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Forebrain overexpression of type 1 adenylyl cyclase promotes molecular stability and behavioral resilience to physical stress

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ABSTRACT

The ability to cope with stress is essential for emotional stability and mental health. It is also hypothesized that factors promoting resilience to stress may offer treatment strategies for maladaptive disorders such as anxiety and depression. Here, we find that physical restraint reduces the expression of type 1 adenylyl cyclase (*Adcy1*), a neurospecific synaptic enzyme that positively regulates the cAMP signaling cascade. Conversely, an increase of forebrain *Adcy1* expression in transgenic mouse (i.e., *Adcy1*^{tg} mouse) predisposes individuals to molecular stability and behavioral resilience. Transgenic overexpression of *Adcy1* prevents the physical restraint-induced down-regulation of brain-derived neurotrophic factor (*BDNF*) and neuropeptide Y (NPY). Further, *Adcy1*^{tg} mice maintain regular locomotive activity in novelty exploration and voluntary wheel running following physical restraint. *Adcy1*^{tg} mice show higher corticosterone and lower basal glucocorticoid receptor (GR) expression, along with a higher MR (mineralocorticoid receptor) to GR ratio in the hippocampus. Further, *Adcy1*^{tg} mice show reduced immobility under acute physical stress conditions in the forced swimming test and are more sensitive to the antidepressant desipramine. Our results demonstrate a novel function of *Adcy1* in stress coping and suggest *Adcy1* as a potential target to antagonize stress vulnerability and promote antidepressant efficacy.

1. Introduction

The psychological impact of stress affects mental health and brain function. An active stress-coping process is essential to maintain appropriate behavioral adaptation (Franklin et al., 2012). It is recognized that excessive vulnerability and lack of necessary resilience to stress often lead to severe psychopathologies, such as anxiety and depression (Franklin et al., 2012; Faye et al., 2018). While molecular alterations in vulnerable individuals may suggest novel aspects of functional pathology, recent efforts to identify resilience factors have had significant impacts on understanding mechanisms underlying active stress-coping strategies (Russo et al., 2012; Cathomas et al., 2019), which may lead to new therapeutic approaches for the treatment of stress-related psychopathology (Southwick et al., 2005).

Psychopathology associated with altered stress responses and maladaptive disorders often affects many behavioral domains. Among them, cognitive impairments along with structural and molecular alterations associated with synaptic plasticity are prevalent (Marsden, 2013), and to a significant degree, impact mental health and daily functioning in patients (Cantone et al., 2017). Interestingly, downstream targets of various antidepressants often play an essential role in regulating plasticity and cognitive functions (van Calker et al., 2018). However, whether factors contributing to cognitive enhancement can promote behavioral stability and resilience to stress remains to be determined.

We previously demonstrated that neuron-specific transgenic mice overexpressing *Adcy1* in the forebrain (*Adcy1*^{tg} mice) show enhanced hippocampal long-term potentiation (LTP) and hippocampus-dependent memory, including object recognition memory (Wang et al., 2004) and

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spatial memory (Zhang and Wang, 2013). At the molecular level, *Adcy1* activity directly impinges on cAMP and the ERK½-CREB (extracellular signal-regulated kinase ½-cAMP responsive element binding protein) signaling cascade (Wang et al., 2004), whose function is implicated in molecular and behavioral aspects of cognition (Kandel, 2012), stress and anxiety (Wand, 2005) and depression (Marsden, 2013). Interestingly, reduction of the Adcy-cAMP-ERK½-CREB signaling cascade associates with affective disorders and stress; increase of this signaling cascade associates with antidepressant treatment (Dowlatshahi et al., 1999; Jensen et al., 2000; Thome et al., 2000; Duman et al., 2007; Qi et al., 2008; Li et al., 2009). However, the causal role of *Adcy* and cAMP-regulated signaling in stress coping and particularly resilience remains unknown and elusive.

In this study, we find that physical restraint down-regulates the *Adcy1* level along with a reduction in motivation behavior such as novelty exploration and wheel running. To determine the causal relevance of the stress-induced decrease of *Adcy1*, we examined the effects of *Adcy1* overexpression on stress coping. Enhanced expression in *Adcy1*^{tg} mice eliminates the molecular responses to stress by stabilizing the expression of *BDNF*, which supports certain aspects of resilience (Taliaz et al., 2011; Notaras and van den Buuse, 2020). Consistently, *Adcy1*^{tg} mice maintain a normal level of novelty exploration and wheel running following physical restraint. Moreover, under acute stress conditions in the forced swimming test, *Adcy1*^{tg} mice show less immobility and are more sensitive to a sub-threshold dose of the antidepressant desipramine. Our data demonstrate a sufficient function of the elevated *Adcy1* and cAMP signaling in promoting molecular and behavioral stability. As ADCY1 is an enzyme rather than a structure protein and only expressed in the central nervous system (CNS) (Xia et al., 1993; Wang and Zhang, 2012), our data further suggest ADCY1 as a potential target for the treatment of stress maladaptation.

2. Methods

2.1. Animals

The *Adcy1*^{tg} mice, which overexpress *Adcy1* driven by the *α-CaMKII* promoter in the forebrain, are in the C57BL/6 background, as described in previous studies (Wang et al., 2004; Zhang and Wang, 2013). For all experiments in this study, we used 2.5- to 3.5-month old male littermate mice. All mice had *ad libitum* access to water and food and were housed under 12 h dark/light cycle. All manipulations complied with the guidelines and approved by the Institutional Animal Care and Use Committee at Michigan State University.

2.2. Physical restraint and sample collection

The mouse was placed in ventilated 50-ml polypropylene tubes and restrained for 2 h. For blood and brain sample collection, mice were sacrificed by rapid decapitation immediately after the physical restraint procedure. Trunk blood was collected into heparinized tubes. Plasma was separated by centrifugation at 3000 rpm for 5 min at 4 °C and stored at −80 °C for corticosterone measurement. Hippocampus and prefrontal cortex were rapidly removed and stored at −80 °C for RNA and protein measurement.

2.3. Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from brain tissues using the TRIzol method (Invitrogen) according to the manufacturer's instruction. One μg of RNA was reverse transcribed using the SuperScript III Reverse transcription kit (Invitrogen) followed by qPCR using Bio-Rad iCycler. The primers for amplification of *Adcy1* are 5′-AAACACAGTCAATGTGGCCAGTCG-3′ and 5′-ACTTTGCCTCTGCACACAAACTGG-3'; primers for *BDNF* exon I isoform are 5′-AGTCTCCAGGACAGCAAAGC-3′ and 5′-GCCTTCATGCAACCGAAGTA-3'; primers for *BDNF* exon IV isoform are 5′-CTCCGCCATGCAATTTCCAC-3′ and 5′-GCCTTCATGCAACCGAAGTA-3'; primers for total *BDNF* are 5′-GCGGCAGATAAAAAGACTGC-3′ and 5′-TCAGTTGGCCTTTGGATACC-3'; primers for *NPY* are 5′-AGAGATCCAGCCCTGAGACA-3′ and 5′-TCACCACATGGAAGGGTCTT-3'; primers for *GR* (glucocorticoid receptor) are 5′-GTGAGTTCTCCTCCGTCCAG-3′ and 5′-TACAGCTTCCACACGTCAGC-3'; primers for *MR* (mineralcorticoid receptor) are 5′-GCAGATCAGCCTTCAGTTCG-3′ and 5′-CTCATCTCCTCAAACGCAGC-3'; primers for *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) are 5′-TCCATGACAACTTTGGCATTGTGG-3′ and 5′-GGATGCAGGGATGATGTTCT-3'. The Value of Ct for each gene was determined and normalized to that of housekeeping gene *GAPDH*. Results are expressed as mean fold changes compared with the unstressed wild type (WT) controls using the $2^{-\Delta\Delta\ Ct}$ method.

2.4. Novel environment exploration in open field chamber

Immediately after the 2-h physical restraint, mice were placed in the center of an open field chamber (Coulbourn Instruments, Whitehall, PA) and allowed to explore freely for 30 min. The overall locomotive exploratory activity in the whole open field arena, as well as in the center area, was recorded and analyzed with the TruScan Photo Beam Activity System (Coulbourn Instruments).

2.5. Voluntary wheel-running activity in home cage

The metal wheel with a diameter of 11.5 cm and equipped with a digital magnetic counter was placed in standard rat cages (47× 26 × 14.5 cm). The maximum running speed, total running distance, and total running time were recorded. Data on daily activity were collected every morning at 9 a.m. The baseline level of wheel-running activity was recorded for 7 days without physical restraint procedure. Following the baseline activity recording, wheel-running activity was recorded for 7 days, during which a daily 2-h physical restraint was imposed between 10 a.m. and 12 p.m. Then, the physical restraint was withdrawn; animals were allowed to recover from daily stress; wheel-running activity was recorded for 7 days.

2.6. Forced swimming test (FST) and tail suspension test (TST)

For FST, the mouse was placed in a clear plastic cylinder (13 cm diameter and 23 cm height) filled with clear water at 23–25 °C. For TST, the mouse was hung with the tail attached to a hook on the ceiling of a box (20 cm width, 20 cm depth, and 30 cm height). During the 6 min test, immobility was recorded for the last 4 min or the entire 6 min. For FST, immobility was defined as the absence of any horizontal or vertical movement in the water except for minor movements required for the mouse to keep its head above the surface. For TST, immobility was defined as a lack of struggle movement.

2.7. Western blot

Brain tissues were first homogenized in buffer H, followed by protein concentration determination (Bio-rad). Samples were then separated by SDS-PAGE, transferred to a nitrocellulose membrane, and incubated with antibodies against ADCY1 (Sigma, 1:1000), pERK½ (Cell Signaling, 1:2000), ERK½ (Cell Signaling, 1:1000), and β-actin (Sigma, 1:10000) overnight at 4 °C. Immuno signal was detected with IRDye 800CW-conjugated goat anti-rabbit or IRDye 680RD-conjugated goat anti-mouse antibody (LI-COR, 1:5000) using the Odyssey Imaging system. The signal intensity of the detected protein was quantified using ImageJ (NIH, MD, USA).

2.8. Plasma corticosterone levels

Plasma corticosterone was measured according to the manufacturer's instructions using a corticosterone enzyme immunoassay kit (Cayman Chemicals, Ann Arbor, MI, USA).

2.9. Statistical analysis

All data are expressed as mean±SEM. Two-tailed Student's t-test was used for analyzing data from two experimental groups. Two-way ANOVA followed by *post hoc* pairwise comparison was used to analyze data that involve two factors (e.g., treatment and genotype). Novelty exploration activity in the open field and voluntary wheel-running activity in the home cage were analyzed by three-way ANOVA with repeated measures. All statistical analyses were performed with the SPSS software.

3. Results

3.1. Stress down-regulates Adcy1 along with stress-related genes in mouse hippocampus and prefrontal cortex

ADCY1 is a major Ca^{2+}-stimulated ADCY, and couples Ca^{2+} to cAMP production in the central nervous system (CNS) (Wang and Zhang, 2012). Consistent with that cAMP signaling regulates both cognitive and emotional function, we found robust expression of *Adcy1* mRNA in the mouse hippocampus and prefrontal cortex (PFC). Interestingly, a 2-h physical restraint stress significantly decreased the level of *Adcy1* mRNA in both brain regions (Fig. 1a).

Previous studies demonstrate that Ca^{2+}-stimulated ADCY supports activity-dependent transcription of cAMP/CREB (cAMP responsive element binding protein) target genes in the hippocampus (Zheng et al., 2012, 2016). The function of cAMP/CREB target genes, particularly *BDNF* and *NPY* (Higuchi et al., 1988; Tao et al., 1998; Pandey, 2003; Zheng et al., 2012), is also strongly implicated in stress response, antidepressant effects, and pathological alterations in depression and anxiety (Khundakar and Zetterstrom, 2006; Eaton et al., 2007; Duric et al., 2010). For the activity-dependent regulation of *BDNF* expression, it is known that isoforms of different exon-containing transcripts may be tailored to distinct stimulation and show brain region-specific pattern (Aid et al., 2007). Among them, exon 1- and 4-containing *BDNF* mRNA level is particularly sensitive to neuronal stimulation (Tao et al., 1998; Zheng et al., 2016). Here, we found that the levels of exon 1- and exon 4-containing *BDNF* mRNA, whose transcription is differentially regulated in hippocampal and cortical neurons (Zheng et al., 2011, 2016), are decreased by physical restraint in the hippocampus (Fig. 1b) and PFC (Fig. 1c), respectively, but not in both regions. The level of total *BDNF* mRNA, which is comprised of 9 different exon-containing isoforms (Aid et al., 2007), was significantly reduced by physical restraint in the hippocampus (Fig. 1b) but not PFC (Fig. 1c). The physical restraint reduced *NPY* mRNA in the hippocampus (Fig. 1d) but not PFC (Fig. 1e). These data demonstrate that physical restraint stress is sufficient to down-regulate *Adcy1* along with certain plasticity- and stress-related genes.

3.2. Overexpression of Adcy1 is sufficient to support molecular stability following physical restraint stress

To determine the functional relevance of the restraint-induced *Adcy1* reduction, we examined whether *Adcy1* overexpression can antagonize the stress-induced molecular alterations. We generated $Adcy1^{tg}$ mice, in which the transgenic gene is under the control of the *α-CaMKII* promoter and overexpressed in forebrain regions (Wang et al., 2004). Compared to the wild type (WT) mice, the $Adcy1^{tg}$ mice show a significant increase of ADCY1 in both hippocampus and PFC (Fig. 2a). Consistent with that cAMP stimulates ERK½ (extracellular signal-regulated kinase ½) activity (Morozov et al., 2003), which is implicated in regulating the cAMP/-CREB target genes (Zheng et al., 2011) and mood disorders (Gourley et al., 2008; Duric et al., 2010), pERK½ (phosphorylated ERK½) level is increased in the hippocampus and PFC of $Adcy1^{tg}$ mice (Fig. 2b). We found that *Adcy1* overexpression blocks the restraint-induced reduction of total and exon 1-containing *BDNF* in the hippocampus (Fig. 2c) and

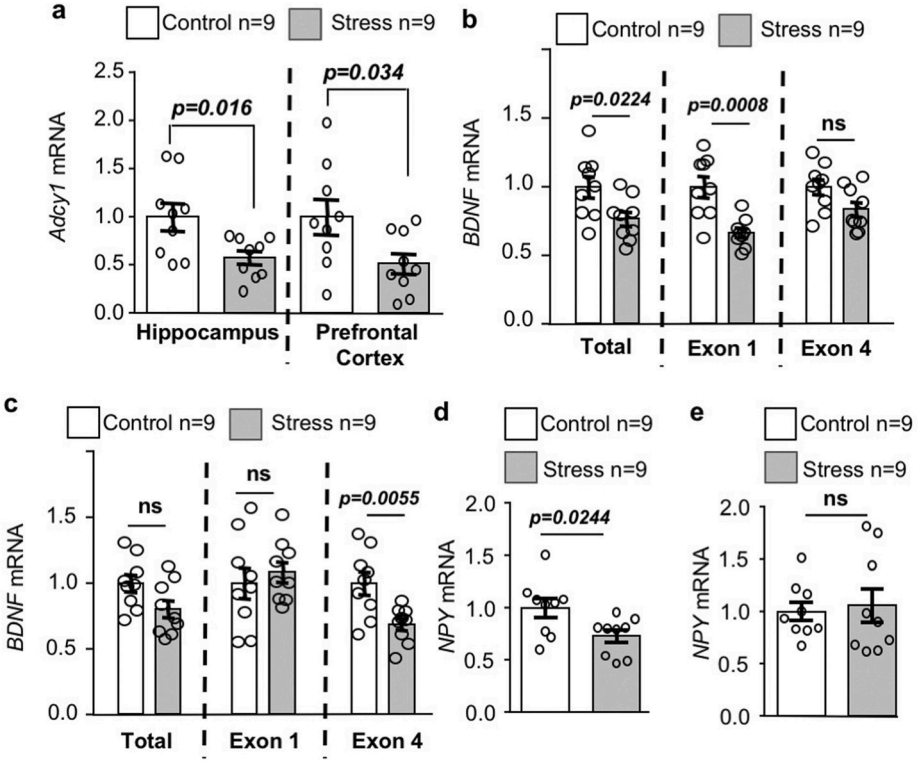

Fig. 1. Physical restraint causes a reduction of *Adcy1* along with stress-related genes in the mouse brain. After a 2-h physical restraint stress, the hippocampus and prefrontal cortex were harvested from wild type mice. The mRNA level of *Adcy1* (**a**), *BDNF* (**b** and **c**), and *NPY* (**d** and **e**) in control and stressed mice was determined by quantitative RT-PCR and normalized to the level of *GAPDH*. For *BDNF*, isoform-specific primers were used to amplify and detect exon 1-containing, exon 4-containing, and total mRNA. The relative level in the control groups was defined as 1. Data reported as mean ± SEM, along with the individual data points, are presented for changes in the hippocampus (**a**, **b**, and **d**) and prefrontal cortex (**a**, **c**, and **e**). The *p* value was determined by two-tailed Student's t-test. ns: not significant (i.e. $p > 0.05$).

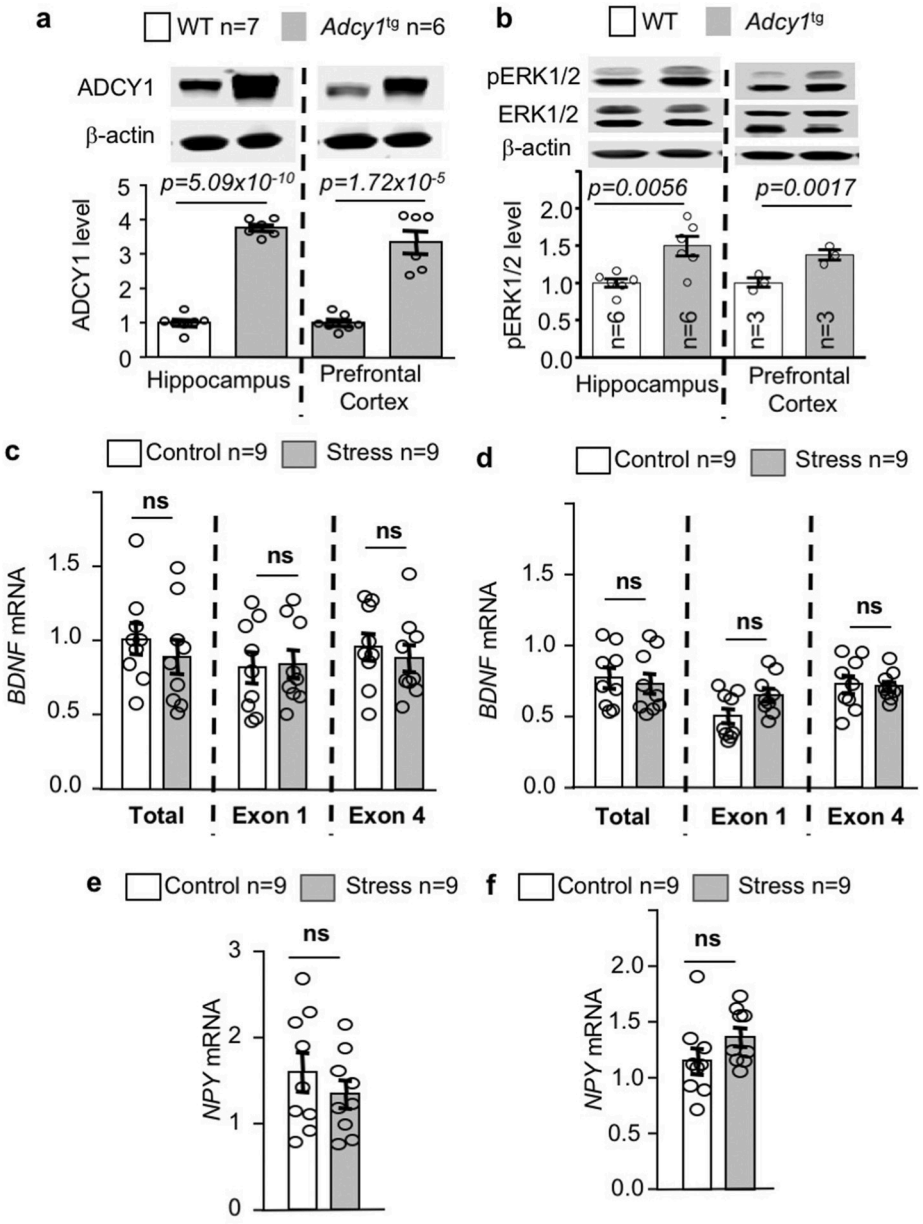

Fig. 2. Overexpression of *Adcy1* prevents the reduction of *BDNF* and *NPY* expression after physical restraint. **a** and **b.** Hippocampus and prefrontal cortex were dissected from the wild type (WT) and *Adcy1*^{tg} mice. The level of ADCY1 protein (**a**) and pERK½ (**b**) was determined by Western blot. The levels of ADCY1 and pERK½ were normalized to the level of β-actin and total ERK½, respectively. The relative level of these molecules was defined as 1 in the WT samples. From **c** to **f**. After a 2-h physical restraint stress, the hippocampus (**c** and **e**) and prefrontal cortex (**d** and **f**) were harvested from the *Adcy1*^{tg} mice. The mRNA level of *BDNF* (total and exon 1- and exon 4-containing mRNA isoforms) (**c** and **d**) and *NPY* (**e** and **f**) in the control and stressed *Adcy1*^{tg} mice was determined by quantitative RT-PCR and normalized to the level of *GAPDH*. All data are reported as mean ± SEM along with individual data points. The *p* value was determined by two-tailed Student's t-test. ns: not significant (i.e. $p > 0.05$).

exon 4-containing *BDNF* in PFC (Fig. 2d) of *Adcy1*^{tg} mice. Physical restraint failed to affect NPY mRNA in the hippocampus (Fig. 2e) and PFC (Fig. 2f) of *Adcy1*^{tg} mice. Interestingly, comparing to the unstressed WT mice, the unstressed *Adcy1*^{tg} mice showed lower basal level of *BDNF* mRNA (1±0.056 in WT versus 0.774±0.070 in *Adcy1*^{tg} mice for total mRNA, $p = 0.031$; 1±0.118 in WT versus 0.505±0.053 in *Adcy1*^{tg} mice for exon 1 mRNA, $p = 0.001$; 1±0.086 in WT versus 0.73±0.06 in *Adcy1*^{tg} mice for exon 4 mRNA, $p = 0.021$) in PFC but not hippocampus. Overexpression of *Adcy1* causes a higher basal level of *NPY* mRNA in the hippocampus (1±0.09 in WT versus 1.60 ±0.227 in *Adcy1*^{tg} mice, $p = 0.025$) but not PFC.

3.3. Overexpression of Adcy1 attenuates behavioral alteration following physical restraint stress

In addition to the molecular alterations, we observed a significant reduction in exploratory activity in a novel open field chamber following the 2-h physical restraint (Fig. 3a–e). For the non-stressed control groups, WT and *Adcy1*^{tg} mice showed comparable locomotion activity (Fig. 3a and b). Following physical restraint, WT but not *Adcy1*^{tg} mice showed decreased exploratory activity in the open field arena (Fig. 3a and b; genotype effect: $F_{1,36} = 9.795$, $p = 0.003$; stress effect: $F_{1,36} = 15.834$, $p < 0.0001$; stress X genotype interaction: $F_{1,36} = 18.902$, $p < 0.0001$). We further analyzed the exploratory activity in the center area of the open field arena, which may reflect certain aspects of anxiety. There was no difference in the non-stressed groups (Fig. 3c and d). Both WT and *Adcy1*^{tg} mice showed reduction of locomotion activity in the center area following physical restraint (Fig. 3c and d; genotype effect: $F_{1,36} = 5.964$, $p = 0.02$; stress effect: $F_{1,36} = 21.613$, $p < 0.0001$; stress X genotype interaction: $F_{1,36} = 1.054$, $p = 0.311$). However, following stress, *Adcy1*^{tg} mice showed more center activity than WT mice (Fig. 3c and d); the relative reduction of center activity in response to stress was significantly less in the *Adcy1*^{tg} mice (Fig. 3e). These results indicate that the exploratory behavior was less affected in *Adcy1*^{tg} mice.

We next examined the effect of physical restraint stress on voluntary wheel running, which reflects certain aspect of motivation that is decreased by stress (DeVallance et al., 2017; Muguruza et al., 2019). During the first week with no stress, *Adcy1*^{tg} and WT mice showed

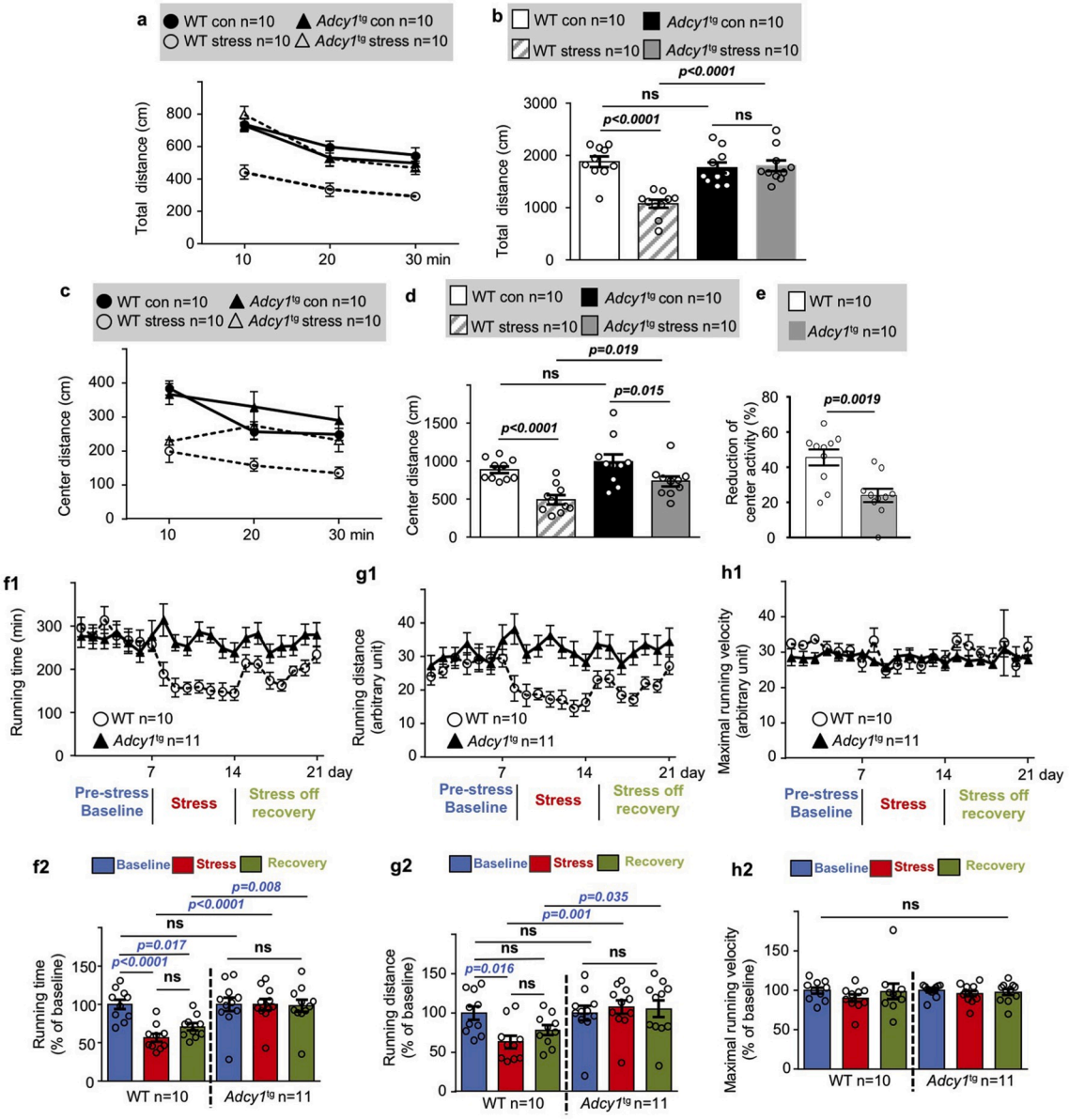

Fig. 3. Exploratory and voluntary running behavior in *Adcy1*^{tg} mice is less affected by physical restraint. From **a** to **e**, wild type (WT) and *Adcy1*^{tg} mice were placed in a novel open field arena immediately after the 2 h-restraint stress and allowed to freely explore for 30 min. Locomotion activity (i.e., travel distance) in the whole open field arena (**a** and **b**) and the center area (**c** and **d**) was recorded. Activity in each of the 10-min bin is presented in **a** and **c**. Accumulative activity during the whole 30-min testing is presented in **b** and **d**. Relative fold reduction of the center activity following the physical restraint is presented in **e**. From **f** to **h**, the running time (**f**), running distance (**g**), and maximal running velocity (**h**) of wild type (WT) and *Adcy1*^{tg} mice were recorded daily for 3 weeks. The baseline activity was recorded during the 1st pre-stress week for mice without exposure to physical restraint. During the 2nd week, mice were subjected to the daily 2-h physical restraint. During the 3rd week, no physical restraint was imposed, and mice were allowed to recover from stress. Data for each day are presented in **f1**, **g1**, and **h1**. The averaged daily activity during the baseline week, stress week, and recovery week is normalized to that of the baseline value and presented in **f2**, **g2**, and **h2**. Data are expressed as mean ± SEM along with individual data points. Data in **a, c, f1, g1, and h1** were analyzed by three-way ANOVA with repeated measures. Data in **b**, **d, f2, g2, and h2** were analyzed by two-way ANOVA followed by *post-hoc* pairwise comparison. Data in **e** were analyzed by two-tailed Student's t-test. ns: not significant (i.e. $p > 0.05$).

comparable baseline running activity including running time, running distance, and maximal speed (Fig. 3f–h). During the second week, the same mice were subjected to a daily 2-h episode of restraint stress. Compared with the baseline activity, running time (Fig. 3f) and distance (Fig. 3g) were sharply decreased in WT but not *Adcy1*^{tg} mice. There was significant difference between WT and *Adcy1*^{tg} mice for daily (Fig. 3f1; genotype effect: $F_{1,57} = 12.748$, $p = 0.001$; treatment effect: $F_{2,57} = 5.248$, $p = 0.008$; treatment X genotype interaction: $F_{2,57} = 5.019$, $p = 0.01$) (Fig. 3g1; genotype effect: $F_{1,57} = 19.326$, $p < 0.0001$; treatment effect: $F_{2,57} = 1.163$, $p = 0.320$; treatment X genotype interaction: $F_{2,57} = 2.936$, $p = 0.061$) and averaged activity (Fig. 3f2; genotype effect: $F_{1,57} = 16.640$, $p < 0.0001$; treatment effect: $F_{2,57} = 5.061$, $p = 0.009$; treatment X genotype interaction: $F_{2,57} = 4.773$, $p = 0.012$) (Fig. 3g2; genotype effect: $F_{1,57} = 10.895$, $p = 0.002$; treatment effect: $F_{2,57} = 1.385$, $p = 0.259$; treatment X genotype interaction: $F_{2,57} = 3.216$, $p = 0.047$). During the third week with stress withdrawal, WT mice exhibited gradual but not instant recovery in running wheel activity toward the pre-stress baseline level (Fig. 3f1 and 3g1). The averaged daily activity in WT mice during the recovery week was still lower and not different from the averaged daily activity during the stress week (Fig. 3f2 and 3g2); the activity on the last 2 days of the recovery week was recovered to the baseline level. Comparing to the relative baseline

level (100±6.46%), the running time on the 6th and 7th day of the recovery week was 73.70±5.81% ($p = 0.0073$) and 84.36±4.93% ($p = 0.070$), respectively. The running distance on the 6th and 7th day was 78.28±6.79% ($p = 0.063$) and 98.59±6.98% ($p = 0.900$), respectively. The maximal running speed in WT and *Adcy1*^{tg} mice remained constant and not altered by the physical restraint (Fig. 3h1; genotype effect: $F_{1,57} = 1.166$, $p = 0.285$; treatment effect: $F_{2,57} = 1.019$, $p = 0.367$; treatment X genotype interaction: $F_{2,57} = 0.302$, $p = 0.740$) (Fig. 3h2; genotype effect: $F_{1,57} = 0.175$, $p = 0.678$; treatment effect: $F_{2,57} = 1.023$, $p = 0.366$; treatment X genotype interaction: $F_{2,57} = 0.280$, $p = 0.757$).

3.4. Overexpression of Adcy1 affects corticosteroids and MR/GR ratio

Stress hormone and brain activity may mutually affect each other. Brain activity may control the production and release of corticosteroids through the HPA (hypothalamic-pituitary-adrenal) axis. On the other hand, corticosteroids may modulate neuronal function through the activation of the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) (Herman et al., 2016). Interestingly, we found that, without the restraint stress, *Adcy1*^{tg} mice showed higher plasma corticosterone level than WT mice (Fig. 4a). Following acute restraint, corticosterone increased in both WT and *Adcy1*^{tg} mice to higher and comparable levels (Fig. 4a). Notably, the fold increase of corticosterone in response to stress was lower in *Adcy1*^{tg} mice (Fig. 4b).

Dysregulation of GR and MR has been implicated in depression and anxiety disorder (Medina et al., 2013). We found that, without the physical restraint, the basal level of GR mRNA is lower in the hippocampus (Fig. 4c) and PFC (Fig. 4f) of *Adcy1*^{tg} mice. WT and *Adcy1*^{tg} mice showed comparable MR mRNA levels in the hippocampus and PFC (Fig. 4d and g). Following the physical restraint, GR but not MR mRNA was reduced in WT PFC (Fig. 4f) but not hippocampus (Fig. 4c). Restraint failed to reduce GR mRNA in the PFC (Fig. 4f) and hippocampus (Fig. 4c) of *Adcy1*^{tg} mice. We further found that overexpression of *Adcy1* causes an increase of MR/GR ratio, of which a reduced ratio has been implicated in certain aspects of stress response and mood disorder, in the hippocampus (Fig. 4e) but not PFC (Fig. 4h).

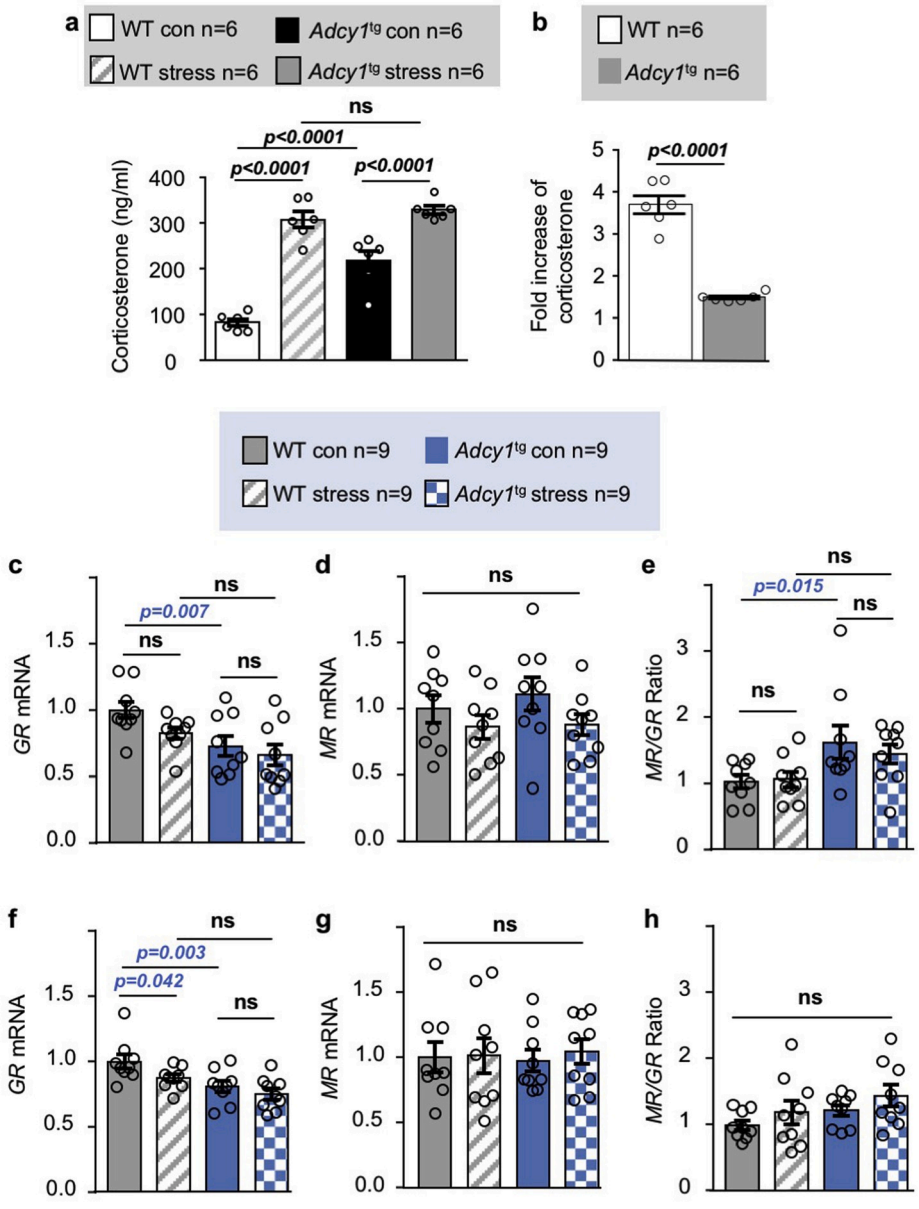

Fig. 4. The level of corticosterone and GR and MR mRNA expression level in wild type and *Adcy1*^{tg} mice. Wild type (WT) and *Adcy1*^{tg} mice were subjected to a 2-h physical restraint. **a** and **b**. Following the physical stress, the level of corticosterone was measured in control and stressed groups. **a**. Corticosterone level in unstressed and stressed WT and *Adcy1*^{tg} mice. **b**. fold increase of corticosterone in response to the physical restraint in WT and *Adcy1*^{tg} mice. Data are reported as the mean ± SEM along with the individual data point. The p values are determined by two-way ANOVA (genotype effect: $F_{1,20} = 26.030$, $p < 0.0001$; treatment effect: $F_{1,20} = 122.067$, $p < 0.0001$; genotype X treatment interaction: $F_{1,20} = 14.136$, $p = 0.001$) followed by pairwise comparison in **a**, and two-tailed Student's t-test in **b**. ns: not significant (i.e. $p > 0.05$). From **c** to **h**. Following the physical restraint, hippocampus (**c**, **d**, and **e**) and prefrontal cortex (**f**, **g**, and **h**) were harvested. The mRNA level of GR (**c** and **f**) and MR (**d** and **g**) in control and stressed mice was determined by quantitative RT-PCR. The ratio of MR to GR is presented in **e** and **h**. Data are reported as the mean ± SEM along with the individual data points. The p values were determined by two-way ANOVA (**c**. genotype effect: $F_{1,32} = 10.533$, $p = 0.003$; stress effect: $F_{1,32} = 3.201$, $p = 0.083$; genotype X stress interaction: $F_{1,32} = 0.629$, $p = 0.434$. **d**. genotype effect: $F_{1,32} = 0.398$, $p = 0.533$; stress effect: $F_{1,32} = 3.344$, $p = 0.077$; genotype X stress interaction: $F_{1,32} = 0.233$, $p = 0.633$. **e**. genotype effect: $F_{1,32} = 8.904$, $p = 0.005$; stress effect: $F_{1,32} = 0.156$, $p = 0.696$; genotype X stress interaction: $F_{1,32} = 0.435$, $p = 0.514$. **f**. genotype effect: $F_{1,32} = 13.509$, $p = 0.001$; stress effect: $F_{1,32} = 4.785$, $p = 0.036$; genotype X stress interaction: $F_{1,32} = 0.642$, $p = 0.429$. **g**. genotype effect: $F_{1,32} = 0.001$, $p = 0.975$; stress effect: $F_{1,32} = 0.158$, $p = 0.693$; genotype X stress interaction: $F_{1,32} = 0.083$, $p = 0.766$. **h**. genotype effect: $F_{1,32} = 3.169$, $p = 0.085$; stress effect: $F_{1,32} = 2.553$, $p = 0.120$; genotype X stress interaction: $F_{1,32} = 0.009$, $p = 0.924$.) followed by *post hoc* pairwise comparison. ns: not significant (i.e. $p > 0.05$).

3.5. *Adcy1*^{tg} mice show reduced immobility in the forced swimming test and increased response to the antidepressant desipramine

Immobility in forced swimming test (FST) and tail suspension test (TST) reflects adaptive responses to acute physical stress. Behavior outcome in FST and TST has also been used to test the efficacy of antidepressants (Porsolt et al., 1978). We found that *Adcy1*^{tg} mice show less immobility than WT mice in both FST (Fig. 5a) and TST (Fig. 5b). In the FST, administration of 20 mg/kg desipramine caused a reduction of immobility in *Adcy1*^{tg} but not WT mice (Fig. 5a). In the TST, which is more sensitive to antidepressant (Cryan et al., 2005), 20 mg/kg desipramine reduced immobility in both *Adcy1*^{tg} and WT mice (Fig. 5b). These data demonstrate that *Adcy1* overexpression sensitizes the response to the antidepressant desipramine in certain despair behavior outcomes.

4. Discussion

The impact of stress on mental health varies and depends on how individuals respond to stress. Notably, maladaptation to stress may cause disturbance of psychological balance and lead to mood disorders such as anxiety and depression. Although promoting the stress-coping function of the brain has been considered as an emerging treatment strategy, the molecular mechanism underlying stress resilience remains mostly unexplored. In this study, we identified a new function of *Adcy1* in regulating stress responses. The following unique features of ADCY1 make it a promising target for the treatment of stress-related disorders. First, *Adcy1* is expressed only in the central nervous system (Xia et al., 1993); alteration of *Adcy1* will have little effect on the peripheral tissues. Second, ADCY1 protein is a regulatable enzyme rather than a structure protein, and thus a reasonable drugable target. Third, ADCY1 is enriched in the post-synaptic density (Conti et al., 2007), indicating that it is more responsive to neuronal stimulation. Forth, ADCY1 activity may be linked to and promote antidepressant efficacy. The currently available antidepressants affect serotonergic, noradrenergic or dopaminergic neurotransmission, which is mediated by the activation of multiple G protein-coupled monoamine receptors. Considering that ADCY1 activity is regulated by G protein-coupled receptors (Wang and Zhang, 2012) and *Adcy1*^{tg} mice are more responsive to desipramine treatment, increasing ADCY1 activity offers an innovative strategy that may be equivalent to targeting multiple monoamine receptors simultaneously, leading to more potent resilience effects. Recent studies with ADCY1-specific inhibitors have shown effects on dampening neuropathic pain and alleviating autism-related symptoms (Wang et al., 2011; Sethna et al., 2017). Discovery of ADCY1-specific activators will help to determine the practical value of ADCY1 as a drug target to treat stress-related disorders.

The function of cAMP, the level of which is controlled by counteracting enzymatic activities from ADCY and phosphodiesterase (PDE), has been implicated in stress-related physiology. Comparing to the Ca^{2+}-stimulated ADCY1, ADCY5 activity is inhibited by Ca^{2+} (Halls and Cooper, 2011). Regarding stress-related behavior, *Adcy5* knockout mice show lower basal level anxiety and antidepressant phenotype (Kim et al., 2008; Krishnan et al., 2008) but display significant worsening of physical and mental health following physical restraint stress (Kim and Han, 2009). Comparing to the cAMP production enzyme ADCY, PDE degrades cAMP. PDE-4D reduction causes benefit effects in the chronic mild stress model (Wang et al., 2015); PDE-4B reduction causes anxiogenic and despair-like behavior (Zhang et al., 2008). These studies suggest that, possibly due to different regulatory property and tissue-specific expression of the cAMP enzymes, general enhancement of cAMP level may not always promote resilience and antagonize anxiety/despair. Regarding how ADCY1 is specifically relevant to stress coping, we found that physical stress reduces the mRNA level of *Adcy1*, and overexpression of *Adcy1* causally stabilizes behavior outcomes after physical stress. Due to technical limitation, we were not able to detect

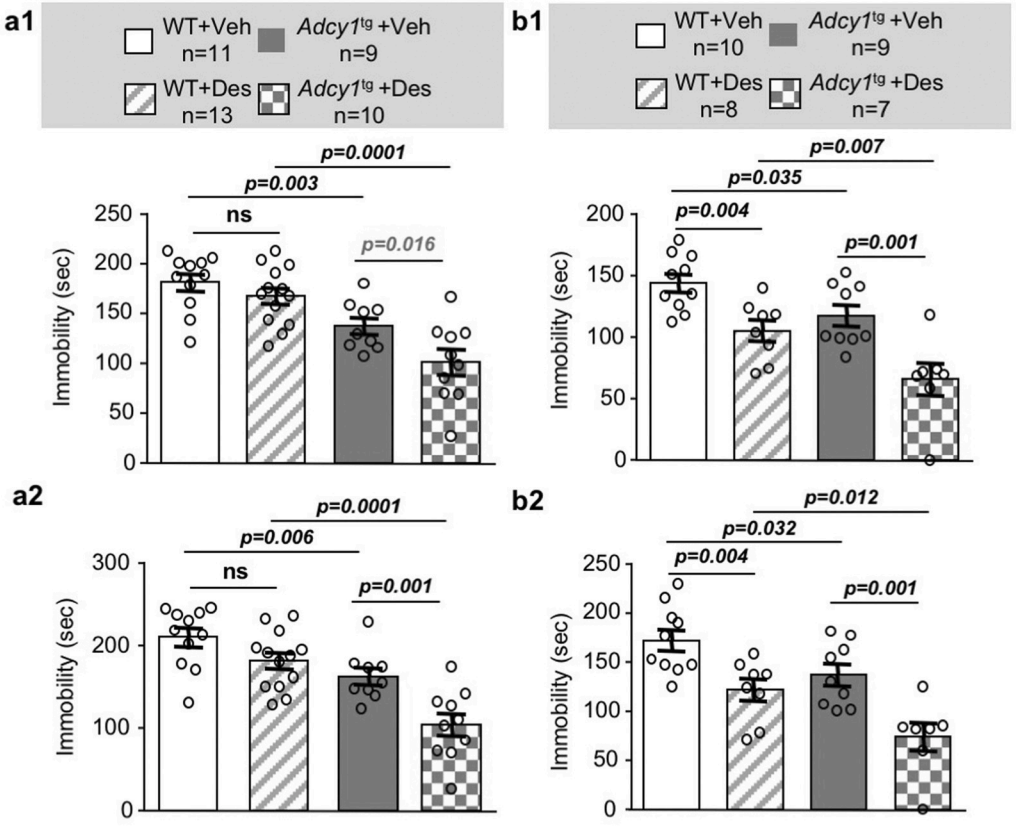

Fig. 5. Overexpression of *Adcy1* causes hypersensitivity to the antidepressant desipramine. Wild type (WT) and *Adcy1*^{tg} mice were subjected to forced swimming test (FST) (**a**) or tail suspension test (TST) (**b**) following ip injection with 20 mg/kg desipramine (Des) or vehicle (Veh). Time spent in immobility (i.e. lack of movement) during the last 4 min (**a1** and **b1**) or the entire 6 min (**a2** and **b2**) was recorded, and is presented as mean ± SEM. The p values were determined by two-way ANOVA (**a1**. genotype effect: $F_{1,39} = 33.077$, $p < 0.0001$; treatment effect: $F_{1,39} = 6.825$, $p = 0.013$; genotype X treatment interaction: $F_{1,39} = 1.366$, $p = 0.255$. **a2**. genotype effect: $F_{1,39} = 30.589$, $p < 0.0001$; treatment effect: $F_{1,39} = 15.078$, $p < 0.0001$; genotype X treatment interaction: $F_{1,39} = 1.761$, $p = 0.192$. **b1**. genotype effect: $F_{1,30} = 13.069$, $p < 0.001$; treatment effect: $F_{1,30} = 24.328$, $p < 0.0001$; genotype X treatment interaction: $F_{1,30} = 0.458$, $p = 0.504$. **b2**. genotype effect: $F_{1,30} = 12.226$, $p < 0.001$; treatment effect: $F_{1,30} = 23.195$, $p < 0.0001$; genotype X treatment interaction: $F_{1,30} = 0.264$, $p = 0.611$.) followed by *post-hoc* pairwise comparison. ns: not significant (i.e. $p > 0.05$).

changes in cAMP and ADCY activity following stress. This is mainly due to the existence of multiple ADCY and PDE isoforms, which may be increased or decreased or unchanged following stress. Consistent with our findings, a previous study found that *Adcy1*^{tg} mice not only show reduced immobility in the FST and TST but also display anxiolytic behavior (Chen et al., 2015), which is exhibited by more occupancy in the open arms of the elevated plus maze and less avoidance of the predator odor. Although whether these phenotypes reflect better stress coping or defective behavioral inhibition or risk taking may need further determination, this study, which compares behavior before and after stress experience, supports that *Adcy1* overexpression alters stress coping and promotes behavioral resilience.

It has been implicated that cAMP positively regulates ERK½ activity *in vivo* (Morozov et al., 2003). Here, we found that overexpression of *Adcy1* is associated with increased pERK½ in both hippocampus and PFC. It is known that the inhibition of ERK½ produces depression-like behaviors, and animal models of depression display disrupted ERK½ (Duman et al., 2007; Qi et al., 2009). Conversely, reduction of the mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1), which negatively regulates ERK½ signaling, causes resilience to stress (Duric et al., 2010). Additionally, systemic administration of antidepressant reversed the disrupted ERK½ pathway in depression-like animals (Duman et al., 2007; Qi et al., 2008). Interestingly, this study found that the elevated ERK½ activity in *Adcy1*^{tg} mice is associated with molecular stability and behavior resilience after physical stress.

BDNF is a known cAMP target gene and considered as a potential therapeutic target of antidepressants and stress-related disorders. BDNF expression level is sensitive to stress. Previous studies and our results show that stress reduces BDNF mRNA in the hippocampus, but behaviorally resilient animals do not necessarily express higher basal BDNF mRNA (Duman and Monteggia, 2006). Interestingly, *Adcy1* overexpression causes a reduction of basal BDNF mRNA in PFC but not hippocampus, indicating a tissue-specific effect on BDNF expression. A similar tissue-specific effect on BDNF expression is demonstrated by a stress-induced decrease in the hippocampus and an increase in nucleus accumbens (Eisch et al., 2003). Nevertheless, the behaviorally resilient but not susceptible animals show molecular stability and lack stress-induced BDNF alteration (Krishnan et al., 2007). Relevant to this phenomenon, overexpression of *Adcy1* blocks the reduction of BDNF mRNA following physical stress.

A critical finding of this study is that overexpression of *Adcy1* causes concurrent elevation of basal corticosteroid level and behavioral resilience to stress. With the higher basal level, the *Adcy1*^{tg} mice responded to stress with a less fold-increase of corticosteroid. These results suggest that the higher basal corticosteroid desensitizes stress response and plays a protective role. Although chronicle high stress hormones may alter brain function and contribute to the pathopsychological outcome of anxiety and mood disorders, the physiological elevation of stress hormone is an adaptive response to stress and important for stress-coping. It is recognized that, within a certain range, an increase in stress hormones may be beneficial. For example, administration of corticosterone before acute stress prevents the subsequent onset of anxiety in rodent (Rao et al., 2012). Clinical study also found that, after receiving post-trauma corticosteroids, patients with acute stress symptoms show a reduced risk of developing PTSD (post-traumatic stress disorder) (Zohar et al., 2011).

The mechanism underlying the higher corticosteroid level in *Adcy1*^{tg} mice is not clear. One speculation is that the higher basal NPY level in *Adcy1*^{tg} mice may affect the HPA axis, and in turn, regulate corticosteroid release. This possibility is supported by that intracerebroventricular injection of NPY leads to an increase of plasma corticosterone (Small et al., 1997). Alternatively, as NPY expression is not only upregulated by cAMP but also by glucocorticoid (Higuchi et al., 1988), the higher corticosteroid level may also increase of NPY gene expression in the brain (Shimizu et al., 2008). In addition to the higher corticosteroid level, the elevated expression of NPY in *Adcy1*^{tg} mice may also contribute to behavior resilience. As a therapeutic target, NPY shows altered expression in mood disorders (Enman et al., 2015). Supportively, intranasal spray and local administration of NPY attenuates stress-induced hyperarousal and increases social behavior, respectively (Silveira Villarroel et al., 2018; Nwokafor et al., 2019).

The higher plasma corticosteroid level in *Adcy1*^{tg} mice indicates an altered function of the HPA axis. Among distinct forebrain regions, the hippocampus inhibits HPA activity over a wide range of corticosteroid concentrations. Corticosteroid actions in the hippocampus, mediated by two receptors (i.e. GR and MR), regulate HPA activity through negative feedback and also influence behavioral responses to stress. Particularly, GR may terminate the HPA axis activation in response to stress, whereas MR may control activation threshold (de Kloet et al., 2007). Thus, a well-balanced GR and MR function is essential for a healthy mental state. Notably, a lower MR/GR ratio is found in suicide victims with a history of major depression as well as in animals subjected to chronic stress (Lopez et al., 1998; Gadek-Michalska et al., 2013). Previous reports show that GR overexpression in the forebrain causes anxiogenic effects (Wei et al., 2004), and GR deficiency leads to anxiolytic effects (Tronche et al., 1999). Our data show that overexpression of *Adcy1* causally reduces GR expression, which may, in turn, elevate basal HPA activity as implicated by the higher corticosteroid level in the *Adcy1*^{tg} mice. As there is no transcription compensation of MR, the MR/GR ratio is higher in the *Adcy1*^{tg} mice.

ADCY and cAMP regulate neural development and synaptic plasticity (Kandel, 2012; Nicol and Gaspar, 2014), which may affect stress coping at the system level (Pittenger and Duman, 2008; Han and Nestler, 2017). Previous studies found that the overall neuron and synapse density are normal in the *Adcy1*^{tg} hippocampus (Wang et al., 2004). Supportively, *Adcy1*^{tg} mice show normal basal neural transmission (Wang et al., 2004). A calcium imaging study with free-behaving mice found that neuronal firing in *Adcy1*^{tg} mice is normal but shows enhancement when animals respond to external stimuli (such as foot shocks during contextual memory training) (Chen et al., 2015). Consistently, *Adcy1*^{tg} mice show enhanced responses to high-frequency stimulation and display enhanced synaptic potentiation (Wang et al., 2004). These lines of evidence support that the effects of *Adcy1* overexpression are more likely due to enhanced signaling after neuronal stimulation rather than structural and developmental alterations.

5. Conclusions

In summary, previous research has found dynamic changes in the cAMP signaling cascade following stress and antidepressant treatment as well as in anxiety and mood disorders. Our study presents evidence to support that overexpression of the Ca^{2+}-stimulated *Adcy1*, which directly causes cAMP increase (Wang et al., 2004), promotes stress resilience and sensitizes antidepressant response.

Significance statement

How individuals cope with stress is critical for mental health. The cAMP signaling cascade has been implicated in the molecular responses to stress and antidepressant as well as in anxiety and mood disorders. However, it is unclear whether enhancement of cAMP contributes to protective functions against stress. This study provides evidence to show that overexpression of *Adcy1*, which directly elevates cAMP level, leads to molecular stability and behavioral resilience against physical stress and also sensitizes antidepressant response. These results implicate a mechanism underlying resilience and suggest *Adcy1* as a potential target for the treatment of stress-related disorders.

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Author contribution

HW initiated the study. All authors contributed to the experimental design and discussion of the data outcome. MY, QD, and MZ performed the experiments. MY, QD and HW wrote the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

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