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A genetic basis for friendship? Homophily for membrane-associated PDE11A-cAMP-CREB signaling in CA1 of hippocampus dictates mutual social preference in male and female mice

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Abstract

Although the physical and mental benefits of friendships are clear, the neurobiological mechanisms driving mutual social preferences are not well understood. Studies in humans suggest friends are more genetically similar, particularly for targets within the 3',5'-cyclic adenosine monophosphate (cAMP) cascade. Unfortunately, human studies can not provide conclusive evidence for such a biological driver of friendship given that other genetically-related factors tend to co-segregate with friendship (e.g., geographical proximity). As such, here we use mice under controlled conditions to test the hypothesis that homophily in the cAMP-degrading enzyme phosphodiesterase 11A4 (PDE11A4) can dictate mutual social preference. Using C57BL/6J and BALB/cJ mice in 2 different behavioral assays, we showed that mice with 2 intact alleles of *Pde11a* prefer to interact with *Pde11* wild-type (WT) mice of the same genetic background over knockout (KO) mice or novel objects; whereas, *Pde11* KO mice prefer to interact with *Pde11* KO mice over WT mice or novel objects. This mutual social preference was seen in both adult and adolescent mice, and social preference could be eliminated or artificially elicited by strengthening or weakening PDE11A homodimerization, respectively. Stereotactic delivery of an isolated PDE11A GAF-B domain to the mouse hippocampus revealed the membrane-associated pool of PDE11A-cAMP-CREB signaling specifically within the CA1 subfield of hippocampus is most critical for regulating social preference. Our study here not only identifies PDE11A homophily as a key driver of mutual social preference across the lifespan, it offers a paradigm in which other mechanisms can be identified in a controlled fashion.

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Keywords

mouse; cAMP; cGMP; phosphodiesterase; cyclic nucleotide; social behavior; social approach; social interaction; hippocampus; friendship

Friendships—relationships characterized by compatibility and mutual reciprocity[1]—provide both physical and mental benefits to those involved. For example, a stronger social network decreases all-cause mortality risk[2–4] as well as risk of mortality from cardiovascular disease, diabetes, Alzheimer's disease, and chronic lower respiratory diseases[3]. Further, greater social engagement protects against age-related cognitive decline[5–7] and depression[8–13]. While the physical and mental benefits of friendship are clear, the neurobiological mechanisms driving mutual social preferences are not well understood.

Some studies suggest there may be molecular mechanisms driving mutual social preference. Friends are more genetically similar than non-friends, with friends demonstrating genetic "relatedness" at the level of 4th cousins[14]. Several genes have been associated with friendship in humans, including homophily for the dopamine D2 receptor (D2Rs) and odorant receptors, both of which transduce signals via the intracellular 3',5'-cyclic adenosine monophosphate (cAMP) cascade[15, 16]. These genetic findings complement those from mechanistic studies in rodents showing cAMP signaling in brain regions like the striatum and hippocampus regulates not only social interactions but also how social isolation impacts the brain[17–23]. Although the studies described above point to genetic similarities in the cAMP cascade as a potential molecular mechanism that promotes friendship in humans, there are alternative explanations for why friends are more genetically related than non-friends. Factors such as social inequality or racial stratification may influence genetic homophily due to societal constructs limiting the choices available for friendship selection[16]. In addition, friendships typically develop among individuals who are geographically near each other[24], and geographical proximity is associated with genetic similarity[25, 26]. Thus, to definitively establish a molecular mechanism underlying mutual social preference, more controlled studies are required.

Fortunately, long-term, non-mating relationships are seen across a wide variety of species[27], suggesting the mechanisms underlying mutual social preference are evolutionarily conserved. Here, we use mouse models to test the hypothesis that genetic similarities within the cAMP-hydrolyzing enzyme phosphodiesterase 11A (PDE11A) may be a key neurobiological mechanism underlying mutual social preference. Several PDE families are known to influence social interactions in rodents[28–30], but PDE11A is particularly interesting in the context of mutual social preference. First, PDE11A expression in the brain is enriched in neurons of the ventral hippocampal formation, a brain region critical to social behaviors[31], and deletion of *Pde11a* alters gene expression in the oxytocin signaling cascade (i.e., a key regulator of social bonding[32]). Second, *Pde11a* has been genetically associated with major depressive disorder in humans[33–35] and appears to be a key molecular mechanism by which social isolation shapes subsequent social behavior in mice[20]. Third—and of greatest interest to the present topic—when given the choice of interacting with either a novel *Pde11a* wild-type (WT) mouse or its *Pde11a* knockout (KO) littermate, mice with

two WT alleles preferred to interact with the *Pde11a* WT mice while the *Pde11a* KO mice preferred to interact with the KO mice[20]. This led us to suggest that PDE11A regulates mutual social preference; however, this report was limited to a single mouse model and a single behavioral paradigm. Here, we use multiple mouse models and behavioral paradigms to explicitly test the hypothesis that homophily in PDE11A function can dictate mutual social preference.

RESULTS

To test the hypothesis that PDE11A genotype dictates mutual social preference, here we compare and contrast behavior of *Pde11a* wild-type (WT), heterozygous (HT) and knockout (KO) mice on either a C57BL/6J background (as originally reported[20, 29]) or a BALB/cJ background. The choice of the BALB/cJ background as a comparator strain was driven by the fact that the BALB/cJ and C57BL/6J strains differ in their sequence for PDE11A4. Whereas the C57BL/6J strain encodes an alanine at amino acid 499, the BALB/cJ encodes a threonine, leading to increased expression of PDE11A4 in the BALB/cJ hippocampus as well as increased homodimerization and accumulation of PDE11A4 *in vitro*[36].

Validation of BALB/cJ knockout (KO) mice.

Consistent with our previous *in vitro* findings[36], here we show that PDE11A4 protein is compartmentalized very differently in the hippocampus of C57BL/6J mice vs. BALB/cJ mice (Figure 1A). Whereas, PDE11A4 expression is found throughout neuronal cell bodies in the ventral subiculum of young adult C57BL/6J mice as previously described[31], it is also found to accumulate in filamentous linear structures in ventral subiculum of young adult male and female BALB/cJ mice (Figure 1A). This suggests PDE11A may function somewhat differently between the 2 strains given that the location of a PDE is just as important to its overall function as is its catalytic activity[37]. After backcrossing the *Pde11a* KO from the largely C57BL/6J background to a BALB/cJ background, we confirmed a lack of PDE11A4 protein expression in both the ventral hippocampus (Figure 1B; see Figure S1 for images of full-length blots) and dorsal hippocampus (Figure 1C) of the BALB/cJ KO. We also confirmed reduced expression in the *Pde11a* heterozygous BALB/cJ mice (HT; Figure 1B–C). Importantly, deletion of PDE11A does not trigger a compensatory upregulation of PDE2A (WT, 0.92 ±0.14 A.U.; HT, 0.95 ±0.16 A.U.; KO, 0.92 ±0.14 A.U.; $F(2,21)=0.007$, $P=0.99$), the closest-related PDE with substantial expression in the hippocampus[38, 39].

Social approach behavior of Pde11a WT and KO mice on a BALB/cJ background varies as a function of the stimulus mouse Pde11a genotype.

Previously, we showed that male, but not female, *Pde11a* KO mice on a C57BL/6J background exhibited significantly reduced social approach relative to *Pde11a* WT mice when given a choice between a novel object vs a novel *Pde11a* WT mouse[20, 29]. Here, we show that while male and female *Pde11a* WT and HT mice on the BALB/cJ background exhibited significant social approach behavior by spending more time with the novel *Pde11a* WT mouse vs. a novel object, male and female *Pde11a* KO mice did not (Figure 2B).
To determine if deletion of PDE11A reduces all social approach behavior, or only social

approach toward *Pde11a* WTs, we repeated the experiment with a new group of mice and replaced the novel *Pde11a* WT mouse with a novel KO mouse. We found that both male and female *Pde11a* HT and KO mice exhibited significant social approach behavior by spending more time interacting with the novel *Pde11a* KO mouse vs the novel object; however, the *Pde11a* WT mice did not (Figure 2C). Together, these data suggest 1) the impact of PDE11A deletion on this behavior is stronger on the BALB/cJ background in that it affects both males and females compared to only males on the C57BL/6J background and 2) that PDE11A deletion does not reduce all social approach but rather shifts social preferences.

Pde11a genotype of stimulus and subject mice must match for C57BL/6J and BALB/cJ mice to exhibit social preference for one mouse over another.

Previously, we showed that while adult male and female C57BL/6J mice prefer to interact with adult *Pde11a* WT vs KO mice, adult *Pde11a* KO mice preferred to interact with adult *Pde11a* KO vs WT mice (all mice on a C57BL/6J background[20]). Here we replicate and extend these findings. Although adult male and female C57BL/6J mice preferred to interact with adult *Pde11a* WT vs KO mice on a C57BL/6J background during the last 5 minutes of the session (Figure 3B), C57BL/6J mice showed no such preference for *Pde11a* WT over KO mice on the BALB/cJ background (Figure 3D), consistent with the fact that the protein sequence for PDE11A differs between C57BL/6J and BALB/cJ mice (see above). Similarly, adult male and female BALB/cJ mice preferred to interact with adult *Pde11a* WT vs. KO mice on the BALB/cJ background (Figure 3E) but not *Pde11a* WT vs. KO mice on the C57BL/6J background (Figure 3G). On both the C57BL/6J background (Figure 3D) and the BALB/cJ background (Figure 3F), adult *Pde11a* KO mice preferred to interact with adult *Pde11a* KO vs WT mice on the same genetic background. Together, these data suggest that in adult mice, *Pde11a* genotype dictates mutual social preference.

To determine if this effect occurred in younger mice, we tested social preference of adolescent subject mice on the BALB/cJ background. We found that male and female adolescent *Pde11a* WT mice preferred to investigate adult same-sex *Pde11a* WT vs KO mice (Figure 3H) and male and female adolescent *Pde11a* KO mice preferred to investigate adult same-sex *Pde11a* KO vs WT mice (all on the BALB/cJ background; Figure 3J). Importantly, adolescent *Pde11a* HT mice showed no such preference between *Pde11a* WT and KO mice (Figure 3I), again consistent with the fact that they did not share an identical genotype with either stimulus mouse.

Pde11a genotype-dependent social preference cannot be explained by overt differences in locomotor activity of stimulus mice nor preference for the social odor of one genotype over another.

To determine if *Pde11a* genotype-dependent social preferences could be due to overt differences in the locomotor activity of the stimulus mice, videos from experiments presented in Figure 3B, 3D, 3E and 3F were hand scored with regard to the number of times the stimulus mice poked their noses through the perforated holes. This endpoint was chosen since the cylinders have a solid top to keep the mice inside the cylinder, which precludes our ability to track their movement in the videos. There was no difference in the extent to which *Pde11a* WT versus KO mice poked their nose out of the cylinders

in response to adult C57BL/6J subject mice (Figure 4A), adult *Pde11a* KO subject mice on the C57BL/6J background (Figure 4B), adult BALB/cJ subject mice (Figure 4C), or adult *Pde11a* KO subject mice on the BALB/cJ background (Figure 4D). To determine if the *Pde11a* genotype-dependent social preferences could be driven by a preference for one social odor over another, BALB/cJ mice and *Pde11a* KO mice on the BALB/cJ background were given the opportunity to investigate wooden beads saturated with the scent of either novel WT mice or novel KO mice on the BALB/cJ background. Both the BALB/cJ mice and the *Pde11a* KO mice on the BALB/cJ background can distinguish the scent of a *Pde11a* WT stimulus mouse from that of a KO stimulus mouse in that they dishabituate from one scent to the other (Figure 4E–F). Subject mice do not, however, appear to prefer the scent of one genotype over the other as they spend approximately the same amount of time investigating each scent and equally habituate to each scent over 2 training trials. *Pde11a* KO mice on the C57BL/6J background (n=4/sex) also demonstrate this pattern of behavior (data not shown; 2-way RM ANOVA effect of genotype: $F(1,7)=0.001$, $P=0.976$; effect of trial: $F(1,7)=26.48$, $P=0.001$).

PDE11A appears to regulate mutual social preference via membrane-associated pools of cAMP in CA1 of hippocampus.

PDE11A4 is expressed in neurons of the CA1 and subiculum subfields of the hippocampus along with the amygdalohippocampal area[29, 31]. Previously, we determined that PDE11A4 specifically within CA1 is the critical pool that regulates social memory formation[40]. Therefore, we tested the hypothesis that PDE11A4 specifically within CA1 is also key to regulating mutual social preferences. To test this hypothesis, we stereotatically infused into stimulus mice a virus that expressed either mCherry alone (negative control; note 2 mice did not actually express the mCherry construct but were included as “mock” controls; Figure 5A–B) or an mCherry-tagged version of the PDE11A4 GAF-B domain (viral expression was verified in all GAF-B stimuli mice). We chose to disrupt PDE11A4 function using this isolated GAF-B construct for 2 reasons. First, the GAF-B domain is where the BALB/cJ point mutation resides and is the domain required for PDE11A4 homodimerization[36]. Second, by binding to PDE11A4 monomers and disrupting PDE11A4 homodimerization, the isolated GAF-B construct preferentially degrades membrane-associated PDE11A4[36], which is the pool of PDE11A that is reduced by social isolation[20]. Indeed, we were able to verify that the GAF-B construct disrupts PDE11A4 expression in a compartment-specific manner within CA1 (Figure 5D–E). Across the first and last 5-minute epoch, sex-matched unsurgerized C57BL/6J subject mice spent significantly more time investigating *Pde11a* WT stimulus mice on the C57BL/6J background with intact PDE11A4 homodimerization (i.e., mCherry treated) vs. those with disrupted PDE11A4 homodimerization (i.e., GAF-B treated; Figure 5F).

In vitro evidence suggests PDE11A4 may hydrolyze cAMP with a higher K_m and V_{max} than cGMP[41, 42]; therefore, we next determined if deletion of PDE11A would reduce total cAMP-PDE activity in the ventral hippocampus more so than cGMP-PDE activity. Consistent with our initial report in mice on the C57BL/6J background[29], we found that deletion of PDE11A reduced cAMP-PDE activity in the ventral hippocampus of BALB/cJ mice but not cGMP-PDE activity (Figure 5G). To confirm this reduction in cAMP-PDE

activity would be sufficient to increase output of the cAMP pathway in the ventral hippocampus, we next measured total cAMP response element binding protein (CREB) in the nucleus of ventral hippocampus. Elevations in cyclic nucleotides activate kinases that phosphorylate CREB, which causes CREB to translocate to the nucleus where it facilitates the transcription of a subset of genes. Consistent with this fact, we found that deletion of PDE11A increased the amount of total CREB located in the nuclear fraction of ventral hippocampi in both C57BL/6J and BALB/cJ mice (Figure 5H). Interestingly, the effect sizes for both the reduction in cAMP-PDE and the increase in nuclear CREB are about twice as high in the *Pde11a* KO mice on the BALB/cJ background vs. *Pde11a* KO mice on the C57BL/6J background, consistent with the much higher levels of PDE11A4 expression that are found in BALB/cJ vs. C57BL/6J mice[36]. Together, these data suggest that PDE11A homophily dictates mutual social preference due to its ability to regulate membrane-associated pools of cAMP specifically within the CA1 subfield of hippocampus.

DISCUSSION

In this study, we showed that—across the lifespan—mice prefer to interact with other mice that are homophilic for *Pde11a* function versus those that are not. Using mice of 2 different genetic backgrounds in 2 different behavioral assays, we showed that mice with 2 intact alleles of *Pde11a* prefer to interact with *Pde11a* WT mice of the same genetic background over KO mice; whereas, *Pde11a* KO mice prefer to interact with *Pde11a* KO mice over WT mice (Figure 2–3). This mutual social preference was seen in both adult and adolescent mice and could be eliminated or artificially elicited by using stimulus mice with strengthened or weakened PDE11A homodimerization, respectively. More specifically, C57BL/6J mice failed to exhibit social preference for *Pde11a* WT mice on the BALB/cJ background (and vice versa) that harbor a mutation that strengthens homodimerization[36] (Figure 3D) and alters subcellular trafficking of PDE11A (Figure 1A). Further, C57BL/6J mice showed social preference for C57BL/6J mice treated with a control virus versus C57BL/6J mice treated with a virus expressing an isolated GAF-B domain that decreases PDE11A4 homodimerization[36] (Figure 5F). The fact that C57BL/6J mice exhibited a social preference for the control mice vs. the GAF-B treated mice suggests PDE11A4 is regulating mutual social preference through an acute regulation of membrane-associated pools of cAMP-CREB signaling as opposed to a prolonged effect on the developmental trajectory of circuits.

PDE11A4 may be unique relative to other PDEs in its regulation of social behaviors. This may not be surprising given that various PDE families are differentially regulated in the brain across the lifespan (e.g., hippocampal PDE11A increases whereas PDE9A and PDE10A decrease [43–45]), and no two PDEs share the exact same regional distribution or subcellular compartmentalization in the brain[39, 46, 47]. To our knowledge, PDE10A is the only other PDE that has been shown to alter baseline social interactions, with deletion/inhibition of PDE10A increasing sociability of male mice[18, 28] and increased expression of PDE10A (via knockdown of its cognate microRNA) reducing sociability of mice[48]. Thus, PDE10A appears to regulate social approach in the opposite—if not a completely different—manner than PDE11A (i.e., data in Figure 2A would typically be interpreted as reduced sociability). That said, PDE1B, PDE2A, PDE4, PDE5A, and PDE9A inhibitors

reverse changes in social behaviors observed in rodent models of schizophrenia and neurodevelopmental disorders (e.g., [30, 49–51]). Note that we hesitate to characterize the social behaviors of these mouse models as “social deficits” since our findings show that behavior interpreted as a social approach deficit (as in Figure 2A) can, in fact, simply reflect a change in social preference when more thoroughly tested (as revealed by data in Figures 2B and 3). In these disease models, PDE inhibitors were delivered systemically. As such, it is not possible to know where in the brain these drugs were acting since these PDE families each have a widespread distribution in the rodent brain[39]. In terms of subcellular localization, it is interesting to note that while PDE11A4 is distinctly enriched in cytosolic versus membrane fractions, both PDE2A and PDE10A are enriched in the membrane versus the cytosol[38]. Despite this relative enrichment, social isolation specifically decreases PDE11A4 expression within the membrane compartment of the VHIPP without affecting PDE2A or PDE10A expression[20, 52]. Together, these data suggest PDE11A is unique relative to other PDE families both in terms of regulating social behaviors and in terms of mediating the effects of social experiences on the brain.

The fact that homophily in PDE11A function seems to drive mutual social preference in mice via the cAMP cascade aligns well with previous studies in humans showing genetic homophily among friends for the DRD2 gene[15] and odorant receptors[14], both of which couple to the cAMP cascade. Mouse studies, of course, can be conducted in a more controlled setting than human studies. Thus, mouse studies do not suffer from the constraints that face human studies in this area. As noted above, factors such as geographical distance, social inequality, or racial stratification may influence genetic homophily by limiting the choices available for friendship selection[16]. Factors such as physical proximity are controlled for in rodent studies, thus, the mutual social preference demonstrated herein cannot be explained away by such influences. Social stratification based on gender is also common in humans, where females tend to be friends with other females and males tend to be friends with other males[53, 54]. Importantly, we saw that PDE11A function dictated mutual same-sex social preference in both male and female mice. It will be of interest to future studies to determine if PDE11A homophily also influences opposite-sex social preference, or if heterophily (i.e., “opposites attract”) might be preferred. Indeed, human studies have also reported genetic heterophily in immune-related genes among friends, which has been suggested to offer a survival benefit[14–16].

It remains to be determined what trait or behavior is being altered by PDE11A function that drives the observed mutual social preference. In humans, genes play a role in regulating interests and hobbies, which tend to be shared by friends[55]. Alcoholism has a genetic basis and increases the likelihood of participating in activities involving alcohol[56, 57]. Such participation puts people in more frequent contact with other alcoholics or heavy drinkers, thus, increasing their chances of become friends. Indeed, friends of alcoholics tend to also be heavy drinkers[58]. Here, we showed that the mutual social preference being driven by PDE11A homophily does not appear to be due to a preference for some gross locomotor phenotype that differs between WT and KO mice nor a preference for the social odors that differ between WT vs. KO mice (Figure 4). Ultrasonic vocalizations are a key mode of social communication in mice that are known to vary in mice with differing genotypes[59, 60]. Thus, it will be of interest to future studies to determine if mice with differing PDE11A

function may emit different ultrasonic vocalizations that drive the observed differences in mutual social preference.

Several studies have suggested that BALB/cJ mice are less sociable than other mouse strains, particularly during adolescence (for review, see[61]); however, we see similar levels of social interaction with both the BALB/cJ and C57BL/6J mice when using stimulus mice of the same strain. It is notable that several reports suggesting BALB/cJ mice are less social measured interactions between a BALB/cJ mouse and a mouse from a different strain[28, 62–64]; whereas, a study that tested social approach behavior in subject mice using stimulus mice of the same strain actually showed increased social exploration in BALB/c versus C57BL/6J mice[65]. The difference between our observations and those of past studies using the 3-compartment chamber may also be related to the fact that we record time spent specifically investigating the cylinder as opposed to time spent in each chamber as a whole. Indeed, one report suggested the reduced sociability noted in BALB/cJ mice was more pronounced when measuring time spent in the chamber versus investigating the cylinder[64]. Taking our results together, we would suggest that BALB/cJ mice may have differences in social preferences relative to other mouse strains, as opposed to social deficits per se.

As more molecular mechanisms underlying mutual social preference are identified, it may become possible to develop a biomarker panel that predicts such compatibility. Such a biomarker would have far reaching implications. The ability to predict mutual social preference could significantly improve healthcare, given patient-therapist and patient-doctor compatibility is known to improve outcomes for patients[66, 67]. Such a biomarker might also hold implications for predicting romantic compatibility, thereby lowering divorce rates and the associated negative impact on financial, emotional, and psychosocial wellbeing[68–71]. More generally speaking, a better understanding of molecular mechanisms underlying mutual social preference could lead to novel treatments for patients with neuropsychiatric disorders where social functioning is impaired (e.g., schizophrenia[72], autism[73], and PSTD[74]). Our study here not only identifies PDE11A homophily as a key driver of mutual social preference, it offers a paradigm in which other mechanisms can be identified in a controlled fashion.

METHODS:

Subjects.

The *Pde11a* mouse line, previously described[29, 40], was originally obtained from Deltagen (San Mateo, CA) and then maintained on a mixed C57BL6 background (99.8% multiple C57BL/6 substrains and 0.2% 129P2/OlaHsd as per the MiniMUGA panel (Transnetyx, Cordova TN). The *Pde11a* KO was then backcrossed to BALB/cJ for 8 generations, ultimately generating a *Pde11a* KO line that is ~98.8% BALBc/J (as per MiniMUGA). C57BL/6J mice and some BALB/cJ mice were purchased from Jax and allowed to acclimate to the facility for at least 1 week prior to testing; other BALB/cJ mice were bred onsite from stock originally obtained from Jax. See figure legends for specific n's/experiment, which were based on past experience with these assays. 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 the University of South Carolina and the University of Maryland, Baltimore. For additional information, see the Supplemental Methods.

Experimental Procedures.

All studies were conducted in a blinded and counterbalanced fashion. Immunofluorescence and Western Blotting were conducted as previously described[31, 44]. Social Approach/Social Avoidance (SASA) and Social Preference were also assessed as previously described[20] (note: the "social preference assay" was previously named the "mouse psychiatry assay"). Social Odor Detection and Preference was evaluated using a modification of our previously published social odor recognition test[31, 40]. PDE Activity was measured based on[75], with some modification. Stereotactic surgeries targeting the CA1 subfields of hippocampus were conducted as previously described[40]. For additional methodological details, see the Supplemental Methods.

Data analysis.—Data were collected with an automated system or by an experimenter blind to treatment and were analyzed by parametric or nonparametric statistics, as appropriate, using Sigmaplot 11.2 as previously described[20, 40, 44] (see Supplemental Methods more specific details). Both males and females were included in each group (females data points located toward the left of each histogram and male data points are located toward the right), but not in sufficient number to power an analysis of the effect of sex with the exception of the experiment presented in Figure 3G. In cases of significant ANOVAs, *post hoc* analyses were conducted using the Student-Newman-Keuls Method. As previously described (e.g., [40, 44, 45]), data points >2 standard deviations from the mean were considered statistical outliers and removed from analyses (outliers/total data points: Figure 3E, 2/52; Figure 5E, 3/56; Figure 5F, 1/32; Figure 5G, 2/32).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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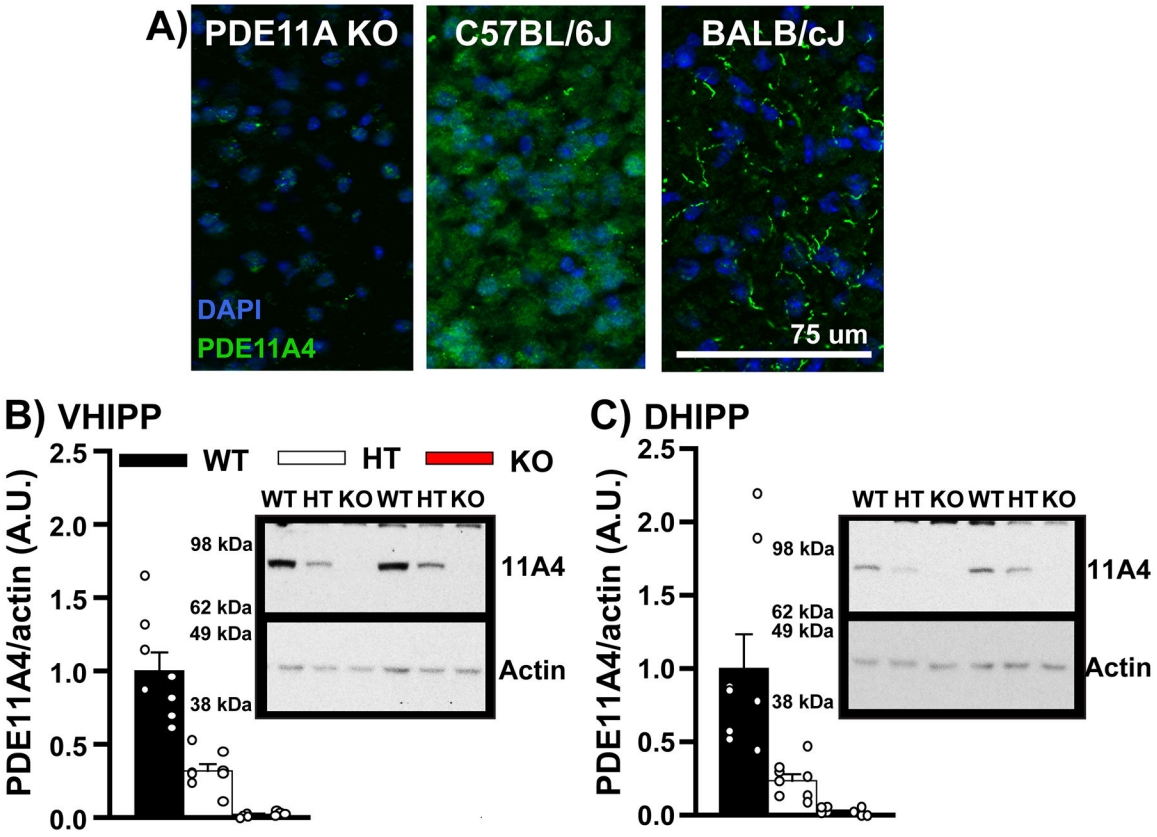

Figure 1. PDE11A4 protein trafficking differs in BALB/cJ vs. C57BL/6J mice; however, genetic deletion similarly affects total PDE11A4 expression levels on both genetic backgrounds.

A) Immunofluorescence of sagittal sections of the ventral subiculum using an antibody recognizing PDE11A4 (green) and a nuclear stain (blue; DAPI) shows PDE11A4 in this brain region is trafficked diffusely throughout neuronal cell bodies in C57BL/6J mice as previously described[31], yet also accumulates into filamentous linear structures in BALB/cJ mice. This pattern is consistent with our previous *in vitro* work showing that a PDE11A point mutation harbored in the BALB/cJ sequence strengthens homodimerization and increases accumulation of the protein relative to the C57BL/6J sequence[36]. The PDE11A knockout was backcrossed from a largely C57BL/6J background to a BALB/cJ background for 8 generations, yielding a line that was 98.8% BALB/cJ. In both B) ventral hippocampus (n=4/genotype/sex) and C) dorsal hippocampus (n=4/genotype/sex), PDE11A heterozygous mice demonstrate only ~20–25% of PDE11A4 protein expression that is seen in wild-type mice. This matches levels of expression detected in HT mice on the C57BL/6J background, and suggests that PDE11A4 drives its own expression[20]. PDE11A KO mice on the BALB/cJ background show no PDE11A4 protein expression as expected. VHIPP—ventral hippocampus, DHIPP—dorsal hippocampus, WT—wild-type, HT—heterozygote, KO—knockout. Data plotted as individual points (females towards left of each bar, males towards right) and expressed as mean ±SEM. Histogram stretch, brightness, and contrast of images adjusted for graphical clarity.

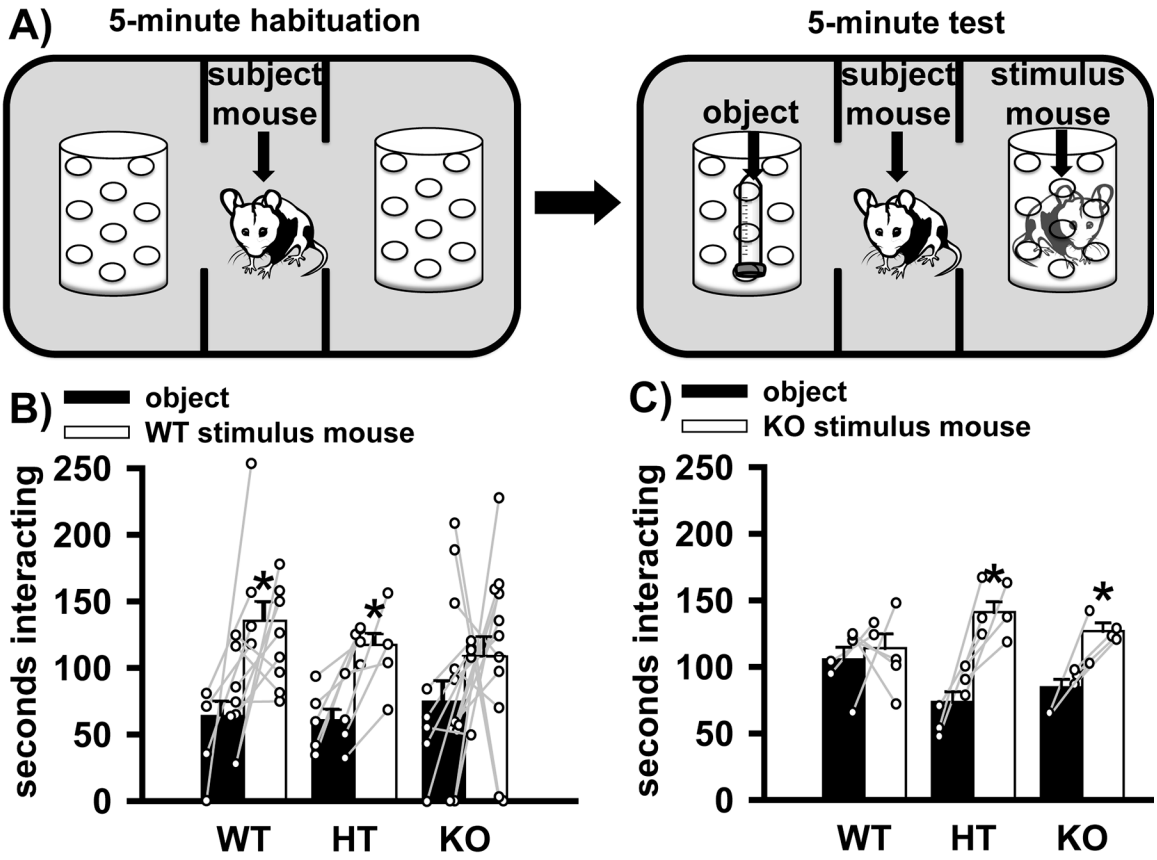

Figure 2. Mice spend significantly more time investigating a novel mouse vs. a novel object only when the novel mouse is of the same *Pde11a* genotype.

A) Mice from the BALB/cJ backcrossing were first habituated for 5 minutes to the 3-compartment chamber that housed a perforated plexiglass cylinder at either end. A novel object was then placed in one cylinder while a novel *Pde11a* wild-type (WT) or knockout (KO) stimulus mouse from the BALB/cJ backcrossing were placed in the other cylinder. Mice were then allowed to explore for an additional 5 minutes with the time spent directly interacting with the cylinders measured. B) When given a choice between a novel object vs. a novel *Pde11a* WT stimulus mouse, *Pde11a* WT subject mice (F, n=4; M, n=8) and heterozygous subject mice (HT; F, n=5; M, n=4) explore the novel WT stimulus mouse significantly longer than the object (2-way RM ANOVA fails normality; paired t-test WT: $t(11)=-3.024$, $P=0.011$; paired t-test HT: $t(8)=-3.799$, $P=0.005$); whereas, their *Pde11a* KO littermates (F, n=5; M, n=11) do not show a significant preference (paired t-test KO: $t(15)=-1.236$, $P=0.235$). C) In contrast, when given a choice between a novel object vs. a novel *Pde11a* KO stimulus mouse, *Pde11a* KO subject mice (F, n=2; M, n=3) and HT subject mice (n=3/sex) explore the novel KO mouse significantly longer than the object; whereas, their *Pde11a* WT littermates (F, n=2; M, n=4) show no such preference (2-way RM ANOVA genotype x cylinder: $F(2,14)=3.919$, $P=0.045$; *Post hoc* object vs. new KO: KO, $P=0.022$; HT, $P=0.0006$; WT, $P=0.5795$). Data plotted as individual points (females towards left of each bar, males towards right) and expressed as mean ±SEM. *vs. object, $P=0.022–0.0006$.

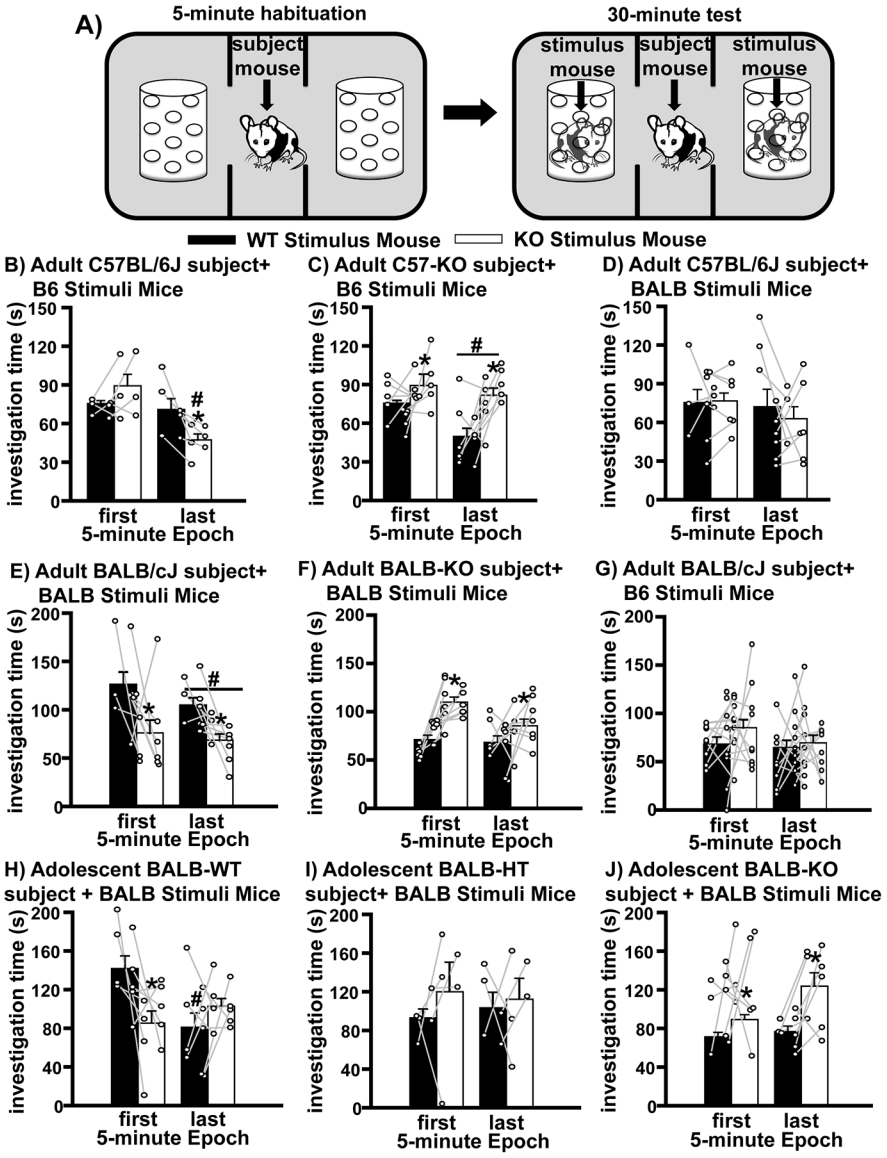

Figure 3. *Pde11a* genotype dictates mutual social preference in adult and adolescent mice in the social preference test.

A) Subject mice were first habituated for 5 minutes to the 3-compartment chamber that housed a perforated plexiglass cylinder at either end. A novel *Pde11a* wild-type (WT) and novel knockout (KO) stimulus mouse from either the C57BL/6J line or the BALB/cJ line was then placed in each cylinder, and subject mice were allowed to interact with these stimulus mice for 30 minutes. The time each subject mouse spent directly interacting with the cylinders was then scored during the first and last 5 –minute epoch of that 30-minute exploration. B) When adult C57BL/6J subject mice (n=3/sex) are given a choice between a *Pde11a* wild-type (WT) versus a knockout (KO) stimulus mouse from the C57BL/6J background, C57BL/6J subject mice prefer to interact with the *Pde11a* WT stimulus mouse during the last 5 minutes (2-way RM ANOVA fails normality; paired t-test for WT stimulus mouse vs. KO stimulus mouse during 'last': $t(5)=3.145$, $P=0.0255$; paired t-test for first vs. last 5-minutes interacting with KO stimulus mouse: $t(5)=-7.501$, $P=0.0007$). C) In contrast,

when adult *Pde11a* KO subject mice on the C57BL/6J background (n=5/sex) are given a choice between a *Pde11a* WT versus a KO stimulus mouse from the C57BL/6J background, they prefer to interact with the KO stimulus mouse across epochs (2-way RM ANOVA effect of stimulus mouse: F(1,9)=13.065, P=0.0056; effect of epoch: F(1,9)=8.71, P=0.0162). D) Interestingly, adult C57BL/6J subject mice (n=8/sex) did not show a preference for *Pde11a* WT versus *Pde11a* KO stimulus mice from the BALB/cJ background (2-way RM ANOVA effect of stimulus mouse: F(1,8)=0.195, P=0.671), suggesting the PDE11A4 point mutation that exists between C57BL/6J and BALB/cJ mice is sufficient to influence social preference. This suggestion is supported by the fact that E) adult BALB/cJ subject mice (F, n=3; M, n=6) did prefer to interact with a *Pde11a* WT versus *Pde11a* KO stimulus mouse from the BALB/cJ background across epochs (2-way RM ANOVA effect of stimulus mouse: F(1,8)=20.036, P=0.0021; effect of epoch: F(1,8)=6.275, P=0.0367); whereas, F) adult *Pde11a* KO subject mice (F, n=6; M, n=7) from the BALB/cJ background preferred to interact with a *Pde11a* KO versus *Pde11a* WT stimulus mouse from the BALB/cJ background across epochs (2-way RM ANOVA effect of stimulus mouse: F(1,12)=20.05, P=0.0005) and G) adult BALB/cJ mice (n=9/sex) showed no preference for *Pde11a* WT versus KO stimulus mice from the C57BL/6J background (3-way RM ANOVA effect of stimulus mouse: F(1,32)=3.00, P=0.093). H) Adolescent *Pde11a* WT subject mice from the BALB/cJ background (F, n=4; M, n=5) also preferred to interact with a *Pde11a* WT versus *Pde11a* KO stimulus mouse from the BALB/cJ background, but only during the first 5 minutes (2-way RM ANOVA effect of stimulus mice x epoch: F(1,8)=11.277, P=0.001; *Post hoc*: WT vs. KO stimulus mouse within first 5-minute epoch, P=0.0265; first vs. last 5-minute epoch with WT stimulus mouse, P=0.0008). I) In concert with data shown in Figure 2, adolescent *Pde11a* HT subject mice from the BALB/cJ background (F, n=3; M, n=2) showed no preference between the adult *Pde11a* WT versus KO stimulus mice from the BALB/cJ background (2-way RM ANOVA effect of stimulus mouse: F(1,4)=0.345, P=0.589), possibly because neither was a perfect genetic match. J) Similar to their adult counterparts, adolescent *Pde11a* KO subject mice from the BALB/cJ background (n=5/sex) preferred to interact with a *Pde11a* KO versus *Pde11a* WT stimulus mouse from the BALB/cJ background across epochs (2-way RM ANOVA effect of stimulus mouse: F(1,7)=6.516, P=0.038). Data plotted as individual points (females towards left of each bar, males towards right) and expressed as mean ±SEM. *vs. WT cylinder, P= 0.038–0.0006; #vs. first 5-minute epoch, P=0.0367–0.0007.

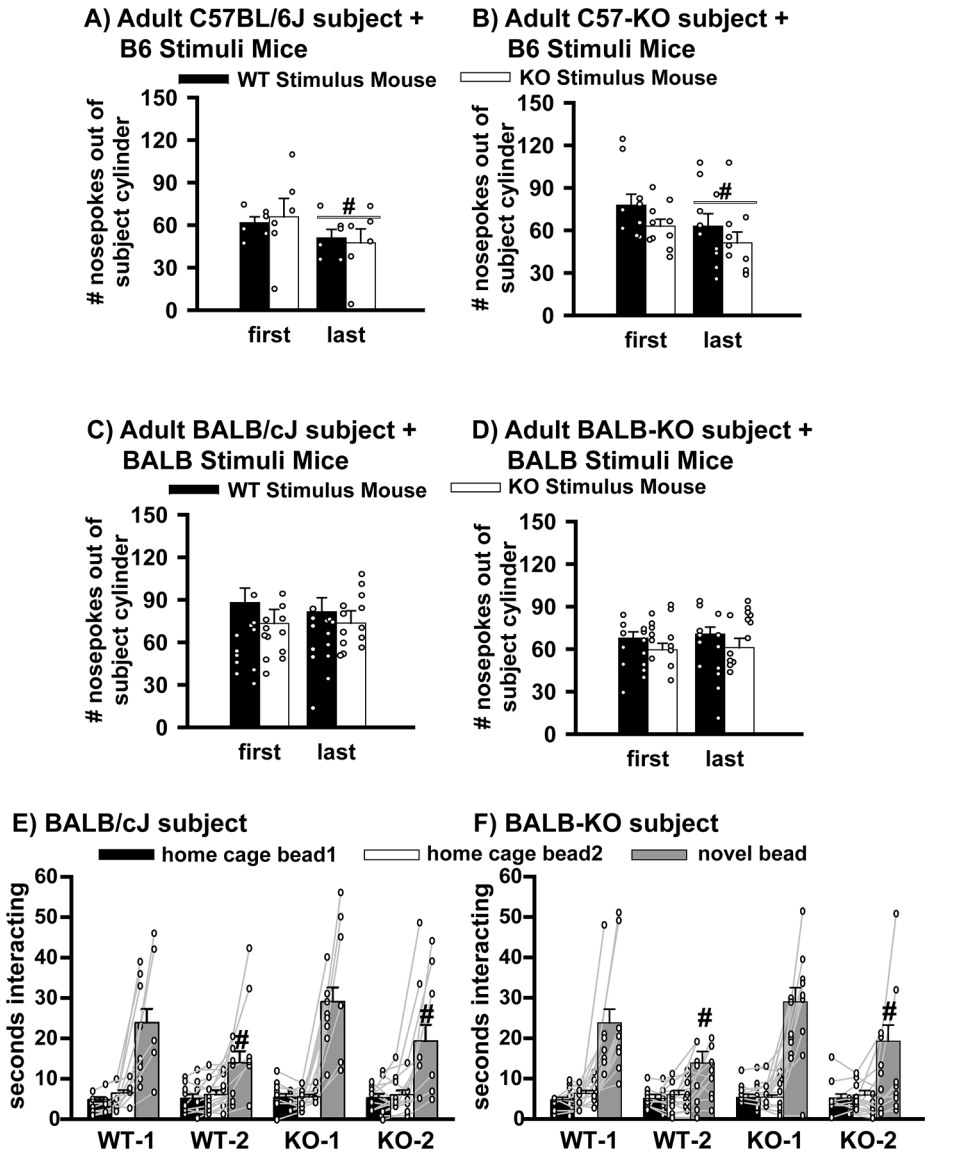

Figure 4. *Pde11a* genotype-dependent social preference cannot be explained by overt differences in locomotor activity of stimulus mice nor social odor preference of subject mice.

To determine if the *Pde11a* genotype-dependent effect observed in the social preference assay could be due to overt differences in the locomotor activity of stimulus mice, videos from experiments presented in Figure 3A, 3B, 3D and 3E were hand scored with regard to the number of times the stimulus mice poked their noses through the perforated holes. There was no difference in the extent to which *Pde11a* WT versus KO stimulus mice poked their nose out of the cylinders in response to A) adult C57BL/6J subject mice (2-way RM ANOVA effect of cylinder: $F(1,5)=0.0009$, $P=0.977$; effect of epoch: $F(1,5)=8.224$, $P=0.0351$), B) adult *Pde11a* KO subject mice on the C57BL/6J background (2-way RM ANOVA effect of cylinder: $F(1,9)=1.786$, $P=0.214$; effect of epoch: $F(1,9)=9.445$, $P=0.0133$), C) adult BALB/cJ subject mice (2-way RM ANOVA fails normality; paired t-test for 'first': $t(8)=1.426$, $P=0.192$; paired t-test for 'last': $t(8)=0.728$, $P=0.487$) or D) adult *Pde11a* KO subject mice on the BALB/cJ background (2-way RM ANOVA effect of

cylinder: F(1,12)=2.175, P=0.166). To determine if the *Pde11a* genotype-dependent effect in the social preference assay could be driven by a preference for each genotype's social odor, wooden beads were saturated with the scent of either novel WT stimulus mice or novel KO stimulus mice and the duration of investigation for each scent was recorded across 2 trials. E) Although BALB/cJ subject mice (F, n=9; M, n=6) can distinguish the scent of *Pde11a* WT stimulus mice from that of KO stimulus mice, they do not appear to prefer the scent of *Pde11a* WT stimulus mice over that of KO stimulus mouse as they spend approximately the same amount of time investigating each scent and equally habituate to each scent over 2 training trials (2-way RM ANOVA fails normality; Friedman RM ANOVA on Ranks effect of trial within novel: Chi-square(3)=27.08, P=0.000006; *Post hoc*: WT-1 vs. WT-2 and KO-1 vs. KO-2, P<0.05; WT-1 vs. KO-1 or WT-2 vs. KO-2, P="not significant"). F) A similar pattern is observed with *Pde11a* KO subject mice (F, n=6; M, n=7) on the BALB/cJ background (2-way RM ANOVA effect of genotype: F(1,17)=2.683, P=0.12; effect of trial: F(1,17)=113.769, P<0.000001). Data plotted as individual points (females towards left of each bar, males towards right) and expressed as mean ±SEM. #vs. first/T1, P=0.0351–0.0133.

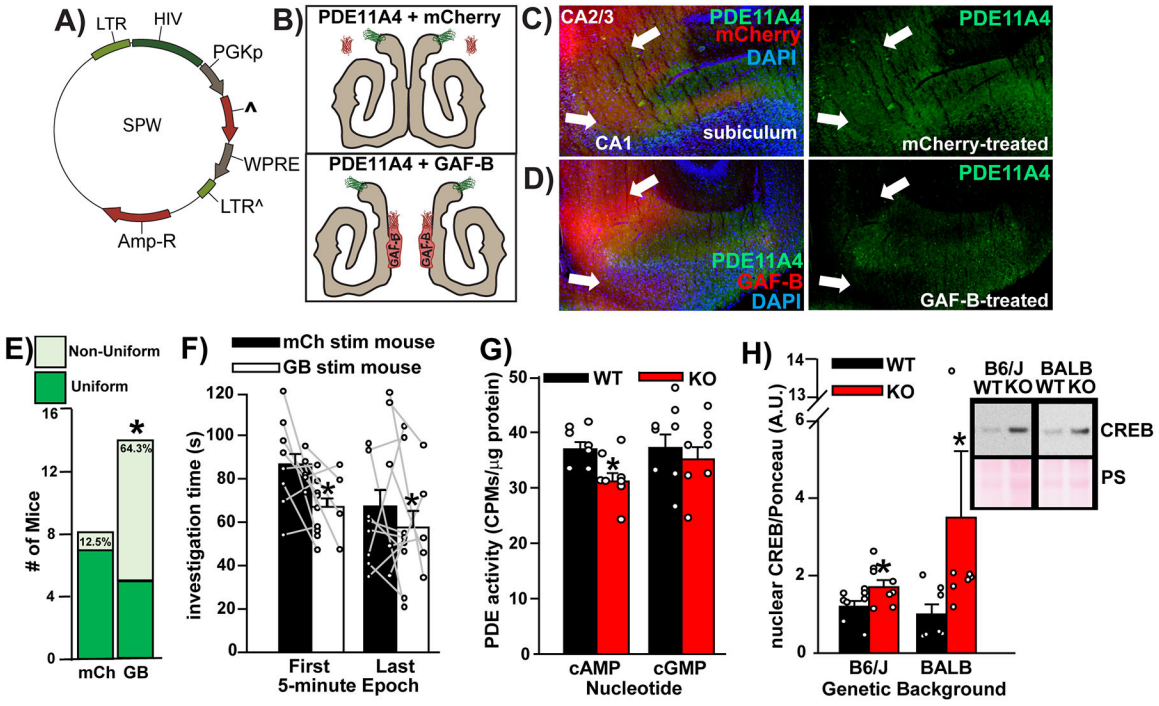

Figure 5. PDE11A appears to regulate social preference via membrane-associated pools of cAMP signaling in CA1 of hippocampus.

To identify the specific hippocampal subfield in which PDE11A regulates mutual social preference, we selectively disrupted PDE11A signaling in the CA1 region of hippocampus in male and female adult *Pde11a* WT stimulus mice on the C57BL/6J background by stereotatically infusing A) a virus that expressed the PDE11A4 GAF-B domain—the domain required for PDE11A4 homodimerization (^--insertion site). B) By acting as a negative sink, the isolated GAF-B construct reduces PDE11A4 homodimerization and preferentially degrades membrane-associated PDE11A4, as shown in[36]. C) Lentiviral expression of mCherry does not disrupt expression of PDE11A4 in ventral CA1. D) In contrast, lentiviral expression of the isolated mCherry-tagged GAF-B construct does reduce PDE11A4 in ventral CA1, particularly in dendrites and axons. Note, solid arrows are shown on the left triple-overlay and right PDE11A4-only images to indicate areas of high viral expression. E) Qualitative assessment of the staining pattern observed in mCherry-treated (mCh; n=4/sex) vs GAFB-treated mice (GB; n=7/sex) by an experimenter blind to treatment confirmed the GAF-B construct increases the patchiness (i.e., the non-uniformity) of PDE11A4 staining across the dendritic, cell body, and axonal layers of CA1 (Rank Sum: T(8,14)=63.0, P=0.024). F) Across the first and last 5-minute epoch of the social preference test, sex-matched adult C57BL/6J subject mice (n=9 females, n=5 males) preferred to interact with *Pde11a* WT stimulus mice on the C57BL/6J background that were treated with the mCherry virus (negative control; note: 2 mice did not actually express mCherry but were included as “mock” controls) vs. those stimulus mice treated with the mCherry-tagged GAF-B construct (effect of stimulus mouse: F(1,10)=7.04, P=0.0173). G) To determine if PDE11A preferentially regulates cAMP vs. cGMP *in vivo*, we measured cAMP-PDE and cGMP-PDE activity in ventral hippocampi of BALB/cJ *Pde11a* WT vs. KO mice. Consistent with previous observations on the C57BL/6J background[29], *Pde11a* KO mice on

the BALB/cJ background (n=3 females and 5 males/genotype) show a 15.7% reduction in ventral hippocampal cAMP-PDE activity relative to *Pde11a* WT mice ($t(13)=2.91$, $P=0.0121$), but equivalent cGMP-PDE activity relative to WT mice ($t(14)=0.63$, $P=0.539$). H) This reduction in cAMP-PDE activity is sufficient to increase output of the cAMP pathway as evidenced by the fact that *Pde11a* KO mice show higher levels of total CREB protein in the nuclear fraction of the ventral hippocampus relative to WT mice on both the C57BL/6J ($t(14)=-2.177$, $P=0.0471$) and BALB/cJ background (fails normality; Rank Sum test: $T(7,7)=34.00$, $P=0.0175$). mCh—mCherry, GB—isolated GAF-B domain tagged with mCherry, n=4/genotype/sex/background. Data plotted as individual points (females towards left, males towards right) and expressed as mean ±SEM. Histogram stretch and gamma of images adjusted for graphical clarity. *vs mCh or WT, P=0.047–0.012.