Calcium Sensitive Adenylyl Cyclases in Depression and Anxiety: Behavioral and Biochemical Consequences of Isoform Targeting

Vaishnav Krishnan[1], **Ami Graham**[1], **Michelle S. Mazei-Robison**[1], **Diane C. Lagace**[1], **Kyoung-Shim Kim**[2], **Shari Birnbaum**[1], **Amelia J. Eisch**[1], **Pyung-Lim Han**[2], **Daniel R. Storm**[3], **Venetia Zachariou**[1,4], and **Eric J. Nestler**[1]

[1]*Departments of Psychiatry and Neuroscience, The University of Texas Southwestern Medical Center (UTSWMC), Dallas, TX*

[2]*Division of Nanosciences and Brain Disease Research Institute, Ewha Womans University School of Medicine, Seoul, Republic of Korea*

[3]*Department of Pharmacology, The University of Washington, Seattle, WA*

[4]*Department of Basic Sciences, University of Crete, Heraklion, Crete, Greece*

Abstract

Background—Adenylyl cyclases (ACs) represent a diverse family of enzymes responsible for the generation of cAMP, a key intracellular second messenger. Ca^{2+}/calmodulin-stimulated AC1 and AC8 isoforms, as well as the calcium-inhibited AC5 isoform, are abundantly expressed within limbic regions of the central nervous system. This study examines the contribution of these AC isoforms to emotional behavior.

Methods—Male and female AC1/8 double knockout mice (DKO) and AC5 knockout mice (AC5KO) were examined on a series of standard laboratory assays of emotionality. Mice were also assayed for hippocampal cell proliferation and for changes in BDNF signaling in the nucleus accumbens, amygdala, and hippocampus, three forebrain structures involved in the regulation of mood and affect.

Results—AC5KO mice showed striking anxiolytic and antidepressant phenotypes on standard behavioral assays. In contrast, AC1/8 DKO mice were hypoactive, exhibited diminished sucrose preference, and displayed alterations in neurotrophic signaling, generally consistent with a prodepressant phenotype. Neither line of mice displayed alterations in hippocampal cell proliferation.

Conclusions—These data illustrate the complex manner in which Ca^{2+}/calmodulin-stimulated adenylyl cyclases contribute to emotional behavior. In addition, they support the possibility that a selective AC5 antagonist would be of therapeutic value against depression and anxiety disorders.

Keywords

AC1; AC8; AC5; nucleus accumbens; hippocampus; knockout mice; calcium-regulated adenylyl cyclases

INTRODUCTION

Adenylyl cyclases (ACs) catalyze the synthesis of cAMP (cyclic adenosine monophosphate), which in turn activates PKA (protein kinase A) signaling. Of the nine transmembrane isoforms (AC1–AC9) that have been identified in mammals (1,2), Ca^{2+}-regulated isoforms are particularly relevant to psychiatry, as they provide a crucial link between neuronal activity and intracellular cAMP (3). Ca^{2+}-stimulated AC activity within limbic regions of the CNS is constituted by AC1 and AC8 (4), both of which are activated by Ca^{2+}/calmodulin (5). Studies of mice that lack AC1 and/or AC8 have revealed an essential role for these enzymes in several neurobiological processes including long-term memory, sensitivity to drugs of abuse, and chronic pain (6–10). In contrast, physiological Ca^{2+} concentrations inhibit AC5 (11), a striatal-enriched isoform important for behavioral responses to morphine (12). AC5 knockout (AC5KO) mice show resistance against oxidative and genotoxic stresses; they also display an extended lifespan, reduced aging-induced cardiomyopathy, and improved bone quality (13), strongly implicating AC5 inhibition as an effective target against aging (14).

This study explores the relevance of Ca^{2+}-regulated AC activity to mood and anxiety disorders by characterizing the performance of AC5KO and AC1/8 double knockout (DKO) mice and their controls on a series of standard behavioral assays. We complement these results with measures of hippocampal proliferation and neurotrophic signaling. Instead of examining the roles of AC1 and AC8 separately, we examine DKO mice since a simultaneous AC1/8 deletion is required to abolish Ca^{2+}-stimulated AC activity within the CNS (8,10), and several phenotypic changes related to loss of Ca^{2+}-stimulated AC activity have only been observed in DKOs (6,7,10).

METHODS AND MATERIALS

Mice

Mice were: i) housed in groups of 3–4, ii) 8–12 weeks old, iii) fed *ad libitum*, and iv) housed at 23–25°C (lights ON between 0700–1900 hrs). AC5+/− breeder pairs were used to generate AC5KO mice and wildtype (WT) littermate controls (12,15). AC1/8 DKO mice obtained through intercrosses between AC1+/−;AC8+/− doubly heterozygous breeders (16,17) were employed to establish DKO breeding colonies in which all the offspring were DKO genotype. To control for genetic background, WT breeding colonies of the same lineage were simultaneously established. For immunoblotting and immunohistochemistry experiments, only males were employed.

Behavioral Testing

AC5KO and AC1/8DKO mice displayed no evidence of grossly abnormal behavior such as ataxia, seizures, stereotypy, etc., and displayed normal sensory and motor reflexes (see Supplementary Methods and Materials for detailed behavioral procedures). Testing was performed in the light phase using established procedures (18, 19). Locomotor activity was measured in a novel cage with photocells that measured total beam breaks during twelve 10 min intervals. Videotracking (Ethovision) was employed for elevated plus maze and open field testing (5 min), whereas dark-light testing (10 min) employed photocell fitted chambers. For social interaction testing, socially isolated mice (2–3 weeks isolation) were tested in a two-trial paradigm (20): in the first trial (2.5 min), mice were allowed to explore a square open field arena containing an empty wire-mesh cage. During the second trial (2.5 min), mice were reintroduced into this arena now containing an unfamiliar "social target" mouse (c57bl6, Jackson Labs) within the cage. Videotracking measured the time spent in an "interaction zone" – a 15 × 30 cm area surrounding the target enclosure during both "target absent" and "target

present" conditions. Sucrose preference at increasing sucrose concentrations was measured through a two bottle choice paradigm (18).

BrdU Immunohistochemistry

Bromo-deoxyuridine (BrdU) labeling and cell counting was performed as described (21). 2 hrs following a single injection of BrdU (150 mg/kg), mice were perfused and fixed brains were sectioned (30 µm) and processed for BrdU with a rat monoclonal anti-BrdU antibody (1:400, Accurate).

Immunoblotting

Tissue punches of nucleus accumbens (NAc), amygdala, and hippocampus were obtained from 1 mm-thick fresh brain slices, frozen on dry ice, and subsequently lysed and sonicated in a lysis buffer (20 mM HEPES, 0.4 M NaCl, 20% glycerol, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM EGTA, 1% NP40, 50 mM DTT, 0.1% protease and phosphatase inhibitors [Sigma]), and then centrifuged at 14,000 rpm for 15 min. Supernatant protein concentrations were determined using the Bradford Assay (Biorad), following which 40 µg of protein were electrophoresed on 4–15% SDS gradient gels (Biorad). Proteins were transferred to PVDF membranes, washed thoroughly in 1x Tris-buffered saline with 0.1% Tween-20 (TBS-T), and subsequently blocked in a solution of 5% w/v nonfat dry milk in TBS-T for 1 hr at room temperature. Membranes were incubated in primary antibody: anti-$BDNF_{N-20}$ (1:200; Santa Cruz), anti-actin (1:10,000; MP Biomedicals), anti-TrkB (1:5000; Upstate), anti-AKT, anti-phosphoAKT$_{S473}$, anti-ERK1/2, anti-phosphoERK1/2$_{T202\ Y204}$, anti-PLCγ, and anti-phosphoPLCγ$_{Y783}$ (1:500, Cell Signaling). After further washes, membranes were incubated with peroxidase-labeled goat anti-rabbit or horse anti-mouse IgG (1:40,000; Vector, Burlingame, CA). Bands were visualized with SuperSignal West Dura substrate (Pierce, Rockford, IL) and quantified with Scion Image (NIH).

Statistical Analyses

Unpaired, two-tailed Student's t tests were utilized for comparisons between WT and KO groups (BrDU counts, immunoblotting). For behavioral testing, two-way ANOVAs were used to examine significant main effects or interactions (*genotype × sucrose concentration, genotype × target, or genotype × time interval*). Significant post-hoc effects were revealed by Bonferroni tests; effects were considered significant at $p < 0.05$.

RESULTS

AC1/8 DKO and AC5KO Mice Display Contrasting Locomotor Habituation Phenotypes

Male AC5KO mice were more hyperactive than their WT counterparts (Figure 1A), and this hyperactivity was limited to the last 50 min of testing. In contrast, AC1/8 DKO mice demonstrated a clear hypoactive phenotype (Figure 1C), with prominent differences observed in the last hour of testing. Female mice of either genotype did not demonstrate statistically significant changes in locomotor activity (ANOVA), although did display similar trends (Figure 1B, D). Thus, AC1/8 DKO and AC5KO mice do not differ from WT controls in the acute locomotor response to novelty, but rather do display opposite locomotor habituation phenotypes.

AC5KO Mice Display a Prominent Anxiolytic Phenotype

We next examined AC5KO and AC1/8 DKO mice on tests of exploratory behavior (Figure 2, Supplementary Figures S1 and S2). In the elevated plus maze, AC5KO mice spent a significantly greater amount of time in the open arms of the maze (Figure 2A), whereas AC1/8 DKO mice were no different from their WT counterparts. Similar results were obtained on the

light-dark test, where AC5KO mice displayed greater exploration of the light side (Figure 2B). However, in the open field test, both AC5KO and AC1/8 DKO mice performed comparably to WTs (Figure 2C), suggesting that the striking anxiolytic effects of AC5 disruption are limited to certain types of anxiety-provoking situations.

Immobility, Social Interaction, and Sucrose Preference

The FST gauges the duration of immobility during an inescapable situation, a measure sensitive to antidepressant treatments and genetic manipulations (19,22). AC5KO mice displayed a reduction in immobility (antidepressant-like behavior), with particularly striking effects in females (Figure 3A). Interestingly, male AC1/8DKO mice also displayed reduced immobility (Figure 3B), while female DKO mice performed identically to WT. We also examined these mice on a modified social interaction test (Supplementary Figure S3A–C). During the presence of a social cue, AC5KO mice were significantly less interactive than WT mice (Figure 3C), an effect that was not observed in females (Figure 3D). AC1/8 DKO displayed the opposite phenotype: they interacted considerably more than their WT counterparts (Figure 3E, F), suggesting that disrupting these two types of Ca^{2+}-regulated AC activities may confer opposite effects on social behavior.

Many chronic stresses produce a stable reduction in the preference for a sweet solution, interpreted as a form of anhedonia (18,23); this reduction is reversed by chronic antidepressant treatments (24). When compared with WT mice, AC5KO mice displayed normal sucrose preference (Figure 4A, B) as well as comparable levels of fluid intake (Figure 4A, B insets), except at higher concentrations where KO mice drank significantly higher volumes. This pattern cannot be explained by body weight, which is normal in AC5KO mice at the ages tested. In contrast, AC1/8 DKO mice displayed a prominent reduction in sucrose preference (Figure 4C, D). This cannot be explained by alterations in gustation, which is normal in these mice (6). Total fluid intake was reduced in AC1/8 DKO mice (Figure 4C, D insets), possibly partly to the significant reductions in body weight observed in DKO mice (Supplementary Figure S3D).

Hippocampal Cell Proliferation is Not Altered in AC KO Mice

Decreased hippocampal cell proliferation has been postulated as a key mechanism for stress-related pathology, with antidepressants causing the opposite effect (25). AC5KO and AC1/8 DKO mice were assayed for changes in levels of proliferation using BrDU as an S-phase marker. BrDU+ cells were enriched in the subgranular zone of the granule cell layer (Figure 5B). There were no significant differences in the number of BrDU+ cells in either AC5KO or AC1/8 DKO mice compared to their respective controls (Figure 5A).

Altered BDNF Signaling in AC KO Mice

Abnormalities in BDNF (brain-derived neurotrophic factor) signaling are thought to represent a candidate pathophysiological mechanism for depression (26); postmortem studies of depressed individuals reveal decreased BDNF protein levels in hippocampus (27) and increased BDNF levels in NAc (18), a forebrain structure involved in reward-related processes. Through immunoblotting, we probed samples of hippocampus, NAc, and the amygdala, which plays a prominent role in anxiety-related processes (28), from AC1/8 DKO and AC5KO mice for abnormalities in BDNF and downstream signaling. In NAc, AC1/8 DKO mice displayed strong inductions of neurotrophic signaling (Figure 6A), including increased levels of the full-length BDNF receptor, TrkB.F (tropomyosin-related kinase B), phospho-AKT, and phospho-PLCγ (phosholipase Cγ). In contrast, NAc from AC5KO mice only displayed a reduction in levels of total PLCγ. Amygdala samples from AC5KO mice displayed significant reductions in both BDNF and TrkB.F levels (Figure 6B). In hippocampus (Figure 6C), AC1/8 DKO mice

displayed significantly lower levels of phospho-ERK1/2 (extracellular signal-related kinase 1,2), whereas AC5KO mice showed reduced phospho-AKT levels.

DISCUSSION

Achieving a detailed understanding of the biology of individual AC isoforms *in vivo* has been challenging: in addition to complex and redundant tissue distribution patterns, catalytic domains of AC isoforms are highly homologous, which has precluded the generation of isoform-specific antibodies. ACs have also been neglected as pharmacological targets: current small molecule modulators of AC function such as forskolin act on virtually all ACs (29). However, important insights have come from examining phenotypes of specific AC KO mice, an approach which may provide insight into any therapeutic potential of isoform-specific small-molecule modulators. Given the contribution of calcium-regulated ACs to memory and addiction-related processes, we examined AC5KO and AC1/8 DKO mice in paradigms of depression and anxiety, two common and complex psychiatric syndromes.

Our data highlight a novel role for AC5 in the regulation of affective behavior. While male AC5KO mice displayed hyperactivity, this effect was only observed during the "habituation phase", and therefore does not confound the results of other assays, which can be sensitive to changes in novelty-induced hyperactivity. AC5KO mice displayed a strong anxiolytic phenotype in two of three standard assays, as well as an antidepressant phenotype in the FST. Together, these features resemble other mouse models of "manic-like" behavior such as *Clock* mutant mice (30,31), which display hyperactivity as well as hyperhedonic, anxiolytic, and antidepressant phenotypes. Indeed, the mood stabilizer lithium has been shown to inhibit AC5 *in vitro* (32), as well as AC7, another isoform that produces an antidepressant phenotype when genetically eliminated in mice (33). Interestingly, levels of BDNF and TrkB were reduced within the amygdala, an important neural substrate for anxiety, while transgenic overexpression of BDNF in this region causes potent anxiogenic effects (34). We also observed decreased levels of phospho-AKT in hippocampus, and AKT is known to phosphorylate and inhibit GSK3β (glycogen synthase kinase 3β). Transgenic mice overexpressing a constitutively active form of GSK3β in broad forebrain regions also display a manic phenotype (35). Further studies will be important to discriminate the "antidepressant" versus "pro-manic" effects of AC5 disruption, and to more causatively link our molecular changes with the observed behavior phenotypes.

AC1/8 DKO mice displayed a more complex phenotype, and since the generation of an appropriately large number of DKO mice required non-littermate comparisons, we acknowledge that these behavioral changes could be a result of different maternal effects. While male AC1/8 DKO mice displayed reduced immobility in the FST, both males and female DKO mice displayed lower body weights and strong deficits in sucrose preference, abnormalities often observed in mouse models of depression (18,24). Depressed patients display reductions in platelet adenylyl cyclase activity (36), which is predominantly constituted by AC3 (37), an isoform structurally and functionally similar to AC1 and AC8 (11). Immunoblotting of NAc revealed strong activation of BDNF signaling in the DKOs, which is also observed in mice after chronic social defeat (18). Additionally, we observed a significant reduction in ERK phosphorylation in the hippocampus. Such an effect has also been observed after prolonged corticosterone exposure (38), and systemic pharmacological inhibition of ERK1/2 produces prodepressant effects (39). DKO mice did not display abnormalities in hippocampal cell proliferation, an important negative finding in light of the critical role of AC1 and AC8, and subgranular zone proliferation, in memory (10). Our social interaction tests revealed that DKO mice socially interacted more than WTs. Social interaction is a complex behavior, mediated by several components including anxiety, social dominance, and motivation (40). In contrast to AC1/8 DKO mice, male AC5KO mice displayed a social deficit, and these opposite findings

correlate with levels of PLCγ signaling within the NAc. Further studies are clearly required to dissect the molecular basis for these social phenotypes.

In summary, constitutive knockout of AC1 and AC8 produces a deficit in sucrose reward, weight loss, and decreased locomotor activity. Together with previous studies that demonstrate altered drug reward and robust memory deficits in AC1/8 DKO mice (see Introduction), these findings suggest that inhibitors of Ca^{2+}/calmodulin-stimulated ACs would have largely adverse behavioral consequences. In contrast, knockout of AC5 causes strong antidepressant and anxiolytic phenotypes. Indeed, novel pharmacotherapies aimed at selectively antagonizing AC5 function would promote longevity and protect against oxidative stress (13). Our findings suggest that such agents would also promote resilience against depression and anxiety disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

REFERENCES

1. Bernatchez R, Belkacemi L, Rassart E, Daoud G, Simoneau L, Lafond J. Differential expression of membrane and soluble adenylyl cyclase isoforms in cytotrophoblast cells and syncytiotrophoblasts of human placenta. Placenta 2003;24:648–657. [PubMed: 12828923]

2. Hanoune J, Pouille Y, Tzavara E, Shen T, Lipskaya L, Miyamoto N, et al. Adenylyl cyclases: structure, regulation and function in an enzyme superfamily. Molecular and cellular endocrinology 1997;128:179–194. [PubMed: 9140089]

3. Wang H, Storm DR. Calmodulin-regulated adenylyl cyclases: cross-talk and plasticity in the central nervous system. Molecular pharmacology 2003;63:463–468. [PubMed: 12606751]

4. Conti AC, Maas JW Jr, Muglia LM, Dave BA, Vogt SK, Tran TT, et al. Distinct regional and subcellular localization of adenylyl cyclases type 1 and 8 in mouse brain. Neuroscience 2007;146:713–729. [PubMed: 17335981]

5. Li S, Lee ML, Bruchas MR, Chan GC, Storm DR, Chavkin C. Calmodulin-stimulated adenylyl cyclase gene deletion affects morphine responses. Molecular pharmacology 2006;70:1742–1749. [PubMed: 16914643]

6. Maas JW Jr, Vogt SK, Chan GC, Pineda VV, Storm DR, Muglia LJ. Calcium-stimulated adenylyl cyclases are critical modulators of neuronal ethanol sensitivity. J Neurosci 2005;25:4118–4126. [PubMed: 15843614]

7. Vadakkan KI, Wang H, Ko SW, Zastepa E, Petrovic MJ, Sluka KA, et al. Genetic reduction of chronic muscle pain in mice lacking calcium/calmodulin-stimulated adenylyl cyclases. Molecular pain 2006;2:7. [PubMed: 16503978]

8. Wei F, Qiu CS, Kim SJ, Muglia L, Maas JW, Pineda VV, et al. Genetic elimination of behavioral sensitization in mice lacking calmodulin-stimulated adenylyl cyclases. Neuron 2002;36:713–726. [PubMed: 12441059]

9. Wei F, Vadakkan KI, Toyoda H, Wu LJ, Zhao MG, Xu H, et al. Calcium calmodulin-stimulated adenylyl cyclases contribute to activation of extracellular signal-regulated kinase in spinal dorsal horn neurons in adult rats and mice. J Neurosci 2006;26:851–861. [PubMed: 16421305]

10. Wong ST, Athos J, Figueroa XA, Pineda VV, Schaefer ML, Chavkin CC, et al. Calcium-stimulated adenylyl cyclase activity is critical for hippocampus-dependent long-term memory and late phase LTP. Neuron 1999;23:787–798. [PubMed: 10482244]

11. Willoughby D, Cooper DM. Organization and Ca2+ regulation of adenylyl cyclases in cAMP microdomains. Physiological reviews 2007;87:965–1010. [PubMed: 17615394]

12. Kim KS, Lee KW, Lee KW, Im JY, Yoo JY, Kim SW, et al. Adenylyl cyclase type 5 (AC5) is an essential mediator of morphine action. Proceedings of the National Academy of Sciences of the United States of America 2006;103:3908–3913. [PubMed: 16537460]

13. Yan L, Vatner DE, O'Connor JP, Ivessa A, Ge H, Chen W, et al. Type 5 adenylyl cyclase disruption increases longevity and protects against stress. Cell 2007;130:247–258. [PubMed: 17662940]

14. Chester JA, Watts VJ. Adenylyl cyclase 5: a new clue in the search for the "fountain of youth"? Sci STKE 2007;2007:pe64. [PubMed: 18029912]

15. Lee KW, Hong JH, Choi IY, Che Y, Lee JK, Yang SD, et al. Impaired D2 dopamine receptor function in mice lacking type 5 adenylyl cyclase. J Neurosci 2002;22:7931–7940. [PubMed: 12223546]

16. Wong AH, Gottesman II, Petronis A. Phenotypic differences in genetically identical organisms: the epigenetic perspective. Hum Mol Genet 2005;14(Spec No 1):R11–R18. [PubMed: 15809262]

17. Zachariou V, Liu R, Laplant Q, Xiao G, Renthal W, Chan GC, et al. Distinct Roles of Adenylyl Cyclases 1 and 8 in Opiate Dependence: Behavioral, Electrophysiological, and Molecular Studies. Biological psychiatry. 2008

18. Krishnan V, Han M-H, Graham DL, Berton O, Renthal W, Russo SJ, et al. Molecular Adaptations Underlying Susceptibility and Resistance to Social Defeat in Brain Reward Regions. Cell 2007;131:391–404. [PubMed: 17956738]

19. Monteggia LM, Luikart B, Barrot M, Theobold D, Malkovska I, Nef S, et al. Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. Biological psychiatry 2007;61:187–197. [PubMed: 16697351]

20. Gemelli T, Berton O, Nelson ED, Perrotti LI, Jaenisch R, Monteggia LM. Postnatal loss of methyl-CpG binding protein 2 in the forebrain is sufficient to mediate behavioral aspects of Rett syndrome in mice. Biological psychiatry 2006;59:468–476. [PubMed: 16199017]

21. Lagace DC, Fischer SJ, Eisch AJ. Gender and endogenous levels of estradiol do not influence adult hippocampal neurogenesis in mice. Hippocampus 2007;17:175–180. [PubMed: 17286277]

22. Lucki I, Dalvi A, Mayorga AJ. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. Psychopharmacology 2001;155:315–322. [PubMed: 11432695]

23. Willner P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 2005;52:90–110. [PubMed: 16037678]

24. Strekalova T, Gorenkova N, Schunk E, Dolgov O, Bartsch D. Selective effects of citalopram in a mouse model of stress-induced anhedonia with a control for chronic stress. Behav Pharmacol 2006;17:271–287. [PubMed: 16572005]

25. Sahay A, Hen R. Adult hippocampal neurogenesis in depression. Nature neuroscience 2007;10:1110–1115.

26. Martinowich K, Manji H, Lu B. New insights into BDNF function in depression and anxiety. Nature neuroscience 2007;10:1089–1093.

27. Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. Brain research 2005;136:29–37. [PubMed: 15893584]

28. Ressler KJ, Mayberg HS. Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. Nature neuroscience 2007;10:1116–1124.

29. Hanoune J, Defer N. Regulation and role of adenylyl cyclase isoforms. Annual review of pharmacology and toxicology 2001;41:145–174.

30. McClung CA, Sidiropoulou K, Vitaterna M, Takahashi JS, White FJ, Cooper DC, et al. Regulation of dopaminergic transmission and cocaine reward by the Clock gene. Proceedings of the National Academy of Sciences of the United States of America 2005;102:9377–9381. [PubMed: 15967985]

31. Roybal K, Theobold D, Graham A, DiNieri JA, Russo SJ, Krishnan V, et al. Mania-like behavior induced by disruption of CLOCK. Proceedings of the National Academy of Sciences of the United States of America 2007;104:6406–6411. [PubMed: 17379666]

32. Mann L, Heldman E, Shaltiel G, Belmaker RH, Agam G. Lithium preferentially inhibits adenylyl cyclase V and VII isoforms. Int J Neuropsychopharmacol 2008:1–7.

33. Hines LM, Hoffman PL, Bhave S, Saba L, Kaiser A, Snell L, et al. A sex-specific role of type VII adenylyl cyclase in depression. J Neurosci 2006;26:12609–12619. [PubMed: 17135423]

34. Govindarajan A, Rao BS, Nair D, Trinh M, Mawjee N, Tonegawa S, et al. Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. Proceedings of the National Academy of Sciences of the United States of America 2006;103:13208–13213. [PubMed: 16924103]

35. Prickaerts J, Moechars D, Cryns K, Lenaerts I, van Craenendonck H, Goris I, et al. Transgenic mice overexpressing glycogen synthase kinase 3beta: a putative model of hyperactivity and mania. J Neurosci 2006;26:9022–9029. [PubMed: 16943560]

36. Hines LM, Tabakoff B. Platelet adenylyl cyclase activity: a biological marker for major depression and recent drug use. Biological psychiatry 2005;58:955–962. [PubMed: 16095566]

37. Katsel PL, Tagliente TM, Schwarz TE, Craddock-Royal BD, Patel ND, Maayani S. Molecular and biochemical evidence for the presence of type III adenylyl cyclase in human platelets. Platelets 2003;14:21–33. [PubMed: 12623444]

38. Gourley SL, Wu FJ, Kiraly DD, Ploski JE, Kedves AT, Duman RS, et al. Regionally Specific Regulation of ERK MAP Kinase in a Model of Antidepressant-Sensitive Chronic Depression. Biological psychiatry. 2007

39. Duman CH, Schlesinger L, Kodama M, Russell DS, Duman RS. A role for MAP kinase signaling in behavioral models of depression and antidepressant treatment. Biological psychiatry 2007;61:661–670. [PubMed: 16945347]

40. Sousa N, Almeida OF, Wotjak CT. A hitchhiker's guide to behavioral analysis in laboratory rodents. Genes, brain, and behavior 2006;5:5–24.
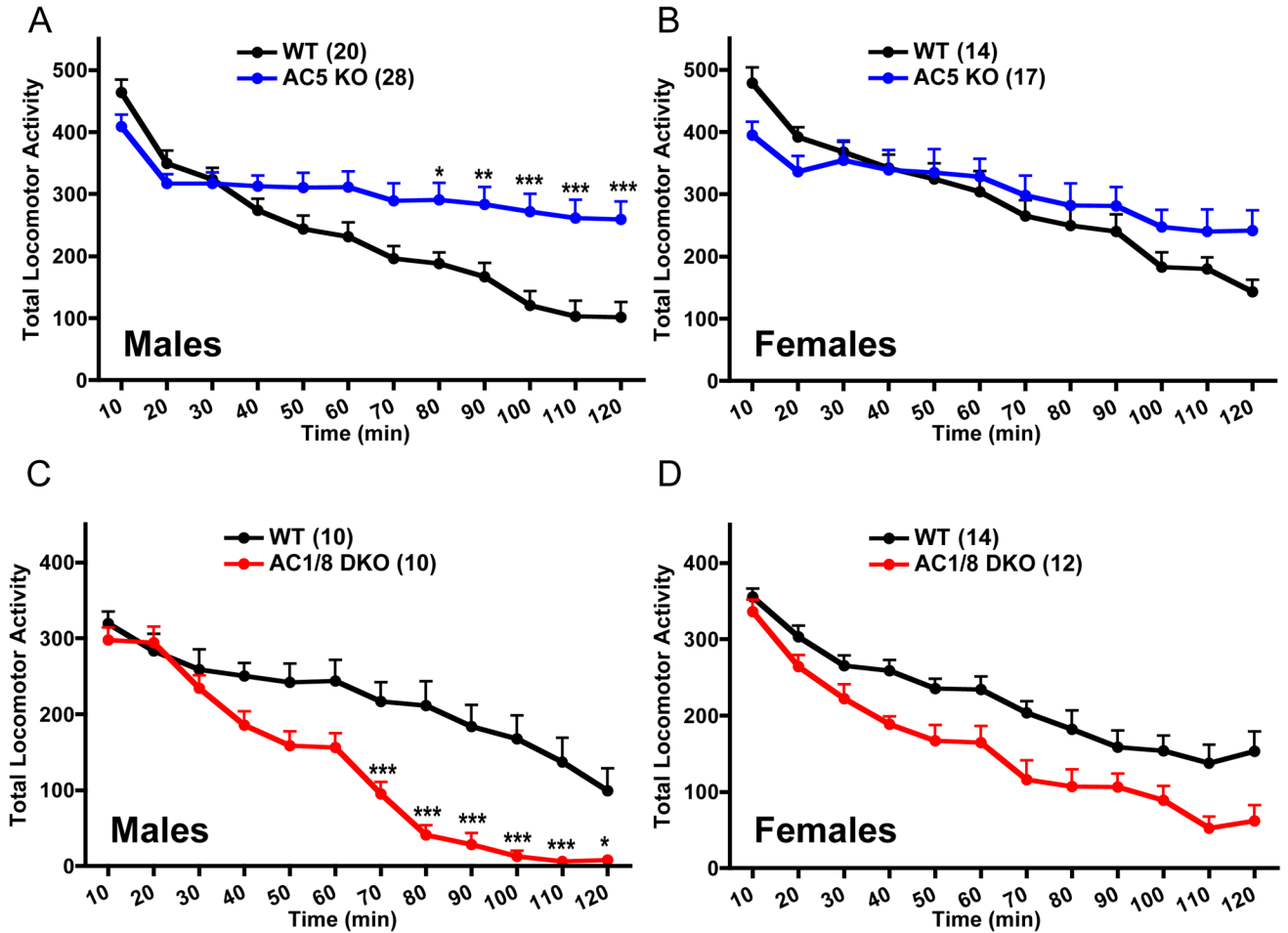
Figure 1.
Male AC5KO and AC1/8 DKO mice display opposite phenotypes on measures of locomotor habituation. **A**: Male AC5KO mice were more active (*genotype × time interval$_{RM}$* interaction, $F_{11,506} = 18.18$, p<0.0001), whereas **B**: female AC5KO and WT mice demonstrated similar locomotor habituation behavior (*time interval$_{RM}$* main effect, $F_{11,319} = 49.94$, p<0.0001). **C**: Male AC1/8DKO mice were less active (*genotype × time interval$_{RM}$* interaction, $F_{11, 198} = 10.78$, p<0.0001), whereas **D**: AC1/8DKO females were as active as WT mice (*time interval$_{RM}$* main effect, $F_{11,264} = 65.78$, p<0.0001). Data are presented as means+SEM, with * indicating significant post hoc differences (*:p<0.05, **:p<0.01, ***:p<0.001). (RM: repeated measure).
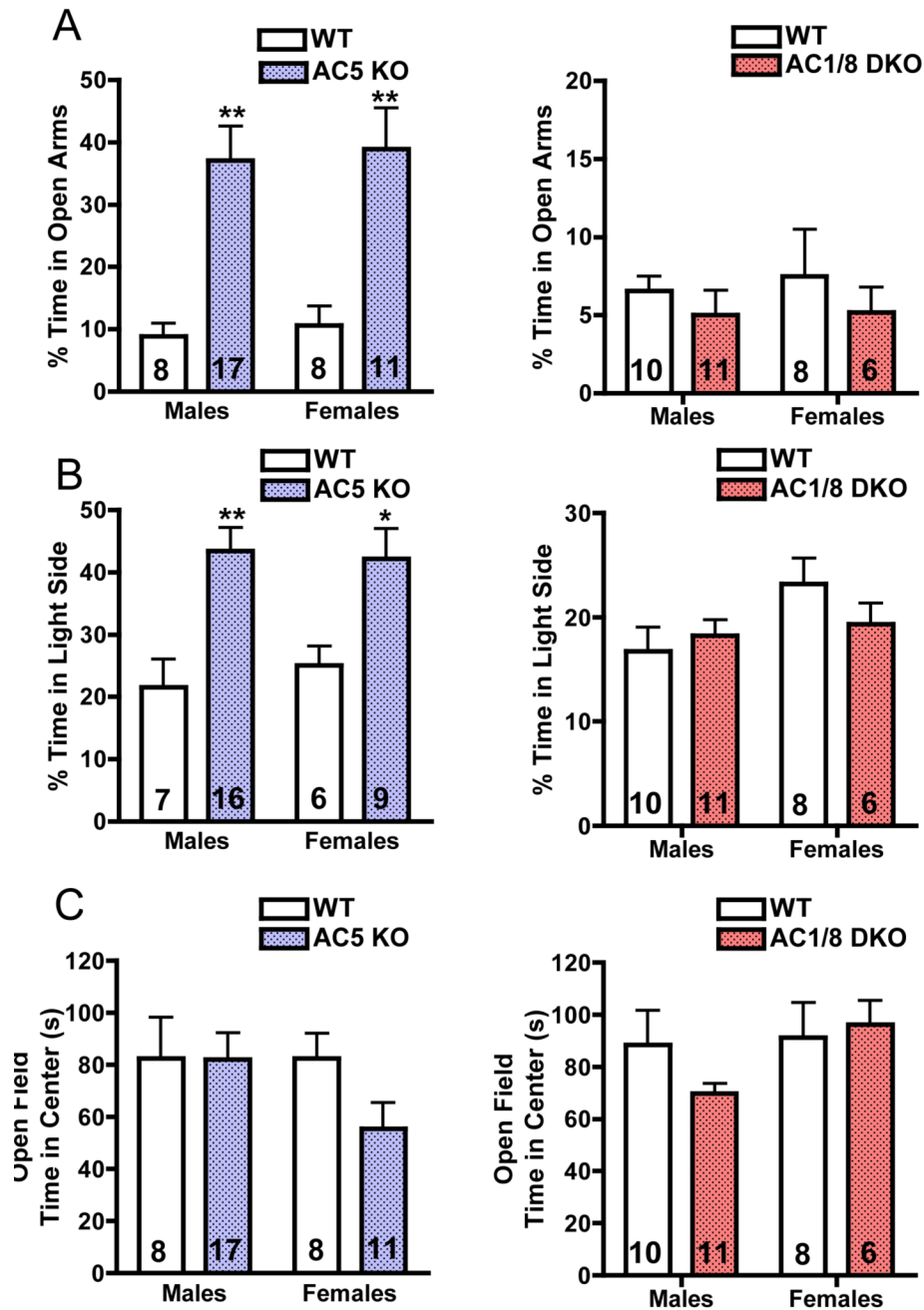
Figure 2.
AC5KO mice display a prominent anxiolytic phenotype. **A**: On the elevated plus maze test, AC5KO mice spent significantly greater time on the open arms of the plus maze (*genotype* main effect, $F_{1,40} = 23.33$, p<0.0001), while AC1/8 DKO mice were no different from their WT controls (*genotype*, $F_{1,31} = 0.04$, p>0.5). **B**: Similar results were obtained in the dark-light test, where AC5KO mice explored the light compartment significantly more than their WT controls (*genotype* main effect, $F_{1,34} = 16.99$, p<0.01), while AC1/8 DKO mice performed comparably to WT mice (*genotype*, $F_{1,30} = 0.30$, p>0.5). **C**: The open field tests did not reveal anxiety phenotypes in either AC5KO (*genotype*, $F_{1,40} = 1.28$, p>0.2) or AC1/8 DKO

(*genotype*, $F_{1,31} = 0.41$, p>0.5). Data are presented as means + SEM (sample size denoted within bars), with * indicating significant posthoc differences (*: p<0.05, **:p<0.01).
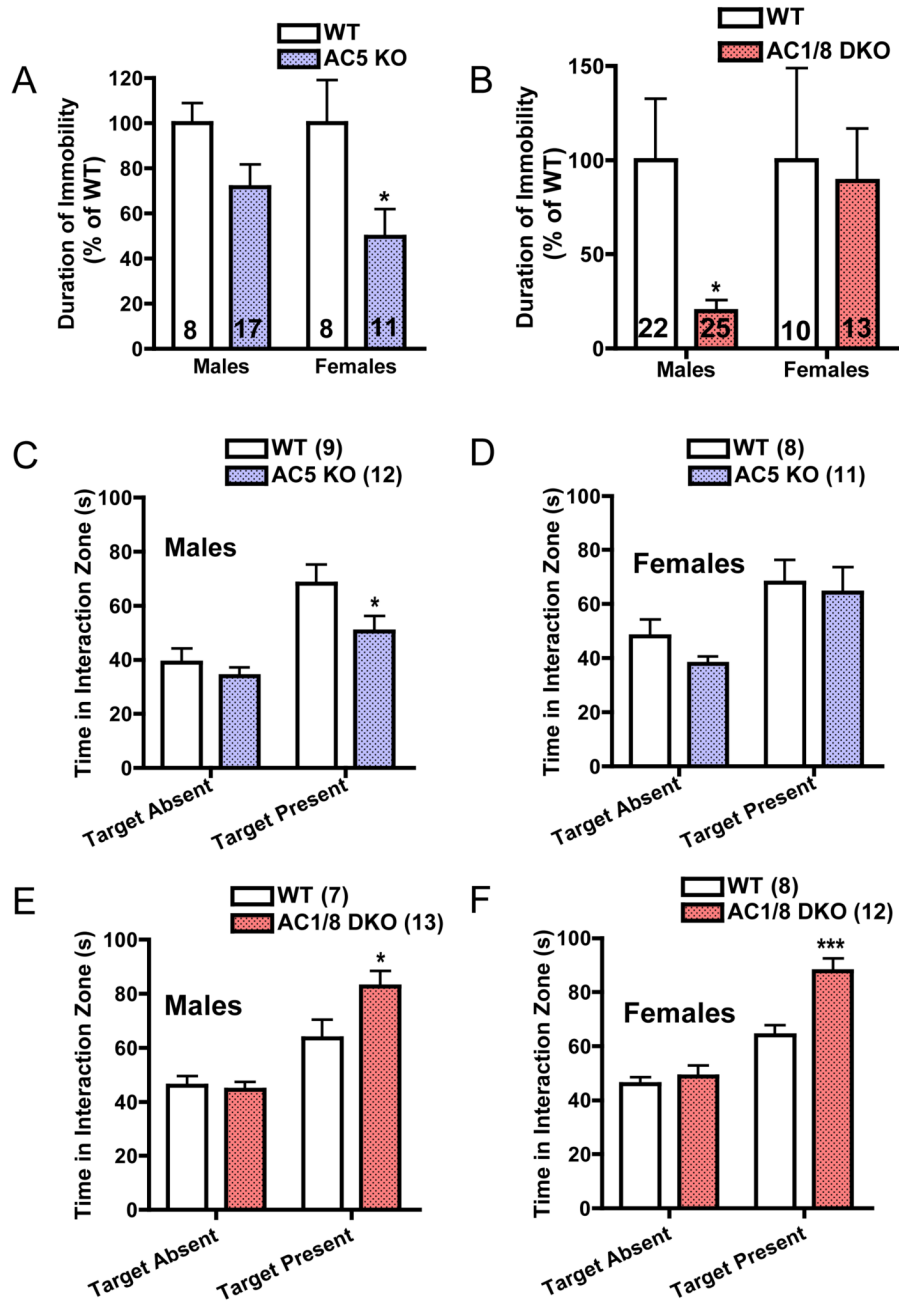
Figure 3.
A,B: Durations of immobility during the last 4 min period of forced swim testing (FST, 6 min test) measured by an experienced observer blind to condition. AC5KO mice spent significantly less time immobile than their wild-type counterparts (*genotype* main effect, $F_{1,39} = 9.71$, p<0.01), with a prominent phenotype emerging in females. AC1/8DKO mice also displayed an decreased immobility on the FST (*genotype* main effect, $F_{1,71} = 3.993$, p<0.05), an effect mainly observed in males. **C, D**: In a two-trial social interaction test (see Methods and Materials), male AC5KO mice were significantly less interactive than WT mice (*genotype* × *target* interaction, $F_{1,38} = 4.68$, p<0.05). This effect was not observed in females (*genotype* × *target* interaction, $F_{1,38} = 0.91$, p>0.3). **E, F**: AC1/8DKO mice were significantly more

interactive than WT mice, and this effect was observed both in males (*genotype × target* interaction, $F_{1,36} = 2.92$, $p<0.05$) and females (*genotype × target* interaction, $F_{1,36} = 9.78$, $p<0.01$). Data are presented as means + SEM (sample size denoted within bars), with * indicating significant post-hoc differences (*:$p<0.05$, ***:$p<0.001$).
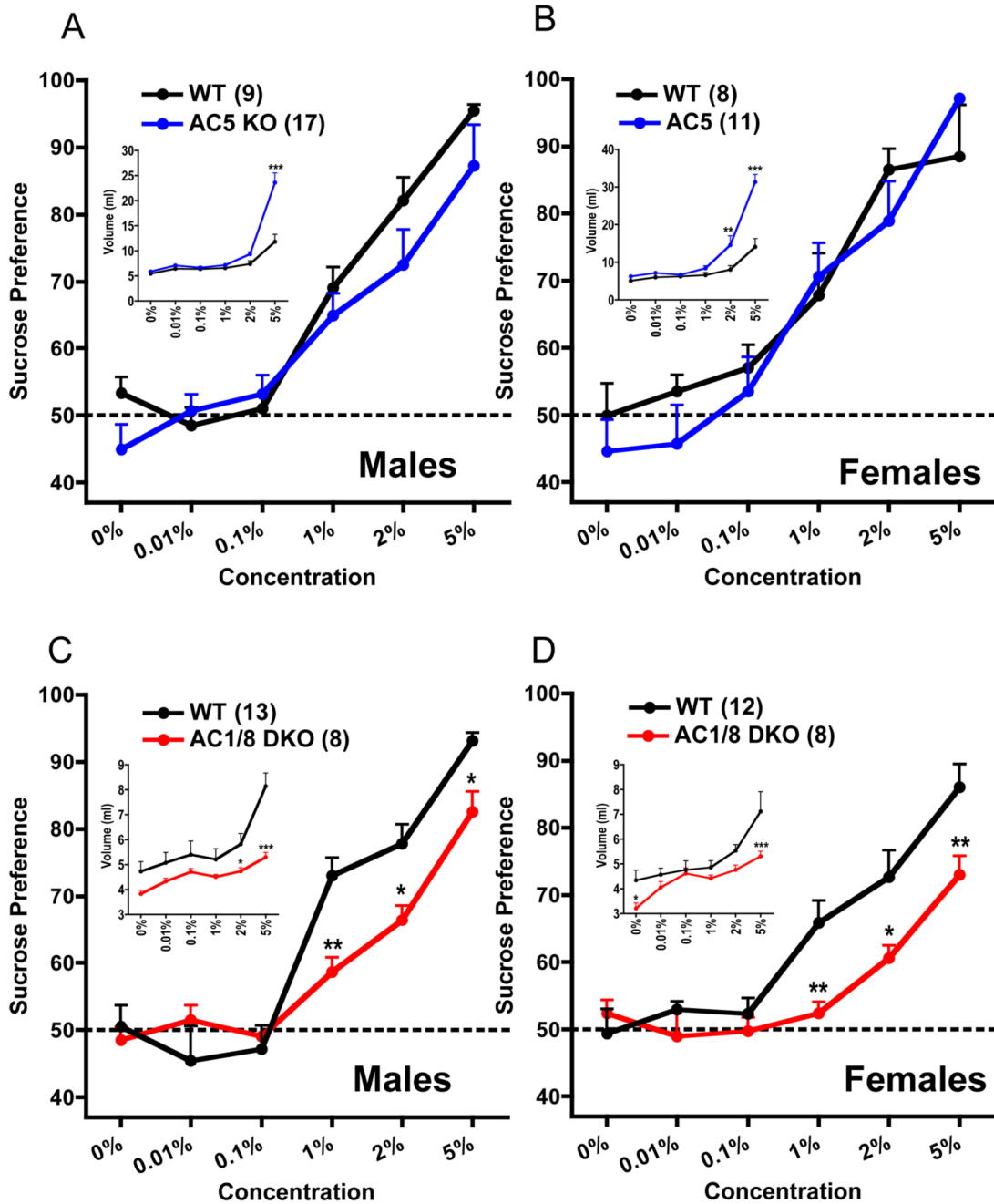
Figure 4.
Sucrose preference data for male and female AC5KO and AC1/8DKO mice depicted as a function of sucrose concentration in bottle "A" (*x* axis, [w/v]), with inset graphs displaying fluid volume intake. **A**: Male AC5KO and WT mice showed comparable sucrose preference (*genotype × concentration* interaction, $F_{5,144} = 0.74$, $p>0.5$), and displayed increased fluid intake at higher sucrose concentrations ($F_{5,144} = 13.20$, $p<0.0001$). **B**: Female AC5KO mice also displayed sucrose preference scores similar to WT (*genotype × concentration* interaction, $F_{5,102} = 0.85$, $p>0.5$), as well as increased fluid intake at higher sucrose concentrations ($F_{5,102} = 12.85$, $p<0.0001$). **C**: Male AC1/8DKO mice exhibited significantly lowered sucrose preference (*genotype × concentration* interaction, $F_{5,114} = 4.59$, $p<0.001$) together with overall

decreases in fluid intake (*genotype* main effect, $F_{1,113} = 53.18$, p<0.0001). **D**: Female AC1/8DKO mice demonstrated significantly lowered sucrose preference (*genotype × concentration* interaction, $F_{5,108} = 2.98$, p<0.05), with reduced overall fluid intake (*genotype* main effect, $F_{1,107} = 22.85$, p<0.0001). Data are presented as means ± SEM, with * indicating significant post-hoc differences (*:p<0.05, **:p<0.01, ***:p<0.001).
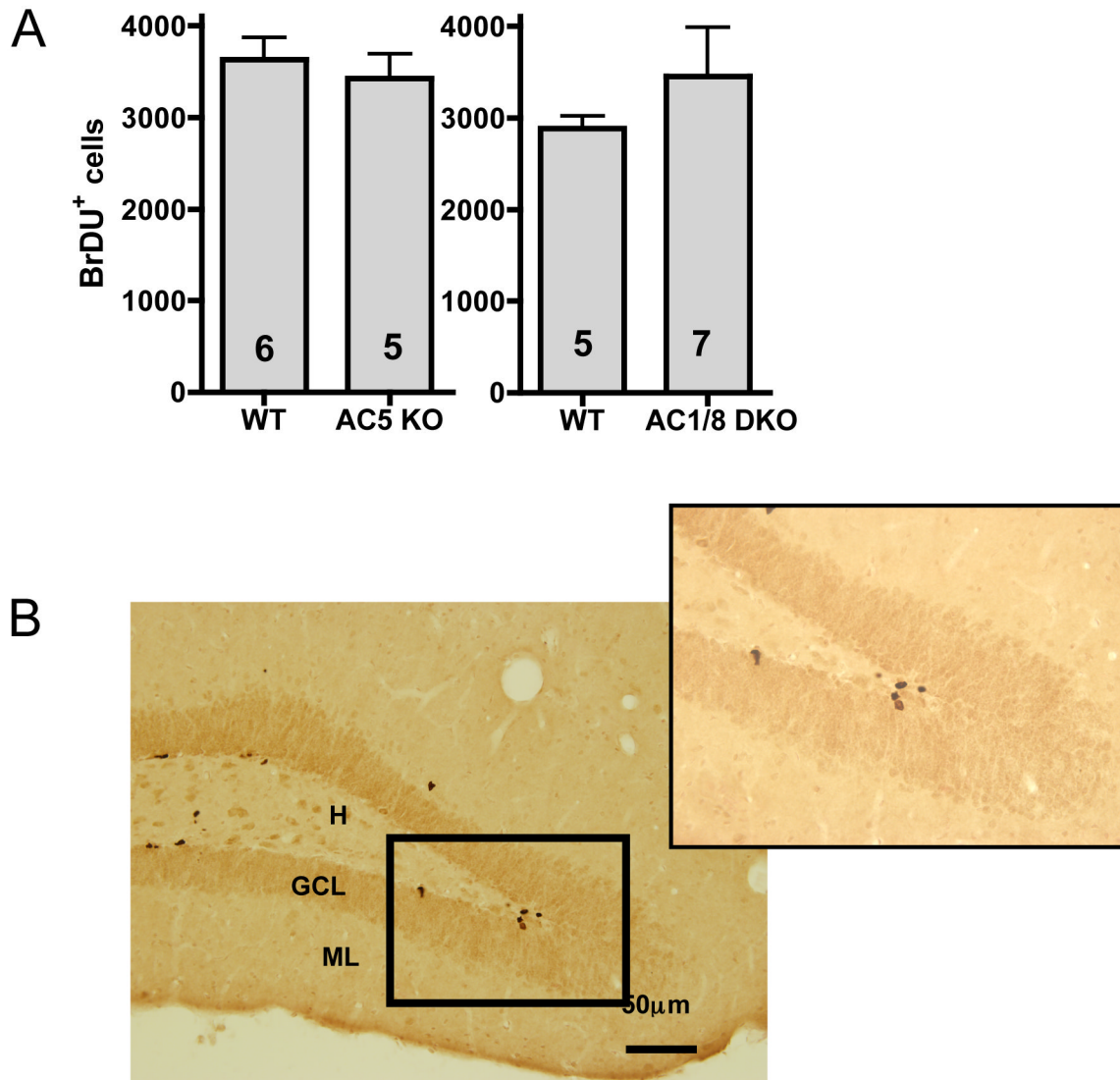
Figure 5.
A: Neither AC5KO nor AC1/8 DKO mice showed abnormal rates of hippocampal proliferation, as measured by the total number of BrDU immunopositive cells found in the dentate gyrus (DG) subgranular zone (AC5: $t_9 = 0.57$, $p > 0.5$, AC1/8: $t_{10} = 0.87$, $p > 0.3$, sample size denoted within bars). **B**: Representative photomicrograph of a coronal section through the hippocampus showing darkly stained BrDU labeled cell clusters in the apex of the DG (20X). Inset: 40X magnified view. Abbreviations: H (Hilus), GCL (Granule Cell Layer), ML (Molecular Layer).
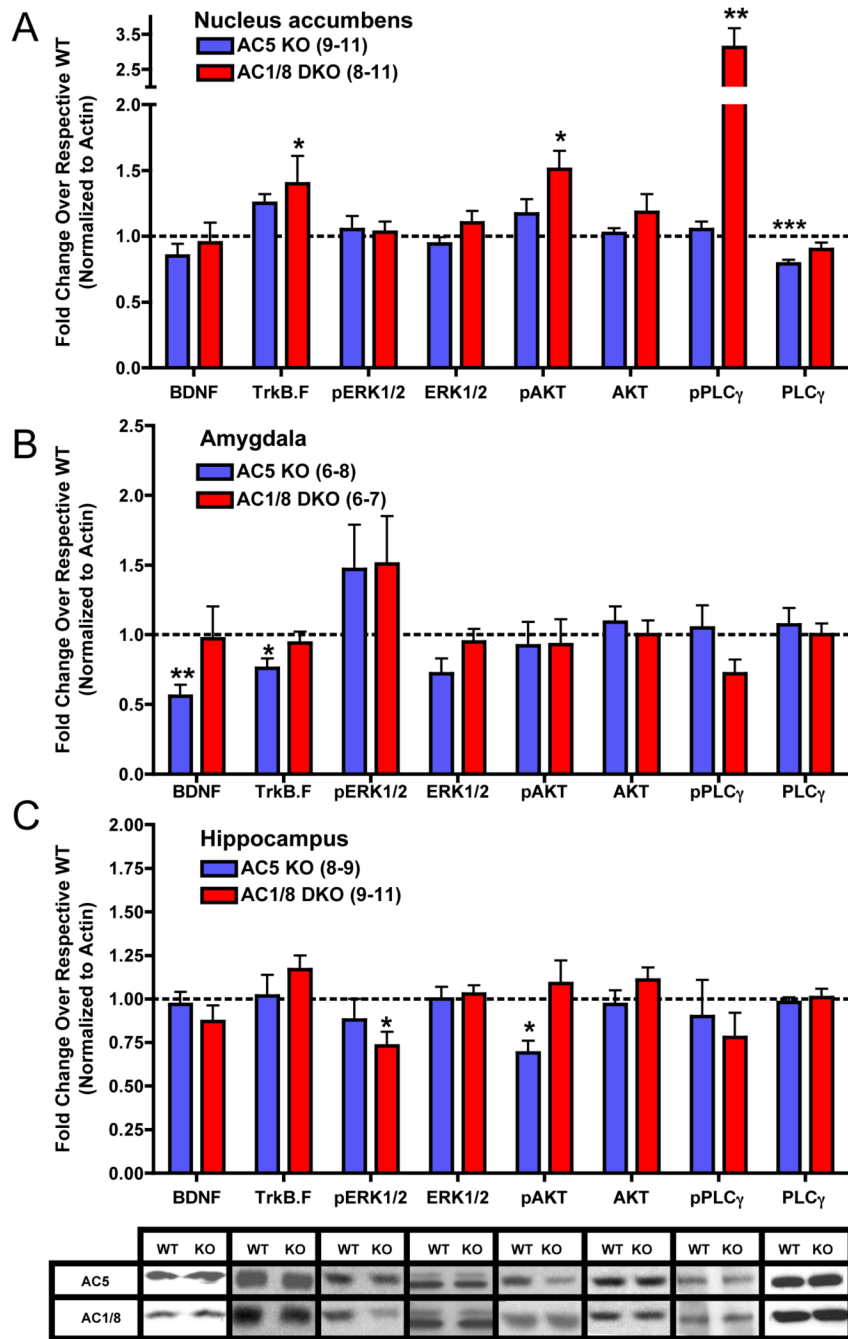
Figure 6.
Effects on BDNF signaling within the NAc, amygdala and hippocampus. **A–C**: Optical density measurements for each sample were first normalized to the respective β-actin band intensity; group averages were then normalized to WT. Representative bands are shown for hippocampal samples. Data are presented as means ± SEM, with * indicating significant differences in comparison to WT controls (*:$p<0.05$, **:$p<0.01$, ***:$p<0.001$). Abbreviations: BDNF (brain-derived neurotrophic factor), TrkB.F (full-length tropomyosin-related kinase B), ERK1/2 (extra-cellular signal regulated kinase 1/2), AKT (thymoma viral proto-oncogene), PLCγ (phospholipase Cγ), pERK1/2, pAKT, and p PLCγ (phosphorylated forms of these proteins).