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A potential role for the acid-sensing T cell death associated gene-8 (TDAG8) receptor in depression-like behavior

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Abstract

Inflammation has been suggested to contribute to the pathophysiology of depression. The T cell death associated gene-8 (TDAG8) receptor is a proton-sensing G-protein-coupled receptor (GPCR) expressed on immune cells in both the CNS and periphery. Previous work has shown modulation of inflammation by the TDAG8 receptor, with pro-inflammatory responses reported in the central nervous system (CNS). Given the link between depression and inflammation, the aim of the present study was to investigate the role of TDAG8 in depression relevant behaviors. Mice deficient in TDAG8 (TDAG8^{-/-}) were tested in the forced swim test (FST) and sucrose preference paradigm. TDAG8 deficiency resulted in significant attenuation of immobility in the FST as compared to wild type TDAG8 (TDAG8^{+/+}) mice. These differences were not due to alterations in motor activity evoked by TDAG8 deficiency as TDAG8^{+/+} and TDAG8^{-/-} mice displayed similar activity in the home cage or in a novel context. TDAG8^{-/-} mice showed significantly higher consumption of sucrose compared to wild type mice although sucrose preference was not significantly different between genotypes. Collectively, our results support the involvement of the TDAG8 receptor in behavioral response relevant to depression. Further investigation is required to validate TDAG8 as a novel target linking inflammation and depression.

Keywords

TDAG8; T cell death associated gene-8; Depression; Behavior

1. Introduction

According to the World Health Organization (WHO), over 350 million people worldwide suffer from depression. Depression is a leading cause of disability and results in a decreased quality of life and major economic burden^{1–4}. Despite the high prevalence and significant

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morbidity of depression the underlying etiology is still unclear. Although antidepressant therapeutics, such as monoamine reuptake inhibitors, are available, a large number of depression patients, approximated to be as high as 50%, do not optimally respond to treatment^{5–8}.

Evidence from clinical and preclinical studies over the past couple decades have provided valuable insights into the role of neurotransmitter abnormalities contributing to depressive pathophysiology^{9,10}. However, there is still an urgent need to investigate underlying mechanisms that can lead to novel targets of intervention and treatment. Recent evidence suggests that dysregulated immune responses may play a role in depression^{11–16}. Depression patients have increased circulating pro-inflammatory cytokines including TNF alpha (TNF- α) interleukin 6 (IL-6), and IL-1 β ^{17–22}. These cytokines are mediators of sickness behavior, and may be responsible for depression associated physiological and behavioral changes which mimic the somatic symptoms of sickness behavior^{23–25}. Depressive-like behaviors can be induced in rodents by administration of pro-inflammatory cytokines or by lipopolysaccharide (LPS) which promotes cytokine release^{26,27}. Interestingly, selective serotonin reuptake inhibitors (SSRIs) have been found to have anti-inflammatory effects²⁸. Pretreatment with SSRIs fluoxetine and paroxetine attenuated LPS-induced increases in TNF α in mice²⁹. Treatment of depressive subjects with SSRIs has led to reduced concentration of pro-inflammatory cytokine TNF α ¹⁸. Collectively, these studies suggest an association of increased pro-inflammatory cytokines with depression. Therefore targets that modulate inflammation are of interest to the pathophysiology of depression.

The T cell death associated gene-8 (TDAG8) receptor is a member of the proton-sensing G-protein-coupled receptor (GPCR) family which also includes the G2 accumulation receptor (G2A), ovarian cancer G-protein receptor 1 (OGR-1), and G-protein-coupled receptor 4 (GPR4)³⁰. TDAG8 was first identified by its increased mRNA expression during T-cell receptor engagement mediated thymocyte apoptosis³⁰. Activation of TDAG8 via extracellular acidosis leads to release of cyclic adenosine 5'-monophosphate (cAMP) in cells transfected with mouse and human TDAG8 cDNA^{31–35}. TDAG8 is expressed on immune cells in both the CNS and periphery^{30,35,36}. Although the functional role of TDAG8 is indistinct³⁷, it has been shown to regulate the release of pro-inflammatory cytokines^{38,39} and has been reported to facilitate inflammation-related to hyperalgesia and allergic airway pathophysiology^{38,40,41}.

In the current study we explored the role of TDAG8, a receptor regulating inflammation, in depression-like behaviors using TDAG8 deficient mice. Since increased inflammation is associated with depressive-like behaviors, we hypothesized that mice lacking TDAG8 will elicit a reduction in these behaviors as compared to wild type mice.

2. Methods

2.1 Animals

TDAG8^{-/-} mice, a generous gift from Dr. Owen Witte, UCLA, were generated on a BALB/c background strain as described before³⁷. Briefly, TDAG8 expression was eliminated by replacing a 2.5 kb fragment containing exon 2-derived TDAG8 coding

sequences with a 3.4-kb fragment encoding promoter-less internal ribosomal entry site EGFP sequence. All experiments reported here were performed on 16–24 week male mice carrying wild-type (TDAG8 ^{+/+}) or knockout (TDAG8 ^{-/-}) allele. Mice were housed under constant temperature (23–28°C) conditions and a 14 h-light, 10 h-dark cycle (lights on at 0600 h). Behavioral experiments were performed between the hours of 8am–1pm. Food and water were provided *ad libitum*. All studies were conducted under research protocols approved by the Institutional Animal Care and Use Committees of University of Cincinnati, in vivariums accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

2.2 Sucrose Preference

Sucrose preference models loss of interest or pleasure, a key symptom of depression. Sucrose intake and preference were assessed on singly housed mice as previously described⁴² with modifications. Mice were allowed to acclimate for 3 days to two water bottles. On the fourth day water was replaced with sucrose solution (1%) in one of the bottles for five days. The bottles were reversed daily to control for side preference. Sucrose and water intake, body weight and food consumption were measured daily. Sucrose preference was calculated based on the ratio of sucrose solution consumed and total liquid consumed in a 24hr period and normalized to body weight.

2.3 Forced Swim Test

The forced swim test was performed as previously described^{43,44}. Briefly, mice were placed into a beaker (30cm height × 20cm diameter) filled approximately 20cm with water (24 ± 2 °C). Mice were tested for 6 minutes and behavior was videotaped. Swimming (moving limbs in an active manner and making circular movements around the apparatus) and immobility (no active movements or floating in the water without struggling) were scored every 5 seconds for each animal. The total counts for each behavior were summed for individual animals for the entire testing period and averaged within each genotype.

2.4 Locomotor activity

Twenty-four hour home cage activity was measured as previously described⁴², using the SmartFrame stainless steel cage rack frame (Lafayette Instrument Company, Lafayette, IN, USA) with infrared photo beam interruption sensors (7X and 15Y). Data was then analyzed using the hmm100 motor monitor software. Locomotor activity was also monitored in a novel context.

2.5 Statistics

Planned comparisons with two-tailed Student's t-test were used across experiments to determine statistical significance between genotypes ($p < 0.05$). Two way repeated measures ANOVA and Holm-Sidak's post hoc test were used for home cage locomotor activity. Prism software was used to analyze all data (GraphPad Software, Inc., La Jolla, CA)

3. Results

3.1 TDAG8^{-/-} mice show decreased immobility in FST

The FST was used to assess despair-like behavior in TDAG8^{+/+} and TDAG8^{-/-} mice. TDAG8^{-/-} mice show significantly less immobility in the FST (Fig. 1A, $t(22)=2.295$, $p=0.0316$, $n=12/\text{genotype}$) as compared to TDAG8^{+/+} mice. Consistent with this, swimming behavior was significantly higher in TDAG8^{-/-} mice in comparison with wild type controls (Fig. 1B, $t(22)=2.295$, $p=0.0316$). To determine whether altered swimming behavior in knockout mice results from differences in activity due to receptor deficiency, motor activity in the home cage or under novelty was assessed. As shown in Fig 2, home cage and novelty-induced locomotor activity was not impacted by TDAG8 deficiency. For home cage activity, two way repeated measures ANOVA revealed a significant effect of time ($F_{(22,308)}=9.578$, $p<0.0001$, $n=8/\text{genotype}$). However, no significant effect of genotype ($F_{(1,14)}=0.3031$, $p=0.5906$) was observed. Exposure to a novel context also revealed no significant difference between TDAG8^{+/+} and TDAG8^{-/-} mice (Fig. 2B, $t(34)=0.132$, $p=0.8958$, $n=17-19/\text{genotype}$).

3.2 TDAG8 deficiency does not alter sucrose preference

To assess whether hedonic drive is regulated by TDAG8 we assessed sucrose preference in TDAG8^{+/+} and TDAG8^{-/-} mice. TDAG8^{+/+} and TDAG8^{-/-} mice showed equivalent preference to a 1% sucrose solution (Fig. 3A, $t(13)=0.9660$, $p=0.3517$, $n=12/\text{genotype}$). Interestingly, TDAG8-deficient mice showed a higher consumption of sucrose (Fig. 3B, $t(13)=2.377$, $p=0.0335$), however, no differences were observed in water consumption (Fig. 3C, $t(13)=0.1999$, $p=0.8446$). There were no differences in the amount of chow intake between the two genotypes (Fig. 3D, $t(21)=0.3289$, $p=0.7455$).

4. Discussion

Despite having a high prevalence and significant morbidity and mortality, the mechanisms underlying depression are still unclear. As an initial study, we sought to determine the role of TDAG8, an immunomodulatory GPCR, in depression-like behavior. Using TDAG8 deficient versus wild type mice we observed a significant genotype effect in the forced swim test, a behavioral measure for despair-like depression related behavior. Lack of TDAG8 receptor led to decreased immobility and increased swimming behavior. TDAG8 did not impact hedonic drive as tested by sucrose preference test, although TDAG8^{-/-} mice showed significantly higher sucrose consumption as compared to wild type mice. Although preliminary, our findings suggest that the TDAG8 receptor can modulate behavioral responses relevant to depression, but has no effect on basal hedonic drive.

Despite extensive investigation, the pathophysiological causes of depression as well as, the mechanism underlying the contribution of inflammatory processes and action of antidepressant effects remain elusive. Therefore, it is difficult to explain the antidepressant-like effects of the TDAG8 deficiency, especially since the functional role of the TDAG8 receptor is not well known. Involvement of the TDAG8 receptor in behavioral response to the forced swim test manipulation is intriguing. The antidepressant-like behavior of TDAG8^{-/-} mice cannot be accounted for by changes in spontaneous activity since there are

no differences in activity between TDAG8^{+/+} and TDAG8^{-/-} mice under familiar and novel environments. The temperature of water used in the current study was slightly below the thermoneutral zone of mice (30–32°C)⁴⁵. Although this temperature is widely used in FST studies^{46–48}, we cannot rule out a potential contribution of cold stress toward the observed behaviors.

One potential link between TDAG8 and behavior may be the modulation of pro-inflammatory cytokines by the receptor. Abundant expression of TDAG8 is observed centrally and peripherally in innate immune cells such as macrophages and more recently, microglia in the CNS^{49,50}. Previous studies in rodent models have shown that TDAG8 may play an inflammatory role in allergic airway disease⁴¹ and inflammatory pain as development of hyperalgesia is enhanced in a TDAG8-dependent manner³⁸. Presence of TDAG8 on microglia opens up alternative mechanistic possibilities for linking inflammation and behavior. The link between innate immune cells in the brain and depression is recently being recognized^{51,23}. Microglia are a major source of pro-inflammatory cytokines such as IL-1 β , TNF- α and IL-6 in the CNS, that have all been implicated in depression-like behaviors rodents^{26,52,53}. IL-1 β and TNF α -deficient mice display improved performance in the FST^{54,55}. Additionally, infusion of LPS and IL-1 β were effective at increasing FST immobility in mice^{56,57}. However, there are other reports showing that induction of inflammation via LPS did not increase FST immobility in rats⁵⁸ and rats exposed to the FST did not show increases in IL-1 mRNA expression in several brain regions⁵⁹. Previous studies have also reported that inhibition of pro-inflammatory cytokines by antidepressant fluoxetine produces reduced immobility in the FST²⁹. It is possible that TDAG8 deficient mice have a reduced inflammatory tone that contributes to improved performance in the forced swim test.

Mechanisms underlying activation of acid sensor TDAG8 and depressive behavior warrant further investigation. Interestingly, our observations are similar to those observed for the acid-sensing ion channel 1-(ASIC1a), another sensor for detecting acidosis. Similar to TDAG8, ASIC1a null mice demonstrate significantly decreased immobility in the FST⁶⁰. This suggests that changes in pH, specifically acidosis may contribute to these behaviors, although how this occurs is currently unclear. Since HPA axis response to stress was not impacted by ASIC deficiency, glucocorticoids do not appear to be recruited⁶⁰. Another possibility is that forced activity or exercise during FST may evoke pH fluctuations⁶¹ that may be translated into behavioral responses. Elevated extracellular acidification can lead to release of pro-inflammatory cytokines such as IL-1 β from innate immune cells⁶². Association of acid-evoked pro-inflammatory responses and TDAG8 recruitment with observed behavioral responses is relevant and needs to be investigated.

While behavior in the FST was altered by TDAG8 deficiency, no effects were observed in preference to sucrose, a measure of hedonic drive, at least under baseline conditions tested in the current study. It is possible that exposure to chronic stress prior to assessment of sucrose preference, a model that results in reduced sucrose preference simulating anhedonia⁶³, may reveal differences between TDAG8^{+/+} and TDAG8^{-/-} mice. In this regard, acid sensor ASIC1a deficient mice elicit significantly attenuated sucrose preference following exposure to chronic mild stress⁶⁰. An interesting observation is the increase in

sucrose intake in TDAG8^{-/-} mice. The lack of differences in sucrose preference may be a result of increased sucrose consumption in the absence of reduced intake of water (since water consumption was not different between genotypes). Although contributory factors underlying these observations are not clear at present, they suggest a potential role of TDAG8 in regulation of fluid homeostasis and metabolism, as an association of pH with water intake has been reported in previous studies⁶⁴. Although outside the scope of this study, future investigation of this link is warranted.

Our observations suggest that the acid-sensing, immunomodulatory TDAG8 receptor is of potential interest to depression and open up exciting future studies related to TDAG8, inflammation and depression. Interaction of TDAG8 with chronic stress, an insult that promotes depression-like behavior and microglial activation^{63,65} will be important. Direct measurement of cytokines will highlight differences in immune function and may reveal important downstream effects of TDAG8 activation. Administration of commonly used antidepressant compounds and immune modulators such as LPS in TDAG8^{+/+} and TDAG8^{-/-} mice are necessary for mechanistic understanding of antidepressant-like characteristics of TDAG8 deficient mice.

In conclusion, our results suggest that the TDAG8 receptor is involved in depression-like behavior and following further validation, may provide a novel target linking inflammation and depression.

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References

1. Greden JF. The burden of disease for treatment-resistant depression. *J Clin Psychiatry*. 2001; 62:26–31+35. [PubMed: 11480881]
2. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. *Lancet*. 1997; 349:1498–504. [PubMed: 9167458]
3. Lépine JP, Briley M. The increasing burden of depression. *Neuropsychiatr Dis Treat*. 2011; 7:3–7. [PubMed: 21750622]
4. Miret M, Ayuso-Mateos JL, Sanchez-Moreno J, Vieta E. Depressive disorders and suicide: Epidemiology, risk factors, and burden. *Neurosci Biobehav Rev*. 2013; 37:2372–4. [PubMed: 23313644]
5. Bobo WV, et al. Randomized comparison of selective serotonin reuptake inhibitor (escitalopram) monotherapy and antidepressant combination pharmacotherapy for major depressive disorder with melancholic features: a COMED report. *J Affect Disord*. 2011; 133:467–76. [PubMed: 21601287]
6. Khan A, Faucett J, Lichtenberg P, Kirsch I, Brown WA. A systematic review of comparative efficacy of treatments and controls for depression. *PLoS One*. 2012; 7:e41778. [PubMed: 22860015]
7. Souery D, Papakostas GI, Trivedi MH. Treatment-resistant depression. *J Clin Psychiatry*. 2006; 67 (Suppl 6):16–22. [PubMed: 16848672]
8. Trivedi MH, et al. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. *Am J Psychiatry*. 2006; 163:28–40. [PubMed: 16390886]

9. Werner FM, Coveñas R. Classical neurotransmitters and neuropeptides involved in major depression in a multi-neurotransmitter system: a focus on antidepressant drugs. *Curr Med Chem*. 2013; 20:4853–8. [PubMed: 24083608]
10. Werner FM, Coveñas R. Classical neurotransmitters and neuropeptides involved in major depression: a review. *Int J Neurosci*. 2010; 120:455–70. [PubMed: 20583898]
11. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008; 9:46–56. [PubMed: 18073775]
12. Rosenblat JD, Cha DS, Mansur RB, McIntyre RS. Inflamed moods: a review of the interactions between inflammation and mood disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2014; 53:23–34. [PubMed: 24468642]
13. Pace TWW, Miller AH. Cytokines and glucocorticoid receptor signaling. Relevance to major depression. *Ann N Y Acad Sci*. 2009; 1179:86–105. [PubMed: 19906234]
14. Iwata M, Ota KT, Duman RS. The inflammasome: pathways linking psychological stress, depression, and systemic illnesses. *Brain Behav Immun*. 2013; 31:105–14. [PubMed: 23261775]
15. Maes M, et al. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis*. 2009; 24:27–53. [PubMed: 19085093]
16. Maier SF, Watkins LR, Fleshner M. Psychoneuroimmunology: The interface between behavior, brain, and immunity. *Am Psychol*. 1994; 49:1004–1017. [PubMed: 7818221]
17. Anisman H, Ravindran AV, Griffiths J, Merali Z. Interleukin-1 beta production in dysthymia before and after pharmacotherapy. *Biol Psychiatry*. 1999; 46:1649–55. [PubMed: 10624546]
18. Tuglu C, Kara SH, Caliyurt O, Vardar E, Abay E. Increased serum tumor necrosis factor-alpha levels and treatment response in major depressive disorder. *Psychopharmacology (Berl)*. 2003; 170:429–33. [PubMed: 12955291]
19. Kim YK, et al. Cytokine imbalance in the pathophysiology of major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007; 31:1044–53. [PubMed: 17433516]
20. Yang K, et al. Levels of serum interleukin (IL)-6, IL-1beta, tumour necrosis factor-alpha and leptin and their correlation in depression. *Aust N Z J Psychiatry*. 2007; 41:266–73. [PubMed: 17464708]
21. Dowlati Y, et al. A meta-analysis of cytokines in major depression. *Biol Psychiatry*. 2010; 67:446–57. [PubMed: 20015486]
22. Howren MB, Lamkin DM, Suls J. Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosom Med*. 2009; 71:171–86. [PubMed: 19188531]
23. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. *Nat Rev Neurosci*. 2008; 9:46–56. [PubMed: 18073775]
24. Schiepers OJG, Wichers MC, Maes M. Cytokines and major depression. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005; 29:201–17. [PubMed: 15694227]
25. Felger JC, Lotrich FE. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. *Neuroscience*. 2013; 246:199–229. [PubMed: 23644052]
26. Kaster MP, Gadotti VM, Calixto JB, Santos ARS, Rodrigues ALS. Depressive-like behavior induced by tumor necrosis factor- α in mice. *Neuropharmacology*. 2012; 62:419–26. [PubMed: 21867719]
27. Tarr AJ, Chen Q, Wang Y, Sheridan JF, Quan N. Neural and behavioral responses to low-grade inflammation. *Behav Brain Res*. 2012; 235:334–41. [PubMed: 22898545]
28. Tynan RJ, et al. A comparative examination of the anti-inflammatory effects of SSRI and SNRI antidepressants on LPS stimulated microglia. *Brain Behav Immun*. 2012; 26:469–79. [PubMed: 22251606]
29. Ohgi Y, Futamura T, Kikuchi T, Hashimoto K. Effects of antidepressants on alternations in serum cytokines and depressive-like behavior in mice after lipopolysaccharide administration. *Pharmacol Biochem Behav*. 2013; 103:853–9. [PubMed: 23262300]
30. Choi JW, Lee SY, Choi Y. Identification of a putative G protein-coupled receptor induced during activation-induced apoptosis of T cells. *Cell Immunol*. 1996; 168:78–84. [PubMed: 8599842]

31. Ishii S, Kihara Y, Shimizu T. Identification of T cell death-associated gene 8 (TDAG8) as a novel acid sensing G-protein-coupled receptor. *J Biol Chem.* 2005; 280:9083–7. [PubMed: 15618224]
32. Ludwig M, et al. Proton-sensing G-protein-coupled receptors. 2003; 425:93–98.
33. Radu CG, Nijagal A, McLaughlin J, Wang L, Witte ON. Differential proton sensitivity of related G protein-coupled receptors T cell death-associated gene 8 and G2A expressed in immune cells. *Proc Natl Acad Sci U S A.* 2005; 102:1632–7. [PubMed: 15665078]
34. Wang JQ, et al. TDAG8 is a proton-sensing and psychosine-sensitive G-protein-coupled receptor. *J Biol Chem.* 2004; 279:45626–33. [PubMed: 15326175]
35. McGuire J, et al. Acid-sensing by the T cell death-associated gene 8 (TDAG8) receptor cloned from rat brain. *Biochem Biophys Res Commun.* 2009; 386:420–5. [PubMed: 19501050]
36. Kyaw H, et al. Cloning, characterization, and mapping of human homolog of mouse T-cell death-associated gene. *DNA Cell Biol.* 1998; 17:493–500. [PubMed: 9655242]
37. Radu CG, et al. Normal immune development and glucocorticoid-induced thymocyte apoptosis in mice deficient for the T-cell death-associated gene 8 receptor. *Mol Cell Biol.* 2006; 26:668–77. [PubMed: 16382156]
38. Chen YJ, Huang CW, Lin CS, Chang WH, Sun WH. Expression and function of proton-sensing G-protein-coupled receptors in inflammatory pain. *Mol Pain.* 2009; 5:39. [PubMed: 19602228]
39. Okajima F. Regulation of inflammation by extracellular acidification and proton-sensing GPCRs. *Cell Signal.* 2013; 25:2263–71. [PubMed: 23917207]
40. Huang CW, et al. Nociceptors of dorsal root ganglion express proton-sensing G-protein-coupled receptors. *Mol Cell Neurosci.* 2007; 36:195–210. [PubMed: 17720533]
41. Kottyan LC, et al. Eosinophil viability is increased by acidic pH in a cAMP- and GPR65-dependent manner. *Blood.* 2009; 114:2774–82. [PubMed: 19641187]
42. Vollmer EL, et al. Attenuated stress-evoked anxiety, increased sucrose preference and delayed spatial learning in glucocorticoid-induced receptor-deficient mice. *Genes Brain Behav.* 2013; 12:241–9. [PubMed: 23088626]
43. Can A, et al. The mouse forced swim test. *J Vis Exp.* 2012:e3638.10.3791/3638 [PubMed: 22314943]
44. Solomon MB, et al. Deletion of forebrain glucocorticoid receptors impairs neuroendocrine stress responses and induces depression-like behavior in males but not females. *Neuroscience.* 2012; 203:135–43. [PubMed: 22206943]
45. Karp CL. Unstressing intemperate models: how cold stress undermines mouse modeling. *J Exp Med.* 2012; 209:1069–74. [PubMed: 22665703]
46. Pliakas AM, et al. Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. *J Neurosci.* 2001; 21:7397–7403. [PubMed: 11549750]
47. Solomon MB, et al. The selective glucocorticoid receptor antagonist CORT 108297 decreases neuroendocrine stress responses and immobility in the forced swim test. *Horm Behav.* 2014; 65:363–71. [PubMed: 24530653]
48. Slattery DA, Cryan JF. Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nat Protoc.* 2012; 7:1009–14. [PubMed: 22555240]
49. Jin Y, et al. Inhibition of interleukin-1 β production by extracellular acidification through the TDAG8/cAMP pathway in mouse microglia. *J Neurochem.* 2014; 10.1111/jnc.12661
50. Vollmer LL, Bailey L, Li K-Y, Lewkowich I, Putnam RW, Sah R. Microglial acid-sensing t-cell death associated gene-8 (tdag8) receptor: Linking neuroinflammation and panic responses. *Soc Neurosci Abstr.* 2013
51. Bergink V, Gibney SM, Drexhage HA. Autoimmunity, inflammation, and psychosis: a search for peripheral markers. *Biol Psychiatry.* 2014; 75:324–31. [PubMed: 24286760]
52. Lawson MA, McCusker RH, Kelley KW. Interleukin-1 beta converting enzyme is necessary for development of depression-like behavior following intracerebroventricular administration of lipopolysaccharide to mice. *J Neuroinflammation.* 2013; 10:54. [PubMed: 23634700]
53. Chourbaji S, et al. IL-6 knockout mice exhibit resistance to stress-induced development of depression-like behaviors. *Neurobiol Dis.* 2006; 23:587–94. [PubMed: 16843000]

54. Koo JW, Duman RS. IL-1beta is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci U S A*. 2008; 105:751–6. [PubMed: 18178625]
55. Simen BB, Duman CH, Simen AA, Duman RS. TNFalpha signaling in depression and anxiety: behavioral consequences of individual receptor targeting. *Biol Psychiatry*. 2006; 59:775–85. [PubMed: 16458261]
56. Godbout JP, et al. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. *FASEB J*. 2005; 19:1329–31. [PubMed: 15919760]
57. Dunn AJ, Swiergiel AH. Effects of interleukin-1 and endotoxin in the forced swim and tail suspension tests in mice. *Pharmacol Biochem Behav*. 2005; 81:688–93. [PubMed: 15982728]
58. Deak T, et al. Behavioral responses during the forced swim test are not affected by anti-inflammatory agents or acute illness induced by lipopolysaccharide. *Behav Brain Res*. 2005; 160:125–34. [PubMed: 15836907]
59. Deak T, Bellamy C, D'Agostino LG. Exposure to forced swim stress does not alter central production of IL-1. *Brain Res*. 2003; 972:53–63. [PubMed: 12711078]
60. Coryell MW, et al. Acid-sensing ion channel-1a in the amygdala, a novel therapeutic target in depression-related behavior. *J Neurosci*. 2009; 29:5381–8. [PubMed: 19403806]
61. Aoi W, Marunaka Y. Importance of pH Homeostasis in Metabolic Health and Diseases: Crucial Role of Membrane Proton Transport. *Biomed Res Int*. 2014; 2014:598986. [PubMed: 25302301]
62. Rajamäki K, et al. Extracellular acidosis is a novel danger signal alerting innate immunity via the NLRP3 inflammasome. *J Biol Chem*. 2013; 288:13410–9. [PubMed: 23530046]
63. Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)*. 1997; 134:319–329. [PubMed: 9452163]
64. Badgaiyan RD, Mandal MB. Alterations of ventricular pH alter water intake and food consumption in rats. *Physiol Behav*. 1990; 47:489–492. [PubMed: 2359758]
65. Walker AJ, et al. Stress, Inflammation, and Cellular Vulnerability during Early Stages of Affective Disorders: Biomarker Strategies and Opportunities for Prevention and Intervention. *Front psychiatry*. 2014; 5:34. [PubMed: 24782789]

Highlights

- The role of the TDAG8 receptor in depression relevant behaviors was studied
- TDAG8^{-/-} mice were tested in the forced swim test (FST) and sucrose preference
- TDAG8 deficiency significantly attenuated immobility in the FST
- There were no significant genotype differences in sucrose preference
- The TDAG8 receptor may be recruited in behavioral responses relevant to depression

Fig. 1. Forced swim test (FST). (A) Deficiency of TDAG8 significantly reduced immobility in the FST (* $p=0.0316$). (B) Swimming behavior was higher in TDAG8^{-/-} (-/-) mice as compared to TDAG8^{+/+} (+/+) mice (* $p=0.0316$) (n=12/group).

Fig. 2.

Effects of TDAG8 deficiency on motor activity (A) Home cage motor activity showed no significant differences between TDAG8^{+/+} ($+/+$) and TDAG8^{-/-} ($-/-$) mice ($p=0.5906$, $n=8$ /group). (B) Upon exposure to novel context no significant differences in motor activity were observed between genotypes ($p=0.8958$, $n=17-19$ /group).

Fig. 3. Sucrose preference test. (A) TDAG8^{-/-} (-/-) mice elicited no significant differences in sucrose preference as compared TDAG8^{+/+} (+/+) mice ($p=.4857$) (B) Consumption of sucrose was significantly higher in TDAG8^{-/-} mice than control mice (* $p=.0335$). (C) TDAG8^{-/-} and TDAG8^{+/+} mice consumed an equivalent amount of water ($p=.8446$). (D) No significant difference in chow intake was observed between genotypes ($p=.7455$) ($n=12$ /group).