, pointing to a role for PDE4B in the late signaling processes required for long-term memory persistence [24]. The impaired cAMP signaling in the hippocampus due to PDE4B may impair the very late-phase consolidation via poor coordination of the late-phase protein transcription required for long-term memory persistence due to a dysregulation in CREB activation. Given that the fear conditioning phenotype of PDE4B[Y358C] was replicated by rolipram treatment, which does not interfere with the interaction with DISC1, it was suggested that reduced contextual freezing is a result of PDE4B dysfunction rather than the disruption of the DICS1/PDE4B complex [24].

Although the information outlined above suggests that PDE4B activity is critical to the formation of memory, behavioral animal studies have provided contradictory evidence. In step down passive avoidance and the Morris water test, two tests that measure hippocampal based memory, PDE4B^{-/-} mice showed no significant change in either short- or long-term memory, in fear conditioning or in acquisition [61,64]. Electrophysiology readings examining excitatory post-synaptic potentials from PDE4B^{-/-} mice hippocampal slice samples did however identify an increase in the proliferation of neuronal cells in the hippocampal dentate gyrus along with enhanced basal postsynaptic responses and long-term depression [61]. It is possible that the loss of protein in other regions of the hippocampus and in the brain as a whole caused by the knockout of PDE4B could affect the neuronal circuitry within the CA1 region in turn leading to synaptic plasticity. Potentially, the treatment with specific PDE4B inhibitors could provide therapeutic relief for patients with persistent fear memories and post-traumatic stress disorders through the alteration of PDE4B activity.

4. The DISC1-PDE4B Signalosome in Schizophrenia

The PDE4 family is the most intensively examined family of PDEs in the context of neurological disorders. Over the years it has been firmly established that PDE4 family members are widely and differentially expressed throughout the brain [35]. In particular, all of the five PDE4B isoforms are found within the brain and this is the predominant sub-family in the amygdala, hypothalamus, and striatum [18,36,48]. Both, the amygdala and the hypothalamus, are key regions in the control of anxiety and responses to stress [65] and it is not surprising that PDE4B is of particular importance in these areas. The role of PDE4B in the field of molecular psychiatry was brought to prominence because of its link with the DISC1 gene [23,66].

The DISC1 gene is a potential susceptibility factor for psychiatric illnesses, which has been shown to be disrupted by a balanced chromosomal translocation (t(1;11)) in a number of cases of schizophrenia [23,67,68]. The t(1;11) translocation results in the production of an abnormal product due to the fusion of the DISC1 gene on chromosome 1 with a disrupted gene on chromosome 11 [69]. From this translocation, the expression of the three possible chimeric transcripts, designated; CP1, CP60, and CP69, resulted in a profoundly altered, deleterious function [69]. Given this information, it has been reported that the reduction in DISC1 function and expression is consistent with a haploinsufficient disease.

DISC1 dysregulation is not the sole contributing factor to Schizophrenia susceptibility. Initial genetic evidence for PDE4B's role in Schizophrenia was discovered in two cousins, both harboring the same translocation disrupting PDE4B and been diagnosed with the psychiatric disorder [23]. In patients with the balanced (1;16) translocation there was an accompanied ~50% reduction in the protein expression of the long PDE4B1 isoform [23]. Reduction in the levels of PDE4B in the postmortem brains of schizophrenia patients prompted the question whether PDE4B may be a common component in a variety of neurological disorders. This notion was supported by recent work suggesting that novel single nucleotide polymorphisms (SNPs) in PDE4B are associated with an increase incidence of schizophrenia within the general population [70].

To determine the molecular function of PDE4B in schizophrenia signaling processes, research groups have investigated the DISC1–PDE4B complex. From transfected HEK293 cell lysates, successful co-immunoprecipitation of DISC1 and PDE4B1, PDE4B3 and the short form PDE4B2 was achieved, suggesting that a common DISC1 binding site is present in these three isoforms [23]. These findings were confirmed with endogenously expressed proteins from human neuroblastoma-derived cell lines SH-SY5Y and LAN5 as well as in primary rat hippocampal cells. The DISC1 interactome was investigated using yeast-two hybrid (Y2H) technology, which also identified PDE4B as an interacting protein [71]. Truncation of PDE4B1 by removal of the UCR2 domain resulted in a loss of DISC1 interaction. This suggested that the specific interaction domain was in the UCR2 domain of PDE4 isoforms, which was confirmed using the UCR2 domain alone to co-immunoprecipitate DISC1 [23]. Peptide array analysis using full length long-forms PDE4D5 and PDE4B1, overlaid with DISC1, identified two common sites in the UCR2 domain [66]. However, PDE4B1 arrays overlaid with a [^{35}S]methionine radiolabelled-DISC1 also highlighted a specific binding site for DISC1 in the catalytic domain of PDE4B1 [66]. Cell penetrating peptides containing the sequence of the two common sites were unable to disrupt the interaction providing further support that PDE4B14B1 utilizes isoform specific binding regions. This concept was further supported utilizing point mutations in the PDE4B1 specific binding sites, which led to reduced DISC1 binding to PDE4B in both human and mouse variants [66]. Reciprocal arrays identified two common sites on DISC1 crucial to the binding of both PDE4B and PDE4D subfamilies; however, a further three sites were specific to PDE4B1 only. These residues were confirmed to be crucial to the interaction through the loss of binding between PDE4B1 and an N-terminally truncated DISC1 [66]. Intriguingly, the super-short isoform PDE4B5 was also found to interact with DISC1 meaning that the interaction motif must lie in the residual part of the truncated UCR2 or in an unknown site within the catalytic domain [34]. The presence of unique binding sites for PDE4B1 supported the notion that it has higher affinity for DISC1 than PDE4D5.

It is noteworthy that the interaction of PDE4B and DISC1 is dynamic and is thought to be regulated by cellular levels of cAMP via a PKA mediated process. Treatment with forskolin (IC$_{50}$ 41 nM) and the non-specific PDE inhibitor 3-isobutyl-1-methylxanthine (IBMX; IC$_{50}$ values of 6.5 ± 1.2, 26.3 ± 3.9 and 31.7 ± 5.3 µM for PDE3, 4 and 5 respectively) led to a marked decrease in amount of DISC1 precipitated with PDE4B [23]. These results suggested a potential model for the interaction, which involved phosphorylation of PDE4B by PKA that resulted in the dissociation from DISC1, in turn, allowing an activated PDE4B long-form pool to reduce local cAMP levels [23]. However, this was refuted by Murdoch et al. [66], who reported no changes in the interaction between DISC1 and PDE4B under differing cAMP concentrations.

What is the relevance of the interaction between DISC1 and PDE4B for signal transduction within the brain? A complex has been identified that contains Nuclear Distribution Factor-E-like (NDEL1) and its orthologue NDE1, Lissencephaly (LIS1), DISC1, and PDE4B. This complex is considered to have a crucial role in neuronal migration due to its localization at the centrosome [29,72,73]. The centrosome is in control of neuronal cell polarity and symmetry, the regulation of the neural progenitor pool and, crucially, the production of new neurons [74]. Additionally, the DISC1 and NDEL1 complex is responsible for the recognition of signals setting the migration pattern limits of neurons in adult brains [75]. Phosphorylation of NDEL1/NDE1 by PKA is known to modulate its protein-protein interactions as well as its subcellular localization [76–79]. The PDE4B/DISC1 interaction influences the PKA dependent phosphorylation of NDE1/NDEL1 at threonine-131/2 (T131/2), which can alter its interaction with LIS1 [76]. Phosphorylation at this site is proposed to modulate the production and positioning of neurons, neurite outgrowth, and LIS1-dependant synaptic function [29,76,80,81]. Reductions in PDE4B, like that seen with translocation mutations, would result in increased cAMP levels and subsequent activation of PKA activity. Given the discussed role of PKA phosphorylation in controlling NDE1/NDEL1s interactions, targeting PDE4B provides an interesting therapeutic strategy in this setting. Altered protein interactions within the multiprotein complex could drastically change neuron-signaling leading to progression of psychiatric disorders. Collectively, these data indicate that

dysregulation of the PDE4B/DISC1interaction with NDE1/NDEL1/LIS1 complex induces the psychiatric disorder phenotype. This multiprotein complex identifies a new therapeutic avenue for numerous psychiatric disorders including schizophrenia.

Currently, pan-PDE4 pharmacological inhibitors, such as rolipram, successfully generate neuroprotective and neurogenerative benefit however, an investigation of knock-out PDE4 mice has shown that individual PDE subtypes play a distinct and non-overlapping role [13]. Behavioral studies comparing PDE4B$^{-/-}$ male mice to their PDE4B$^{+/+}$ littermates revealed a moderate anxiogenic-like behavior measured by a decrease in immobility during forced swim test, reduced head-dipping in the hole board test as well as decrease exploration and rears in the open-field test [64]. However, the PDE4B$^{-/-}$ mice performed as wild type (WT) mice in both passive avoidance tests and in fear conditioning, as well as showing unaltered reactions in a shock test [61,64,82]. Moreover, these mice also show a lack of inhibitory effects on conditioned avoidance responses when treated with Rolipram [83]. Specific inhibition of the catalytic domain of PDE4B using a PDE4B^{Y358C} mutant mouse resulted in an increased phosphorylation of CREB, decreased binding to DISC1 accompanied by increased DISC1 and β-arrestin expression in both the hippocampus and amygdala. These data points to a possible mechanism for compensatory changes designed to compensate for lack of PDE4B activity [24]. PDE4B^{Y358C} mice also displayed decreased anxiety, increased cognitive enhancement, enhanced neurogenesis and impaired depotentiation [24]. Interestingly, these mice displayed decreased contextual fear memory at 7 days post fear conditioning, which was also "phenocopied" using pharmacological inhibition of PDE4 with rolipram [24]. In terms of treatment, chronic use of antidepressants including serotonin reuptake inhibitors, such as citalopram and paroxetine, led to an upregulation in both PDE4B and PDE4A in the cerebral cortex [84]. In addition, chronic cocaine administration has been shown to downregulate the levels of PDE4B within the nucleus accumbens, a dopamine target area, leading to a profound inhibition of the reward actions of psychostimulants [84]. Therefore, modulation of the levels of PDE4B through specific antidepressant use may influence both motivation and reward.

The research outlined above highlights the utility of strategies designed to inhibit specific isoforms or sub-families of PDE4. Targeting PDE4B activity or PDE4B-containing signalosomes may provide a novel route for the development of novel compounds that treat CNS disease without causing the side effects produced by pan-PDE4 inhibition, such as emesis and nausea [85].

5. PDE4B in Neuroinflammation

It is widely known that the PDE4 subfamily is heavily involved in the regulation of inflammatory cell activation [86–88]. Toll-like receptors (TLRs) within cells initiate responses to numerous pathogen-associated molecular patterns and host derived molecules [89,90]. The activation of TLRs initiates a downstream signaling cascade leading to the upregulation of a wide variety of target genes including chemokines, cytokines, and other inflammatory molecules [89,91,92]. Lipopolysaccharides (LPS) stimulate TLR4, which in turn drives activation of both Myeloid differentiation primary response 88 (MyD88) and Toll/interleukin-1 receptor-domain-containing adapter-inducing interferon-β (TRIF)-dependent pathway (Figure 2). Consequentially, there is the activation of numerous downstream pathways such as the nuclear factor κB (NF-κB), extracellular-signal regulated kinase 1/2 (ERK1/2) pathways, as well as the interferon regulatory factor 3 (IRF3) pathway, a transcriptional factor involved in expression of inflammatory cytokines (Figure 2) [90]. The stimulation of TLR4 by LPS and production of other regulators including cAMP elevators drives the expression of the interleukin-1 receptor antagonist (IL-1Ra) in both monocytes and macrophages. IL-1Ra is concomitantly produced in response to inflammatory stimuli and functions to downregulate pro-inflammatory cytokines such as tumor necrosis factor α (TNF-α) [93]. The complexity of this pathway driving proinflammatory-signaling means that dysregulation at any of the key regulators can lead to a prolonged inflammatory response, which if left unchecked, can create detrimental effects.
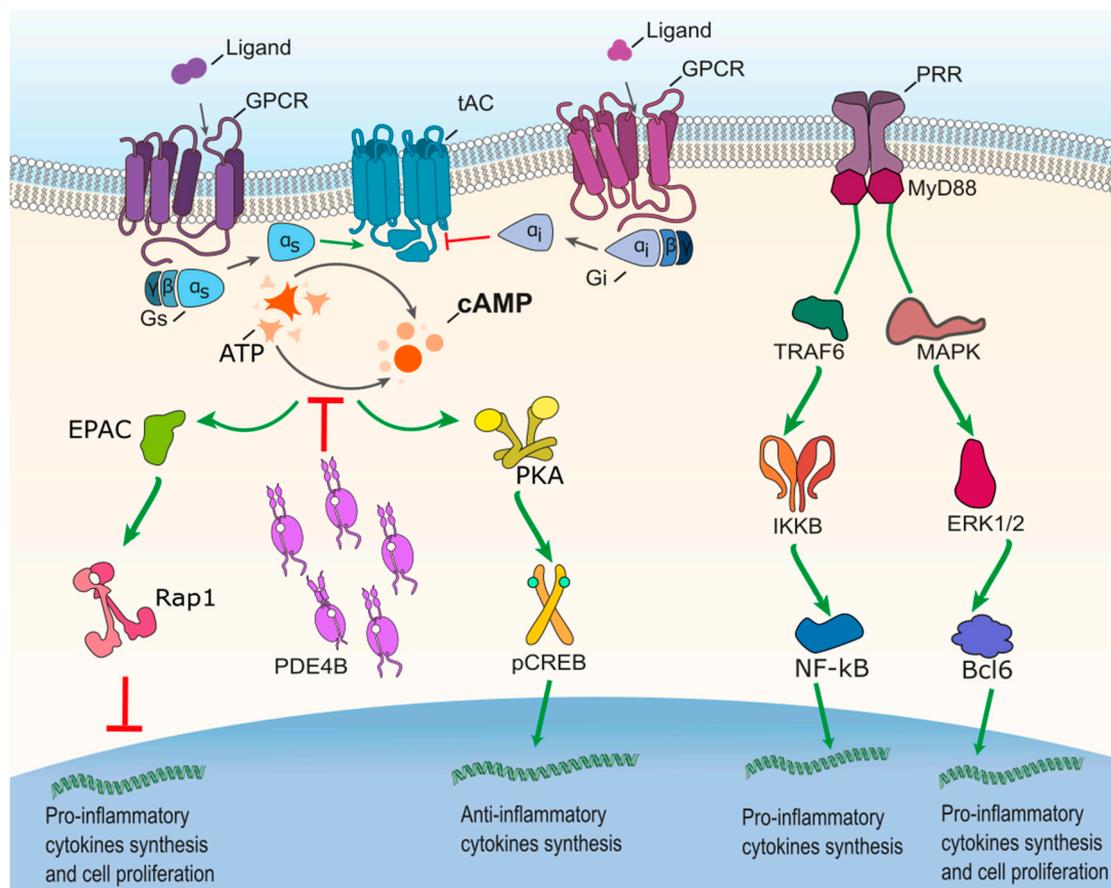
Figure 2. Schematic of inflammatory signaling pathways, PDE4B is involved in the production of inflammatory and anti-inflammatory cytokines through its action in degrading cAMP. PKA and Epac are activated by high cellular cAMP resulting in the phosphorylation of cAMP-responsive element binding protein (CREB) and activating transcription factor 1 (ATF1) leading to the generation of anti-inflammatory cytokines. Transcriptional activity of NF-κB can be regulated by PKA through modulation of its interaction with CREB. Phosphorylation of PKA can inhibit the synthesis and activity of B-cell lymphoma 6 protein (Bcl-6) and NF-κB–mediated proinflammatory cytokines. In addition, the activation of Epac leads to small GTPases (Rap1) blocking the release of pro-inflammatory cytokines. These pathways show PDE4 to be a viable target for reducing inflammation.

As mentioned above, cAMP can regulate endogenous inflammatory response mechanisms, with increasing cAMP concentrations promoting anti-inflammatory effects [94]. For example, augmented cAMP signaling is responsible for the upregulation of numerous inflammatory markers including Arginase 1 (Arg1) [95]. Specific inhibition of the cAMP-specific PDE4 significantly alters the functions of specialized inflammatory cells [96–98]. Wang and colleagues [86] identified PDE4B2 to be the most dominant isoform within human neutrophils, leukocytes, astrocytes and monocytes representing 95–100% of total cellular PDE4. PDE4B2 has been identified to control and regulate neutrophil accumulation as well as, importantly, TNF production [99,100]. In the context of inflammation, PDE4B2 is of particular importance because of its control over various inflammatory stimuli including induction by LPS and inhibition by Interleukin (IL) 10 4 [86,87,101]. Furthermore, increased expression in PDE4B resulted in a pro-inflammatory phenotype in neutrophils, macrophages, and microglia. This concept has been further strengthened following the discovery of increased PDE4B2 expression in experimental models of neuroinflammation diseases [87,101]. For example, PDE4B2 was found to exhibit elevated expression within infiltrating T cells, macrophages, and microglia following inflammatory induction of experimental autoimmune encephalomyelitis (EAE) in Lewis rats [31].

Microglia and macrophages are known to express multiple isoforms of PDE sub-families PDE4A, B, C, and D; however, PDE4B was identified to be the primary subfamily involved in the LPS response [87,99]. This was confirmed in PDE4B^{-/-} mice that exhibited a retarded TNFα response to LPS in both peripheral blood leukocytes as well as in peritoneal macrophages [99,102]. LPS specifically upregulates PDE4B expression and the resulting TNFα reduction is dependent on the cAMP/PKA pathway. No other cAMP-specific PDE was able to rescue this phenotype and in LPS-induced shock it was only PDE4B^{-/-} animals that were protected [99,102]. There is a lack of evidence surrounding genetic ablation of PDE4B in microglia; however, the general pharmacological inhibition of PDE4 with rolipram after TLR/LPS stimulation resulted in the reduction of proinflammatory cytokines, including tissue factor-1 (TF1) and TNFα, genes that are positively regulated by nuclear factor kappa B (NF-κB), as well as proinflammatory mediators, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) [103]. In fact, PDE4B^{-/-} mice showed a >50% reduction in both the levels of TNF-α mRNA and protein in macrophages, which was not seen with either PDE4A^{-/-} or PDE4D^{-/-} mice [99]. It has been established that the release of proinflammatory cytokines, TNFα, and IL-1β lead to a dramatic reduction in microglial cellular cAMP levels in conjunction with a marked increase in PDE4 activity and expression levels [87]. This finding was accompanied by the demonstration, using a cyclic AMP-Glo™ Assay (Promega), that PDE4 inhibition via rolipram or RNAi knockdown of PDE4B2 in microglia prevented the reduction of cAMP after TNF-α activation [87]. In addition, Alzheimer's disease (AD) model mice, which are deficient in PDE4B, show decreased TNFα expression in response to inflammatory stimuli. Furthermore, Ghosh and colleagues [103] were able to reverse the cAMP reduction created by TNFα by inhibiting PDE4 activity with rolipram.

Interestingly, after TNFα stimulation there was an increase in the levels of ERK1/2 activity ERK phosphorylation [38], and subsequently, an increased activation of PDE4B2, and other short isoforms, greatly reducing the cAMP levels thereby reducing inflammatory signals [87].

As described, elevation of intracellular cAMP downregulates a range of multifaceted immune cell functions including the expression of TNFα, interferon-γ (IFN-γ) and numerous ILs [104–108]. There is increasing evidence to support the hypothesis that an immune challenge by LPS creates depressive-like behavior, possibly related to the upregulated PDE4 activity that is associated with a neuroinflammatory response. Traumatic Brain Injury (TBI) is a serious clinical problem that can leave patients with high levels of cognitive impairment and lasting TBI-related deficiencies reducing both life quality and expectancy [25,109–111]. During an acute inflammatory response, cAMP levels are significantly repressed post-injury and remain so for several days [30,112]. Such a reduction in cAMP signaling severely attenuates CREB activation, which is a master regulator of pathways critical to memory and learning as well as an acute up-regulator of PDE4B2. Targeting TBI-directed inflammation has been identified as a promising strategy for reducing histopathological damage and cognitive deficits. As mentioned, PDE4B2 has been implicated in both the activation and regulation of inflammatory cells including microglia and neutrophils [87,101]. Within 24 h of a TBI, there is an immediate and extensive infiltration of the brain by neutrophils leading to pathophysiological symptoms, including neuroinflammation, [113] as well as elevated levels of PDE4B2 in both the injured cortex and the hippocampus [25]. Interestingly in models of inflammatory lung disease, PDE4B^{-/-} and PDE4D^{-/-} neutrophils also showed markedly decreased chemotaxis in response to chemokine (C-X-C motif) ligand 1 (CXCL1 or KC) and levels of macrophage inflammatory protein-2 (MIP-2) [100]. This effect was comparable to the PDE4 inhibition by rolipram, but was not additive. Importantly, this data underpinned the idea that both PDE4B and PDE4D share a complementary, non-redundant role in the control of neutrophil function [100], which provides precedent for the mechanism in the brain.

More evidence of PDE4B involvement was uncovered when PDE4B^{-/-} mutant mice[-] and mice treated with A33 (2- (4-phenyl)acetic acid, a selective PDE4B inhibitor with an IC$_{50}$ = 27 nM [30,114,115]) showed a reduction in the levels of neutrophil accumulation after 24 h, but not 3 h after treatment. This suggests that the initial neutrophil response is not driven by PDE4B, but peak accumulation may be [25]. A33 selectivity of PDE4B is derived from a single amino polymorphism in the C-terminus,

which inhibits its activity by keeping it locked in the inactive conformation. Following the inhibition of PDE4B by A33, cAMP levels were not reduced, leading to an increase in CREB phosphorylation and subsequent activation of anti-apoptotic genes, such as B cell lymphoma 2 [116]. Additionally, it is suggested that PDE4B2 inhibition decreases Integrin beta chain-2 (CD18) expression within the circulating neutrophils. CD18 expression is a crucial mediator for neutrophil infiltration [117,118]. Therefore, CD18 reduction in circulating neutrophils leads to the reduction in infiltrating neutrophils in the brain [100]. Such data suggest specifically targeting PDE4B is a valid therapeutic target for reducing the peak accumulation of neutrophil post TBI. As such, further investigations of TBI in model systems of the central nervous system (CNS) have already identified the use of PDE4 inhibitors, such as rolipram, and the elevation of cAMP levels, to be an effective strategy for reducing inflammation and promoting recovery [119,120]. Pre-injury treatment with rolipram has clearly been shown to rescue the cAMP signaling deficits in conjunction with reduced inflammation after TBI as well as reduced neuronal loss in the cortex and l CA3 region, the hippocampal region that receives inputs from the entorhinal cortex [22]. Although administration of rolipram post-TBI confirmed these concepts, exacerbation of the cortical contusion volume and atrophy was observed, resulting in prolonged brain injury [112,121]. Pan-PDE4 inhibitors have been used to reduce TNF levels and increase the accumulation of neutrophils in mouse models of CNS-related inflammatory disorders including TBI [122]. The issue with the general inhibition of PDE4 is that the benefits of acute treatment are overshadowed by vascular perturbations, shown by increased hemorrhage after TBI [22,25]. As PDE4B plays a crucial role in the modulation of inflammation and the subsequent TBI pathophysiology, adult male Sprague Dawley rats were induced with a moderate parasagittal fluid-percussion brain injury before being treated with either A33 or vehicle control [25]. Treatment after injury resulted in a pronounced reduction in microglial activation markers and neutrophil infiltration at 3 h and 24 h post injury. Specificity for PDE4B in this regard is under continuing investigation. Goto and colleagues [123] created a novel and potent *N*-propylacetamide derivative, known as compound 31b, for PDE4B (IC$_{50}$ = 7.5 nM) and TNFα (IC$_{50}$ = 9.8 nM) inhibition in mouse splenocytes. Although this compound has not been investigated in the brain, it can reduce anti-inflammatory activity in lung inflammation mouse models induced by LPS, which may be translated in the brain [123].

It is evident that PDE4B has a major role in neuroinflammation caused by injury, and it is also clear that the enzyme plays a critical role in the alcohol priming effect, leading to exacerbated responses of immune cells to LPS [124–128]. Work performed by McClain and colleagues [125] identified that there was a decrease in cellular cAMP levels, which had a pathological role in ethanol priming, which lead to an increase in the LPS-inducible TNFα expression. This is in part due to the knowledge that alcohol-induced, gut-mediated peripheral endotoxemia has a significant role in neuroinflammation and glial cell activation. Alcohol fed C57Bl/6 wild type (WT) mice displayed both induced peripheral endotoxin and increased PDE4B expression in the brain [129,130]. This was accompanied by activation of both microglia and astrocytes, as well as a significant increase in levels of inflammatory cytokines and markers, including TNFα, as previously described, Monocyte chemoattractant protein-1, IL-17, and COX-2 [127,129–131]. Microglial cells activated by alcohol displayed increased levels of only the PDE4B sub-family [129], and this was validated by experiments where a single intraperitoneal administration of endotoxin significantly upregulated PDE4B expression in glial cells, as well as distinct CNS expression of numerous inflammatory mediators [49,50]. Interestingly, alcohol feeding in mice results in increased serum endotoxin levels with elevated cluster of differentiation 14 (CD14), causing gut dysfunction accompanied by a movement of microbial products, leading to a systemic innate immune response [129]. Accompanying the increase in endotoxin production was the activation of alcohol-induced inflammatory changes in the brain, including the activation of glial cells [129]. Furthermore, there was both an increase in TLR4 and PDE4B expression in both microglia and total brain homogenate. Another noteworthy point is that rolipram was found to significantly reduce alcohol intake and alcohol preference in mice [130,132]. Functional implications of *PDE4B* expression in alcohol-affected brains are related to the decrease in total cAMP levels that triggers loss of immune

homeostasis, reductions in pro-inflammatory mediator release, and a diminution in the release of anti-inflammatory cytokines, such as IL-10 [133]. The pivotal role of PDE4B in alcohol promotion of inflammation has also been strongly supported by work in PDE4B$^{-/-}$ mice that do not displaying the symptomatic decrease in cAMP that usually accompanies alcohol ingestion [129]. In conjunction with this, the inhibition of PDE4B activity by PDE4 inhibitors—rolipram (1 and 5 mg/kg) and piclamilast (IC$_{50}$: 2 nM; 1 mg/kg)—led to a marked reduction in the production of inflammatory cytokines as well as in the activation of glial cells [133].

6. Conclusions

We have reviewed the role of PDE4B in brain development, brain function, and the in the pathophysiology of numerous brain-related diseases. Our take-home message is that the regulation, localization, and expression levels of PDE4B isoforms are crucial to the maintenance of healthy brain function. We feel it is apparent that a more targeted approach to treatment of diseases of the brain mentioned here could be achieved by pharmacological interventions that specifically attenuate activity of PDE4B isoforms or the discretely localized signaling complexes that contain this PDE4 sub-family. Unfortunately, the similarity of the structure of the catalytic/regulatory domains between PDE4B isoforms means that this level of selectivity has not been possible for small molecule inhibitors to date, and peptide disruptors that can specifically dislodge single PDE4B forms are unlikely to be able to cross the blood–brain barrier unless used in conjunction with novel transporter technology, such as nanoligand technology or BBB shuttle peptides [134–136]. We look forward to further exciting developments that signify new roles for the PDE4B sub-family in the brain that may offer tractable, mechanism-based therapeutic avenues.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Francis, S.H.; Busch, J.L.; Corbin, J.D. cGMP-Dependent Protein Kinases and cGMP Phosphodiesterases in Nitric Oxide and cGMP Action. *Pharmacol. Rev.* **2010**, *62*, 525–563. [CrossRef] [PubMed]
2. Xu, Y.; Zhang, H.-T.; O'Donnell, J.M. Phosphodiesterases in the Central Nervous System: Implications in Mood and Cognitive Disorders. In *Phosphodiesterases as Drug Targets*; Francis, S.H., Conti, M., Houslay, M.D., Eds.; Springer: Berlin/Heidelberg, Germany, 2011; pp. 447–485.
3. Sakamoto, K.; Karelina, K.; Obrietan, K. CREB: A multifaceted regulator of neuronal plasticity and protection. *J. Neurochem.* **2011**, *116*, 1–9. [CrossRef] [PubMed]
4. Kelly, M.P.; Adamowicz, W.; Bove, S.; Hartman, A.J.; Mariga, A.; Pathak, G.; Reinhart, V.; Romegialli, A.; Kleiman, R.J. Select 3′,5′-cyclic nucleotide phosphodiesterases exhibit altered expression in the aged rodent brain. *Cell. Signal.* **2014**, *26*, 383–397. [CrossRef] [PubMed]
5. Beavo, J.A.; Brunton, L.L. Cyclic nucleotide research—Still expanding after half a century. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 710–717. [CrossRef] [PubMed]
6. Nikolaev, V.O.; Lohse, M.J. Monitoring of cAMP Synthesis and Degradation in Living Cells. *Physiology* **2006**, *21*, 86–92. [CrossRef] [PubMed]
7. Willoughby, D.; Cooper, D.M.F. Live-cell imaging of cAMP dynamics. *Nat. Methods* **2008**, *5*, 29–36. [CrossRef]
8. Baillie, G.S. Compartmentalized signalling: Spatial regulation of cAMP by the action of compartmentalized phosphodiesterases. *FEBS J.* **2009**, *276*, 1790–1799. [CrossRef]
9. Edwards, H.V.; Christian, F.; Baillie, G. cAMP: Novel concepts in compartmentalised signalling. *Semin. Cell Dev. Boil.* **2012**, *23*, 181–190. [CrossRef]
10. Francis, S.H.; Blount, M.A.; Corbin, J.D. Mammalian Cyclic Nucleotide Phosphodiesterases: Molecular Mechanisms and Physiological Functions. *Physiol. Rev.* **2011**, *91*, 651–690. [CrossRef]

11. McCahill, A.C.; Huston, E.; Li, X.; Houslay, M. PDE4 Associates with Different Scaffolding Proteins: Modulating Interactions as Treatment for Certain Diseases. In *Protein-Protein Interactions as New Drug Targets*; Klussmann, E., Scott, J., Eds.; Springer: Berlin/Heidelberg, Germany, 2008; pp. 125–166.

12. Bender, A.T.; Beavo, J.A. Cyclic Nucleotide Phosphodiesterases: Molecular Regulation to Clinical Use. *Pharmacol. Rev.* **2006**, *58*, 488–520. [CrossRef]

13. Conti, M.; Beavo, J. Biochemistry and Physiology of Cyclic Nucleotide Phosphodiesterases: Essential Components in Cyclic Nucleotide Signaling. *Annu. Rev. Biochem.* **2007**, *76*, 481–511. [CrossRef] [PubMed]

14. Zaccolo, M.; Di Benedetto, G.; Lissandron, V.; Mancuso, L.; Terrin, A.; Zamparo, I. Restricted diffusion of a freely diffusible second messenger: Mechanisms underlying compartmentalized cAMP signalling. *Biochem. Soc. Trans.* **2006**, *34*, 495–497. [CrossRef]

15. Terrin, A.; Di Benedetto, G.; Pertegato, V.; Cheung, Y.-F.; Baillie, G.; Lynch, M.J.; Elvassore, N.; Prinz, A.; Herberg, F.W.; Houslay, M.; et al. PGE1 stimulation of HEK293 cells generates multiple contiguous domains with different [cAMP]: Role of compartmentalized phosphodiesterases. *J. Cell Boil.* **2006**, *175*, 441–451. [CrossRef] [PubMed]

16. Oliveira, R.F.; Terrin, A.; Di Benedetto, G.; Cannon, R.C.; Koh, W.; Kim, M.; Zaccolo, M.; Blackwell, K.T. The Role of Type 4 Phosphodiesterases in Generating Microdomains of cAMP: Large Scale Stochastic Simulations. *PLoS ONE* **2010**, *5*, e11725. [CrossRef] [PubMed]

17. Francis, S.H.; Houslay, M.D.; Conti, M. (Eds.) Phosphodiesterase Inhibitors: Factors That Influence Potency, Selectivity, and Action. In *Phosphodiesterases as Drug Targets*; Springer: Berlin/Heidelberg, Germany, 2011; pp. 47–84.

18. Pérez-Torres, S.; Miró, X.; Palacios, J.M.; Cortés, R.; Puigdoménech, P.; Mengod, G. 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.* **2000**, *20*, 349–374. [CrossRef]

19. Wachtel, H. Characteristic behavioral alterations in rats induced by rolipram and other selective adenosine cyclic 3′,5′-monophosphate phosphodiesterase inhibitors. *Psychopharmacology* **1982**, *77*, 309–316. [CrossRef] [PubMed]

20. O'Donnell, J.M.; Zhang, H.-T. Antidepressant effects of inhibitors of cAMP phosphodiesterase (PDE4). *Trends Pharmacol. Sci.* **2004**, *25*, 158–163. [CrossRef] [PubMed]

21. Souness, J.E.; Aldous, D.; Sargent, C. Immunosuppressive and anti-inflammatory effects of cyclic AMP phosphodiesterase (PDE) type 4 inhibitors. *Immunopharmacology* **2000**, *47*, 127–162. [CrossRef]

22. Atkins, C.; Oliva, A.A.; Alonso, O.F.; Pearse, D.D.; Bramlett, H.M.; Dietrich, W.D. Modulation of the cAMP signaling pathway after traumatic brain injury. *Exp. Neurol.* **2007**, *208*, 145–158. [CrossRef]

23. Millar, J.K. DISC1 and PDE4B Are Interacting Genetic Factors in Schizophrenia That Regulate cAMP Signaling. *J. Physiol.* **2005**, *310*, 1187–1191. [CrossRef]

24. McGirr, A.; Lipina, T.V.; Mun, H.-S.; Georgiou, J.; Al-Amri, A.H.; Ng, E.; Zhai, D.; Elliott, C.; Cameron, R.T.; Mullins, J.; et al. Specific Inhibition of Phosphodiesterase-4B Results in Anxiolysis and Facilitates Memory Acquisition. *Neuropsychopharmacology* **2015**, *41*, 1080. [CrossRef] [PubMed]

25. Wilson, N.M.; Gurney, M.E.; Dietrich, W.D.; Atkins, C. Therapeutic benefits of phosphodiesterase 4B inhibition after traumatic brain injury. *PLoS ONE* **2017**, *12*, e0178013. [CrossRef] [PubMed]

26. Tibbo, A.J.; Tejeda, G.S.; Baillie, G. Understanding PDE4's function in Alzheimer's disease; a target for novel therapeutic approaches. *Biochem. Soc. Trans.* **2019**, *47*, 1557–1565. [CrossRef] [PubMed]

27. Blokland, A.; Schreiber, R.; Prickaerts, J. Improving Memory: A Role for Phosphodiesterases. *Curr. Pharm. Des.* **2006**, *12*, 2511–2523. [CrossRef]

28. Blokland, A.; Menniti, F.S.; Prickaerts, J. PDE Inhibition and cognition enhancement. *Expert Opin. Ther. Pat.* **2012**, *22*, 349–354. [CrossRef]

29. Bradshaw, N.J.; Ogawa, F.; Antolin-Fontes, B.; Chubb, J.E.; Carlyle, B.C.; Christie, S.; Claessens, A.; Porteous, D.J.; Millar, J.K. DISC1, PDE4B, and NDE1 at the centrosome and synapse. *Biochem. Biophys. Res. Commun.* **2008**, *377*, 1091–1096. [CrossRef]

30. Titus, D.; Furones, C.; Kang, Y.; Atkins, C. Age-dependent alterations in cAMP signaling contribute to synaptic plasticity deficits following traumatic brain injury. *Neuroscience* **2013**, *231*, 182–194. [CrossRef]

31. Reyes-Irisarri, E.; Sanchez, A.J.; García-Merino, J.A.; Mengod, G. Selective Induction of cAMP Phosphodiesterase PDE4B2 Expression in Experimental Autoimmune Encephalomyelitis. *J. Neuropathol. Exp. Neurol.* **2007**, *66*, 923–931. [CrossRef]

32. Houslay, M.D. PDE4 cAMP-specific phosphodiesterases. *Prog. Nucleic Acid Res. Mol. Biol.* **2001**, *69*, 249–315.

33. Houslay, M.; Baillie, G.; Maurice, D.H. cAMP-Specific Phosphodiesterase-4 Enzymes in the Cardiovascular System. *Circ. Res.* **2007**, *100*, 950–966. [CrossRef]

34. Cheung, Y.-F.; Kan, Z.; Garrett-Engele, P.; Gall, I.; Murdoch, H.; Baillie, G.; Camargo, L.M.; Johnson, J.M.; Houslay, M.; Castle, J.C. PDE4B5, a Novel, Super-Short, Brain-Specific cAMP Phosphodiesterase-4 Variant Whose Isoform-Specifying N-Terminal Region Is Identical to That of cAMP Phosphodiesterase-4D6 (PDE4D6). *J. Pharmacol. Exp. Ther.* **2007**, *322*, 600. [CrossRef] [PubMed]

35. Fatemi, S.; King, D.P.; Reutiman, T.J.; Folsom, T.D.; Laurence, J.A.; Lee, S.; Fan, Y.-T.; Paciga, S.A.; Conti, M.; Menniti, F.S. PDE4B polymorphisms and decreased PDE4B expression are associated with schizophrenia. *Schizophr. Res.* **2008**, *101*, 36–49. [CrossRef] [PubMed]

36. Sheperd, M. Molecular cloning and subcellular distribution of the novel PDE4B4 cAMP-specific phosphodiesterase isoform. *Biochem. J.* **2003**, *370*, 429–438. [CrossRef] [PubMed]

37. Cedervall, P.; Aulabaugh, A.; Geoghegan, K.F.; McLellan, T.J.; Pandit, J. Engineered stabilization and structural analysis of the autoinhibited conformation of PDE4. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E1414–E1422. [CrossRef] [PubMed]

38. Baillie, G.; MacKenzie, S.J.; McPhee, I.; Houslay, M. Sub-family selective actions in the ability of Erk2 MAP kinase to phosphorylate and regulate the activity of PDE4 cyclic AMP-specific phosphodiesterases. *Br. J. Pharmacol.* **2000**, *131*, 811–819. [CrossRef] [PubMed]

39. Houslay, M.; Adams, D.R. PDE4 cAMP phosphodiesterases: Modular enzymes that orchestrate signalling cross-talk, desensitization and compartmentalization. *Biochem. J.* **2003**, *370*, 1–18. [CrossRef]

40. Xie, M.; Blackman, B.; Scheitrum, C.; Mika, D.; Blanchard, E.; Lei, T.; Conti, M.; Richter, W. The upstream conserved regions (UCRs) mediate homo- and hetero-oligomerization of type 4 cyclic nucleotide phosphodiesterases (PDE4s). *Biochem. J.* **2014**, *459*, 539–550. [CrossRef]

41. Richter, W.; Conti, M. Dimerization of the type 4 cAMP-specific phosphodiesterases is mediated by the upstream conserved regions (UCRs). *J. Boil. Chem.* **2002**, *277*, 40212–40221. [CrossRef]

42. Richter, W.; Conti, M. The oligomerization state determines regulatory properties and inhibitor sensitivity of type 4 cAMP-specific phosphodiesterases. *J. Boil. Chem.* **2004**, *279*, 30338–30348. [CrossRef]

43. Richter, W.; Menniti, F.S.; Zhang, H.-T.; Conti, M. PDE4 as a target for cognition enhancement. *Expert Opin. Ther. Targets* **2013**, *17*, 1011–1027. [CrossRef]

44. Alberini, C.M. Transcription Factors in Long-Term Memory and Synaptic Plasticity. *Physiol. Rev.* **2009**, *89*, 121–145. [CrossRef] [PubMed]

45. Wood, M.A.; Kaplan, M.P.; Park, A.; Blanchard, E.J.; Oliveira, A.M.M.; Lombardi, T.L.; Abel, T. Transgenic mice expressing a truncated form of CREB-binding protein (CBP) exhibit deficits in hippocampal synaptic plasticity and memory storage. *Learn. Mem.* **2005**, *12*, 111–119. [CrossRef] [PubMed]

46. Sweatt, J.D. Toward a Molecular Explanation for Long-Term Potentiation. *Learn. Mem.* **1999**, *6*, 399–416. [CrossRef] [PubMed]

47. Frey, U.; Huang, Y.; Kandel, E. Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* **1993**, *260*, 1661–1664. [CrossRef] [PubMed]

48. Cherry, J.A.; Davis, R.L. Cyclic AMP phosphodiesterases are localized in regions of the mouse brain associated with reinforcement, movement, and affect. *J. Comp. Neurol.* **1999**, *407*, 287–301. [CrossRef]

49. Johansson, E.M.; Reyes-Irisarri, E.; Mengod, G. Comparison of cAMP-specific phosphodiesterase mRNAs distribution in mouse and rat brain. *Neurosci. Lett.* **2012**, *525*, 1–6. [CrossRef]

50. Reyes-Irisarri, E.; Pérez-Torres, S.; Miró, X.; Martinez, E.; Puigdomènech, P.; Palacios, J.M.; Mengod, G. Differential distribution of PDE4B splice variant mRNAs in rat brain and the effects of systemic administration of LPS in their expression. *Synapse* **2008**, *62*, 74–79. [CrossRef]

51. Ahmed, T.; Frey, J.U. Expression of the specific type IV phosphodiesterase gene PDE4B3 during different phases of long-term potentiation in single hippocampal slices of rats in vitro. *Neuroscience* **2003**, *117*, 627–638. [CrossRef]

52. Bliss, T.V.P.; Collingridge, G.L. A synaptic model of memory: Long-term potentiation in the hippocampus. *Nature* **1993**, *361*, 31–39. [CrossRef]

53. Winder, D.G.; Mansuy, I.M.; Osman, M.; Moallem, T.M.; Kandel, E.R. Genetic and pharmacological evidence for a novel, intermediate phase of long-term potentiation suppressed by calcineurin. *Cell* **1998**, *92*, 25–37. [CrossRef]

54. Wong, S.T.; Athos, J.; Figueroa, X.A.; Pineda, V.V.; Schaefer, M.L.; Chavkin, C.C.; Muglia, L.J.; Storm, D.R. Calcium-stimulated adenylyl cyclase activity is critical for hippocampus-dependent long-term memory and late phase LTP. *Neuron* **1999**, *23*, 787–798. [CrossRef]

55. Ahmed, T.; Frey, S.; Frey, J. Regulation of the phosphodiesterase PDE4B3-ISOTYPE during long-term potentiation in the area dentata in vivo. *Neuroscience* **2004**, *124*, 857–867. [CrossRef] [PubMed]

56. Frey, J.U.; Morris, R.G.M. Weak before strong: Dissociating synaptic tagging and plasticity-factor accounts of late-LTP. *Neuropharmacology* **1998**, *37*, 545–552. [CrossRef]

57. Ahmed, T.; Frey, J. Phosphodiesterase 4B (PDE4B) and cAMP-level regulation within different tissue fractions of rat hippocampal slices during long-term potentiation in vitro. *Brain Res.* **2005**, *1041*, 212–222. [CrossRef] [PubMed]

58. Matzel, L.D.; Townsend, D.A.; Grossman, H.; Han, Y.R.; Hale, G.; Zappulla, M.; Light, K.; Kolata, S. Exploration in outbred mice covaries with general learning abilities irrespective of stress reactivity, emotionality, and physical attributes. *Neurobiol. Learn. Mem.* **2006**, *86*, 228–240. [CrossRef] [PubMed]

59. Light, K.R.; Kolata, S.; Hale, G.; Grossman, H.; Matzel, L.D. Up-regulation of exploratory tendencies does not enhance general learning abilities in juvenile or young-adult outbred mice. *Neurobiol. Learn. Mem.* **2008**, *90*, 317–329. [CrossRef] [PubMed]

60. Saab, B.J.; Georgiou, J.; Nath, A.; Lee, F.J.S.; Wang, M.; Michalon, A.; Liu, F.; Mansuy, I.M.; Roder, J.C. NCS-1 in the Dentate Gyrus Promotes Exploration, Synaptic Plasticity, and Rapid Acquisition of Spatial Memory. *Neuron* **2009**, *63*, 643–656. [CrossRef]

61. Rutten, K.; Wallace, T.L.; Works, M.; Prickaerts, J.; Blokland, A.; Novak, T.J.; Santarelli, L.; Misner, D.L. Enhanced long-term depression and impaired reversal learning in phosphodiesterase 4B-knockout (PDE4B−/−) mice. *Neuropharmacology* **2011**, *61*, 138–147. [CrossRef]

62. LeDoux, J.E. Emotion Circuits in the Brain. *Annu. Rev. Neurosci.* **2000**, *23*, 155–184. [CrossRef]

63. Li, Y.; Liu, H.; Li, X.; Bao, G.; Tao, Y.; Wu, Z.; Xia, P.; Wu, C.; Li, B.; Ma, L. Regulation of amygdalar PKA by β-arrestin-2/phosphodiesterase-4 complex is critical for fear conditioning. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 21918–21923. [CrossRef]

64. Zhang, H.-T.; Huang, Y.; Masood, A.; Stolinski, L.R.; Li, Y.; Zhang, L.; Dlaboga, D.; Jin, S.-L.C.; Conti, M.; O'Donnell, J.M. Anxiogenic-like behavioral phenotype of mice deficient in phosphodiesterase 4B (PDE4B). *Neuropsychopharmacology* **2008**, *33*, 1611–1623. [CrossRef] [PubMed]

65. Deutch, A.; Charney, D.S. A Functional Neuroanatomy of Anxiety and Fear: Implications for the Pathophysiology and Treatment of Anxiety Disorders. *Crit. Rev. Neurobiol.* **1996**, *10*, 419–446. [CrossRef] [PubMed]

66. Murdoch, H. Isoform-Selective Susceptibility of DISC1/Phosphodiesterase-4 Complexes to Dissociation by Elevated Intracellular cAMP Levels. *J. Neurosci. Res.* **2007**, *27*, 9513–9524. [CrossRef] [PubMed]

67. Blackwood, D.; Fordyce, A.; Walker, M.; Clair, D.S.; Porteous, D.J.; Muir, W. Schizophrenia and Affective Disorders—Cosegregation with a Translocation at Chromosome 1q42 That Directly Disrupts Brain-Expressed Genes: Clinical and P300 Findings in a Family. *Am. J. Hum. Genet.* **2001**, *69*, 428–433. [CrossRef]

68. Harrison, P.J.; Weinberger, D.R. Schizophrenia genes, gene expression, and neuropathology: On the matter of their convergence. *Mol. Psychiatry* **2005**, *10*, 40–68. [CrossRef]

69. Eykelenboom, J.E.; Briggs, G.J.; Bradshaw, N.J.; Soares, D.; Ogawa, F.; Christie, S.; Malavasi, E.L.; Makedonopoulou, P.; Mackie, S.; Malloy, M.P.; et al. A t(1;11) translocation linked to schizophrenia and affective disorders gives rise to aberrant chimeric DISC1 transcripts that encode structurally altered, deleterious mitochondrial proteins. *Hum. Mol. Genet.* **2012**, *21*, 3374–3386. [CrossRef]

70. Feng, Y.; Cheng, D.; Zhang, C.; Li, Y.; Zhang, Z.; Wang, J.; Shi, Y. Association of PDE4B Polymorphisms with Susceptibility to Schizophrenia: A Meta-Analysis of Case-Control Studies. *PLoS ONE* **2016**, *11*, e0147092. [CrossRef]

71. Camargo, L.M.; Collura, V.; Rain, J.-C.; Mizuguchi, K.; Hermjakob, H.; Kerrien, S.; Bonnert, T.P.; Whiting, P.J.; Brandon, N.J. Disrupted in Schizophrenia 1 Interactome: Evidence for the close connectivity of risk genes and a potential synaptic basis for schizophrenia. *Mol. Psychiatry* **2006**, *12*, 74. [CrossRef]

72. Wynshaw-Boris, A. Lissencephaly and LIS1: Insights into the molecular mechanisms of neuronal migration and development. *Clin. Genet.* **2007**, *72*, 296–304. [CrossRef]

73. Brandon, N.J.; Handford, E.; Schurov, I.; Rain, J.-C.; Pelling, M.; Duran-Jimeniz, B.; Camargo, L.; Oliver, K.; Beher, D.; Shearman, M.; et al. Disrupted in Schizophrenia 1 and Nudel form a neurodevelopmentally regulated protein complex: Implications for schizophrenia and other major neurological disorders. *Mol. Cell. Neurosci.* **2004**, *25*, 42–55. [CrossRef]

74. Higginbotham, H.R.; Gleeson, J.G. The centrosome in neuronal development. *Trends Neurosci.* **2007**, *30*, 276–283. [CrossRef] [PubMed]

75. Duan, X.; Chang, J.H.; Ge, S.; Faulkner, R.; Kim, J.Y.; Kitabatake, Y.; Liu, X.-B.; Yang, C.-H.; Jordan, J.D.; Ma, D.K.; et al. Disrupted-In-Schizophrenia 1 Regulates Integration of Newly Generated Neurons in the Adult Brain. *Cell* **2007**, *130*, 1146–1158. [CrossRef] [PubMed]

76. Bradshaw, N.J.; Soares, D.C.; Carlyle, B.C.; Ogawa, F.; Davidson-Smith, H.; Christie, S.; Millar, J.K. PKA Phosphorylation of NDE1 Is DISC1/PDE4 Dependent and Modulates Its Interaction with LIS1 and NDEL1. *J. Neurosci.* **2011**, *31*, 9043. [CrossRef] [PubMed]

77. Toyo-Oka, K.; Sasaki, S.; Yano, Y.; Mori, D.; Kobayashi, T.; Toyoshima, Y.Y.; Tokuoka, S.M.; Ishii, S.; Shimizu, T.; Muramatsu, M.; et al. Recruitment of katanin p60 by phosphorylated NDEL1, an LIS1 interacting protein, is essential for mitotic cell division and neuronal migration. *Hum. Mol. Genet.* **2005**, *14*, 3113–3128. [CrossRef]

78. Shen, Y.; Li, N.; Wu, S.; Zhou, Y.; Shan, Y.; Zhang, Q.; Ding, C.; Yuan, Q.; Zhao, F.; Zeng, R.; et al. Nudel Binds Cdc42GAP to Modulate Cdc42 Activity at the Leading Edge of Migrating Cells. *Dev. Cell* **2008**, *14*, 342–353. [CrossRef]

79. Hebbar, S.; Mesngon, M.T.; Guillotte, A.M.; Desai, B.; Ayala, R.; Smith, D.S. Lis1 and Ndel1 influence the timing of nuclear envelope breakdown in neural stem cells. *J. Cell Boil.* **2008**, *182*, 1063. [CrossRef]

80. Greenwood, J.S.; Wang, Y.; Estrada, R.C.; Ackerman, L.; Ohara, P.T.; Baraban, S.C. Seizures, enhanced excitation, and increased vesicle number in Lis1 mutant mice. *Ann. Neurol.* **2009**, *66*, 644–653. [CrossRef]

81. Mori, D.; Yamada, M.; Mimori-Kiyosue, Y.; Shirai, Y.; Suzuki, A.; Ohno, S.; Saya, H.; Wynshaw-Boris, A.; Hirotsune, S. An essential role of the aPKC–Aurora A–NDEL1 pathway in neurite elongation by modulation of microtubule dynamics. *Nat. Cell Biol.* **2009**, *11*, 1057. [CrossRef]

82. Siuciak, J.; McCarthy, S.A.; Chapin, D.S.; Martin, A.N.; Harms, J.F.; Schmidt, C.J. Behavioral characterization of mice deficient in the phosphodiesterase-10A (PDE10A) enzyme on a C57/Bl6N congenic background. *Neuropharmacology* **2008**, *54*, 417–427. [CrossRef]

83. Siuciak, J.; Chapin, D.S.; McCarthy, S.A.; Martin, A.N. Antipsychotic profile of rolipram: Efficacy in rats and reduced sensitivity in mice deficient in the phosphodiesterase-4B (PDE4B) enzyme. *Psychopharmacology* **2007**, *192*, 415–424. [CrossRef]

84. Takahashi, M.; Terwilliger, R.; Lane, C.; Mézes, P.S.; Conti, M.; Duman, R.S. Chronic Antidepressant Administration Increases the Expression of cAMP-Specific Phosphodiesterase 4A and 4B Isoforms. *J. Neurosci.* **1999**, *19*, 610. [CrossRef] [PubMed]

85. Li, H.; Zuo, J.; Tang, W. Phosphodiesterase-4 Inhibitors for the Treatment of Inflammatory Diseases. *Front. Pharmacol.* **2018**, *9*, 1048. [CrossRef] [PubMed]

86. Wang, P.; Wu, P.; Ohleth, K.M.; Egan, R.W.; Billah, M.M. Phosphodiesterase 4B2 Is the Predominant Phosphodiesterase Species and Undergoes Differential Regulation of Gene Expression in Human Monocytes and Neutrophils. *Mol. Pharmacol.* **1999**, *56*, 170–174. [CrossRef] [PubMed]

87. Ghosh, M.; Garcia-Castillo, D.; Aguirre, V.; Golshani, R.; Atkins, C.; Bramlett, H.M.; Dietrich, W.D.; Pearse, D.D. Proinflammatory cytokine regulation of cyclic AMP-phosphodiesterase 4 signaling in microglia in vitro and following CNS injury. *Glia* **2012**, *60*, 1839–1859. [CrossRef] [PubMed]

88. Flora, G.; Joseph, G.; Patel, S.; Singh, A.; Bleicher, D.; Barakat, D.; Louro, J.; Fenton, S.; Garg, M.; Bunge, M.B.; et al. Combining Neurotrophin-Transduced Schwann Cells and Rolipram to Promote Functional Recovery from Subacute Spinal Cord Injury. *Cell Transplant.* **2013**, *22*, 2203–2217. [CrossRef] [PubMed]

89. Kielian, T. Toll-like receptors in central nervous system glial inflammation and homeostasis. *J. Neurosci. Res.* **2006**, *83*, 711–730. [CrossRef]

90. Kawai, T.; Akira, S. The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. *Nat. Immunol.* **2010**, *11*, 373–384. [CrossRef]

91. Kaisho, T.; Akira, S. Pleiotropic function of Toll-like receptors. *Microbes Infect.* **2004**, *6*, 1388–1394. [CrossRef]

92. Kopp, E.; Medzhitov, R. Recognition of microbial infection by Toll-like receptors. *Curr. Opin. Immunol.* **2003**, *15*, 396–401. [CrossRef]

93. Marsh, C.B.; Moore, S.A.; Pope, H.A.; Wewers, M.D. IL-1ra suppresses endotoxin-induced IL-1 beta and TNF-alpha release from mononuclear phagocytes. *Am. J. Physiol. Cell. Mol. Physiol.* **1994**, *267*, L39–L45. [CrossRef]

94. Borysiewicz, E.; Fil, D.; Dlaboga, D.; O'Donnell, J.M.; Konat, G.W. Phosphodiesterase 4B2 gene is an effector of Toll-like receptor signaling in astrocytes. *Metab. Brain Dis.* **2009**, *24*, 481. [CrossRef]

95. Gray, M.J.; Poljakovic, M.; Kepka-Lenhart, D.; Morris, S. Induction of arginase I transcription by IL-4 requires a composite DNA response element for STAT6 and C/EBPβ. *Gene* **2005**, *353*, 98–106. [CrossRef] [PubMed]

96. Barnette, M.S. Phosphodiesterase 4 (PDE4) inhibitors in asthma and chronic obstructive pulmonary disease (COPD). In *Progress in Drug Research*; Szmuszkovicz, J., Ed.; Birkhäuser Basel: Basel, Switzerland, 1999; pp. 193–229.

97. Torphy, T.J. Phosphodiesterase Isozymes. *Am. J. Respir. Crit. Care Med.* **1998**, *157*, 351–370. [CrossRef] [PubMed]

98. Essayan, D.M. Cyclic nucleotide phosphodiesterase (PDE) inhibitors and immunomodulation. *Biochem. Pharmacol.* **1999**, *57*, 965–973. [CrossRef]

99. Jin, S.-L.C.; Lan, L.; Zoudilova, M.; Conti, M. Specific Role of Phosphodiesterase 4B in Lipopolysaccharide-Induced Signaling in Mouse Macrophages. *J. Immunol.* **2005**, *175*, 1523. [CrossRef]

100. Ariga, M.; Neitzert, B.; Nakae, S.; Mottin, G.; Bertrand, C.; Pruniaux, M.P.; Jin, S.-L.C.; Conti, M. Nonredundant Function of Phosphodiesterases 4D and 4B in Neutrophil Recruitment to the Site of Inflammation. *J. Immunol.* **2004**, *173*, 7531. [CrossRef]

101. Shepherd, M.C.; Baillie, G.; Stirling, D.I.; Houslay, M. Remodelling of the PDE4 cAMP phosphodiesterase isoform profile upon monocyte-macrophage differentiation of human U937 cells. *Br. J. Pharmacol.* **2004**, *142*, 339–351. [CrossRef]

102. Jin, S.-L.C.; Conti, M. Induction of the cyclic nucleotide phosphodiesterase PDE4B is essential for LPS-activated TNF-α responses. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 7628–7633. [CrossRef]

103. Ghosh, M.; Aguirre, V.; Wai, K.; Felfly, H.; Dietrich, W.D.; Pearse, D.D. The Interplay between Cyclic AMP, MAPK, and NF-kB Pathways in Response to Proinflammatory Signals in Microglia. *BioMed Res. Int.* **2015**, *2015*, 18. [CrossRef]

104. Chan, S.C.; Li, S.-H.; Hanifin, J.M. Increased Interleukin-4 Production by Atopic Mononuclear Leukocytes Correlates with Increased Cyclic Adenosine Monophosphate—Phosphodiesterase Activity and Is Reversible by Phosphodiesterase Inhibition. *J. Investig. Dermatol.* **1993**, *100*, 681–684. [CrossRef]

105. Krause, D.S.; Deutsch, C. Cyclic AMP directly inhibits IL-2 receptor expression in human T cells: Expression of both p55 and p75 subunits is affected. *J. Immunol.* **1991**, *146*, 2285.

106. Maghazachi, A.A. Tumor Necrosis Factor-α Is Chemokinetic for Lymphokine-Activated Killer Cells: Regulation by Cyclic Adenosine Monophosphate. *J. Leukoc. Biol.* **1991**, *49*, 302–308. [CrossRef] [PubMed]

107. Novak, T.J.; Rothenberg, E.V. cAMP inhibits induction of interleukin 2 but not of interleukin 4 in T cells. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 9353–9357. [CrossRef] [PubMed]

108. Prabhakar, U.; Lipshutz, D.; Bartus, J.O.; Slivjak, M.J.; Smith, E.F.; Lee, J.C.; Esser, K.M.; Iii, E.F.S. Characterization of cAMP-dependent inhibition of LPS-induced TNFα production by rolipram, a specific phosphodiesterase IV (PDE IV) inhibitor. *Int. J. Immunopharmacol.* **1994**, *16*, 805–816. [CrossRef]

109. Whitnall, L.; McMillan, T.M.; Murray, G.; Teasdale, G.M. Disability in young people and adults after head injury: 5–7 year follow up of a prospective cohort study. *J. Neurol. Neurosurg. Psychiatry* **2006**, *77*, 640. [CrossRef] [PubMed]

110. Moretti, L.; Cristofori, I.; Weaver, S.; Chau, A.; Portelli, J.N.; Grafman, J. Cognitive decline in older adults with a history of traumatic brain injury. *Lancet Neurol.* **2012**, *11*, 1103–1112. [CrossRef]

111. Cuthbert, J.P.; Pretz, C.R.; Bushnik, T.; Fraser, R.T.; Hart, T.; Kolakowsky-Hayner, S.A.; Malec, J.F.; O'Neil-Pirozzi, T.M.; Sherer, M. Ten-Year Employment Patterns of Working Age Individuals After Moderate to Severe Traumatic Brain Injury: A National Institute on Disability and Rehabilitation Research Traumatic Brain Injury Model Systems Study. *Arch. Phys. Med. Rehabil.* **2015**, *96*, 2128–2136. [CrossRef]

112. Atkins, C.; Cepero, M.L.; Kang, Y.; Liebl, D.J.; Dietrich, W.D. Effects of early rolipram treatment on histopathological outcome after controlled cortical impact injury in mice. *Neurosci. Lett.* **2013**, *532*, 1–6. [CrossRef]

113. Soares, H.; Hicks, R.; Smith, D.; McIntosh, T. Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury. *J. Neurosci.* **1995**, *15*, 8223. [CrossRef]

114. Naganuma, K.; Omura, A.; Maekawara, N.; Saitoh, M.; Ohkawa, N.; Kubota, T.; Nagumo, H.; Kodama, T.; Takemura, M.; Ohtsuka, Y.; et al. Discovery of selective PDE4B inhibitors. *Bioorganic Med. Chem. Lett.* **2009**, *19*, 3174–3176. [CrossRef]

115. Fox, D.; Burgin, A.B.; Gurney, M.E. Structural basis for the design of selective phosphodiesterase 4B inhibitors. *Cell. Signal.* **2014**, *26*, 657–663. [CrossRef] [PubMed]

116. Meller, R.; Minami, M.; Cameron, J.A.; Impey, S.; Chen, D.; Lan, J.-Q.; Henshall, D.C.; Simon, R. CREB-Mediated Bcl-2 Protein Expression after Ischemic Preconditioning. *J. Cereb. Blood Flow Metab.* **2005**, *25*, 234–246. [CrossRef]

117. Zhou, X.; Gao, X.-P.; Fan, J.; Liu, Q.; Anwar, K.N.; Frey, R.S.; Malik, A.B. LPS activation of Toll-like receptor 4 signals CD11b/CD18 expression in neutrophils. *Am. J. Physiol. Cell. Mol. Physiol.* **2005**, *288*, L655–L662. [CrossRef]

118. Lynn, W.A.; Raetz, C.R.; Qureshi, N.; Golenbock, D.T. Lipopolysaccharide-induced stimulation of CD11b/CD18 expression on neutrophils. Evidence of specific receptor-based response and inhibition by lipid A-based antagonists. *J. Immunol.* **1991**, *147*, 3072. [PubMed]

119. Ghosh, M.; Pearse, D.D. Cyclic amp-specific pdes: A promising therapeutic target for cns repair. *Transl. Neurosci.* **2010**, *1*, 101–105. [CrossRef]

120. Lorenowicz, M.J.; Fernandez-Borja, M.; Hordijk, P.L. cAMP Signaling in Leukocyte Transendothelial Migration. *Arterioscler. Thromb. Vasc. Biol.* **2007**, *27*, 1014–1022. [CrossRef]

121. Oliva, A.A., Jr.; Kang, Y.; Furones, C.; Alonso, O.F.; Bruno, O.; Dietrich, W.D.; Atkins, C. Phosphodiesterase isoform-specific expression induced by traumatic brain injury. *J. Neurochem.* **2012**, *123*, 1019–1029. [CrossRef] [PubMed]

122. Baye, J. Roflumilast (daliresp): A novel phosphodiesterase-4 inhibitor for the treatment of severe chronic obstructive pulmonary disease. *J. Formul. Manag.* **2012**, *37*, 149–161.

123. Goto, T.; Shiina, A.; Yoshino, T.; Mizukami, K.; Hirahara, K.; Suzuki, O.; Sogawa, Y.; Takahashi, T.; Mikkaichi, T.; Nakao, N.; et al. Identification of the fused bicyclic 4-amino-2-phenylpyrimidine derivatives as novel and potent PDE4 inhibitors. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3325–3328. [CrossRef]

124. Gobejishvili, L.; Barve, S.; Joshi-Barve, S.; McClain, C.J. Enhanced PDE4B expression augments LPS-inducible TNF expression in ethanol-primed monocytes: Relevance to alcoholic liver disease. American journal of physiology. *Gastrointest. Liver Physiol.* **2008**, *295*, G718–G724. [CrossRef]

125. Gobejishvili, L.; Barve, S.; Joshi-Barve, S.; Uriarte, S.; Song, Z.; McClain, C.J. Chronic ethanol-mediated decrease in cAMP primes macrophages to enhanced LPS-inducible NF-κB activity and TNF expression: Relevance to alcoholic liver disease. *Am. J. Physiol. Liver Physiol.* **2006**, *291*, G681–G688. [CrossRef] [PubMed]

126. Hu, T.-M.; Lee, R.-P.; Lee, C.-J.; Subeq, Y.-M.; Lin, N.-T.; Hsu, B.-G. Heavy Ethanol Intoxication Increases Proinflammatory Cytokines and Aggravates Hemorrhagic Shock-Induced Organ Damage in Rats. *Mediat. Inflamm.* **2013**, *2013*, 1–9. [CrossRef] [PubMed]

127. Qin, L.; He, J.; Hanes, R.N.; Pluzarev, O.; Hong, J.-S.; Crews, F.T. Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. *J. Neuroinflammation* **2008**, *5*, 10. [CrossRef] [PubMed]

128. Fernandez-Lizarbe, S.; Pascual, M.; Guerri, C. Critical Role of TLR4 Response in the Activation of Microglia Induced by Ethanol. *J. Immunol.* **2009**, *183*, 4733. [CrossRef]

129. Avila, D.V.; Myers, S.A.; Zhang, J.; Kharebava, G.; McClain, C.J.; Kim, H.-Y.; Whittemore, S.R.; Gobejishvili, L.; Barve, S. Phosphodiesterase 4b expression plays a major role in alcohol-induced neuro-inflammation. *Neuropharmacology* **2017**, *125*, 376–385. [CrossRef]

130. Blednov, Y.A.; Benavidez, J.M.; Black, M.; Harris, R.A. Inhibition of phosphodiesterase 4 reduces ethanol intake and preference in C57BL/6J mice. *Front. Neurosci.* **2014**, *8*. [CrossRef]

131. He, J.; Crews, F.T. Increased MCP-1 and microglia in various regions of the human alcoholic brain. *Exp. Neurol.* **2008**, *210*, 349–358. [CrossRef]

132. Hu, W.; Lu, T.; Chen, A.; Huang, Y.; Hansen, R.; Chandler, L.J.; Zhang, H.-T. Inhibition of phosphodiesterase-4 decreases ethanol intake in mice. *Psychopharmacology* **2011**, *218*, 331–339. [CrossRef]

133. Raker, V.; Becker, C.; Steinbrink, K. The cAMP Pathway as Therapeutic Target in Autoimmune and Inflammatory Diseases. *Front. Immunol.* **2016**, *7*, 123. [CrossRef]

134. Wu, L.; Ahmadvand, D.; Su, J.; Hall, A.; Tan, X.; Farhangrazi, Z.S.; Moghimi, S.M. Crossing the blood-brain-barrier with nanoligand drug carriers self-assembled from a phage display peptide. *Nat. Commun.* **2019**, *10*, 4635. [CrossRef]

135. Oller-Salvia, B.; Sanchez-Navarro, M.; Giralt, E.; Teixido, M. Blood–brain barrier shuttle peptides: An emerging paradigm for brain delivery. *Chem. Soc. Rev.* **2016**, *45*, 4690–4707. [CrossRef] [PubMed]

136. Jafari, B.; Pourseif, M.M.; Barar, J.; Rafi, M.A.; Omidi, Y. Peptide-mediated drug delivery across the blood-brain barrier for targeting brain tumors. *Expert Opin. Drug Deliv.* **2019**, *16*, 583–605. [CrossRef] [PubMed]