# Phosphodiesterase 11A in brain is enriched in ventral hippocampus and deletion causes psychiatric disease-related phenotypes

Michele P. Kelly[a,1], Sheree F. Logue[a], Julie Brennan[a], Jonathon P. Day[b], Subha Lakkaraju[a], Lixin Jiang[a], Xiaotian Zhong[a], May Tam[a], Stacey J. Sukoff Rizzo[a], Brian J. Platt[a], Jason M. Dwyer[a], Sarah Neal[a], Virginia L. Pulito[a], Michael J. Agostino[a], Steven M. Grauer[a], Rachel L. Navarra[a], Cody Kelley[a], Thomas A. Comery[a], Richard J. Murrills[a], Miles D. Houslay[b], and Nicholas J. Brandon[a]

[a]Pfizer Research, Princeton, NJ 08852; and [b]Neuroscience and Molecular Pharmacology, Faculty of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, United Kingdom

Edited* by Solomon Snyder, Johns Hopkins University School of Medicine, Baltimore, MD, and approved March 30, 2010 (received for review January 20, 2010)

**Phosphodiesterase 11A (PDE11A) is the most recently identified family of phosphodiesterases (PDEs), the only known enzymes to break down cyclic nucleotides. The tissue expression profile of this dual specificity PDE is controversial, and little is understood of its biological function, particularly in the brain. We seek here to determine if PDE11A is expressed in the brain and to understand its function, using PDE11A$^{-/-}$ knockout (KO) mice. We show that PDE11A mRNA and protein are largely restricted to hippocampus CA1, subiculum, and the amygdalohippocampal area, with a two- to threefold enrichment in the ventral vs. dorsal hippocampus, equal distribution between cytosolic and membrane fractions, and increasing levels of protein expression from postnatal day 7 through adulthood. Interestingly, PDE11A KO mice show subtle psychiatric-disease–related deficits, including hyperactivity in an open field, increased sensitivity to the glutamate *N*-methyl-D-aspartate receptor antagonist MK-801, as well as deficits in social behaviors (social odor recognition memory and social avoidance). In addition, PDE11A KO mice show enlarged lateral ventricles and increased activity in CA1 (as per increased Arc mRNA), phenotypes associated with psychiatric disease. The increased sensitivity to MK-801 exhibited by PDE11A KO mice may be explained by the biochemical dysregulation observed around the glutamate α-amino-3-hydroxy-5-methyl-4-isozazolepropionic (AMPA) receptor, including decreased levels of phosphorylated-GluR1 at Ser845 and the prototypical transmembrane AMPA-receptor–associated proteins stargazin (γ2) and γ8. Together, our data provide convincing evidence that PDE11A expression is restricted in the brain but plays a significant role in regulating brain function.**

cAMP | cGMP | schizophrenia | knockout | mouse model

Phosphodiesterases (PDEs) are the only known enzymes to degrade cyclic nucleotides and thus are integral to the regulation of intracellular signaling (1, 2). PDEs have been implicated in a wide range of biological functions including—but not limited to—sperm and cardiac physiology, platelet aggregation, smooth muscle contractions, behavior, and cognition (1). The most recently discovered of the 11 PDE families is PDE11A (3–5), which catalyzes the hydrolysis of adenosine 3′,5′-cyclic monophosphate (cAMP) and guanosine 3′,5′-cyclic monophosphate (cGMP) equally well (6–8). Little is understood of the biological function of PDE11A to date. What is known suggests that PDE11A may play a role in sperm (9) and adrenal physiology (10).

Perhaps contributing to our limited understanding of PDE11A's biological function is the uncertainty surrounding where, exactly, PDE11A is expressed. With respect to the brain, initial studies failed to identify PDE11A mRNA in samples from an entire brain or whole brain regions (3, 4). This failure is not surprising, given that a later study employing immunohistochemistry reported sporadic, low levels of expression in "neurons" and "glia" [brain region not identified (11)]. If PDE11A is expressed only in a subpopulation of cells within a discrete subregion of the brain, one might not expect to detect expression when analyzing samples prepared from an entire brain (region). Evidence implicating other PDE families in brain function and psychiatric disease (12–30), along with a recent report linking PDE11A to major depressive disorder and antidepressant response (31), suggest that it is important to understand the role of PDE11A in the brain. Using in situ hybridization, quantitative real-time PCR, and Western blot, we clarify here where in the brain PDE11A is expressed and, by using PDE11A-deleted mice, we demonstrate a role for PDE11A in brain function.

## Results

**PDE11A Is Expressed in Brain of Wild-Type Mice and Is Absent in PDE11A KO Mice.** We show here that PDE11A mRNA and protein are expressed in the rodent brain, with the most pronounced expression in hippocampus CA1, subiculum, and the amygdalohippocampal area (mouse mRNA, Fig. 1B2; mouse protein, Fig. 1*D* and Fig. S1; rat mRNA, Fig. S2*B*). Expression of PDE11A protein was also observed in two different samples of human (total) hippocampus (Fig. 1*F*). Interestingly, PDE11A mRNA and protein expression within the mouse hippocampus appears to be two- to threefold higher within the ventral vs. dorsal segment (Fig. 1 *C*–*E*). In addition, expression of PDE11A appears to be developmentally regulated as PDE11A protein expression is low in mouse hippocampus on postnatal day 7 but increases with age (Fig. 1*H* and Fig. S1 *C* and *D*). Finally, subcellular fractionation studies suggest that PDE11A may be in a position to regulate both cytosolic and membrane-proximal pools of cyclic nucleotides because PDE11A protein is equally distributed between these two fractions taken from mouse hippocampus (Fig. 1*G*).

To test the role of PDE11A in brain function, PDE11A KO mice were obtained from Deltagen. Deletion of PDE11A mRNA in KOs was confirmed by quantitative PCR, and deletion of protein was confirmed by Western blotting with multiple antibodies (Fig. 1 and Fig. S1). PDE11A appears to account for a small but significant amount of the total PDE activity in ventral hippocampus

NEUROSCIENCE

Author contributions: M.P.K., M.D.H., and N.J.B. designed research; M.P.K., S.F.L., J.B., J.PD., S.L., L.J., X.Z., M.T., S.J.S.R., B.J.P., J.MD., S.N., V.L.P., M.J.A., S.M.G., R.L.N., and C.K. performed research; M.P.K. and R.J.M. contributed new reagents/analytic tools; M.P.K., S.F.L., J.B., J.PD., L.J., X.Z., M.T., S.J.S.R., B.J.P., J.M.D., S.N., V.L.P., M.J.A., S.M.G., R.L.N., C.K., and T.A.C. analyzed data; and M.P.K. wrote the paper.

Conflict of interest statement: The authors are full-time employees or collaborators of Pfizer Research.

*This Direct Submission article had a prearranged editor.

[1]To whom correspondence should be addressed. E-mail: michele.kelly@pfizer.com

This article contains supporting information online at www.pnas.org/cgi/content/full/1000730107/DCSupplemental.

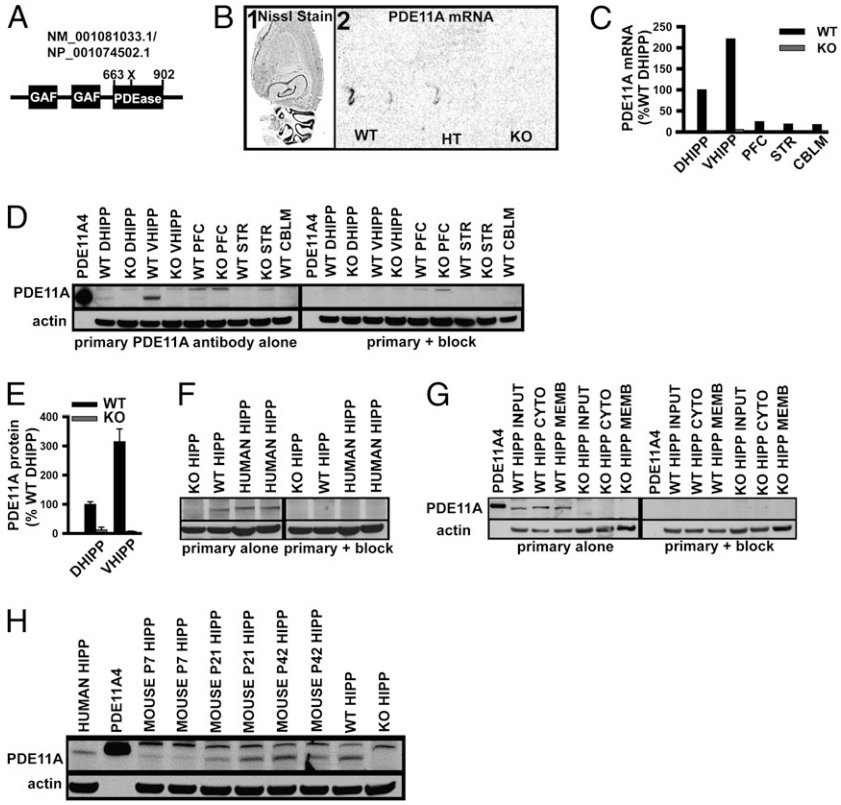


**Fig. 1.** PDE11A is enriched in ventral hippocampus. (*A*) PDE11A knockout (KO) mice from Deltagen were generated using a Lac-Z neo cassette to delete catalytic domain amino acids 693–717 (corresponding to bases 2123–2199 and indicated by "X"). (*B*, 1) Nissl stain of brain section that corresponds to (*B*, 2) autoradiographic in situ hybridizations for PDE11A mRNA. Comparison of the autoradiographs with the Nissl stain suggests that PDE11A mRNA is enriched in ventral hippocampus CA1, subiculum, and the amygdalohippocampal area in wild-type mice (WT) and is absent in knockout mice (KO). Labeling in rat shows an identical pattern (Fig. S2). (*C*) Quantitative PCR analyses of PDE11A mRNA [primers: 2707–2728/2775–2755, normalized to GAPDH mRNA and expressed as percentage of wild-type (%WT) dorsal hippocampus (DHIPP)] and (*D*) Western blotting of PDE11A protein (100 μg total protein; 2 ng purified human PDE11A4-His as positive control, FabGennix 112AP antibody to amino acids 454–468, normalized to actin) confirms that PDE11A mRNA and protein are enriched in WT mouse ventral hippocampus (VHIPP) and absent in KO hippocampus. (*E*) PDE11A protein (normalized to actin and expressed as %WT DHIPP) appears threefold higher in VHIPP vs. DHIPP of WTs. (*F*) PDE11A protein also appears to be expressed in human hippocampus (60 μg total protein; 1 ng purified PDE11A4). (*G*) Fractionation of mouse hippocampus shows that PDE11A expression is equally distributed between cytosolic (CYTO) and membrane (MEMB) fractions in WT mice. (*H*) Finally, Western blotting experiments show that PDE11A protein is developmentally regulated with increasing levels of expression from postnatal day 7 (P7) to adulthood. In all Western blotting experiments, the PDE11A antibody was able to detect purified PDE11A4-His at the appropriate molecular weight (~98 kDa), and the signal disappeared when the primary antibody was preincubated with a blocking peptide or when tested against KO samples, suggesting specificity of the signals observed. In addition, similar results were observed with two additional PDE11A antibodies (Fig. S1). PFC, prefrontal cortex; STR, striatum; CBLM, cerebellum.

because male and female KO mice show an 8% decrease in total cAMP–PDE activity relative to wild-type (WT) littermates [$n$ = 16–19; WT: 100 ± 2.2%; KO: 92.8 ± 1.9%; $F_{(1,34)} = 5.85, P = 0.02$]. Although a trend toward an increase in cGMP was observed in KO males (WT: 0.076 ± 0.006 pmol/mg tissue; KO: 0.093 ± 0.008 pmol/mg tissue; $P = 0.104$), we were unable to detect a significant change in cAMP or cGMP in ventral hippocampus of PDE11A knockout mice. This is not surprising, given the small effect of the KO on total cAMP–PDE activity coupled with the limited resolution of our cyclic nucleotide assays, which in our hands require differences of >50% to detect statistically reliable changes (32).

Deletion of PDE11A does not appear to significantly impact the general health and well-being of the mice. PDE11A KO, heterozygous (HT), and WT littermates are produced and survive in expected Mendelian ratios and do not differ in the SHIRPA assay (SmithKline Beecham Pharmaceuticals-**H**arwell, MCR Mouse Genome Centre and Mammalian Genetics Unit-**I**mperial College School of Medicine at St. Mary's-Royal London Hospital, St. Bartholomew's-**R**oyal London School of Medicine **P**henotype **A**ssessment). Also, PDE11A KO × KO matings are capable of producing and maintaining equivalently sized litters, as are WT × WT matings. Furthermore, consistent with the limited expression pattern of PDE11A in the brain, deletion of PDE11A does not appear to affect vision (as determined by visual cliff), auditory processing (Fig. 2*A*), or motor coordination (Fig. 2*B*).

**PDE11A Knockout Mice Exhibit Subtle Behavioral Abnormalities Consistent with Ventral Hippocampal Dysfunction.** The fact that PDE11A expression is enriched in ventral hippocampus and the fact that its expression is developmentally regulated is particularly striking in the context of psychiatric research. Lesions to the neonatal ventral hippocampus (NVHL) are reported to cause a constellation of disease-related phenotypes in adult rats, including increased locomotion/hyperactivity, increased responsiveness to *N*-methyl-D-aspartate (NMDA) receptor blockade (e.g., with MK-801), and dopaminergic stimulation, as well as social sensorimotor gating (as measured by prepulse inhibition (PPI) of acoustic startle) and hippocampus-dependent memory deficits (33). Given that PDE11A expression is enriched in the ventral hippocampus, we tested adult PDE11A KO mice in paradigms intended to measure behaviors sensitive to ventral hippocampus dysfunction. Consistent with ventral hippocampus dysfunction, PDE11A KO mice are hyperactive in a novel open field, as indicated by a greater distance traveled over the hour-long session (Fig. 2*C*); however, they are able

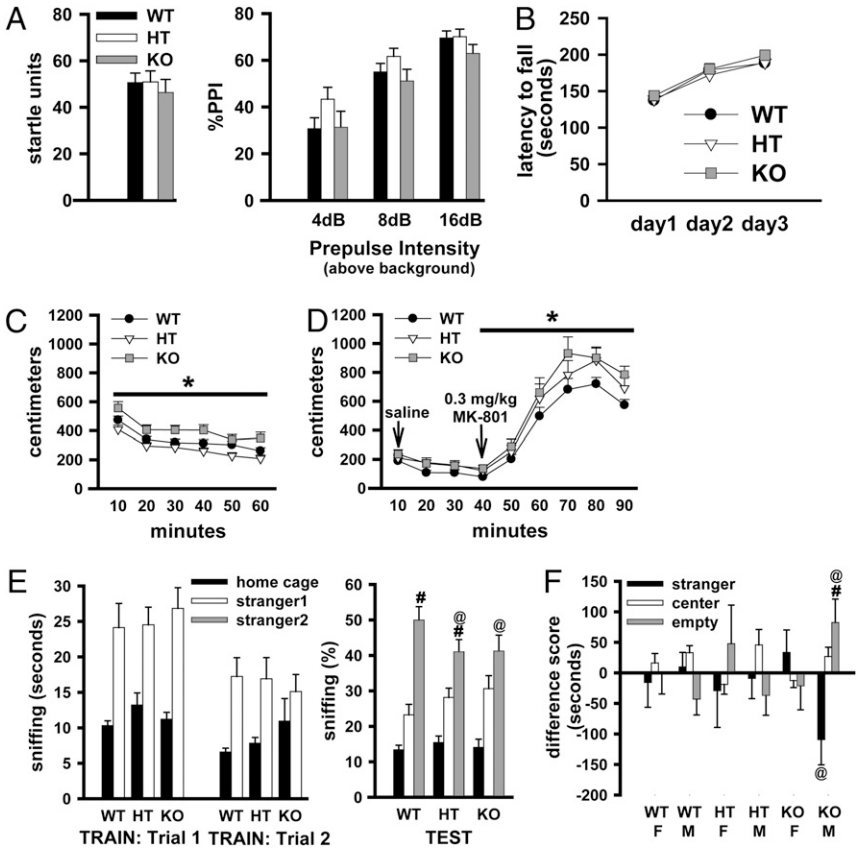


**Fig. 2.** PDE11A knockout mice exhibit subtle behavioral abnormalities consistent with ventral hippocampal dysfunction. (*A*) PDE11A knockout (KO) and heterozygous (HT) mice are normal relative to wild-type (WT) littermates on measures of startle and prepulse inhibition (PPI) of acoustic startle, suggesting intact sensorimotor gating. (*B*) PDE11A KO and HTs also exhibit normal improvement across days of rotarod training (effect of days: $F_{(2,222)} = 130.47$, $P < 0.0001$), suggesting intact motor coordination and procedural learning. (*C*) In contrast, PDE11A KO mice are hyperactive in a novel open field (effect of genotype: $F_{(2,320)} = 4.59$, $P = 0.014$; post hoc KO vs. WT and HT: $P = 0.024$–$0.0005$); however, they do habituate equally over time. (*D*) Following habituation to the open field, PDE11A KO mice do not show hyperactivity after saline injection. PDE11A KO mice do, however, show significantly increased locomotion following injection of 0.3 mg/kg MK-801 ($F_{(2,1653)} = 3.32$, $P = 0.043$; post hoc WT vs. KO: $P = 0.01$; WT vs. HT: $P = 0.064$), suggesting alterations in glutamatergic signaling. PDE11A KO mice also show alterations in socially based behaviors. (*E*) During social odor recognition (SOR) training Trial 1, PDE11A WT, HT, and KO mice spend more time sniffing beads scented with a stranger (stranger1) vs. themselves (home cage; effect of bead: $F_{(1,180)} = 28.12$, $P < 0.0001$) and spend less time sniffing the novel scented beads during Trial 2 (effect of trial: $F_{(1,180)} = 28.03$, $P < 0.0001$). This suggests that the ability to detect, identify, and learn about novel odors remains intact in PDE11A knockout mice. Twenty-four hours later, PDE11A WT mice exhibit a robust memory for the stranger1 bead that they learned about during training, as indicated by a significant difference in the percentage of time spent sniffing stranger1 vs. a bead from novel stranger2 (genotype × bead: $F_{(4,155)} = 2.46$, $P = 0.048$; post hoc within WT, donor 2 vs. donor 1: $P < 0.0001$). PDE11A HT mice also exhibit memory for the stranger1 bead (HT, stranger2 vs. stranger1, $P = 0.015$); however, this memory appears less robust (stranger2 WT vs. stranger2 HT: $P = 0.022$). In contrast, PDE11A KOs fail to show significant recognition memory for stranger1. (*F*) Despite showing a normal approach to a stranger-scented bead during SOR training, PDE11A KO males avoid a chamber containing an actual stranger mouse (genotype × sex × chamber: $F_{(4,102)} = 2.80$, $P = 0.03$; post hoc vs. WT-F and WT-M: $P = 0.049$–$0.013$; vs. stranger: $P = 0.001$). WT, $n = 20$–41; HT, $n = 16$–37; KO, $n = 18$–39. F, females; M, males. Post hoc: WT vs. KO across time—$^{*}P = 0.024$–$0.01$; vs. stranger (1)—$^{\#}P = 0.015$ to $<0.001$; vs. WT within bead/compartment—$^{@}P = 0.022$–$0.013$. Data graphed are mean ± SEM.

to habituate over time (decrease locomotion over time). Given the ability of PDE11A KO to habituate to the open field over time, we also used the open field to test sensitivity to MK-801, as has been reported with the NVHL model (33). Following habituation to the open field, PDE11A KO mice do not show increased reactivity after saline injection, relative to wild-type littermates. PDE11A KO mice do, however, show significantly increased hyperlocomotion following injection of MK-801, relative to wild-type littermates (Fig. 2*D*).

PDE11A KO mice also show subtle alterations in socially based behaviors, including social odor recognition (SOR) (an olfactory recognition test employing wooden beads scented with the odor of a stranger mouse) and social approach/avoidance of a live stranger mouse. During SOR training, PDE11A KO mice distinguish beads scented with a stranger (stranger1) vs. themselves and habituate to the novel scent across trials (Fig. 2*E*), suggesting that their ability to detect, identify, and learn about novel odors remains intact. Twenty-four hours later, PDE11A WT mice exhibit a robust SOR memory for the stranger1 bead, PDE11A HTs exhibit a significant—but reduced—SOR memory, and PDE11A KO mice show no significant SOR memory for the stranger1 bead [memory indicated by a significant difference in the percentage of time spent sniffing a bead from stranger1 vs. a bead from novel stranger2 (Fig. 2*E*)]. It is interesting to note that post-training injection of MK-801, like PDE11A deletion, is capable of disrupting the formation of SOR memory in normal mice. Despite showing normal approach to a stranger-scented bead during SOR training, PDE11A KO males show significant avoidance of an actual stranger male mouse in a social approach/avoidance task (Fig. 2*F*). PDE11A KO mice perform equivalently to age-matched wild-type mice in measures of PPI (Fig. 2*A*), hippocampus-dependent memory (contextual fear conditioning), apomorphine-induced climbing, as well as an anxiety/depression-related behavioral battery (elevated plus maze, stress-induced hyperthermia, four-plate, tail suspension test, forced swim test).

**PDE11A KO Mice Show a Significant Enlargement of the Lateral Ventricles, Deficits in Biochemical Endpoints Related to Glutamatergic Signaling, and Increased Activation of Ventral CA1.** Given that PDE11A KO mice exhibit psychiatric-disease–related behavioral intermediate phenotypes, we determined whether deletion of PDE11A would also lead to disease-relevant neuroanatomical and biochemical findings. Consistent with the subtlety of the behavioral profile, PDE11A KO mice exhibit a small but significant enlargement of the lateral ventricles (~25% increase; Fig. 3). Consistent with the behavioral sensitivity to MK-801 noted above, PDE11A KO mice exhibit alterations around the glutamatergic α-amino-3-hydroxy-5-methyl-4-isozazolepropionic (AMPA) receptor, including a small but significant reduction in the ratio of phosphoGluR1(Ser845):totalGluR1 (an AMPA receptor subunit; Fig. 4*A*). In contrast, PDE11A KOs show normal levels of phosphoGluR2/3(S880/S891):total GluR2/3 (AMPA subunit), phosphoNR2B(Y1336):total NR2B (NMDA receptor subunit), and total NR2A (NMDA subunit). Interestingly, PDE11A KO mice also show a reduction in levels of the transmembrane AMPA receptor regulatory proteins (TARPs) stargazin (γ2) and γ8, but not in the NMDA receptor scaffolding protein PSD-95 (Fig. 4*B* and Fig. S3). Alterations in TARP expression were also observed in prefrontal cortex of PDE11A KO mice (Fig. S4). In addition to hypoglutamatergic signaling, patients with schizophrenia have also been shown to exhibit increased activation of hippocampus CA1 by fMRI (34), possibly due to compensatory losses in GABAergic tone (35), so next we mapped neural activation in PDE11A KO mice using the surrogate molecular marker *Arc* mRNA. PDE11A female and male KO mice, as well as PDE11A HT female mice, show increased Arc mRNA levels in ventral CA1 relative to sex-matched WT littermates (Fig. 4*C*).

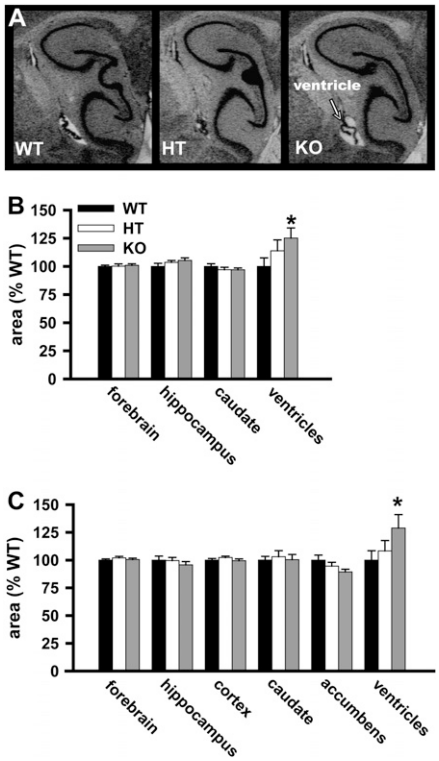


**Fig. 3.** PDE11A knockout mice exhibit significantly enlarged ventricles. (*A*) Thionin-stained sagital sections from brain suggest that PDE11A knockout (KO) mice show limited neuroanatomical alterations relative to wild-type (WT) littermates. PDE11A KO mice exhibit a small, but significant, enlargement of the lateral ventricles in sections taken (*B*) 2.76 mm lateral from Bregma (genotype × region: $F_{(6,126)} = 2.32$, $P = 0.037$) and (*C*) 1.32 mm lateral from Bregma (genotype × region: $F_{(10,205)} = 2.36$, $P = 0.012$). WT, $n = 19$; heterozygous (HT), $n = 15$; KO, $n = 20$. Post hoc vs. WT: $*P < 0.001$. Data graphed are mean ± SEM.

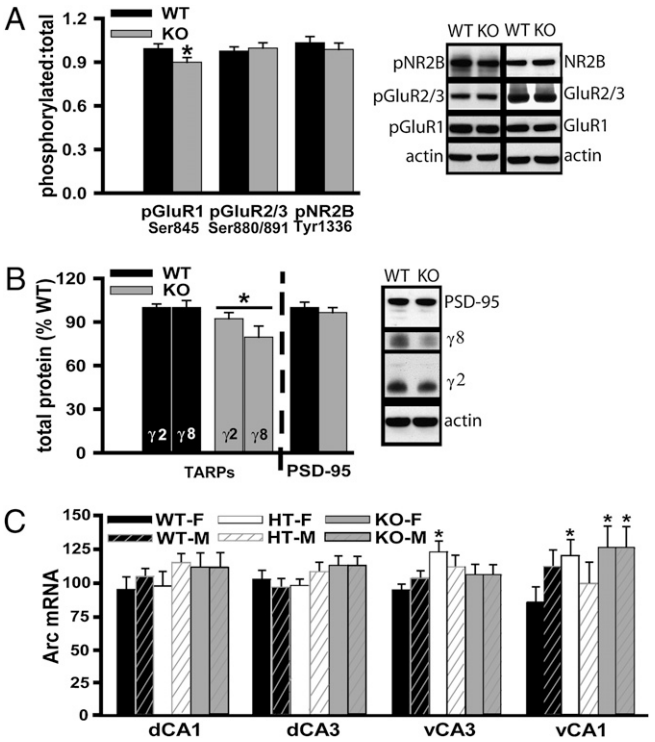


**Fig. 4.** PDE11A KO mice show biochemical alterations consistent with glutamatergic hypofunction. (*A*) Across sexes, PDE11A knockout (KO) mice exhibit a small but significant reduction in the ratio of phosphoGluR1(Ser845):totalGluR1 (an AMPA receptor subunit), relative to wild-type (WT) littermates (effect of genotype: $F_{(1,30)} = 4.32$, $P = 0.046$). In contrast, PDE11A KOs show normal levels of phosphoGluR2/3 (S880/S891):total GluR2/3 (AMPA subunit), phosphoNR2B(Y1336):total NR2B (NMDA receptor subunit), and total NR2A (NMDA subunit). (*B*) Across sexes, PDE11A KO mice also show a reduction in levels of the transmembrane AMPA receptor associated regulatory proteins stargazin (γ2) and γ8 (effect of genotype: $F_{(1,34)} = 5.89$, $P = 0.021$), but not the NMDA receptor scaffolding protein PSD-95. (*C*) Consistent with the disinhibition that would be predicted to occur as a consequence of glutamatergic hypofunction, PDE11A KO mice show increased neural activity in ventral CA1, as mapped by Arc mRNA (genotype × region × sex: $F_{(6,132)} = 2.45$, $P = 0.028$). WT, $n = 19$–20; heterozygous (HT), $n = 16$; KO, $n = 20$. F, female; M, male. Post hoc vs. WT: $*P = 0.046$–$0.01$. Data graphed are mean ± SEM.

## Discussion

We show here that PDE11A is clearly expressed in the brain, with its distribution enriched in ventral hippocampal CA1 and subiculum as well as the amygdalohippocampal area (Fig. 1). We also show that PDE11A protein in the hippocampus contributes ~8% of total cAMP-PDE activity, is equally distributed between cytosolic and membrane fractions, and appears to be developmentally regulated, with increasing levels of expression between P7 and adulthood. Consistent with this restricted pattern of expression, PDE11A KO mice exhibit several subtle psychiatric-disease–related phenotypes indicative of ventral hippocampus dysfunction. PDE11A KO mice also show several subtle anatomical and biochemical phenotypes related to psychiatric disease, including significantly enlarged ventricles, biochemical alterations indicative of glutamatergic hypofunction, and increased activation of CA1. Together, these studies show that PDE11A plays a role in normal brain function and suggest that alterations in PDE11A function may be relevant to psychiatric diseases.

**PDE11A KO Mice Show a Subset of Phenotypes Associated with Dysfunction of the Ventral Hippocampus.** We observe here that deletion of PDE11A results in some, but not all, phenotypes that occur in adults following lesions of the neonatal ventral hippocampus, a manipulation intended to model in rodents aspects of schizophrenia (33). PDE11A KO mice exhibit hyperactivity in an open field, increased sensitivity to NMDA receptor blockade (using MK-801), and impaired social behaviors (Fig. 2). In contrast, PDE11A KO mice show normal sensorimotor gating (as measured by PPI), normal responses to the dopaminergic (indirect) agonists apomorphine and amphetamine, and intact contextual fear conditioning, a hippocampus-dependent memory. Although the latter suggests that PDE11A does not play a role in hippocampus-dependent associative memory, it remains to be determined if this holds true for other forms of hippocampus-dependent cognition, such as spatial or place memory. PDE11A KO mice also tested normal on a battery of anxiety- and depressive-related behaviors. This was surprising, given that there is a reported genetic linkage between PDE11A and major depressive disorder (31). It should be noted, however, that the colony was single-housed following shipment (due to aggression observed across all genotypes), and this manipulation may have shifted behavior to a floor/ceiling in the various anxiety/depressive-related behavioral assays.

There are several potential explanations, none of which are mutually exclusive, of why PDE11A KO mice do not show all phenotypes associated with a complete lesion to the ventral hippocampus. First, PDE11A is not expressed throughout the entire ventral hippocampus, but rather is restricted to CA1 and subiculum. Thus, it is reasonable to expect that deletion of PDE11A would not affect the entirety of ventral hippocampal functioning. Similarly, it is reasonable to hypothesize that deletion of a single molecule within a complex signaling cascade would not achieve the same effect as a complete anatomical lesion. Indeed, we believe that our data lend further support to the hypothesis that different behaviors have separable molecular cascades within a given brain region (i.e., not every molecule in a given brain region plays a role in every behavior dependent on that brain region). Equally likely is the possibility that phenotypes observed in PDE11A KO mice are related to the acute loss of PDE11A signaling (i.e., during adulthood) as opposed to an effect on development—despite the fact that PDE11A is expressed as early as postnatal day 7 in mouse. Indeed, direct infusion of MK-801 and/or lesions in the adult ventral hippocampus produce hyperactivity and impair socially related memory while leaving PPI and some other forms of memory relatively intact (36–38). Resolution of these hypotheses awaits improved tools, such as regulated transgenic systems or PDE11A-specific pharmacological agents.

PDE11A is not the first PDE family implicated in brain function and psychiatric disease. The PDE4 family has received, perhaps, the most attention in the context of behavior, cognition, and psychiatric illness. PDE4 has been implicated as a player in the pathophysiology of schizophrenia and mood disorders through linkage studies as well as through its interaction with the schizophrenia candidate gene disrupted in schizophrenia 1 (*DISC1*) (12–13). Genetic deletion of PDE4 isoforms in mice affects cognitive, depressive-related, and anxiety-related behaviors (14–16). Furthermore, PDE4 inhibitors improve sensorimotor gating, strengthen memory, and reduce depressive-related behaviors in rodents (e.g., 17–23). The PDE10A family has also received much attention in recent years, particularly in the context of schizophrenia because genetic deletion or inhibition of PDE10A results in antipsychotic-like effects in rodents (24–26). Deletion or inhibition of PDE1, -2, and -5 have also been shown to affect behavior and cognition (27–29), and up-regulation of PDE1 has been shown in a mouse model of schizophrenia (30). It will be of interest to future studies to determine if the deleterious behavioral profile observed in PDE11A KO mice may be due, in part, to an up-regulation of other PDE families.

**PDE11A KO Mice Show Anatomical and Biochemical Dysfunction Relevant to Psychiatric Disease.** Consistent with the psychiatric-disease–related behavioral phenotypes noted above, PDE11A KO mice exhibit significantly enlarged lateral ventricles and biochemical alterations indicative of glutamatergic hypofunction in ventral hippocampus. An enlargement of the lateral ventricles is the most reproducible anatomical finding in brains of schizophrenia patients (39) and is likely due to a combination of developmental deficits and ongoing degeneration (40). Glutamatergic hypofunction is often hypothesized to be a significant contributor to the pathophysiology of schizophrenia. We show here that PDE11A KO mice show lower levels of phospho-GluR1 (Ser845) and of the transmembrane AMPA receptor regulatory proteins stargazin ($\gamma 2$) and $\gamma 8$ in ventral hippocampus, all of which would lead to decreased AMPA receptor function (41). Sex-dependent effects on TARP expression were also noted in prefrontal cortex (Fig. S4). It is interesting to note that ketamine, an NMDA receptor anatagonist that produces many of the same effects as MK-801, has also been shown to decrease phospho-GluR1(Ser-845) (42). Also, GluR1 KO mice exhibit schizophrenia-related behaviors (43), and drugs that increase AMPA receptor signaling have been pursued as treatments for schizophrenia (44). Glutamatergic hypofunction within the hippocampus is thought to lead to disinhibition of GABAergic inhibitory interneurons and, in so doing, produce a net increase in excitation (35). This may explain why increased activation of hippocampus CA1 has been observed by functional MRI in patients with schizophrenia (34). PDE11A KO mice appear to exhibit such an increase in excitation in ventral CA1, as measured by increased levels of *Arc* mRNA, a molecular marker for neuronal activity. As with the behavioral phenotype noted above, it remains to be determined if the anatomical and biochemical phenotypes observed in PDE11A KO mice are due to the acute loss of PDE11A signaling during adulthood or are due to developmental alterations. In conclusion, data presented here suggest that what little PDE11A is expressed in the brain is of functional importance. Furthermore, the subtle phenotypes observed in PDE11A KO mice suggest that PDE11A may be one of several genes playing a role in the multifactorial origin of psychiatric diseases such as schizophrenia.

## Materials and Methods

**Subjects.** Breeding pairs of PDE11A-deleted mice (mixed C57BL6/129SvEv background) were obtained from Deltagen and bred at the Jackson Laboratory, where offspring were group housed. Approximately equal numbers of male and female offspring were then shipped to Wyeth at 4 months of age, and testing was conducted between 4 and 8 months of age. Due to aggression that ensued following shipment of the first cohort (across genotypes), all subjects were single-housed following arrival onsite. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Pub. 85–23, revised 1996) and were fully approved by the Institutional Animal Care and Use Committee of Wyeth Research. See *SI Materials and Methods* for additional details.

**Biochemistry and Behavior.** Histology, in situ hybridization, quantitative PCR, Western blotting, cyclic nucleotides, and PDE activity were measured as previously described (21, 22, 30, 32) as was behavioral output (21–23, 30, 45–49). See *SI Materials and Methods* for additional details.

**Data Analyses.** All data were analyzed for the effect of genotype and sex by ANOVA or repeated measure ANOVA where appropriate. Where the effect of genotype did not differ between sexes, data were graphed collapsed across sexes for the sake of graphic clarity. Significance was determined as $P < 0.05$. See *SI Materials and Methods* for additional details.

**ACKNOWLEDGMENTS.** The authors thank Shannon Elliot for assistance in managing the off-site breeding colony and Youping Huang for help with statistical analyses. This work was supported by grants to M.D.H. from the Medical Research Council (U.K.) (Grant G0600765) and Fondation Leducq (Grant 06CVD02).

1. Bender AT, Beavo JA (2006) Cyclic nucleotide phosphodiesterases: Molecular regulation to clinical use. *Pharmacol Rev* 58:488–520.
2. Conti M, Beavo J (2007) Biochemistry and physiology of cyclic nucleotide phosphodiesterases: Essential components in cyclic nucleotide signaling. *Annu Rev Biochem* 76:481–511.
3. Fawcett L, et al. (2000) Molecular cloning and characterization of a distinct human phosphodiesterase gene family: PDE11A. *Proc Natl Acad Sci USA* 97:3702–3707.
4. Yuasa K, et al. (2000) Isolation and characterization of two novel phosphodiesterase PDE11A variants showing unique structure and tissue-specific expression. *J Biol Chem* 275:31469–31479.
5. Hetman JM, et al. (2000) Cloning and characterization of two splice variants of human phosphodiesterase 11A. *Proc Natl Acad Sci USA* 97:12891–12895.
6. Yuasa K, Kanoh Y, Okumura K, Omori K (2001) Genomic organization of the human phosphodiesterase PDE11A gene. Evolutionary relatedness with other PDEs containing GAF domains. *Eur J Biochem* 268:168–178.
7. Yuasa K, Ohgaru T, Asahina M, Omori K (2001) Identification of rat cyclic nucleotide phosphodiesterase 11A (PDE11A): Comparison of rat and human PDE11A splicing variants. *Eur J Biochem* 268:4440–4448.
8. Weeks JL, II, Zoraghi R, Francis SH, Corbin JD (2007) N-Terminal domain of phosphodiesterase-11A4 (PDE11A4) decreases affinity of the catalytic site for substrates and tadalafil, and is involved in oligomerization. *Biochemistry* 46:10353–10364.
9. Wayman C, et al. (2005) Phosphodiesterase 11 (PDE11) regulation of spermatozoa physiology. *Int J Impot Res* 17:216–223.
10. Horvath A, et al. (2006) Adrenal hyperplasia and adenomas are associated with inhibition of phosphodiesterase 11A in carriers of PDE11A sequence variants that are frequent in the population. *Cancer Res* 66:11571–11575.
11. D'Andrea MR, et al. (2005) Expression of PDE11A in normal and malignant human tissues. *J Histochem Cytochem* 53:895–903.
12. Millar JK, et al. (2005) DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science* 310:1187–1191.
13. Murdoch H, et al. (2007) Isoform-selective susceptibility of DISC1/phosphodiesterase-4 complexes to dissociation by elevated intracellular cAMP levels. *J Neurosci* 27: 9513–9524.
14. Zhang HT, et al. (2002) Antidepressant-like profile and reduced sensitivity to rolipram in mice deficient in the PDE4D phosphodiesterase enzyme. *Neuropsychopharmacology* 27:587–595.
15. Zhang HT, et al. (2008) Anxiogenic-like behavioral phenotype of mice deficient in phosphodiesterase 4B (PDE4B). *Neuropsychopharmacology* 33:1611–1623.
16. Siuciak JA, McCarthy SA, Chapin DS, Martin AN (2008) Behavioral and neurochemical characterization of mice deficient in the phosphodiesterase-4B (PDE4B) enzyme. *Psychopharmacology (Berl)* 197:115–126.
17. Barad M, Bourtchouladze R, Winder DG, Golan H, Kandel E (1998) Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. *Proc Natl Acad Sci USA* 95:15020–15025.
18. O'Donnell JM, Frith S (1999) Behavioral effects of family-selective inhibitors of cyclic nucleotide phosphodiesterases. *Pharmacol Biochem Behav* 63:185–192.
19. Houslay MD, Schafer P, Zhang KYJ (2005) Keynote review: Phosphodiesterase-4 as a therapeutic target. *Drug Discov Today* 10:1503–1519.
20. Zhang HT, et al. (2005) Effects of the novel PDE4 inhibitors MEM1018 and MEM1091 on memory in the radial-arm maze and inhibitory avoidance tests in rats. *Psychopharmacology (Berl)* 179:613–619.
21. Kelly MP, et al. (2007) Constitutive activation of Galphas within forebrain neurons causes deficits in sensorimotor gating because of PKA-dependent decreases in cAMP. *Neuropsychopharmacology* 32:577–588.
22. Kelly MP, et al. (2009) Developmental etiology for neuroanatomical and cognitive deficits in mice overexpressing Galphas, a G-protein subunit genetically linked to schizophrenia. *Mol Psychiatry* 14:398–415, 347.
23. Kanes SJ, et al. (2007) Rolipram: A specific phosphodiesterase 4 inhibitor with potential antipsychotic activity. *Neuroscience* 144:239–246.
24. Siuciak JA, et al. (2006) Inhibition of the striatum-enriched phosphodiesterase PDE10A: A novel approach to the treatment of psychosis. *Neuropharmacology* 51: 386–396.
25. Siuciak JA, et al. (2006) Genetic deletion of the striatum-enriched phosphodiesterase PDE10A: Evidence for altered striatal function. *Neuropharmacology* 51:374–385.
26. Schmidt CJ, et al. (2008) Preclinical characterization of selective phosphodiesterase 10A inhibitors: A new therapeutic approach to the treatment of schizophrenia. *J Pharmacol Exp Ther* 325:681–690.
27. Rutten K, et al. (2007) Time-dependent involvement of cAMP and cGMP in consolidation of object memory: Studies using selective phosphodiesterase type 2, 4 and 5 inhibitors. *Eur J Pharmacol* 558:107–112.
28. Siuciak JA, et al. (2007) Behavioral and neurochemical characterization of mice deficient in the phosphodiesterase-1B (PDE1B) enzyme. *Neuropharmacology* 53: 113–124.
29. Masood A, Nadeem A, Mustafa SJ, O'Donnell JM (2008) Reversal of oxidative stress-induced anxiety by inhibition of phosphodiesterase-2 in mice. *J Pharmacol Exp Ther* 326:369–379.
30. Kelly MP, et al. (2008) Constitutive activation of the G-protein subunit Galphas within forebrain neurons causes PKA-dependent alterations in fear conditioning and cortical Arc mRNA expression. *Learn Mem* 15:75–83.
31. Wong ML, et al. (2006) Phosphodiesterase genes are associated with susceptibility to major depression and antidepressant treatment response. *Proc Natl Acad Sci USA* 103: 15124–15129.
32. Grauer SM, et al. (2009) Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia. *J Pharmacol Exp Ther* 331:574–590.
33. Tseng KY, Chambers RA, Lipska BK (2009) The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. *Behav Brain Res* 204: 295–305.
34. Schobel SA, et al. (2009) Differential targeting of the CA1 subfield of the hippocampal formation by schizophrenia and related psychotic disorders. *Arch Gen Psychiatry* 66:938–946.
35. Lisman JE, et al. (2008) Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. *Trends Neurosci* 31:234–242.
36. Alvarez P, Wendelken L, Eichenbaum H (2002) Hippocampal formation lesions impair performance in an odor-odor association task independently of spatial context. *Neurobiol Learn Mem* 78:470–476.
37. Bast T, Feldon J (2003) Hippocampal modulation of sensorimotor processes. *Prog Neurobiol* 70:319–345.
38. Levin ED, Sledge D, Baruah A, Addy NA (2003) Ventral hippocampal NMDA blockade and nicotinic effects on memory function. *Brain Res Bull* 61:489–495.
39. Shenton ME, Dickey CC, Frumin M, McCarley RW (2001) A review of MRI findings in schizophrenia. *Schizophr Res* 49:1–52.
40. Berger GE, Wood S, McGorry PD (2003) Incipient neurovulnerability and neuro-protection in early psychosis. *Psychopharmacol Bull* 37:79–101.
41. Morimoto-Tomita M, et al. (2009) Autoinactivation of neuronal AMPA receptors via glutamate-regulated TARP interaction. *Neuron* 61:101–112.
42. Maeng S, et al. (2008) Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiatry* 63:349–352.
43. Wiedholz LM, et al. (2008) Mice lacking the AMPA GluR1 receptor exhibit striatal hyperdopaminergia and 'schizophrenia-related' behaviors. *Mol Psychiatry* 13: 631–640.
44. Arai AC, Kessler M (2007) Pharmacology of ampakine modulators: From AMPA receptors to synapses and behavior. *Curr Drug Targets* 8:583–602.
45. Kelly MP, et al. (2009) The supra-additive hyperactivity caused by an amphetamine-chlordiazepoxide mixture exhibits an inverted-U dose response: Negative implications for the use of a model in screening for mood stabilizers. *Pharmacol Biochem Behav* 92:649–654.
46. Sankoorikal GM, Kaercher KA, Boon CJ, Lee JK, Brodkin ES (2006) A mouse model system for genetic analysis of sociability: C57BL/6J versus BALB/cJ inbred mouse strains. *Biol Psychiatry* 59:415–423.
47. Grauer SM, et al. (2009) WAY-163909, a 5-HT2C agonist, enhances the preclinical potency of current antipsychotics. *Psychopharmacology (Berl)* 204:37–48.
48. Hughes ZA, et al. (2008) WAY-200070, a selective agonist of estrogen receptor beta as a potential novel anxiolytic/antidepressant agent. *Neuropharmacology* 54:1136–1142.
49. Favilla C, Abel T, Kelly MP (2008) Chronic Galphas signaling in the striatum increases anxiety-related behaviors independent of developmental effects. *J Neurosci* 28: 13952–13956.