Neuroscience 335 (2016) 151–169

PDE11A REGULATES SOCIAL BEHAVIORS AND IS A KEY MECHANISM BY WHICH SOCIAL EXPERIENCE SCULPTS THE BRAIN

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Abstract—Despite the fact that appropriate social behaviors are vital to thriving in one's environment, little is understood of the molecular mechanisms controlling social behaviors or how social experience sculpts these signaling pathways. Here, we determine if Phosphodiesterase 11A (PDE11A), an enzyme that is enriched in the ventral hippocampal formation (VHIPP) and that breaks down cAMP and cGMP, regulates social behaviors. PDE11 wild-type (WT), heterozygous (HT), and knockout (KO) mice were tested in various social approach assays and gene expression differences were measured by RNA sequencing. The effect of social isolation on PDE11A4 compartmentalization and subsequent social interactions and social memory was also assessed. Deletion of PDE11A triggered age- and sex-dependent deficits in social approach in specific social contexts but not others. Mice appear to detect altered social behaviors of PDE11A KO mice, because C57BL/6J mice prefer to spend time with a sex-matched PDE11A WT vs. its KO littermate; whereas, a PDE11A KO prefers to spend time with a novel PDE11A KO vs. its WT littermate. Not only is PDE11A required for intact social interactions, we found that 1 month of social isolation vs. group housing decreased PDE11A4 protein expression specifically within the membrane fraction of VHIPP. This isolation-induced decrease in PDE11A4 expression appears functional because social isolation impairs subsequent social approach behavior and social memory in a PDE11A genotype-dependent manner. Pathway analyses following RNA sequencing suggests PDE11A is a key regulator of the oxytocin pathway and membrane signaling, consistent with its pivotal role in regulating social behavior.

Key words: phosphodiesterase, hippocampus, social buffering, social approach, social odor recognition, social memory.

INTRODUCTION

Several neuropsychiatric disorders are associated with deficits in social behaviors (c.f., (Young, 2008)) and no medicines remedy these symptoms. Without acceptable social behaviors, our abilities to attract a mate, acquire resources from society, and establish a safe/secure environment are severely compromised (Ferguson et al., 2002). When an individual lacks proper social behaviors, one is often ostracized (Reidpath et al., 2005). Social isolation worsens mental and physical health and increases mortality, particularly among adolescents, the elderly and the mentally ill (Pompili et al., 2008; Bartels and Pratt, 2009; Dickens et al., 2011; Hawton et al., 2011; Kondo et al., 2013; Slavich and Cole, 2013; Holt-Lunstad et al., 2015; Yang et al., 2016). To make matters worse, social isolation further impairs subsequent social behaviors—thus, creating a vicious cycle (Kogan et al., 2000; Reidpath et al., 2005; Derntl et al., 2011; Shahar-Gold et al., 2013). Despite the fact that appropriate social behaviors are vital to thriving in one's environment (Young, 2008), little is understood of the molecular mechanisms that control social behaviors or how social experiences modify these signaling pathways.

Cyclic nucleotide (cAMP/cGMP) signaling appears to be a molecular mechanism of social behavior that is conserved from amebas (Gregor et al., 2010) to mammals (Bickle, 2008). For example, oxytocin, a neuropeptide that regulates social behaviors (Lukas and Neumann, 2013; Stoesz et al., 2013; Feldman et al., 2015), couples to both the cAMP and cGMP cascades (Cheng et al., 2009; Viero et al., 2010). Further, cAMP-response element binding protein (CREB) and exchange protein activated by cAMP (Epac) are required for various types of social behaviors (Bickle, 2008; Srivastava et al., 2012; Yang et al., 2012). Phosphodiesterases (PDE), the only known enzymes to break down cAMP and cGMP (Francis et al., 2011), also modulate social behaviors (Boess et al., 2004; Grauer et al., 2009; Kelly et al., 2010; Hutson et al., 2011; Liebenberg et al., 2012). Cyclic nucleotide signaling specifically within the ventral hippocampal formation (VHIPP) may be particularly important because VHIPP lesions and knockdown of CREB in the rodent VHIPP impair social behaviors (Kogan et al., 2000; Brightwell et al., 2005; Tseng et al., 2009). This is consistent with the fact that both cyclic nucleotide

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Abbreviations: ANOVA, analysis of variance; CREB, cAMP-response element binding protein; GH, group housed; HT, heterozygous; KO, knockout; PDE11A, Phosphodiesterase 11A; Q-PCR, quantitative PCR; SI, socially isolated; VHIPP, ventral hippocampal formation; WT, wild-type.

http://dx.doi.org/10.1016/j.neuroscience.2016.08.019

signaling deficits (Ebstein et al., 1976; Belmaker et al., 1978; Bowers and Study, 1979; Kafka et al., 1979, 1986; Hoshino et al., 1980; Garver et al., 1982; Gattaz et al., 1983; Kafka and van Kammen, 1983; Memo et al., 1983; Goldberg et al., 1984; Kanof et al., 1986, 1987, 1989; Lerer et al., 1987; Ofuji et al., 1989; Kaiya et al., 1990; Kang, 1990; Young et al., 1991, 1993, 1994; Kaiya, 1992; Cowburn et al., 1994; Avissar et al., 1997; Gurguis et al., 1997, 1999a,b; Rahman et al., 1997; Avissar et al., 2001a,b; Bacchelli et al., 2003; Edmunds et al., 2008; Kelley et al., 2008; Turetsky and Moberg, 2009; Woolfrey et al., 2009; Ji et al., 2011) and abnormalities in the VHIPP (Suddath et al., 1990; Shenton et al., 1992; Rajarethinam et al., 2001; Jessen et al., 2003; Pegues et al., 2003; Lee et al., 2004; Rametti et al., 2007; Rusch et al., 2008; Zhou et al., 2008; Ghose et al., 2009; Nesvaderani et al., 2009; Schobel et al., 2009a,b; Hall et al., 2010; Goldman et al., 2011; Zhang et al., 2011) (referred to as the *anterior* hippocampus in primates) are observed in patients with neuropsychiatric disorders in which social deficits are known to occur.

Phosphodiesterase 11A (PDE11A, specifically isoform PDE11A4) is the only PDE with expression in brain restricted to the hippocampal formation, with a 3–10-fold enrichment in the VHIPP versus dorsal HIPP (Fig. 1A) (Kelly et al., 2010, 2014; Kelly, 2014, 2015; Hegde et al., 2016; Pathak et al., in press) and little to no expression in peripheral organs (c.f., (Kelly, 2015)). PDE11A has been genetically and/or functionally associated with clinical phenotypes related to changes in social function, including major depressive disorder (Wong et al., 2006; Cabanero et al., 2009; Luo et al., 2009), suicide risk (an inactivating mutation, (Coon et al., 2013)), and lithium responsivity (Couzin, 2008; Kelsoe, 2010; Mertens et al., 2015; Pathak et al., in press). Previously, we showed that reducing PDE11A expression was sufficient to reduce social approach behavior toward a novel mouse in adult male mice, but not female mice (Kelly et al., 2010). Here, we determine if social context and age differentially affect the manifestation of social approach deficits in PDE11A mutant mice and if social experience feeds back to shape the brain and subsequent behavior via PDE11A4. We show that PDE11A4 is a key regulator of social behaviors and the oxytocin signaling pathway, and that social experience shapes the brain, at least in part, by altering PDE11A4 compartmentalization.

EXPERIMENTAL PROCEDURES

Subjects

PDE11A knockout (KO) mice were originally developed by Deltagen (San Mateo, CA, USA), and are maintained on a mixed C57BL/6J-C57BL/6N-129S6 background, as previously described (Kelly et al., 2010; Hegde et al., 2016). PDE11A mice were bred onsite in heterozygous (HT) × HT matings, with same-sex wild-type (WT), HT, and KO littermates weaned together into the same cage at P28 (3–5/cage; but see "mouse psychiatrist" assay below). C57BL/6J mice were either obtained from Jackson Laboratories or bred onsite and were also group housed (3/cage). For social isolation studies, C57BL/6J mice were single-housed for 1 month and mice from the PDE11A colony were single-housed for 1–2 months. Approximately equal numbers of male and female offspring were tested between P28 and P42 for adolescent studies and 2–9 months of age for adult studies. In all experiments, PDE11A KO mice were compared to sex-matched littermates. Animals are housed on a 12:12-h light:dark cycle with unlimited access to food and water. See figure legends for experimental n's. 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.

In situ hybridization, quantitative PCR (Q-PCR) and Western blotting

In situ hybridization, quantitative PCR (Q-PCR) and Western blotting were performed as previously described (Kelly et al., 2010, 2014). *In situ* hybridization for PDE11A was performed using an S35-labeled antisense probe (5′-gccacctgtctggagatctcccacggtttggt cacggc-3′). Q-PCR was performed using pre-designed gene-specific oligonucleotide primer-probe sets (Table 1; Integrated DNA Technologies (IDT), Coralville, IA, USA). For Western blotting, antibodies were used to detect PDE11A (Fabgennix PD11-112 Lot 173, 1:500) and actin as a loading control (Sigma #A2066; 1:10,000).

Social approach assay

As we previously described (Kelly et al., 2010), we measured social approach behavior using Brodkin's version of the 3-compartment chamber in which a perforated plexiglass cylinder is placed in either end of the compartment (Sankoorikal et al., 2006). After subjects habituated to the apparatus and empty cylinders for 5 min, a novel object (50 mL conical tube) and either a novel PDE11A WT, a novel C57BL/6J, or a cagemate were placed in the cylinders (position counterbalanced across subjects). Littermates were tested against the same stimulus mice, with order of genotypes tested counterbalanced across littermate groups. In the "cagemate vs. novel mouse" version of the assay, the cagemate remained and the object was first replaced with a novel PDE11A WT and then a novel C57BL/6J. Each approach trial lasted 5 min. For the "new object vs. new PDE11A WT" assay, chamber time was hand scored. For the "new object vs. new C57BL/6J" assay, latency and chamber time were collected using AnyMaze to track center of body, and cylinder data was hand scored. For the cagemate assays, latency and chamber/cylinder time were collected by tracking head position using Ethovision 7.0, with the exception of the adolescent study that was hand scored. For all studies using AnyMaze or Ethovision, locomotor activity was recorded but no effects of genotype were noted (Table 2).

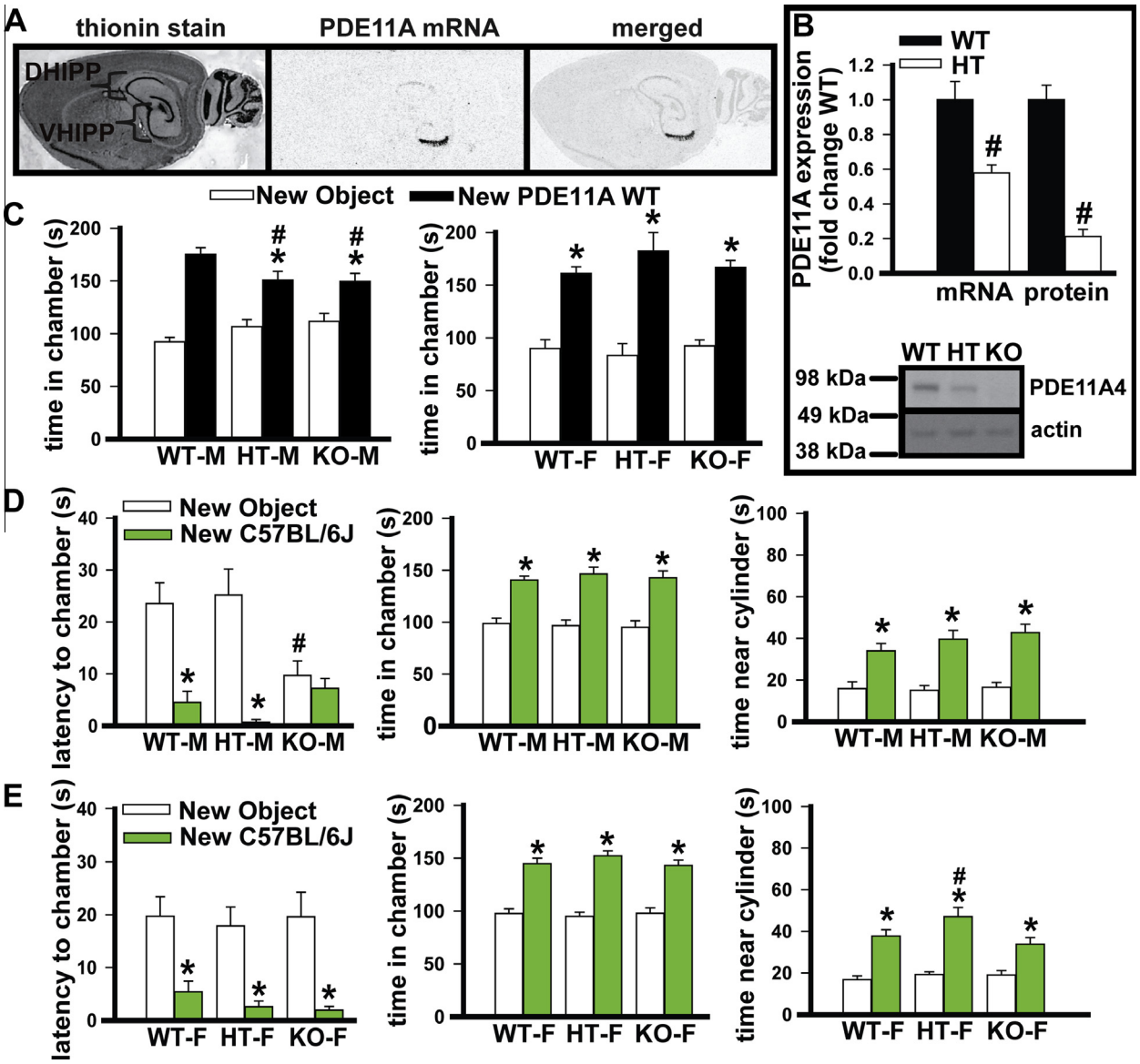

Fig. 1. PDE11A mRNA expression is restricted to the hippocampal formation and deletion alters social approach in a manner that depends on the strain of the stimulus mouse. (A) As previously reported (Kelly et al., 2010, 2014; Kelly, 2014, 2015), PDE11A mRNA is selectively expressed in CA1 (and possibly ventral CA2), subiculum, and the adjacent amygdalohippocampal area (not shown at this level), with a three to 10-fold enrichment in ventral (VHIPP) versus dorsal hippocampus (DHIPP). (B) As expected, PDE11A heterozygous (HT; $n = 6$ (three females)) mice show a 50% reduction in ventral hippocampal (VHIPP) PDE11A mRNA expression relative to wild-type (WT) littermates ($n = 7$ (four females)), as measured by Q-PCR ($t(11) = 3.47$, $P = 0.005$). Note, no signal was detected in samples from PDE11A knockout (KO) mice, proving specificity of the primer/probe sets (data not shown). Surprisingly, however, PDE11A HT mice ($n = 9$ (five females)) show an 80% reduction in VHIPP PDE11A4 protein expression relative to WT littermates ($n = 10$ (five females)), as measured by Western blot ($t(17) = 8.20$, $P < 0.001$; shown in lower panel). Identical results were obtained in dorsal hippocampal samples from these mice as well as whole hippocampus samples from a second cohort of mice (data not shown). The mechanism underlying this disconnect between mRNA and protein remains to be determined, but alterations in social behavior driven by the HT deletion may further drive down PDE11A4 protein expression in the PDE11A HT mice (e.g., see Fig. 5A). (C) (Left) When given a chance to explore a novel object vs. a novel PDE11A WT mouse, adult male PDE11A mutant mice (i.e., HT, $n = 8$, and KO mice, $n = 10$) exhibited reduced social approach relative to that of male WT littermates ($n = 14$; $F(2,26) = 4.50$, $P = 0.021$). (Right) in contrast, female PDE11A WT ($n = 7$), HT ($n = 3$), and KO mice ($n = 9$) spent equivalent amounts of time with a novel mouse from the PDE11A colony ($F(1,16) = 68.77$, $P < 0.001$). This sex-specific phenotype in group-housed mice bred onsite is consistent with our previous report in single-housed mice bred offsite (Kelly et al., 2010). Note: latency to chamber and time near the cylinder were not available for this experiment. (D) Changing the stimulus mouse to a C57BL/6J altered the pattern of behavior seen in PDE11A mutant mice. (Left) male PDE11A KO mice ($n = 18$) failed to show a significant social preference in terms of their latency to approach the new C57BL/6J mouse vs. the new object, having approached the novel object much more quickly than their male HT ($n = 14$) and WT littermates ($n = 20$; effect replicated in two cohorts of mice, combined analysis: $F(2,49) = 4.82$, $P = 0.012$). (Middle) despite this difference in latency to approach, PDE11A KO males exhibited significant social preference in terms of their time in each chamber ($F(1,49) = 55.22$, $P < 0.001$) and (right) time near each cylinder ($F(1,49) = 54.60$, $P < 0.001$). Unlike PDE11A KO males, (E) (left) PDE11A KO females ($n = 17$) showed a significance preference for the new C57BL/6J mouse vs. the new object, as did PDE11A WT and HT females (WT, $n = 27$; HT, $n = 22$), in terms of their (left) latency to approach ($F(1,63) = 31.78$, $P < 0.001$) as well as their (middle) time in chamber ($F(1,63) = 87.15$, $P < 0.001$), and (right) time near the cylinder ($F(2,57) = 4.57$, $P = 0.014$), with PDE11A HT females showing significantly greater social approach on the last measure. *Post hoc*, *vs. object, $P < 0.001$; $^{\#}$vs. WT, $P < 0.05$–0.001.

Table 1. Primers used to assess PDE11A mRNA expression

Gene	Set IDT Cat#	Sequence Name	Sequence
PDE11A exon 12–13	Mm.PT.58.42976146	PrimeTime Probe	5′-/6-FAM/CAG CTC TAC /ZEN/GGG ACC TCG GC/IBFQ/-3′
		PrimeTime Primer 2	GAA CCA ACA ATG CCT TCC AAG
		PrimeTime Primer 1	CCT CAC TCT GAA GGA TCA TCA C
TBP exon 3–4	Mm.PT.58.10867035	PrimeTime Probe	5′-/6-FAM/CA CTC CTG C/Zen/C ACA CCA GCT TCT /IBFQ/-3′
		PrimeTime Primer 2	TTC ACC AAT GAC TCC TAT GAC C
		PrimeTime Primer 1	CAA GTT TAC AGC CAA GAT TCA CG
ACTB exon 4–5	Mm.PT.58.33257376.gs	PrimeTime Probe	5′-/6-FAM/AT TCC ATA C/Zen/C CAA GAA GGA AGG CTG G/IBFQ/-3′
		PrimeTime Primer 2	ATT GGC AAC GAG CGG TT
		PrimeTime Primer 1	AGG TCT TTA CGG ATG TCA ACG
POLR2A exon 22–23	Mm.PT.58.13811327	PrimeTime Probe	5′-/6-FAM/AC CAC CTC T/Zen/T CCT CCT CTT GCA TCT /IBFQ/-3′
		PrimeTime Primer 2	TCG AAT CCG CAT CAT GAA CAG
		PrimeTime Primer 1	GTC AGC ATG TTG GAC TCA ATG

PDE11A was normalized to the average of values for the housekeeping genes TBP, ACTB, and POLR2A. Primer-probe sets were used with 5′ 6-FAM (6-Carboxyfluorescein) fluorophore, 3′ IBFQ (Iowa black FQ) and internal ZEN quenchers (IDT) using iTaq™ Universal Probes Supermix (Bio-Rad, Hercules, CA).

"Mouse psychiatrist" assay

This assay is somewhat similar to the Preference for Typical Social Interaction Test (Shah et al., 2013) but with substantial differences in methodology/design. A C57BL/6J mouse, a "social" mouse strain (Brodkin et al., 2004; Fairless et al., 2012), was first habituated to the social approach apparatus for 5 min. Next, an age- and sex-matched PDE11A WT and either a HT or KO littermate were placed in the two cylinders (i.e., one mouse/cylinder). The position of genotypes in the cylinders was counterbalanced across trials. Each C57BL/6J was tested against a new pair of WT-HT/KO littermates. The C57BL/6J "mouse psychiatrist" was then allowed to assess the WT and HT/KO mice for 30 min. Data were binned in 5-min epochs. This assay was also run using PDE11A KO mice as the "mouse psychiatrist". These PDE11A KO mice were raised in HT x HT matings but were weaned exclusively with other sex-matched KO mice. Chamber data were calculated using AnyMaze, and cylinder data were hand scored.

Biochemical fraction

Animals were sacrificed by cervical dislocation, with individual brain regions immediately dissected onto dry ice and stored at −80 °C. Each biological replicate contained tissue from 2 to 3 mice to provide sufficient starting material. Tissue samples were homogenized, biochemically fractionated, and processed by Western blotting as previously described (Kelly et al., 2010, 2014; Pathak et al., in press).

Social odor recognition

As previously described (Kelly et al., 2010; Hegde et al., 2016), mice were individually placed in a clean home cage and allowed to explore three wooden beads—two from their home cage and one from the cage of a novel mouse strain (C57BL/6J, BALB/cJ, 129S6/SvEv). During a 2-min memory test 24 h after training, mice were again presented with three beads: home cage, bead from training, and a bead from another strain. Time investigating each bead was scored, and "memory" was operationally defined as spending significantly more time investigating the novel versus the familiar odor.

RNA sequencing

Animals were sacrificed by cervical dislocation, with individual brain regions immediately dissected onto dry ice and stored at −80 °C. RNA and library preparation, sequencing, and post-processing of the raw data were performed by the Epigenomics Core at Weill Cornell Medicine. Tissue stored at −80 °C was dry-fractured in a tissueTUBE device using a cryoPREP TM Impactor as per manufacturer recommendations (Covaris, Woburn, MA, USA). Each pulverized sample was resuspended in 350uls of Qiagen RNeasy Plus Mini RTL lysis buffer and RNA extracted as per manufacturer recommendations (Qiagen, Valencia, CA, USA). RNA integrity was assessed using the Lab Chip GX (Perkin Elmer, Waltham, MA, USA), and samples had a quality score > 8.0. TruSeq RNA libraries were prepared using established Illumina methods (Illumina, San Diego, CA, USA, Part #RS-122-2001). Each library was made with one of the TruSeq barcode index sequences and samples were sequenced across three lanes. The pools were clustered at 6.5 pM on a pair end read flow cell and sequenced for 100 cycles on an Illumina HiSeq 2500. Primary processing of sequencing images was done using Illumina's Real Time Analysis software (RTA). CASAVA 1.8.2 software was used to perform image capture, base calling and demultiplexing. Sequences were aligned to the mouse mm10 genome (http://hgdownload.soe.ucsc.edu/goldenPath/mm10/bigZips/) using STAR v2.3.1 (Dobin et al., 2013). Reads were counted using the featureCounts function of the Subreads package (Liao et al., 2013) in R (https://www.R-project.org/) using Gencode M6 GTF (http://www.gencodegenes.org/mouse_stats/archive.html) and summarized at exon, transcript, or gene level. Only reads that were mapped uniquely to the genome were used. Mapping quality (MAPQ) minimum threshold was set at 10. Differential expression analysis was performed in R using the edgeR package (Robinson et al., 2010).The average read depth for the samples was 64 million reads and only genes with at least one count per million average depth

Table 2. There was no significant difference in locomotor activity between genotypes as measured by total centimeters traveled in the social approach chamber

Social approach test	Source	WT-M	HT-M	KO-M	WT-F	HT-F	KO-F
New object vs. new PDE11A WT	Kelly et al. (2010), PNAS	1862 (105)	1970 (86)	1753 (111)	1890 (167)	1542 (186)	2250 (516)
New object vs. new C57BL/6J	Fig. 1D, E	1484 (54)	1576 (68)	1517 (50)	1629 (62)	1645 (79)	1786 (94)
New object vs. cagemate	Fig. 2A	3078 (787)	4299 (1325)	2998 (953)	2525 (658)	3463 (969)	1744 (169)
Cagemate vs. new PDE11A WT	Fig. 2B	3393 (896)	6104 (2243)	3406 (1236)	3231 (826)	7241 (4062)	2297 (379)
Cagemate vs. new C57BL/6J	Fig. 2C	3574 (1113)	6133 (1352)	2753 (757)	2865 (786)	2393 (570)	2682 (600)

Data expressed as mean (SEM).

were considered for differential expression analysis. Raw counts were normalized using the trimmed mean of *m*-values (TMM) method. Dispersion estimates were then calculated using the estimateGLMRobustDisp function (Zhou et al., 2014). The normalized read counts were then fitted to a generalized linear model using the function glmFit (McCarthy et al., 2012). Genewise tests for significant differential expression were performed using the function glmLRT. The *P*-value was then corrected for multiple testing using Benjamini-Hochburg's FDR (Benjamini and Hochberg, 1995). Genes that differed in expression between PDE11A WT and KO mice (FDR $P < 0.05$; Table 3) were examined for enrichment in KEGG and GO pathway using STRING 10 (string-db.org) (Szklarczyk et al., 2015).

Data analyses

Behavioral and biochemical data were collected in a blinded fashion and analyzed using Statistica 9.0. As previously described (Kelly et al., 2010; Kelly, 2014; Pathak et al., 2015), to mitigate non-specific effects related to transfer efficiencies, film exposures, etc. across gels, Western blot data on a given gel were normalized to a reference group (e.g., GH). Data were analyzed by an analysis of variance (ANOVA) or repeated measure ANOVA for genotype and/or housing condition and compartment. With regard to the effect of sex, male and female data in Figs. 1 and 3A were analyzed separately based on our previous sex-specific findings in an "object vs. novel mouse" version of the approach assay. For remaining datasets, sex was included along with genotype in ANOVAs where $n \geqslant 6$/sex/genotype. Data were graphed collapsed across sex unless a significant genotype × sex interaction was detected to best reflect the conclusions of the statistical tests. In cases of significant ANOVAs, *post hoc* analyses were conducted using the Student–Newman–Keuls Method. As previously described (Kelly et al., 2008, 2010; Kelly, 2014; Pathak et al., 2015), statistical outliers >2 standard deviations from the mean were removed from analyses (outliers/total data points: Fig. 1C Left, 2/62; Fig. 1E Right, 6/132; Fig. 2A Left 8/28; Fig. 2A Middle, 7/128; Fig. 2A Right, 7/128; Fig. 2B Left, 3/64; Fig. 2B Middle, 8/128; Fig. 2B Right, 10/128; Fig. 2C Left, 6/128; Fig. 2C Middle, 5/128; Fig. 2C Right 10/128; Fig. 3A Left, 2/109; Fig. 3A Right, 3/56; Fig. 3B, 4/101; Fig. 3C, 3/101; Fig. 3D, 4/101; Fig. 4A, 5/104; Fig. 4B, 4/104; Fig. 4C, 5/104; Fig. 4D, 6/88; Fig. 4E, 1/88; Fig. 4F, 4/88; Fig. 4H, 1/56; Fig. 4I, 1/56). Significance was defined as $P < 0.05$. Data are graphed as mean ± SEM.

RESULTS

Social approach behaviors of PDE11A HT and KO mice depend on the social context

In our previous laboratory (Kelly et al., 2010), we reported that within a colony that was bred offsite and single-housed, male PDE11A KO mice—but not female KO mice—exhibited significantly reduced social approach behavior relative to sex-matched WT littermates. Here, we replicated this sex-specific effect with group-housed (GH) mice bred onsite (Fig. 1C). Next we determined if social approach behavior of PDE11A mutant mice might depend on the social context. Changing the stimulus mouse from a novel PDE11A WT to a novel C57BL/6J mouse (Fig. 1D, E) and/or replacing the alternative option of the novel object with a cagemate (Fig. 2A) altered the social approach deficits exhibited by PDE11A HT and KO mice, in some cases lessening the deficits and in other cases worsening the deficits. Together, these data suggest that social approach deficits exhibited by PDE11A mutant mice differ depending on the social context.

Effects of PDE11A deletion on social approach behavior are age dependent

Previously we showed that PDE11A expression is minimal at postnatal day 7 but dramatically increases to young adulthood levels between postnatal day 21–28 (Hegde et al., 2016). Social approach behaviors become reliably measurable in the mouse around postnatal day 19 (Laviola et al., 1994; Fairless et al., 2012), suggesting PDE11A mutant mice might exhibit social approach deficits early in adolescence. That said, a number of neurodevelopmental insults, including neonatal ventral hippocampal lesions, do not yield immediate behavioral deficits but, rather, require a period of development to occur before deficits fully manifest (e.g., (Tseng et al., 2009)). As such, we determined if adolescent PDE11A HT and KO mice would exhibit similar social approach deficits relative to WT littermates as did their adult counterparts. Adolescent PDE11A HT and KO mice largely performed normally in social approach behaviors where

Table 3. RNA sequencing reveals 169 genes that significantly differ in expression between PDE11A KO versus WT littermates (sorted by KO vs. WT FDR *P*-value)

Gene ID	KO vs. WT			HT vs. WT			KO vs. HT		
	logFC	*P*-Value	FDR *P*-value	logFC	*P*-Value	FDR *P*-value	logFC	*P*-value	FDR *P*-value
Xist	**4.6819**	**2.351E−11**	**1.736E−07**	0.8148	3.052E−01	9.998E−01	**1.9335**	**4.048E−07**	2.990E−03
Mylk	**−0.6646**	**3.077E−08**	**1.515E−04**	−0.2890	1.542E−02	9.443E−01	−0.1878	1.368E−03	2.617E−01
Samd4	**0.6946**	**5.943E−08**	**2.194E−04**	**0.4917**	**1.025E−04**	4.619E−01	0.1015	1.120E−01	8.715E−01
Fgl2	**−1.9915**	**2.400E−07**	**7.088E−04**	−0.7670	3.958E−02	9.998E−01	−0.6122	1.233E−03	2.617E−01
Hivep1	**1.1266**	**3.264E−07**	**8.036E−04**	**0.8016**	**2.502E−04**	4.619E−01	0.1625	1.324E−01	8.955E−01
Syce2	**−1.3233**	**5.799E−07**	**1.224E−03**	−0.5006	4.899E−02	9.998E−01	−0.4113	1.245E−03	2.617E−01
Pitpnm3	**0.8443**	**8.292E−07**	**1.531E−03**	0.4902	3.295E−03	7.487E−01	0.1771	3.853E−02	7.123E−01
Hlf	**0.7903**	**1.064E−06**	**1.746E−03**	0.2144	1.765E−01	9.998E−01	**0.2879**	**2.640E−04**	1.950E−01
Satb1	**0.8303**	**1.264E−06**	**1.867E−03**	0.5077	2.779E−03	7.309E−01	0.1613	6.132E−02	7.703E−01
Asap1	**0.8505**	**1.638E−06**	**2.199E−03**	0.3569	3.840E−02	9.998E−01	0.2468	5.392E−03	4.525E−01
Unc13c	**1.9464**	**2.114E−06**	**2.602E−03**	1.2594	1.634E−03	7.120E−01	0.3435	8.163E−02	8.359E−01
Cacnb4	**0.6627**	**2.398E−06**	**2.724E−03**	0.3364	1.525E−02	9.443E−01	0.1631	1.999E−02	6.357E−01
Mtcl1	**0.8188**	**2.949E−06**	**3.046E−03**	0.2353	1.849E−01	9.998E−01	0.2917	9.939E−04	2.617E−01
Fam26e	**−0.6501**	**3.096E−06**	**3.046E−03**	−0.2762	4.846E−02	9.998E−01	−0.1869	8.562E−03	5.038E−01
Hecw1	**0.7093**	**3.300E−06**	**3.046E−03**	0.3463	2.109E−02	9.852E−01	0.1815	1.467E−02	5.640E−01
St3gal1	**1.0880**	**3.808E−06**	**3.309E−03**	0.6929	2.668E−03	7.297E−01	0.1976	8.500E−02	8.392E−01
Cobl	**1.3253**	**5.535E−06**	**4.542E−03**	**1.0592**	**2.278E−04**	4.619E−01	0.1331	3.443E−01	9.457E−01
Nbl1	**−0.7475**	**6.227E−06**	**4.812E−03**	−0.4266	7.894E−03	8.388E−01	−0.1604	5.065E−02	7.449E−01
Grm8	**1.2196**	**6.516E−06**	**4.812E−03**	**0.8843**	**9.596E−04**	6.450E−01	0.1677	2.027E−01	9.260E−01
Etl4	**0.7987**	**7.032E−06**	**4.946E−03**	0.5497	1.983E−03	7.120E−01	0.1245	1.608E−01	9.132E−01
Rab26	**−0.6809**	**7.532E−06**	**5.057E−03**	−0.2208	1.347E−01	9.998E−01	−0.2301	2.150E−03	3.125E−01
Rora	**0.7188**	**7.926E−06**	**5.090E−03**	0.4800	2.485E−03	7.120E−01	0.1194	1.270E−01	8.897E−01
Mlip	**0.6955**	**1.174E−05**	**7.223E−03**	**0.5119**	**7.089E−04**	5.847E−01	0.0918	2.473E−01	9.415E−01
Il1rapl2	**1.3039**	**1.480E−05**	**8.743E−03**	0.6506	3.221E−02	9.998E−01	0.3267	2.532E−02	6.812E−01
Plcb4	**0.8478**	**1.581E−05**	**8.979E−03**	0.5576	3.578E−03	7.738E−01	0.1451	1.348E−01	8.971E−01
RP24−490B17.1	**2.0479**	**1.765E−05**	**9.435E−03**	0.8881	6.392E−02	9.998E−01	0.5799	1.327E−02	5.401E−01
Cntn4	**0.8808**	**1.840E−05**	**9.435E−03**	0.5997	3.201E−03	7.471E−01	0.1406	1.486E−01	9.053E−01
Vwc2l	**1.8754**	**1.853E−05**	**9.435E−03**	1.3651	1.367E−03	7.120E−01	0.2551	2.188E−01	9.322E−01
Zdhhc22	**1.3832**	**2.075E−05**	**1.021E−02**	0.6864	3.144E−02	9.998E−01	0.3484	2.750E−02	6.839E−01
Frmd7	**−1.1582**	**2.399E−05**	**1.074E−02**	−0.5211	4.737E−02	9.998E−01	−0.3186	2.140E−02	6.517E−01
Pamr1	**0.9322**	**2.317E−05**	**1.074E−02**	0.5266	1.588E−02	9.443E−01	0.2028	6.407E−02	7.763E−01
Dlg1	**0.4058**	**2.361E−05**	**1.074E−02**	0.2297	1.534E−02	9.443E−01	0.0881	6.472E−02	7.763E−01
Cpne7	**−0.9417**	**2.905E−05**	**1.226E−02**	−0.2759	1.905E−01	9.998E−01	−0.3329	2.800E−03	3.504E−01
Nr4a2	**0.9161**	**2.828E−05**	**1.226E−02**	**0.7585**	**6.396E−04**	5.847E−01	0.0788	4.777E−01	9.518E−01
Fut9	**0.6358**	**3.079E−05**	**1.263E−02**	0.3092	4.054E−02	9.998E−01	0.1633	2.764E−02	6.839E−01
Pknox2	**0.5421**	**3.193E−05**	**1.275E−02**	0.1613	1.994E−01	9.998E−01	0.1904	3.439E−03	3.681E−01
Igfbp3	**−0.8821**	**3.446E−05**	**1.339E−02**	0.0503	7.989E−01	9.998E−01	**−0.4662**	**9.618E−06**	4.735E−02
Deptor	**1.1437**	**3.608E−05**	**1.366E−02**	0.7056	1.015E−02	8.766E−01	0.2190	1.002E−01	8.567E−01
Camk2g	**0.4572**	**3.942E−05**	**1.399E−02**	0.0855	4.258E−01	9.998E−01	0.1858	8.338E−04	2.512E−01
Astn2	**0.5271**	**3.796E−05**	**1.399E−02**	0.1916	1.256E−01	9.998E−01	0.1677	8.933E−03	5.095E−01
Lnp	**0.4311**	**3.977E−05**	**1.399E−02**	0.3909	2.466E−04	4.619E−01	0.0201	7.065E−01	9.797E−01
Adra1d	**−0.8237**	**4.383E−05**	**1.407E−02**	−0.3748	5.364E−02	9.998E−01	−0.2245	2.522E−02	6.812E−01
Smad6	**−1.6239**	**4.283E−05**	**1.407E−02**	−0.9034	2.135E−02	9.852E−01	−0.3603	6.840E−02	7.885E−01
Chrna4	**0.8001**	**4.221E−05**	**1.407E−02**	0.4968	1.161E−02	9.421E−01	0.1516	1.176E−01	8.771E−01
Sowahb	**1.4828**	**4.328E−05**	**1.407E−02**	1.0883	2.371E−03	7.120E−01	0.1973	2.776E−01	9.441E−01
Tshz3	**1.3431**	**4.644E−05**	**1.459E−02**	0.6382	4.970E−02	9.998E−01	0.3525	2.655E−02	6.812E−01
Rnf128	**−0.5481**	**5.331E−05**	**1.637E−02**	−0.1518	2.552E−01	9.998E−01	−0.1981	2.978E−03	3.605E−01
Gm23935	**−1.2578**	**5.431E−05**	**1.637E−02**	−0.4726	1.597E−01	9.998E−01	−0.3926	1.825E−02	6.130E−01
Slc17a6	**1.4644**	**5.748E−05**	**1.687E−v02**	0.8239	1.942E−02	9.723E−01	0.3202	7.196E−02	8.035E−01
Pth1r	**1.0572**	**5.825E−05**	**1.687E−02**	**0.8738**	**6.847E−04**	5.847E−01	0.0917	4.763E−01	9.518E−01
Epha10	**0.5337**	**5.999E−05**	**1.698E−02**	0.2302	8.915E−02	9.998E−01	0.1518	2.389E−02	6.784E−01
Hspa1b	**−0.9499**	**6.174E−05**	**1.698E−02**	−0.4208	7.725E−02	9.998E−01	−0.2646	2.445E−02	6.804E−01
Osbpl3	**0.6298**	**6.207E−05**	**1.698E−02**	0.3116	4.510E−02	9.998E−01	0.1591	4.309E−02	7.265E−01
Tusc1	**−0.8527**	**6.390E−05**	**1.716E−02**	−0.1130	5.845E−01	9.998E−01	−0.3699	5.334E−04	2.462E−01
Ubac2	**−0.3748**	**6.566E−05**	**1.732E−02**	−0.2280	1.552E−02	9.443E−01	−0.0734	1.204E−01	8.771E−01
Nova1	**0.6060**	**6.856E−05**	**1.777E−02**	0.2648	7.041E−02	9.998E−01	0.1706	2.498E−02	6.812E−01
Ptgds	**−1.6596**	**7.009E−05**	**1.785E−02**	−0.4602	2.558E−01	9.998E−01	−0.5997	2.959E−03	3.605E−01
Cntnap5a	**0.7787**	**7.409E−05**	**1.797E−02**	0.3909	4.037E−02	9.998E−01	0.1939	4.467E−02	7.329E−01
Cit	**0.9118**	**7.371E−05**	**1.797E−02**	0.6235	6.443E−03	8.209E−01	0.1441	2.035E−01	9.263E−01
Slc20a1	**0.3071**	**7.478E−05**	**1.797E−02**	0.2245	4.194E−03	7.738E−01	0.0413	2.905E−01	9.443E−01

Table 3 (*continued*)

Gene ID	KO vs. WT			HT vs. WT			KO vs. HT		
	logFC	*P*-Value	FDR *P*-value	logFC	*P*-Value	FDR *P*-value	logFC	*P*-value	FDR *P*-value
Lrrtm3	**0.8080**	**7.542E−05**	**1.797E−02**	**0.8092**	**6.359E−05**	4.619E−01	−0.0006	9.949E−01	9.994E−01
Fam53b	**0.5043**	**7.863E−05**	**1.834E−02**	0.0684	5.987E−01	9.998E−01	0.2180	7.599E−04	2.512E−01
Cdon	**0.7665**	**7.947E−05**	**1.834E−02**	0.3634	6.201E−02	9.998E−01	0.2015	3.872E−02	7.130E−01
R3hdm2	**0.3424**	**8.228E−05**	**1.870E−02**	0.1582	6.914E−02	9.998E−01	0.0921	3.418E−02	7.000E−01
Rprml	**−0.8683**	**8.893E−05**	**1.976E−02**	−0.2619	2.362E−01	9.998E−01	−0.3032	6.772E−03	4.713E−01
Gm22405	**−2.2935**	**9.097E−05**	**1.976E−02**	−1.4109	1.486E−02	9.443E−01	−0.4413	1.328E−01	8.955E−01
Fgd2	**1.2255**	**9.010E−05**	**1.976E−02**	0.8777	4.495E−03	7.738E−01	0.1739	2.363E−01	9.398E−01
Zfp365	**0.3791**	**9.500E−05**	**1.976E−02**	0.0822	3.955E−01	9.998E−01	0.1485	1.970E−03	2.984E−01
Obscn	**0.9565**	**9.380E−05**	**1.976E−02**	0.5057	3.871E−02	9.998E−01	0.2254	6.629E−02	7.829E−01
Gm28370	**0.6501**	**9.390E−05**	**1.976E−02**	0.4202	1.352E−02	9.443E−01	0.1150	1.574E−01	9.128E−01
Agps	**0.3853**	**1.036E−04**	**2.125E−02**	0.1543	1.258E−01	9.998E−01	0.1155	2.106E−02	6.480E−01
Snca	**−0.4367**	**1.050E−04**	**2.125E−02**	−0.2042	6.800E−02	9.998E−01	−0.1162	3.756E−02	7.077E−01
Rcan2	**0.4698**	**1.078E−04**	**2.151E−02**	0.3143	9.596E−03	8.688E−01	0.0777	1.996E−01	9.255E−01
Galnt13v	**0.6550**	**1.108E−04**	**2.183E−02**	0.2291	1.673E−01	9.998E−01	0.2130	1.025E−02	5.271E−01
Kdm5d	**−4.6329**	**1.151E−04**	**2.208E−02**	−1.3754	6.847E−02	9.998E−01	−1.6287	1.937E−02	6.282E−01
B930095G15Rik	**0.6217**	**1.147E−04**	**2.208E−02**	0.4885	3.026E−03	7.406E−01	0.0666	4.017E−01	9.478E−01
Stard5	**−0.5076**	**1.270E−04**	**2.406E−02**	−0.3388	1.051E−02	8.974E−01	−0.0844	2.051E−01	9.272E−01
Olfm3	**0.6911**	**1.309E−04**	**2.448E−02**	**0.6636**	**2.247E−04**	4.619E−01	0.0138	8.778E−01	9.924E−01
Tshz2	**1.8146**	**1.336E−04**	**2.466E−02**	0.8524	6.725E−02	9.998E−01	0.4811	3.457E−02	7.013E−01
Rxfp1	**1.7468**	**1.420E−04**	**2.527E−02**	0.6832	1.310E−01	9.998E−01	0.5318	1.641E−02	5.801E−01
Satb2	**1.2285**	**1.390E−04**	**2.527E−02**	0.6804	3.054E−02	9.998E−01	0.2740	8.687E−02	8.402E−01
Yam1	**−1.8381**	**1.415E−04**	**2.527E−02**	**−1.8508**	**1.697E−04**	4.619E−01	0.0064	9.785E−01	9.989E−01
Dcaf12l2	**−0.9540**	**1.438E−04**	**2.529E−02**	−0.2825	2.562E−01	9.998E−01	−0.3358	7.761E−03	4.838E−01
Kcnab3	**0.7636**	**1.541E−04**	**2.677E−02**	0.4629	2.285E−02	9.959E−01	0.1503	1.469E−01	9.053E−01
Gm11914	**−0.7652**	**1.648E−04**	**2.750E−02**	−0.0130	9.484E−01	9.998E−01	**−0.3761**	**2.296E−04**	1.785E−01
Tesc	**−0.6218**	**1.652E−04**	**2.750E−02**	−0.2546	1.118E−01	9.998E−01	−0.1836	2.571E−02	6.812E−01
Slc35e2	**0.4057**	**1.625E−04**	**2.750E−02**	0.2064	5.572E−02	9.998E−01	0.0996	6.372E−02	7.763E−01
Hkdc1	**0.8737**	**1.657E−04**	**2.750E−02**	0.5539	1.801E−02	9.498E−01	0.1599	1.720E−01	9.210E−01
Rnf217	**0.5521**	**1.696E−04**	**2.784E−02**	0.5010	5.547E−04	5.847E−01	0.0255	7.228E−01	9.797E−01
Dcdc2a	**−0.6627**	**1.716E−04**	**2.785E−02**	−0.2704	1.225E−01	9.998E−01	−0.1962	2.578E−02	6.812E−01
Calcrl	**−0.5534**	**1.910E−04**	**2.794E−02**	−0.0688	6.409E−01	9.998E−01	−0.2423	6.780E−04	2.512E−01
Rph3a	**0.5848**	**1.841E−04**	**2.794E−02**	0.0880	5.685E−01	9.998E−01	0.2484	1.260E−03	2.617E−01
Tacc1	**0.4630**	**1.885E−04**	**2.794E−02**	0.1262	3.085E−01	9.998E−01	0.1684	6.032E−03	4.713E−01
Fcgbp	**−1.2194**	**1.743E−04**	**2.794E−02**	−0.3520	2.712E−01	9.998E−01	−0.4337	7.536E−03	4.829E−01
Grm2	**0.9173**	**1.909E−04**	**2.794E−02**	0.3328	1.632E−01	9.998E−01	0.2922	1.665E−02	5.801E−01
Syt2	**1.1424**	**1.802E−04**	**2.794E−02**	0.4946	9.704E−02	9.998E−01	0.3239	3.012E−02	6.878E−01
Plxdc1	**1.2835**	**1.835E−04**	**2.794E−02**	0.7993	1.605E−02	9.443E−01	0.2421	1.538E−01	9.084E−01
Mcc	**0.5805**	**1.899E−04**	**2.794E−02**	0.4434	4.223E−03	7.738E−01	0.0685	3.637E−01	9.457E−01
Dsc3	**2.9992**	**1.850E−04**	**2.794E−02**	2.3767	2.806E−03	7.309E−01	0.3113	4.064E−01	9.479E−01
Brinp3	**0.7647**	**1.800E−04**	**2.794E−02**	0.6185	2.243E−03	7.120E−01	0.0731	4.726E−01	9.510E−01
Epha5	**0.6288**	**1.943E−04**	**2.813E−02**	0.2785	9.477E−02	9.998E−01	0.1751	3.901E−02	7.130E−01
Grik3	**0.9567**	**1.978E−04**	**2.837E−02**	0.6005	1.851E−02	9.518E−01	0.1781	1.559E−01	9.089E−01
Nxph3	**1.2644**	**2.091E−04**	**2.970E−02**	0.8348	1.607E−02	9.443E−01	0.2148	2.023E−01	9.256E−01
Smarcd3	**−0.4134**	**2.275E−04**	**3.171E−02**	−0.1088	3.258E−01	9.998E−01	−0.1523	6.890E−03	4.713E−01
Scn4b	**1.3640**	**2.263E−04**	**3.171E−02**	0.6231	8.663E−02	9.998E−01	0.3705	4.042E−02	7.215E−01
Grb7	**1.9920**	**2.303E−04**	**3.179E−02**	1.4475	6.829E−03	8.209E−01	0.2723	2.977E−01	9.443E−01
Crym	**−0.5688**	**2.336E−04**	**3.195E−02**	−0.3365	2.362E−02	9.998E−01	−0.1162	1.241E−01	8.818E−01
Tbc1d30	**0.4939**	**2.387E−04**	**3.235E−02**	0.2748	3.946E−02	9.998E−01	0.1095	1.030E−01	8.576E−01
Cbln2	**0.9211**	**2.441E−04**	**3.248E−02**	0.4791	5.943E−02	9.998E−01	0.2210	8.262E−02	8.359E−01
Wdr83	**−0.4093**	**2.432E−04**	**3.248E−02**	−0.2675	1.656E−02	9.498E−01	80.0709	2.002E−01	9.255E−01
Cecr2	**0.6876**	**2.490E−04**	**3.284E−02**	0.0912	6.358E−01	9.998E−01	0.2982	1.826E−03	2.900E−01
Vipr1	**0.5246**	**2.558E−04**	**3.314E−02**	0.1278	3.893E−01	9.998E−01	0.1984	7.324E−03	4.822E−01
Gm27177	**4.2839**	**2.545E−04**	**3.314E−02**	2.8819	1.195E−02	9.425E−01	0.7010	1.897E−01	9.218E−01
Mgp	**−1.1996**	**2.700E−04**	**3.468E−02**	−0.8580	8.336E−03	8.610E−01	−0.1708	2.776E−01	9.441E−01
Limk2	**0.2955**	**2.756E−04**	**3.509E−02**	0.1775	3.353E−02	9.998E−01	0.0590	1.560E−01	9.089E−01
Fam217b	**0.2971**	**2.896E−04**	**3.538E−02**	0.1064	1.954E−01	9.998E−01	0.0953	1.996E−02	6.357E−01
Zdhhc14	**−0.4323**	**2.898E−04**	**3.538E−02**	−0.1936	9.898E−02	9.998E−01	−0.1194	4.400E−02	7.328E−01
Ttc30b	**−0.3918**	**2.893E−04**	**3.538E−02**	−0.1829	9.113E−02	9.998E−01	−0.1044	5.473E−02	7.501E−01
Cacng3	**0.4936**	**2.898E−04**	**3.538E−02**	0.2444	6.276E−02	9.998E−01	0.1246	6.737E−02	7.843E−01
Kcnt1	**0.6121**	**2.888E−04**	**3.538E−02**	0.4703	6.106E−03	8.209E−01	0.0709	4.073E−01	9.479E−01
Cdh7	**0.7944**	**2.973E−04**	**3.599E−02**	0.6049	5.484E−03	8.100E−01	0.0948	3.819E−01	9.457E−01

(*continued on next page*)

Table 3 (*continued*)

Gene ID	KO vs. WT			HT vs. WT			KO vs. HT		
	logFC	*P*-Value	FDR *P*-value	logFC	*P*-Value	FDR *P*-value	logFC	*P*-value	FDR *P*-value
Sh3bgrl	**−0.3908**	**3.202E−04**	**3.845E−02**	−0.2139	4.429E−02	9.998E−01	−0.0885	1.032E−01	8.576E−01
Tyro3	**0.3389**	**3.257E−04**	**3.880E−02**	0.0525	5.711E−01	9.998E−01	0.1432	2.374E−03	3.217E−01
Ank1	**0.6262**	**3.296E−04**	**3.894E−02**	0.3612	4.014E−02	9.998E−01	0.1325	1.306E−01	8.922E−01
Rhobtb2	**0.4320**	**3.418E−04**	**4.006E−02**	0.2404	3.852E−02	9.998E−01	0.0958	1.074E−01	8.639E−01
Kif5a	**0.3307**	**3.524E−04**	**4.066E−02**	0.1806	5.095E−02	9.998E−01	0.0750	1.044E−01	8.586E−01
Gm5639	**−1.0324**	**3.520E−04**	**4.066E−02**	−0.7001	1.528E−02	9.443E−01	−0.1661	2.621E−01	9.423E−01
Gatsl2	**0.4026**	**3.590E−04**	**4.110E−02**	0.1782	1.159E−01	9.998E−01	0.1122	4.651E−02	7.355E−01
Arhgef10l	**0.5565**	**3.676E−04**	**4.141E−02**	0.2019	1.723E−01	9.998E−01	0.1773	2.326E−02	6.713E−01
Trim66	**0.8119**	**3.701E−04**	**4.141E−02**	0.5932	8.757E−03	8.649E−01	0.1094	3.261E−01	9.457E−01
Pcdh15	**0.9567**	**3.665E−04**	**4.141E−02**	0.9113	7.126E−04	5.847E−01	0.0227	8.649E−01	9.921E−01
Lama3	**1.1681**	**3.737E−04**	**4.150E−02**	0.5850	7.442E−02	9.998E−01	0.2916	7.112E−02	8.013E−01
Stk3	**−0.3788**	**3.809E−04**	**4.179E−02**	−0.0391	6.976E−01	9.998E−01	−0.1698	1.468E−03	2.617E−01
Prss23	**−0.7360**	**3.820E−04**	**4.179E−02**	−0.5581	6.054E−03	8.209E−01	−0.0889	3.671E−01	9.457E−01
G8m24447	**−1.3571**	**3.849E−04**	**4.180E−02**	−0.9883	1.243E−02	9.425E−01	−0.1844	3.447E−01	9.457E−01
Arap2	**0.5580**	**3.929E−04**	**4.236E−02**	0.2518	1.097E−01	9.998E−01	0.1531	5.159E−02	7.478E−01
Pi15	**−1.0084**	**3.990E−04**	**4.270E−02**	−0.1340	6.215E−01	9.998E−01	−0.4372	2.229E−03	3.149E−01
Galk2	**−0.3509**	**4.069E−04**	**4.317E−02**	−0.0144	8.856E−01	9.998E−01	−0.1682	8.177E−04	2.512E−01
Rapgef3	**0.4784**	**4.092E−04**	**4.317E−02**	0.2501	6.167E−02	9.998E−01	0.1142	8.934E−02	8.430E−01
Arhgef15	**0.6324**	**4.133E−04**	**4.329E−02**	0.3132	7.582E−02	9.998E−01	0.1596	6.946E−02	7.918E−01
Sytl2	**0.5686**	**4.203E−04**	**4.372E−02**	0.2429	1.333E−01	9.998E−01	0.1629	4.235E−02	7.249E−01
Zmat4	**0.5322**	**4.252E−04**	**4.392E−02**	0.1010	4.954E−01	9.998E−01	0.2156	3.267E−03	3.676E−01
Pik3c2b	**0.6788**	**4.317E−04**	**4.398E−02**	0.2323	2.393E−01	9.998E−01	0.2232	2.332E−02	6.713E−01
Rgs6	**0.9177**	**4.299E−04**	**4.398E−02**	0.5679	2.817E−02	9.998E−01	0.1749	1.717E−01	9.210E−01
Cacna2d2	**0.4855**	**4.381E−04**	**4.432E−02**	0.3532	9.762E−03	8.688E−01	0.0662	3.401E−01	9.457E−01
Epb4.1	**0.7239**	**4.545E−04**	**4.532E−02**	0.1620	4.260E−01	9.998E−01	0.2810	5.182E−03	4.424E−01
Dock3	**0.7209**	**4.613E−04**	**4.532E−02**	0.2911	1.533E−01	9.998E−01	0.2149	3.668E−02	7.063E−01
Slc8a3	**0.6069**	**4.511E−04**	**4.532E−02**	0.2673	1.186E−01	9.998E−01	0.1698	4.152E−02	7.240E−01
Ncald	**0.5018**	**4.622E−04**	**4.532E−02**	0.2284	1.039E−01	9.998E−01	0.1367	4.919E−02	7.420E−01
Gm5616	**−0.4293**	**4.634E−04**	**4.532E−02**	−0.2619	3.185E−02	9.998E−01	−0.0837	1.756E−01	9.210E−01
Tmem180	**−0.3773**	**4.738E−04**	**4.604E−02**	−0.1906	7.419E−02	9.998E−01	−0.0933	8.470E−02	8.384E−01
Agap1	**0.3895**	**4.858E−04**	**4.607E−02**	0.1665	1.329E−01	9.998E−01	0.1115	4.560E−02	7.348E−01
Map3k9	**0.7193**	**4.847E−04**	**4.607E−02**	0.3765	6.769E−02	9.998E−01	0.1714	9.319E−02	8.465E−01
Atp10b	**1.2795**	**4.897E−04**	**4.607E−02**	0.6771	6.617E−02	9.998E−01	0.3012	1.031E−01	8.576E−01
Arhgap32	**0.5381**	**4.873E−04**	**4.607E−02**	0.3709	1.611E−02	9.443E−01	0.0836	2.780E−01	9.442E−01
Mapk6	**0.4079**	**4.857E−04**	**4.607E−02**	0.3133	6.890E−03	8.209E−01	0.0473	4.179E−01	9.479E−01
Fibcd1	**−0.8530**	**4.940E−04**	**4.618E−02**	−0.4837	5.282E−02	9.998E−01	−0.1847	1.484E−01	9.053E−01
Cpne2	**−1.2501**	**5.141E−04**	**4.757E−02**	−0.1021	7.650E−01	9.998E−01	−0.5740	1.408E−03	2.617E−01
Gm6788	**−0.5516**	**5.250E−04**	**4.757E−02**	−0.0535	7.368E−01	9.998E−01	−0.2490	1.715E−03	2.814E−01
Gst8m6	**−0.5525**	**5.179E−04**	**4.757E−02**	−0.1977	2.082E−01	9.998E−01	−0.1774	2.568E−02	6.812E−01
Nrros	**−0.5870**	**5.241E−04**	**4.757E−02**	−0.3482	4.270E−02	9.998E−01	−0.1194	1.722E−01	9.210E−01
Gucy1a3	**0.5850**	**5.231E−04**	**4.757E−02**	0.5019	2.507E−03	7.120E−01	0.0416	6.213E−01	9.711E−01
Dock10	**0.6370**	**5.379E−04**	**4.786E−02**	0.0765	6.802E−01	9.998E−01	0.2802	2.561E−03	3.328E−01
Ssbp4	**−0.4606**	**5.317E−04**	**4.786E−02**	−0.1781	1.727E−01	9.998E−01	−0.1412	3.273E−02	6.915E−01
Wwox	**0.4364**	**5.376E−04**	**4.786E−02**	0.3074	1.511E−02	9.443E−01	0.0645	3.053E−01	9.455E−01
Syt6	**−0.7500**	**5.528E−04**	**4.889E−02**	−0.0377	8.615E−01	9.998E−01	−0.3561	1.080E−03	2.617E−01
Nlk	**0.3939**	**5.674E−04**	**4.939E−02**	0.1694	1.339E−01	9.998E−01	0.1122	4.566E−02	7.348E−01
Prrg3	**0.6091**	**5.649E−04**	**4.939E−02**	0.4106	1.976E−02	9.762E−01	0.0992	2.529E−01	9.423E−01
Orai3	**−0.3671**	**5.684E−04**	**4.939E−02**	−0.2533	1.729E−02	9.498E−01	−0.0569	2.767E−01	9.441E−01

KO–PDE11A knockout; WT–PDE11A wild-type; HT–PDE11A heterozygous mouse.

Note: In the HT vs. WT and KO vs. HT analyses, no genes were significant at the level of the whole genome (i.e., all FDR-corrected *P*-values were > 0.05). That said, *P*-values for a number of genes reached significance in both analyses when the stricter Bonferroni correction was applied but limited to the number of genes within this specific gene set (i.e., 0.05/169 = $P < 0.00029$; shown in bold).

adult HT and KO mice showed deficits (Fig. 3). In fact, adolescent PDE11A KO mice demonstrated significant social approach toward their cagemate vs. a novel object when adolescent PDE11A WT and HT littermates demonstrated no such preference (Fig. 3B). Overall, these data suggest deficits observed in the adult mutant mice are age dependent.

A social mouse prefers to spend time with a PDE11A WT versus a PDE11A KO but a PDE11A KO prefers to spend time with another KO

The above experiments suggest that adult PDE11A mutant mice exhibit altered social behaviors in specific social contexts; however, this is based on a human definition of

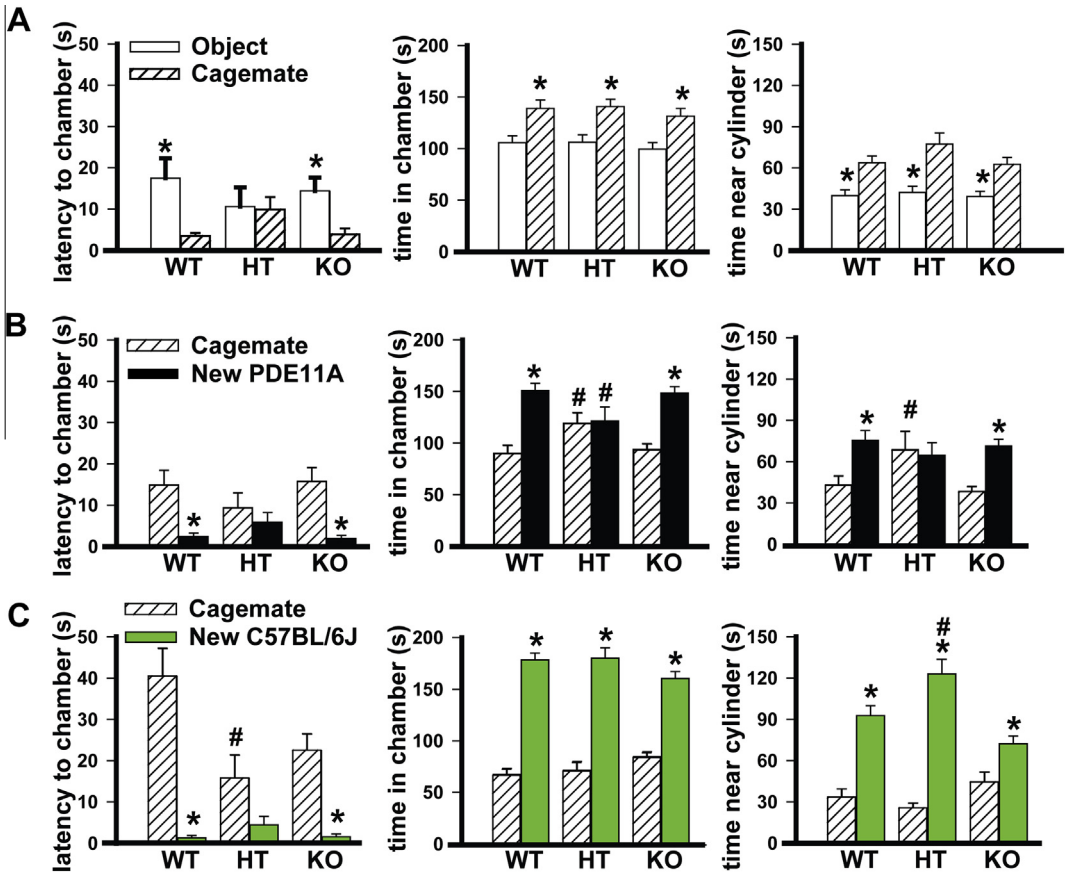

Fig. 2. Replacing the new object with a cagemate aligned social approach behaviors of PDE11A mutant males and females, with PDE11A mutant mice showing context-dependent changes in social approach behavior. (A) Relative to PDE11A wild-type (WT) mice, PDE11A HT and KO mice generally demonstrated intact social approach toward their own cagemate. (Left) an exception exists in that HTs fail to differentiate their latency to approach a cagemate vs. a novel object (latency: $F(1,60) = 5.94$, $P = 0.018$); however, all mice demonstrate significant social preference for the cagemate in terms of (middle) chamber time ($F(1,63) = 15.01$, $P < 0.001$) and (right) cylinder time ($F(1,60) = 32.79$, $P < 0.001$). (B) When given a choice between a cagemate and a novel PDE11A WT, PDE11A KO mice exhibited significant social approach in terms of (left) their latency to approach the chamber ($F(1,24) = 13.75$, $P = 0.001$), (middle) time in chamber ($F(1,25) = 28.39$, $P = 0.001$), and (right) time near the cylinder ($F(1,23) = 20.41$, $P < 0.001$), as did PDE11A WT mice (latency: $F(1,22) = 15.18$, $P < 0.001$; chamber time: $F(1,23) = 16.24$, $P < 0.001$; cylinder time: $F(1,23) = 7.46$, $P = 0.012$). Surprisingly, PDE11A HT mice showed further impairments in social approach such that both male and female HTs failed to demonstrate any preference for the new PDE11A WT over their own cagemate on any measure. Further, PDE11A HT mice spent significantly more time in the chamber of the cagemate than did PDE11A WT and KO mice ($F(2,63) = 5.12$, $P = 0.009$) and significantly less time in the chamber of the new PDE11A WT than did PDE11A WT and KO mice ($F(2,65) = 4.52$, P = 0.015). (C) Similarly, PDE11A HT mice failed to show a significant preference in their latency to approach a new C57BL/6J mouse vs. their own cagemate, unlike the PDE11A WT ($F(1,24) = 31.54$, $P < 0.001$) and KO mice ($F(1,25) = 28.15$, $P < 0.001$). This is due to the fact that PDE11A HT approached their cagemate significantly sooner than did PDE11A WT mice ($F(2,65) = 3.85$, $P = 0.026$). A similar trend was observed in the PDE11A KO mice (*Post hoc*, $P = 0.079$). (Middle) despite these differences in latency to approach, PDE11A WT ($F(1,23) = 85.83$, $P < 0.001$), HT ($F(1,14) = 45.43$, $P < 0.001$), and KO mice ($F(1,26) = 48.12$, $P < 0.001$) all showed a significant preference for the new C57BL/6J mouse over their own cagemate in terms of time spent in each chamber. (Right) PDE11A WT ($F(1,23) = 30.91$, $P < 0.001$), HT ($F(1,13) = 83.04$, $P < 0.001$), and KO mice ($F(1,22) = 6.84$, $P = 0.016$) also showed a significant preference for the new C57BL/6J mouse over their own cagemate in terms of time spent near the cylinder. That said, there was a significant difference among the genotypes in terms of how much time was spent near the cylinder of the C57BL/6J mice ($F(2,63) = 10.90$, $P < 0.001$), with PDE11A HT mice spending significantly more time relative to WT mice and PDE11A KO mice showing a strong trend toward spending less time than WT mice (*Post hoc*, $P = 0.057$). (F, G) WT-F, $n = 14$; HT-F, $n = 9$; KO-F, $n = 15$; WT-M, $n = 12$; HT-M, $n = 7$; KO-M, $n = 11$. *Post hoc*, *vs. cagemate, $P < 0.05$–0.001; $^{\#}$vs. WT, $P = 0.025$–0.004.

what is an appropriate social behavior for a mouse. Therefore, we determined if a C57BL/6J "mouse psychiatrist" would be able to detect abnormal behavior in PDE11A mutant mice. C57BL/6J mice strongly preferred to spend time with PDE11A WT versus KO mice, particularly during the last 5 min of the 30-min session (Fig. 4A–C). In contrast, C57BL/6J mice preferred PDE11A WT over HT mice during the first 5 min, but then switched to prefer the HTs over the WTs during the last 5 min (Fig. 4D–F). In contrast, PDE11A KO mice preferred to spend time with another PDE11A KO vs. a WT mouse, particularly during the final 5 min of the session (Fig. 4G–I). These data suggest other mice are able to detect differences in PDE11A mutant mouse behavior.

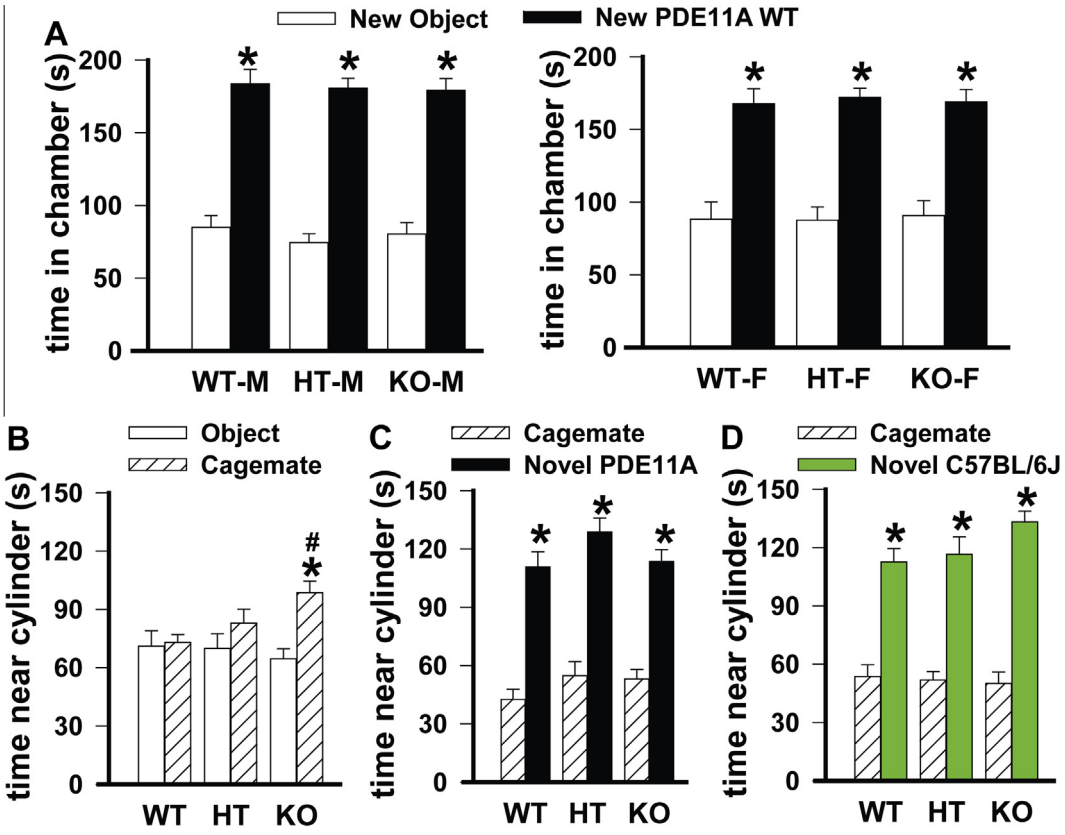

Fig. 3. Adolescent PDE11A heterozygous (HT) and knockout (KO) mice show no deficits in social approach behavior relative to PDE11A wild-type (WT) littermates. (A) Unlike adult male PDE11A HT and KO mice (see Figs. 1 and 2), adolescent PDE11A HT and KO mice show no deficits in social approach relative to PDE11A WT littermates in the "object vs. novel PDE11A" version of the assay (females, $F(2,47) = 162.60$, $P < 0.001$; WT, $n = 9$; HT, $n = 9$; KO, $n = 10$) (males, $F(2,78) = 304.12$, $P < 0.001$; WT, $n = 13$; HT, $n = 13$; KO, $n = 17$). (B) In the presence of a cagemate, male and female adolescent PDE11A HT and KO mice similarly show normal or better social approach behavior relative to sex-matched PDE11A WT littermates in terms of the latency to approach ($F(2,40) = 3.56$, $P = 0.038$), (C) the time spent in the chamber ($F(1,41) = 201.31$, $P < 0.001$), and (D) the time spent near the cylinder ($F(1,42) = 190.30$, $P < 0.001$). 3B-D WT-F, $n = 8$; HT-F, $n = 8$; KO-F, $n = 12$; WT-M, $n = 6$; HT-M, $n = 6$; KO-M, $n = 7$. *Post hoc*, *vs. object or cagemate, $P < 0.01$–0.001; #vs. WT, $P = 0.014$.

Social isolation decreases PDE11A4 protein expression in a subcellular compartment-specific manner

When humans lack proper social behaviors, they are often ostracized. We show this phenomenon extends to mice as well (Fig. 4). As such, we determined if social isolation might alter PDE11A4 protein expression. In biochemically fractionated ventral hippocampi of C57BL/6J mice, 1 month of social isolation decreased PDE11A4 expression in the membrane but not the cytosolic compartment (Fig. 5A).

Isolation impairs subsequent social approach and social odor recognition memory in a PDE11A genotype-dependent manner

Given that social isolation impairs subsequent social behaviors in humans (Kogan et al., 2000; Derntl et al., 2011; Shahar-Gold et al., 2013), we determined if isolation would impair subsequent social behaviors in a PDE11A genotype-dependent manner. If so, this would suggest that the isolation-induced decreases in PDE11A4 expression noted above are of functional consequence. To do so, we compared social behaviors in GH versus single-housed PDE11A WT, HT, and KO mice, reasoning that isolation should induce a dose–response shift across PDE11A genotypes. We tested social approach behavior in male mice in the "novel object vs. novel PDE11A" version of the assay and social odor recognition in both male and female mice. Across the two assays, behavior of socially isolated (SI) WTs shifted toward that of GH HTs and the behavior of SI HTs shifted toward that of GH KOs, with no effect of isolation on PDE11A KO behavior (Fig. 5B, C).

Deletion of PDE11A triggers gene expression changes in the oxytocin pathway

To determine the molecular mechanisms by which PDE11A deletion alters social behavior, we conducted RNA sequencing on VHIPP samples from adult male and female PDE11A WT, HT, and KO mice ($n = 7$/genotype). After FDR correction, 52 genes were down regulated and 117 genes were upregulated in PDE11A KO vs. WT mice (Table 3; Fig. 6). KEGG pathway analyses identified enrichment in only three pathways (Table 4). One of these three pathways is the oxytocin pathway, a major signaling pathway

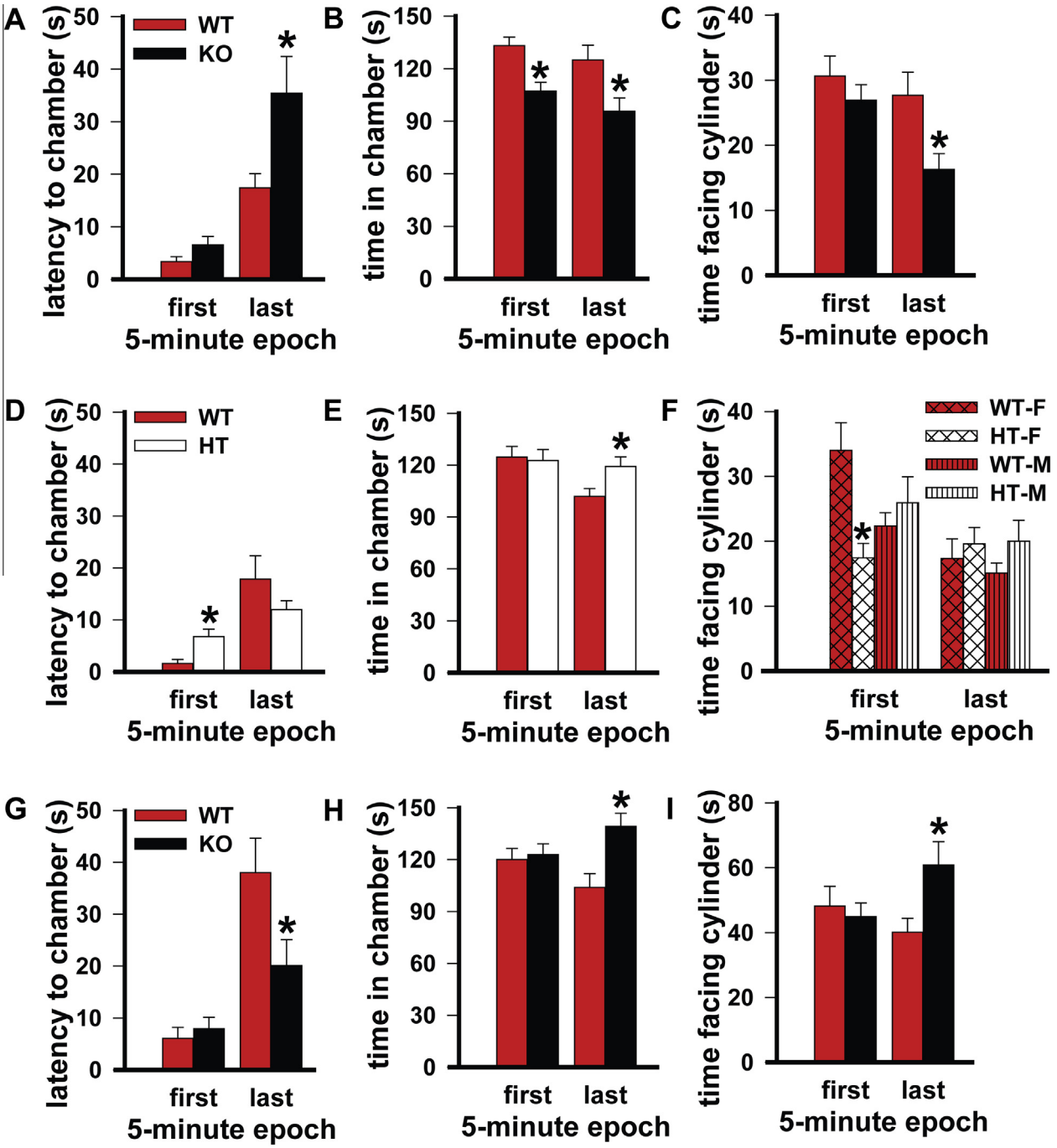

Fig. 4. Social C57BL/6J mice spend more time with a PDE11A wild-type (WT) mouse relative to a PDE11A knockout (KO) mouse; whereas, PDE11A KO mice spend more time with a PDE11A KO mouse relative to a WT mouse. (A) During a 30-min session, C57BL/6J mice (males, $n = 10$; females, $n = 14$) were given access to a sex-matched PDE11A WT and its PDE11A KO littermate (consistent effects observed across two cohorts and combined analyses are shown here). During the last 5 min, C57BL/6J mice approached the chamber of the PDE11A WT significantly faster than that of the KO littermate ($F(1,22) = 4.94$, $P = 0.018$). (B) In both the first ($F(1,23) = 4.37$, $P = 0.048$) and last 5 min ($F(1,23) = 4.94$, $P = 0.036$), C57BL/6J mice spent more time in the chamber of the PDE11A WT versus KO littermate. (C) During the last 5 min, C57BL/6J mice spent more time directly interacting with the cylinder of the PDE11A WT versus KO littermate ($F(1,21) = 7.6$, $P = 0.011$). (D) The pattern of results differed when C57BL/6J mice (males, $n = 12$; females, $n = 10$) were given access to a sex-matched PDE11A WT and its heterozygous (HT) littermate (consistent effects observed across two cohorts and combined analyses are shown here). C57BL/6J mice approached the PDE11A WT significantly faster than the HT littermate only during the first 5 min ($F(1,17) = 8.82$, $P = 0.009$). (E) In the last 5 min, C57BL/6J mice actually spent significantly more time in the chamber of the HT vs. WT littermate ($F(1,19) = 4.61$, $P = 0.045$). (F) Consistent with these effects shown in panels D and E, female C57BL/6J mice spent significantly more time interacting with the cylinder of the female PDE11A WT vs. HT littermate during the first 5 min ($F(1,17) = 9.61$, $P = 0.017$), and yet male and female C57BL/6J mice showed a trend toward spending more time interacting with the cylinder of the HT vs. WT littermate during the last 5 min ($F(1,17) = 4.05$, $P = 0.07$). (G) Interestingly, when PDE11A KO mice (male, $n = 6$; female, $n = 8$) were given access to a novel PDE11A WT and that WT's KO littermate, the PDE11A KO mice approached the novel PDE11A KO mouse chamber more quickly than that of the WT littermate ($F(1,12) = 6.90$, $P = 0.022$) and (H) spent significantly more time with the PDE11A KO mouse vs. the WT littermate in the chamber ($F(1,11) = 6.20$, $P = 0.03$) and (I) at the cylinder ($F(1,11) = 5.31$, $P = 0.042$). *Post hoc*, *vs. WT, $P = 0.048$–0.005.

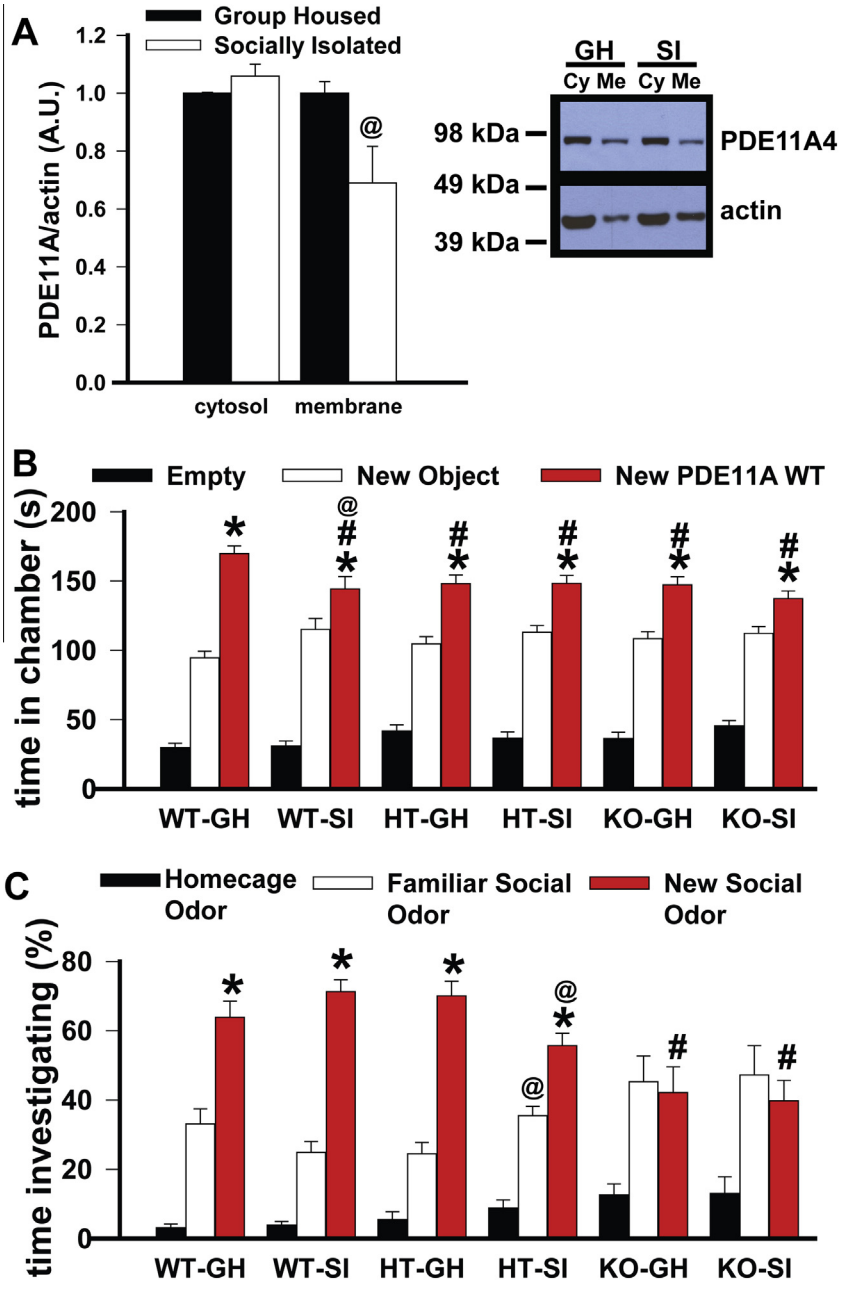

Fig. 5. Isolation-induced decreases in PDE11A4 expression are sufficient to impair subsequent social behavior. (A) Socially isolated (SI) C57BL/6J mice expressed significantly less PDE11A4 protein in the membrane compartment of the ventral hippocampal formation (VHIPP) relative to group-housed (GH) C57BL/6J mice ($F(1,12) = 5.65$, $P = 0.035$; $n = 7$ biological replicates (five males + two females)/group). (B) Isolation, then, induced a dose–response shift across PDE11A genotypes such that isolated PDE11A wild-type (WT) mice exhibited a behavioral profile similar to that of GH PDE11A heterozygous (HT) mice in social approach (assessed in males only, $F(10,156) = 2.61$, $P = 0.006$). WT-GH, $n = 16$; WT-SI, $n = 12$; HT-GH, $n = 14$; HT-SI, $n = 15$; KO-GH, $n = 16$; KO-SI, $n = 11$. (C) Similarly, isolated HTs shifted their behavioral profile toward that of PDE11A knockout (KO) mice in 24-h long-term memory for social odor recognition ($F(2,58) = 5.43$, $P = 0.007$). All males except where noted: WT-GH, $n = 17$; WT-SI, $n = 9$; HT-GH, $n = 17$ (includes three females); HT-SI, $n = 14$ (includes four females); KO-GH, $n = 10$; KO-SI, $n = 5$. $^{\#}$vs. WT-GH, $P < 0.03$–0.001; *vs. novel object/familiar odor, $P \leqslant 0.002$; @vs. GH, $P = 0.019$–0.003.

underlying social behaviors (c.f., (Viero et al., 2010; Ebstein et al., 2012; Lukas and Neumann, 2013; Stoesz et al., 2013; Feldman et al., 2015)) and the effects of social buffering (Chen et al., 2011; Hostinar et al., 2014). Consistent with a role for PDE11A in regulating behavior, the GO Biological Process and GO Cellular compartment most significantly affected by PDE11A deletion was synaptic transmission and the synapse, respectively (Table 4; Fig. 6). Given that social isolation specifically decreased PDE11A4 expression in membrane, it is interesting to note that PDE11A deletion also significantly affected several membrane-related GO pathways (e.g., synaptic membrane, Table 4).

DISCUSSION

Here we showed that PDE11A4, an enzyme enriched in the VHIPP, is a key regulator of social behavior and a key mechanism by which social experience feeds back to shape signaling in the brain. We showed that (i) PDE11A deletion impairs social behaviors of adults much more so than adolescents (Figs. 1–3), (ii) social deficits caused by the loss of PDE11A are sensitive to the social context (Figs. 1, 2 and 4), (iii) social isolation decreases PDE11A4 expression in a subcellular compartment-specific manner (Fig. 5A), (iv) isolation impairs subsequent social behaviors in a PDE11A genotype-dependent manner, suggesting that isolation-induced decreases in PDE11A4 expression do contribute to subsequent impairments in social behaviors (Fig. 5B, C), and (v) deletion of PDE11A4 significantly alters expression of genes in a major signaling pathway underlying social behavior (Table 4, Fig. 6), namely the oxytocin pathway (c.f. (Viero et al., 2010; Ebstein et al., 2012; Lukas and Neumann, 2013; Stoesz et al., 2013)). We believe that social approach deficits measured here in PDE11A mutant mice likely reflect a fundamental deficit in social approach behavior as opposed to a simple failure to detect social novelty because PDE11A HT and KO mice retain the ability to differentiate familiar versus novel social odors when presented via wooden beads, although the consolidation of long-term social memory is impaired (Fig. 5C; (Kelly et al., 2010; Hegde et al., 2016)). Thus, phenotypes exhibited by PDE11A mutant mice are reminiscent of social deficits observed in patients with certain neurodevelopmental disorders (e.g., (Miller et al., 2002; Johnstone et al., 2005; Jahr et al., 2007; Locke et al., 2010; Giacco et al., 2012; Dean et al., 2014)), in that mutant mice initiate social interactions differently relative to WT mice and a social mouse chooses to interact less with the PDE11A KOs.

Although the effect sizes of the social approach deficits noted in the PDE11A mutant mice are arguably limited, they are reproducible and their magnitude is well in line with the sparse nature of PDE11A4 expression in the brain (i.e., limited to a subpopulation of neurons in CA1, subiculum, and the amygdalohippocampal area (Kelly et al., 2010, 2014; Kelly, 2015; Hegde et al., 2016)). The effect of PDE11A deletion on social interactions is clearly dependent on the social context as changing the stimulus mouse strain (i.e., new PDE11A vs. cagemate vs. new C57BL/6J mouse) or the nature of the alternative option (i.e., new object vs. cagemate), distinctly alters the phenotype of PDE11A HT and KO mice. Although PDE11A mutant mice clearly show context-dependent alterations in their social approach behaviors, it remains to be determined what is driving the context specificity of the effects. It may be that deletion of PDE11A affects motivational aspects of task performance. For example, the social approach deficits noted in male PDE11A mutant mice in the ‘‘new object vs. new PDE11A mouse” context may reflect a slight aversion for the new mouse or simply a stronger attraction toward the novel object. Similarly, the fact that male PDE11A KO mice exhibit reduced social approach in the ‘‘object vs. new PDE11A WT” context, but not in the ‘‘cagemate vs. new PDE11A mouse” context, may reflect a positive effect of social buffering in the latter (i.e., the stress-reducing effect elicited by the presence of a conspecific (Ditzen and Heinrichs, 2014; Hostinar et al., 2014; Gobrogge and Wang, 2015)), since it does not appear that a PDE11A mutant mouse is simply more motivated to explore a novel object vs. a familiar mouse (Fig. 2A). Indeed, PDE11A4 is expressed in VHIPP neurons that project to the nucleus accumbens (Cembrowski et al., 2016), suggesting PDE11A4 may be important in regulating how motivated one is to engage in social interactions or how rewarding one finds those social interactions. Whatever motivations drive the altered social interactions of a PDE11A mutant mouse, those alterations appear to be recognizable by another mouse as the ‘‘mouse psychiatrist” assay shows a C57BL/6J mouse prefers to interact with a PDE11A WT mouse over a PDE11A KO mice while a PDE11A KO mouse prefers to interact with another PDE11A KO mouse over a PDE11A WT mouse. The fact that PDE11A KO mice prefer another KO vs. a WT in the ‘‘mouse psychiatrist” assay suggests that a PDE11A KO mouse might even show stronger social approach relative to a WT when given the choice between a novel object and another PDE11A KO mouse. Findings in the ‘‘mouse psychiatrist” assay are consistent with reports in humans showing people tend to associate with like-minded people (Launay and Dunbar, 2015) and individuals with neurodevelopmental or psychiatric illnesses tend to socially withdraw and/or be ostracized (e.g., (Miller et al., 2002; Johnstone et al., 2005; Locke et al., 2010; Giacco et al., 2012; Dean et al., 2014)).

Interestingly, deleting PDE11A changes social behaviors in a manner that does not strictly follow a gene-dosage relationship (Figs. 1, 2 and 4). This suggests that full deletion of PDE11A may trigger a protective compensatory mechanism that partial deletion does not. Consistent with this idea, the RNAseq experiment shows that PDE11A KO mice exhibit a significant reduction relative to PDE11A WT and HT mice in insulin-like growth factor binding protein 3 (IGFBP3), elevated expression of which is associated with chronic loneliness in humans (Cole et al., 2007). Although some questions remain, data presented here strongly argue that PDE11A is a key regulator of social interactions, which is consistent with the fact that PDE11A has been genetically and/or functionally associated with clinical phenotypes related to changes in social function, including major depressive disorder (Wong et al., 2006; Couzin, 2008; Cabanero et al., 2009; Luo et al., 2009; Kelsoe, 2010; Coon et al., 2013), suicide risk (an inactivating mutation, (Coon et al., 2013)), and lithium responsivity (Couzin, 2008; Kelsoe, 2010; Mertens et al., 2015; Pathak et al., in press).

It remains to be determined whether PDE11A is more relevant to social deficits observed in adulthood disorders, such as schizophrenia, or those observed in childhood disorders, such as autism. Adolescent PDE11A mutant mice exhibit normal social approach behaviors in social contexts; whereas, adult PDE11A mutant mice show

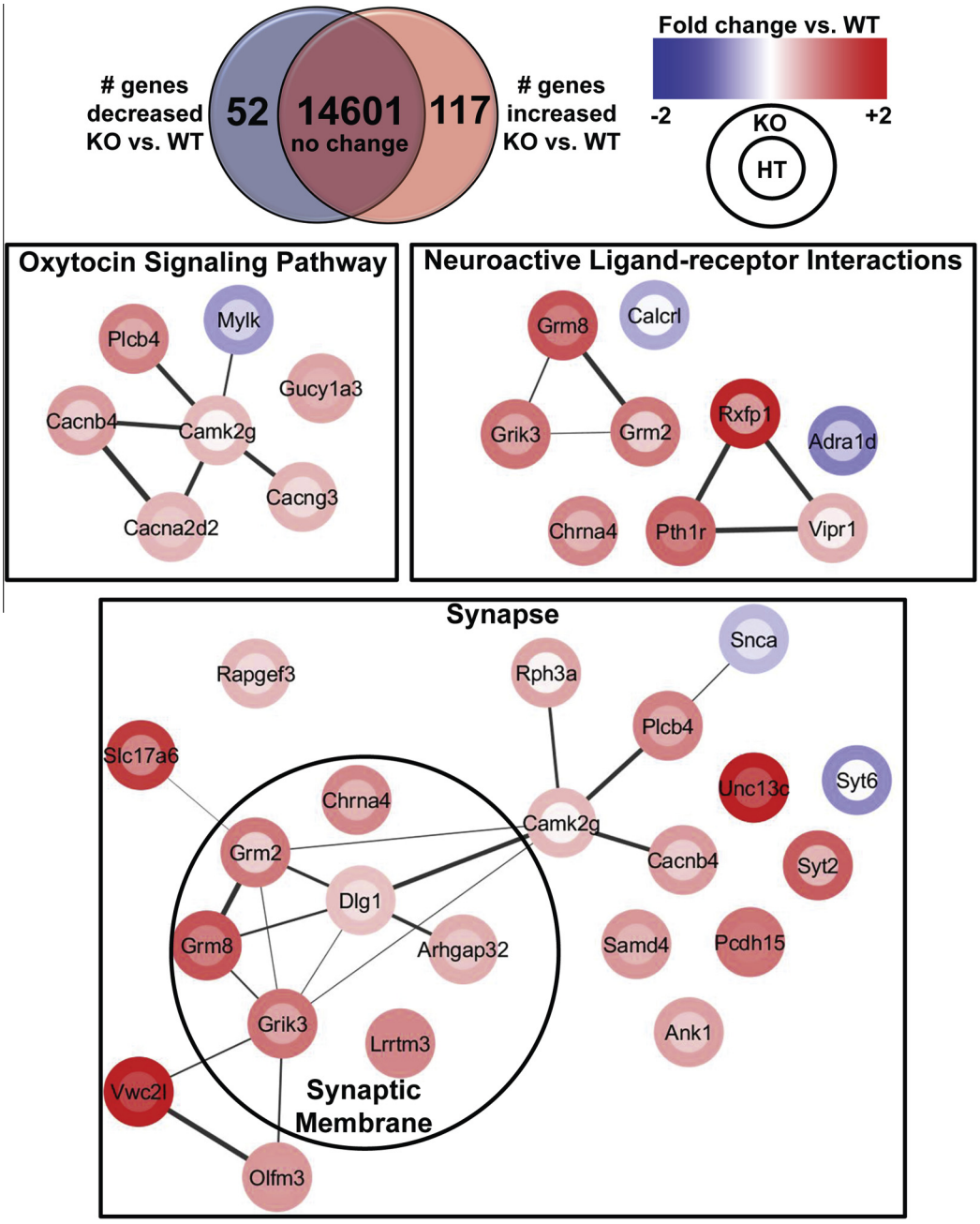

Fig. 6. RNA sequencing of VHIPP reveals 52 downregulated and 116 upregulated genes in PDE11A knockout (KO) mice relative to PDE11A wild-type (WT) littermates, with significant enrichment of genes in pathways related to social behavior. Pathway genes and edges generated by STRING 10 (medium confidence) for the oxytocin (FDR $P = 0.014$), neuroactive-ligand (FDR $P = 0.014$), synapse (FDR $P < 0.001$), and synaptic membrane pathways (FDR $P = 0.038$) are shown here. Nodes are color coded for the log-fold change in gene expression of PDE11A KO mice (outer rim) and PDE11A heterozygous (HT) mice (inner circle) relative to WT littermates. See Table 3 for the complete list of genes that significantly differed between PDE11A KO vs. WT mice that were included in the pathway analyses and Table 4 for the full list of KEGG and GO pathways demonstrating significant gene enrichment. Gene changes in PDE11A HT mice are also detailed in Table 3. $n = 7$ (three males, four females)/genotype).

reduced social approach (Fig. 3). Such an age-dependent trajectory is thought to be relevant in modeling aspects of schizophrenia in rodents (Feleder et al., 2010), since schizophrenia is thought to be caused by neurodevelopmental insults that do not fully manifest until early adulthood. That said, adolescent PDE11A KO mice are impaired in other types of social behaviors. Previously we showed that both adolescent and adult PDE11A mutant mice show significant impairments in consolidating long-term memories involving social information (Kelly et al., 2010; Hegde et al., 2016). This raises the interesting possibility that adolescent PDE11A mutant mice have a fundamental inability to form long-term memories for what is and what is not a socially-acceptable behavior, which ultimately leads to alterations in social approach behavior at a later age. Indeed, adolescents

Table 4. Pathway analyses following RNA sequencing of VHIPP from male and female adult PDE11A wild-type versus knockout mice

GO_id	Term	# Genes	p-value_fdr
KEGG pathways			
4261	Adrenergic signaling in cardiomyocytes	8	1.74E−03
4921	Oxytocin signaling pathway	7	1.37E−02
4080	Neuroactive ligand-receptor interaction	9	1.37E−02
GO biological processes			
GO:0007268	Synaptic transmission	12	2.97E−02
GO:0051239	Regulation of multicellular organismal process	35	2.97E−02
GO:0030001	Metal ion transport	13	2.97E−02
GO:0007196	Adenylate cyclase-inhibiting G-protein-coupled glutamate receptor signaling pathway	3	3.07E−02
GO:0006812	Cation transport	15	3.07E−02
GO:0051056	Regulation of small gtpase-mediated signal transduction	12	3.07E−02
GO:0065008	Regulation of biological quality	36	3.46E−02
GO:0006816	Calcium ion transport	8	4.21E−02
GO Cellular components			
GO:0045202	Synapse	22	2.83E−06
GO:0042995	Cell projection	34	2.20E−05
GO:0097458	Neuron part	25	4.47E−04
GO:0044456	Synapse part	15	1.05E−03
GO:0005886	Plasma membrane	49	1.42E−03
GO:0043005	Neuron projection	21	1.42E−03
GO:0034702	Ion channel complex	10	1.98E−03
GO:1902495	Transmembrane transporter complex	10	2.98E−03
GO:0071944	Cell periphery	48	2.98E−03
GO:1990351	Transporter complex	10	2.98E−03
GO:0008328	Ionotropic glutamate receptor complex	5	4.10E−03
GO:0005623	Cell	107	7.23E−03
GO:0005622	Intracellular	96	8.78E−03
GO:0044464	Cell part	106	9.25E−03
GO:0043235	Receptor complex	9	1.50E−02
GO:0030054	Cell junction	19	1.95E−02
GO:0097060	Synaptic membrane	8	3.84E−02
GO:0042734	Presynaptic membrane	4	4.52E−02
GO:0044424	Intracellular part	92	4.57E−02
GO molecular functions			
GO:0043167	Ion binding	61	3.93E−03
GO:0005543	Phospholipid binding	11	1.70E−02
GO:0001640	Adenylate cyclase inhibiting G-protein-coupled glutamate receptor activity	3	1.70E−02
GO:0008289	Lipid binding	14	1.70E−02
GO:0043169	Cation binding	43	1.70E−02
GO:0030276	Clathrin binding	5	1.70E−02
GO:0046872	Metal ion binding	42	1.79E−02
GO:0098772	Molecular function regulator	19	2.05E−02
GO:0005261	Cation channel activity	9	2.73E−02
GO:0005089	Rho guanyl-nucleotide exchange factor activity	5	2.74E−02
GO:0005088	Ras guanyl-nucleotide exchange factor activity	6	3.99E−02

Pathway analyses conducted using STRING 10.0. $n = 7$/genotype. VHIPP–ventral hippocampal formation.
Note: 5/8 genes included in the "Adrenergic signaling in cardiomyocytes" pathway are represented in the "Oxytocin" pathway.

with intellectual disabilities were found to have lower quality friendships relative to typically developing adolescents, which was predicted by impaired social skills earlier in development (Tipton et al., 2013). It will be of interest to future studies to determine if social interaction deficits observed in adult PDE11A mutant mice are, in fact, directly related to their inability to form social memories.

Our work argues that it may be necessary to not only stimulate PDE11A4 catalytic activity but also to restore PDE11A4 to a specific subcellular localization to treat social deficits. The importance of the compartment-specific effects noted herein in response to social isolation are underscored by the fact that patients with neuropsychiatric and neurodegenerative diseases may have cyclic nucleotide deficits in one subcellular compartment but not another (Rahman et al., 1997; Bonkale et al., 1999; Fields et al., 1999; Chang et al., 2003). It is possible to stimulate PDE11A4 catalytic activity via its GAF-A domain (Jager et al., 2012). Further, it is possible to increase insertion of PDE11A4 into the VHIPP membrane by promoting homodimerization of the protein at its GAF-B domain (Pathak et al., in press). Importantly, PDE11A4 GAF domains are less than 50% homologous to those in other PDE families (84), and PDE11A1–3 do not

contain a full GAF-A domain (Yuasa et al., 2000). This suggests selectivity for PDE11A4 relative to other PDE families, and even other PDE11A isoforms, is possible. Selective targeting of PDE11A4 may reduce side effect potential given its minimal expression outside of the brain (Kelly, 2015).

Acknowledgments—The authors would like to thank Marcelo Wood, Ted Abel, and Igor Roninson for helpful advice on RNA sequencing as well as Alex Gasparian for project management support. Research supported by 1P20GM109091 from NIGMS (Project 1, M.S.; Project 3, M.P.K.), a Research Starter Grant in Pharmacology & Toxicology from the PhRMA Foundation (M.P.K.), an ASPIRE award from the Office of the Vice President for Research from the University of South Carolina (individual awards to M.S. and M.P.K.), a Research Development Fund Award from the University of South Carolina School of Medicine (M.P.K.), a NARSAD Young Investigator Award from the Brain & Behavior Research Foundation (M.P.K.), and 1R01MH101130 from NIMH (M.P.K.). M.P.K. receives consulting fees from ASUBIO, Inc. and Deallus for projects unrelated to the current manuscript. S.H., H.J., D.O., N.P. and M.S. have no financial conflicts to disclose.

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(Accepted 9 August 2016)
(Available online 17 August 2016)