Biological Psychiatry

Archival Report

Comparative Transcriptional Analyses in the Nucleus Accumbens Identifies RGS2 as a Key Mediator of Depression-Related Behavior

Alexia V. Williams, Catherine J. Peña, Stephanie Ramos-Maciel, Abigail Laman-Maharg, Evelyn Ordoñez-Sanchez, Monica Britton, Blythe Durbin-Johnson, Matt Settles, Rebecca Hao, Sae Yokoyama, Christine Xu, Pei X. Luo, Tjien Dwyer, Shanu Bhela, Alexis M. Black, Benoit Labonté, Randal Alex Serafini, Anne Ruiz, Rachael L. Neve, Venetia Zachariou, Eric J. Nestler, and Brian C. Trainor

ABSTRACT

BACKGROUND: Major depressive disorder is one of the most commonly diagnosed mental illnesses worldwide, with a higher prevalence in women than in men. Although currently available pharmacological therapeutics help many individuals, they are not effective for most. Animal models have been important for the discovery of molecular alterations in stress and depression, but difficulties in adapting animal models of depression for females has impeded progress in developing novel therapeutic treatments that may be more efficacious for women.

METHODS: Using the California mouse social defeat model, we took a multidisciplinary approach to identify stress-sensitive molecular targets that have translational relevance for women. We determined the impact of stress on transcriptional profiles in male and female California mouse nucleus accumbens (NAc) and compared these results with data from postmortem samples of the NAc from men and women diagnosed with major depressive disorder.

RESULTS: Our cross-species computational analyses identified *Rgs2* (regulator of G protein signaling 2) as a transcript downregulated by social defeat stress in female California mice and in women with major depressive disorder. RGS2 plays a key role in signal regulation of neuropeptide and neurotransmitter receptors. Viral vector–mediated overexpression of *Rgs2* in the NAc restored social approach and sucrose preference in stressed female California mice.

CONCLUSIONS: These studies show that *Rgs2* acting in the NAc has functional properties that translate to changes in anxiety- and depression-related behavior. Future studies should investigate whether targeting *Rgs2* represents a novel target for treatment-resistant depression in women.

https://doi.org/10.1016/j.biopsych.2022.06.030

Chronic stress is a risk factor for mental illnesses such as anxiety and major depressive disorder (MDD), which are leading causes of disability worldwide (1–3). These disorders place a burden on society by affecting performance in school or work settings, social relationships, and self-care. Although therapies are available, many individuals seeking treatment do not respond completely (4), and the remission rate is approximately 20% (5). Extensive research indicates that the nucleus accumbens (NAc), part of the ventral striatum, is altered in patients with MDD. In humans, reductions in brain volume and brain activity in the ventral striatum are associated with social anhedonia (6) and MDD (7,8). Because stress is a risk factor for depression, rodent social stress models can be used to investigate the impact of stress on brain function.

Social stress reduces social approach behaviors, which are affected by anxiety and depression disorders. This phenotype is modulated in part by the NAc (9). The NAc is important for the processing of rewarding and aversive stimuli (10) and receives dopaminergic, serotonergic, and glutamatergic innervation from nearby regions (11,12). Through connections to motor regions, the NAc aids in the selection and elicitation of directed behavior to salient stimuli (13–15), including both rewarding and aversive cues (16,17). In rodents, chronic stress alters transcription and neuronal morphology in the NAc (9). Ultimately, these changes can affect the functional activity and connectivity of these cells and contribute to depression-like phenotypes.

A limitation of previous rodent social stress studies is that most focused exclusively on males (18). This forms a gap in knowledge because women are more likely to develop MDD than men. Although there has been progress integrating females in preclinical models (19), females are still underrepresented in rodent models of MDD. Recent studies suggest that there are distinct molecular signatures in the NAc and other

 ISSN: 0006-3223

brain regions in men and women with MDD (20,21). Similar findings have been reported in rodents exposed to subchronic variable stress (20,22) or early-life stress (23). These sex-specific effects highlight the need for further development of preclinical models using female rodents that can be used to identify novel molecular targets (24). A challenge for studying molecular mechanisms of social stress in females has been the difficulty in establishing robust protocols in conventional rodents (25). In California mice (*Peromyscus californicus*), both males and females exhibit vigorous aggressive behavior, which allows for both sexes to be exposed to similar levels of social stress in an ethologically valid approach (26).

We took a multidisciplinary approach using the California mouse social defeat model system to assess how social defeat stress (SDS) affects the transcriptome of reward-related brain regions. We used RNA sequencing (RNA-seq) to examine the transcriptional responses to social defeat in the NAc of male and female California mice and compared these data with the transcriptional profiles from postmortem samples of the NAc of patients with MDD. Our analyses identified the G protein regulator *Rgs2* as a transcript downregulated in samples from stressed female mice and women with depression. This protein facilitates the process of GTP (guanosine triphosphate) hydrolysis, which in turn terminates downstream G protein–coupled receptor signaling pathways (27). Replication experiments and viral overexpression of *Rgs2* in the NAc suggest that stress-induced decreases in *Rgs2* in female California mouse NAc contribute to depression- and anxiety-related behavior.

METHODS AND MATERIALS

Full details for all experimental procedures (28,29) are provided in Supplement 1.

Animals and Housing Conditions

All studies on California mice (*P. californicus*) were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the University of California, Davis.

Social Defeat Stress

Mice were randomly assigned to control handling or 3 episodes of social defeat as previously described (30). Behavior tests and brain tissue collection were conducted 2 weeks after the last episode of social defeat.

Sucrose Anhedonia

Sucrose preference was assessed using a two-bottle choice test (30). Mice were habituated for 2 days with 2 bottles of tap water. The next day water in 1 bottle was replaced with 1% sucrose solution for a 24-hour observation period. Percent sucrose preference was calculated as the amount of sucrose solution consumed (in milliliters) over total amount of solution consumed (water and sucrose solution combined, in milliliters).

Social Interaction Test

Social interaction testing was performed as previously described (30,31). We defined time spent in the interaction zone with a target mouse as social approach. Social vigilance was scored during the acclimation and interaction phases by recording the amount of time the focal mouse spent with its head oriented toward the target mouse while outside the interaction zone.

RNA Extraction and RNA-Seq Library Preparation

Adult mice were euthanized 1 day after the final behavioral test. Brains were removed rapidly, and bilateral punches were made from the ventral tegmental area (VTA) (16 gauge), NAc (14 gauge), and prefrontal cortex (PFC) (12 gauge) and flash-frozen in tubes on dry ice. Total RNA was isolated using TriZol reagent (Invitrogen) and purified using RNeasy Micro Kits (Qiagen). Purified RNA was used to prepare libraries using TruSeq RNA Library Prep Kit (RS-122-2001/2; Illumina). VTA, NAc, and PFC samples were prepared from individual animals and sequenced with 125-nucleotide single-end reads at Beckman Coulter Genomics (currently Genewiz). Samples were multiplexed to produce >30 million reads/sample. All reads and RNA-seq files have been deposited and are available through National Center for Biotechnology Information BioProject (ID: PRJNA700778). RNA-seq data from human subjects used for analysis was published previously (20).

RNA-Seq Data Analysis

Raw reads were processed using expHTS (32) to trim low-quality reads and adapter contamination and to remove polymerase chain reaction (PCR) duplicates. The processed reads were aligned to a California mouse brain transcriptome (PRJNA350325) using BWA-MEM (33). The average mapping rate was 90.2%. Read counts per transcript were combined to generate counts per gene. Genes with fewer than 2 counts per million reads in all samples were filtered prior to analysis, leaving 40,634 genes. Differential expression analyses were conducted using the limma-voom Bioconductor pipeline (34,35). Heatmaps were generated using Python, and Gene Ontology analyses on female differential expression analyses were performed using Kolmogorov-Smirnov tests, as implemented in the Bioconductor package topGO, to compare uncorrected differential expression p values for genes annotated with a given term with those not annotated with a given term (36). Full threshold-free differential expression lists were performed using rank-rank hypergeometric overlap (RRHO) (37). Parameters for significant differential expression were set at an uncorrected $p < .05$ and a $\log_2$ fold change $> |1.15|$ between stress comparisons (38,39).

In Situ Hybridization

In situ hybridization was performed as previously described (40). Brains were coronally cryosectioned at 60 μm, fixed, treated with proteinase K, acetylated, permeabilized, and equilibrated in hybridization solution. A riboprobe (0.3 μg/mL) directed against *Rgs2* corresponding to bases 74 to 550 of complementary DNA sequence XM_028884620.1 was hybridized overnight at 65 °C. Slides were then washed and incubated with alkaline phosphatase–conjugated sheep anti-digoxigenin primary antibody (1:1000 dilution; Roche) overnight and then developed in nitro blue tetrazolium and BCIP (Roche) at 37 °C for 24 hours.

Western Blotting

Protein was extracted from NAc punches and then separated using gel electrophoresis and transferred to polyvinylidine fluoride membranes (Bio-Rad), rinsed, and blocked. Membranes were incubated overnight in primary rabbit anti-RGS2 (ab155762; Abcam) in 1 in 1000 dilution at 4 °C that was validated using samples from *Rgs2* knockout mice (Figure S1 in Supplement 1). Membranes were incubated in peroxidase-conjugated antirabbit secondary antibody (1:100 dilution; Vector). Membranes were washed, developed, and imaged on a Bio-Rad ChemiDoc. Blots were probed for β-actin as a loading control (1:1000 dilution; Cell Signaling), and RGS2 protein bands were normalized to their respective β-actin controls.

Real-Time Quantitative PCR

RNA was extracted from NAc tissue punches from SDS male and female mice (n = 5–8 per group) using an RNeasy Micro Kit (74004; Qiagen) and converted to complementary DNA using an iScript cDNA Synthesis Kit (1708891; Bio-Rad). Real-time quantitative PCR was performed using SybrGreen Fast master mix (Applied Biosystems, Thermo Fisher Scientific) on a ViiA 7 Real-Time PCR system (Applied Biosystems, Thermo Fisher Scientific) and analyzed using the $2^{-\Delta\Delta Ct}$ method. For primer sequences, see Table S1 in Supplement 1.

Overexpression of *Rgs2* Within the NAc Through Herpes Simplex Virus–Mediated Gene Transfer

To overexpress RGS2, we used bicistronic p1005 herpes simplex virus expressing either GFP (green fluorescent protein) or GFP and *Rgs2* (Origene). Expression of GFP is driven by a cytomegalovirus promoter while the gene of interest is driven by the IE4/5 promoter (41,42). One week following defeat, mice received one bilateral 0.6 μL injection of either the RGS2 vector or vector containing GFP alone into the NAc core (anterior/posterior: 0.84, medial/lateral: ±1.5, dorsal/ventral: 6.0). One week later, mice were tested for sucrose anhedonia and social interaction. To confirm that expression was limited to the NAc, sections of the NAc were imaged to visualize GFP colocalization using Neurotrace (Thermo Fisher Scientific). RGS2 overexpression was confirmed via Western blotting.

Statistical Analyses

All statistical analyses were performed using R statistical software (R Foundation for Statistical Computing). Normality of data was assessed using Shapiro-Wilk tests. A Fligner-Killeen test was used to assess homogeneity of variance. Two-way analysis of variance (ANOVA) (sex and stress) was used to analyze quantitative PCR data and behavior measures. An unpaired t test was used for Western blot data. One-way ANOVA was used to analyze behavior data for the RGS2 overexpression experiment. After ANOVA analyses that revealed significant interaction effects, we used a priori planned comparisons to test for effects of stress in males and females or RGS2 and GFP controls (43).

RESULTS

Effects of Social Defeat on Male and Female Transcriptional Responses in the NAc of California Mice

We performed behavioral and transcriptional analyses 2 weeks after social defeat or control manipulations (Figure 1A). Similar to previous studies of California mice (38,44), stressed females but not males showed a decrease in social interaction ratio when the target was present (Figure 1B) (stress × sex interaction effect, $F_{1,29} = 4.37$, $p < .05$), and planned comparisons showed an effect of stress in females but not males. There were no differences in the open field phase (Figure 1C). We performed RNA-seq on samples of the NAc from these mice (Table S2 in Supplement 2). We first set more liberal

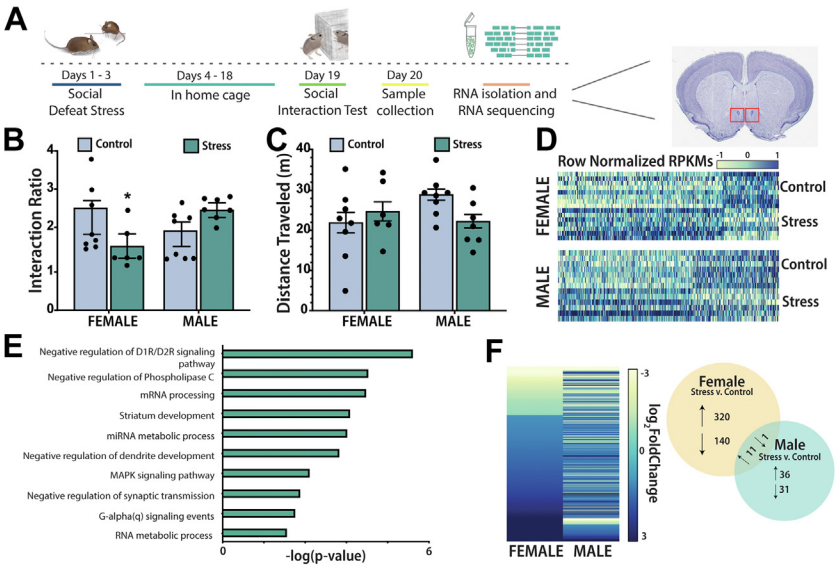

Figure 1. Social defeat stress differentially expresses nucleus accumbens transcriptional patterns in a sex-specific manner. **(A)** Timeline of experiment. Mice were run through social defeat and weeks later, they were run in a social interaction test. The next day, fresh punches of the nucleus accumbens were collected for RNA sequencing. **(B)** Social defeat reduces social approach in females but not in males during the social interaction test. **(C)** No differences were seen in distance traveled during the open field phase. **(D)** Stressed female mice have different average transcript expression (RPKM) patterns compared with control females. **(E)** Highly enriched differently expressed Gene Ontology terms identified from overlapping male and female differentially expressed genes. **(F)** Minimal overlap is present in differentially expressed genes between males and females. *planned comparison $p < .05$ vs. control. Group *ns*: male/control = 8, male/stress = 7, female/control = 8, female/stress = 6. D1R, D_1 receptor; D2R, D_2 receptor; MAPK, mitogen-activated protein kinase; miRNA, microRNA; mRNA, messenger RNA; RPKM, reads per kilobase million.

parameters for identifying alterations in transcription (uncorrected $p < .05$, $\log_2$ fold change $> |0.38|$) to identify broad patterns of transcriptional changes (39,45). Heatmaps plotting normalized transcript expression (reads per kilobase millions [RPKMs]) showed contrasting transcriptional expression patterns in females, in which low-abundance transcripts in control females were more abundant in stressed females, and vice versa (Figure 1D); this pattern was less distinct in males (Figure 1D). Most of the top 10 highly enriched terms following Gene Ontology analyses of differentially expressed transcripts in females are related to dopamine signaling pathways and G_q alpha second messenger signaling cascades (Figure 1E). When comparing these datasets using RRHO, we found more overlap in overexpressed transcripts for males and females (Figure S2 in Supplement 1) and less overlap in transcripts that were reduced after defeat. When stricter parameters were used to identify differentially expressed genes (uncorrected $p < .05$, $\log_2$ fold change $> |1.15|$) (39,46), we found a more robust effect of stress in females, with 320 transcripts elevated in stressed females versus 36 transcripts in males (Figure 1F). In contrast, 140 transcripts were less abundant in females versus 31 transcripts in males. Volcano plots of these data also indicate stronger transcriptional responses in females than in males (Figure S3 in Supplement 1). Together, these results suggest that there may be sex-specific changes in transcriptomic responses in the NAc, with stronger responses occurring in females than in males. For example, only 11 transcripts that were upregulated in stressed females were also upregulated in stressed males (Figure 1F; Table S3 in Supplement 1). In females, we also used RRHO analyses to assess the extent to which effects of stress on transcription in the NAc generalized to the PFC and VTA (9). Transcripts that were more abundant in the NAc of stressed females were also more abundant in the PFC and VTA (Figure S4 in Supplement 1) of stressed females. In contrast, distinct sets of transcripts were decreased by social defeat across the NAc, PFC, and VTA. A weakness of these analyses is that the vast majority of comparisons do not pass false discovery rate thresholds for significance, a common problem for bulk tissue RNA-seq analyses (47). To determine the extent to which the patterns of gene expression in our study generalize across species, we used RRHO analyses to compare male and female California mouse NAc RNA-seq results with the data obtained from the NAc of men and women with MDD (GEO accession number: GSE102556).

Transcriptional Patterns Related to Social Defeat and MDD

Using RRHO, we observed that the effects of SDS on transcriptional responses in female California mice were broadly similar to the differences observed in samples from women with MDD (Figure 2A). This overlap was largely absent in samples from male California mice and samples from men with MDD (Figure 2B). We then identified 17 transcripts present in both stressed female mice and women with MDD with an uncorrected $p < .05$ and log fold change $> |1.15|$ (Figure 2A). One of these transcripts was *Rgs2*. We identified a sex-specific effect of stress on *Rgs2* RPKMs in female California mice (Figure 2C) (sex $\times$ stress interaction effect, $F_{1,29} = 4.762$, $p < .05$), with planned comparisons showing an effect of stress in females but not in males. Importantly, *Rgs2* was not identified as a differentially expressed gene in the NAc of male mice or males with MDD. There was a positive correlation between *Rgs2* expression and social approach in the social interaction test in females (Figure 2D) (Pearson $r = 0.6$, $p < .05$) but not in males (Figure 2E) ($r = 0.098$, $p > .05$). No effects of stress were observed on *Rgs2* RPKMs in the VTA or PFC (Figure S5 in Supplement 1). To determine the robustness of SDS on *Rgs2* expression, we measured gene expression in a set of biological replicates and RGS2 protein in a separate set of samples (Figure 3A).

SDS Reduces *Rgs2* Messenger RNA and RGS2 Protein Expression in the Female NAc

Using in situ hybridization, we confirmed *Rgs2* expression in the NAc (Figure 3B). Social defeat reduced social approach in females but not in males when the target was present (Figure S6A in Supplement 1) (stress $\times$ sex, $F_{1,26} = 5.995$, $p < .05$), but no behavioral change was observed when the

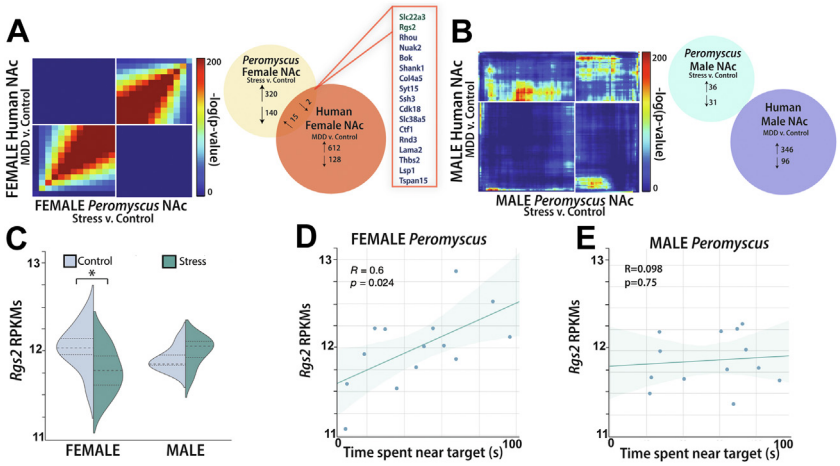

Figure 2. Social defeat stress induces gene expression patterns in the NAc in female California mice that are similar to women with MDD. **(A)** Social defeat stress induces transcriptional patterns in female California mice that are similar to transcriptional patterns observed in women with MDD; one transcript similarly affected in both datasets is *Rgs2*. **(B)** No similarities in differentially expressed genes were observed in stressed male California mice and men with MDD. **(C)** Stress reduces *Rgs2* average expression in a sex-specific manner. **(D, E)** *Rgs2* expression is correlated to social avoidance behavior in female **(D)** but not in male **(E)** California mice. *planned comparison $p < .05$ vs. control. Group *ns*: male/control = 8, male/stress = 7, female/control = 8, female/stress = 8, women/MDD = 13, women/control = 9, men/MDD = 13, men/control = 13. MDD, major depressive disorder; NAc, nucleus accumbens; RPKM, reads per kilobase million.

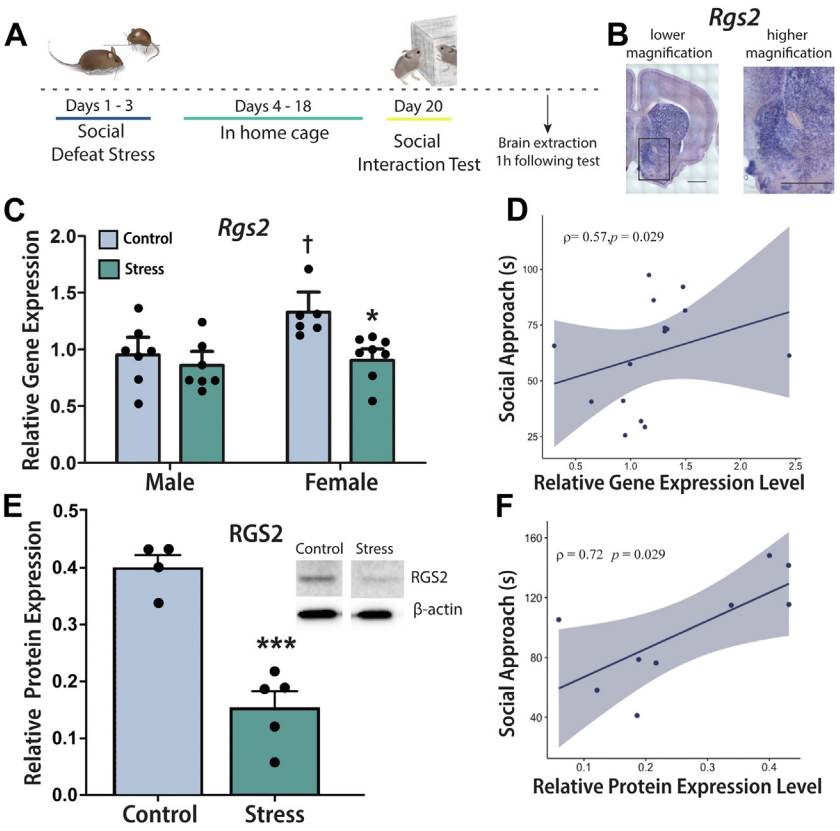

Figure 3. SDS reduces *Rgs2* mRNA and protein expression in the nucleus accumbens of female California mice. **(A)** Timeline of experiment. Male and female California mice were run through control handling or SDS. Two weeks later, mice were run through a social interaction test. Tissue samples were collected 1 hour following behavior testing for different cohorts of mice. **(B)** *Rgs2* expression in the nucleus accumbens was confirmed using in situ hybridization. **(C, D)** SDS reduced *Rgs2* mRNA in females but not in males **(C)**, and *Rgs2* mRNA expression levels are positively correlated with social approach behavior **(D)**. **(E, F)** Stress reduced RGS2 protein levels in females **(E)**, and these protein levels correlate with social approach **(F)**. $^{*}p < .05$ planned comparison vs. female control, $^{***}p < .001$ independent *t* test vs. control, $^{\dagger}p < .05$ planned comparison vs. male control. Quantitative polymerase chain reaction group *ns*: male/control = 7, male/stress = 7, female/control = 6, female/stress = 8. Western blot group *ns*: control = 4, stress = 5. mRNA, messenger RNA; SDS, social defeat stress.

target was absent (Figure S6B in Supplement 1). In these mice, real-time PCR analyses showed that social defeat reduced *Rgs2* messenger RNA (mRNA) in the NAc in females but not in males (Figure 3C) (stress × sex, $F_{1,26} = 4.687$, $p < .05$). While RNA-seq analyses showed no sex differences in *Rgs2* mRNA in control mice, planned comparisons in the real-time PCR cohort showed that *Rgs2* mRNA was higher in control females than in control males ($p < .05$). Similar to the analyses of sequencing data, *Rgs2* mRNA was positively correlated with social approach in females (Figure 3D) (Spearman $\rho = 0.57$, $p = .03$) but not in males ($\rho = -0.27$, $p = .39$). In a separate group of biological replicates, social defeat significantly decreased RGS2 protein expression in females (Figure 3E) ($t_7 = 6.9$, $p < .001$) and RGS2 protein expression level was positively correlated with social approach (Figure 3F) ($\rho = 0.73$, $p = .03$).

Rgs2 Overexpression in the NAc Blocks Depression-like Behavior in Stressed Females

Overexpression of *Rgs2* in the NAc via viral gene transfer was used to assess the effect of increasing *Rgs2* on depression- and anxiety-like phenotypes in stressed females (Figure 4A, B). Viral expression occurred in neurons, and the *Rgs2* virus increased RGS2 protein in the NAc (Figure 4C). There were no differences in sucrose preference prior to SDS (Figure 4D). After defeat, there were significant differences in sucrose preference (one-way ANOVA $F_{2,14} = 36.4$, $p < .001$), with females receiving the *Rgs2* virus in the NAc consuming more sucrose than females receiving GFP (planned comparison $p < .001$), whereas females with misplaced *Rgs2* viral injections did not differ from females receiving GFP. In the social interaction test, there were significant differences in social approach (Figure 4E) ($F_{2,14} = 11.66$, $p < .01$) and social vigilance (Figure 4F) ($F_{2,14} = 36.39$, $p < .01$). Mice that received the *Rgs2* virus in the NAc had higher social approach (planned comparison, $p < .01$) and lower social vigilance (planned comparison, $p < .05$) than females receiving GFP. Mice with misplaced *Rgs2* injections were not different from GFP controls. *Rgs2* overexpression had no effects on behavior during the acclimation phase when the target was absent (Figure 4G, H) (both *p*s $> .05$). During the open field phase of the social interaction test, *Rgs2* overexpression had no effects on distance traveled (Figure 4I) ($p > .05$) or on time spent in the center of the arena (Figure 4J) ($p > .05$).

DISCUSSION

An important question in psychiatry is why rates of depression and anxiety are elevated in women compared with men. There is growing evidence that distinct neurobiological responses can be evoked by stress in women and men. In this study, we demonstrated that SDS in female California mice induces broad patterns of transcriptional changes in the NAc that are correlated with transcriptional patterns reported in postmortem

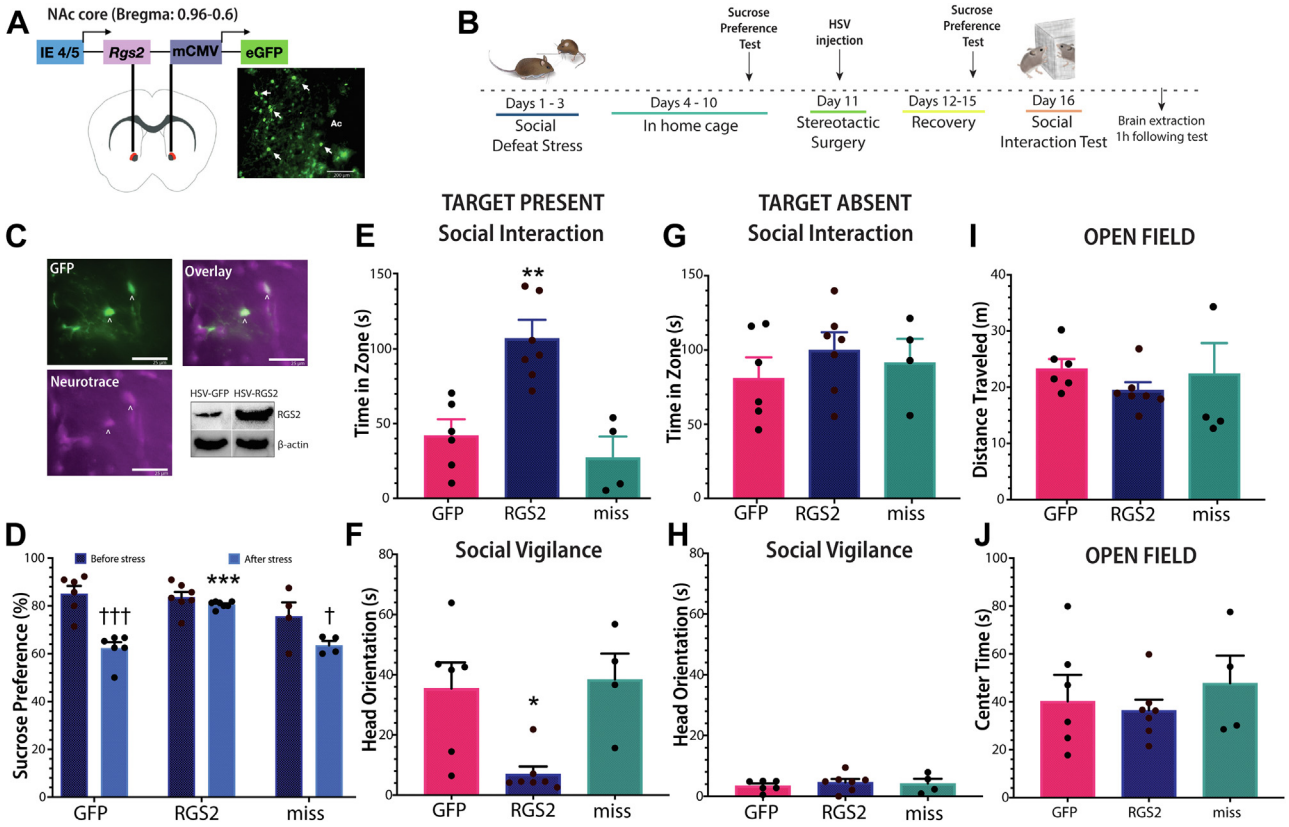

Figure 4. Overexpression of *Rgs2* in the NAc reverses stress-induced depression-like behavior in female California mice. Schematic of placement site and viral construct and image of viral transfection (GFP in NAc). **(A)** Timeline of experiment. Scale bar = 200 μm. **(B)** We took baseline sucrose preference levels from all mice before stress exposure. All females were exposed to social defeat and then 1 week later, viral vectors (GFP or *Rgs2*) were microinjected into the NAc core. Four days later, stressed mice received a sucrose preference test followed by a social interaction test. **(C)** Neurotrace staining shows that GFP expression occurs in neurons and Western blot analysis shows that HSV-RGS2 vectors increase RGS2 protein in the NAc. Scale bar = 25 μm. **(D)** *Rgs2* overexpression reversed anhedonia-like phenotypes in the sucrose preference test. **(E–G)** *Rgs2* overexpression increased social approach **(E)** and decreased social vigilance **(F)** when the target was present but not while the target was absent **(G, H)**. **(I, J)** *Rgs2* overexpression did not alter distance traveled **(I)** or center time **(J)** during the open field phase. $^{*}p < .05$ planned comparison vs. GFP. $^{**}p < .01$ planned comparison vs. GFP. $^{***}p < .001$ planned comparison vs. GFP. $^{\dagger}p < .05$ paired *t* test with baseline (before stress). $^{\dagger\dagger\dagger}p < .001$ paired *t* test with baseline (before stress). Group *n*s: RGS2 = 7, GFP = 6, miss = 4. eGFP, enhanced green fluorescent protein; HSV, herpes simplex virus; IE, immediate early promoter; mCMV, murine cytomegalovirus; miss, misplaced *Rgs2* injections; NAc, nucleus accumbens.

NAc samples collected from women diagnosed with depression. This finding suggests a strong translational potential for female California mice in studying biological mechanisms related to depression and anxiety that are relevant for women. These analyses identified *Rgs2* as a stress-sensitive transcript in the NAc of females. RGS2 protein regulates the activity of neuropeptide and neurotransmitter receptors, and its overexpression blocked stress-induced sucrose anhedonia and social avoidance. Overexpression of *Rgs2* also reduced social vigilance, which is modulated by the bed nucleus of the stria terminalis (48), suggesting that *Rgs2* modulates direct or indirect connections with the extended amygdala. Consistent with prior studies, stressed males did not exhibit social avoidance and had fewer transcriptional changes in the NAc. However, stressed male California mice exhibit alternative phenotypes such as reduced cognitive flexibility (49), suggesting that in males, stress could have stronger effects on transcription outside of the NAc. Thus, when using rodent models to study social stress in both males and females, it is important to consider a broad range of behavioral and neurobiological phenotypes.

Genetic variants of the *RGS2* gene that have less stable *RGS2* mRNA (50) are correlated with increased risk for depression (51), anxiety (52–56), and risk for suicide (57–59). Disruptions in *RGS2* gene expression are also linked to patients with treatment-resistant depression (54), suggesting that *RGS2* may play some role in the lack of efficacy of currently available treatments. These studies included both men and women and adjusted genetic analyses for sex. However, none of these studies tested whether *RGS2* gene variations had stronger associations with health outcomes in women than in men. Preclinical studies in male *Mus musculus* showed that *Rgs2* deletion increased anxiety-like responses and passive coping responses (60,61). These studies had important limitations. Global knockout approaches cannot distinguish whether behavioral changes are due to

developmental effects of *Rgs2* or altered gene function in the adult brain. Our results show that stress-induced decreases in *Rgs2* expression in the adult brain can contribute to depression-like behaviors. Furthermore, *Rgs2* is widely distributed throughout the brain; therefore, global knockout approaches have little precision for identifying the brain circuits mediating *Rgs2* action on social behavior. In addition, behavioral studies have been focused primarily on males. In our quantitative PCR experiment, *Rgs2* expression was higher in control females than in control males, although this difference was not replicated in the RNA-seq dataset. There are few data on RGS2 expression in male and female brains, although higher *Rgs2* expression was reported in female versus male rat brainstem (62). Further study is needed to determine whether baseline differences in *Rgs2* expression are consistent across species or brain regions. Previous work showed that *Rgs2* expression in the brain can be stress sensitive (63), but to our knowledge, no study has tested whether these changes contribute to behavioral outcomes via RGS2 manipulation. Our experiments show that in females, *Rgs2* mRNA and protein in the NAc are decreased by social defeat and that viral overexpression of RGS2 in NAc is sufficient to reduce stress-induced social avoidance, social vigilance, and sucrose anhedonia. These findings agree with clinical findings, suggesting that *Rgs2* is an important modulator for behaviors with translational relevance.

A main function of RGS (regulator of G protein signaling) proteins is to potentiate the process of GTP hydrolysis, which effectively switches off downstream G protein–coupled receptor signaling pathways (27,64,65). Reduced production of RGS2 protein disrupts this process (66), which consequently interferes with the function of neuropeptide and neurotransmitter receptors (67,68). In addition to the GTPase activating action, RGS proteins may modulate G protein–coupled receptor responses through several other mechanisms. For example, they may act as effector antagonists for G protein alpha subunits or as regulators of epigenetic and transcriptional processes (69). RGS2 specifically regulates G_q alpha signaling events (70). Given this distinction, an important question to consider is which receptor signaling pathways within the NAc are being affected by stress-induced reductions in RGS2. Prior findings indicate that RGS2 may regulate dopamine D_1 receptor (D1R)–expressing neurons in the NAc (71–73). Single-cell RNA-seq analyses of the striatum showed that *Rgs2* gene in the striatum clusters significantly with D1R-expressing neurons but not with D2R-expressing neurons (74). Although these data lack anatomical specificity, these suggest that RGS2 could be affecting social behavior through a D1R-driven mechanism within the striatum and potentially specifically within the NAc. Consistent with this hypothesis, D1R agonist infusions in the NAc are sufficient to reduce social approach in unstressed female California mice (75). Confirming whether *Rgs2* modulates signaling pathways in the NAc through a D1R-driven mechanism, or through other receptor signaling systems, will lead to novel insights on a cell type–specific mechanism through which *Rgs2* modulates social behavior and deficits in social behavior that are relevant to stress disorders. Other members of the RGS family have been shown to modulate stress, but they have distinct functions. Prevention of *Rgs7* action reduces anxiety-like behaviors in response to environmental stimuli in male mice (76), whereas deletion of the *Rgs4* gene decreases the efficacy of monoamine-targeting antidepressants and promotes the actions of ketamine (77).

Although *Rgs2* was not differentially expressed in the VTA or PFC, at a broad level, RRHO analyses comparing the NAc with the VTA and PFC detected more overlap in transcripts upregulated by stress than transcripts downregulated by stress. In C57BL/6J mice, unpredictable chronic mild stress induced similar transcriptional changes in the NAc and PFC in males but not in females, while in females, similar gene expression profiles were observed in the NAc and basolateral amygdala (78). In human postmortem samples, RRHO analyses detected little overlap in gene expression across the NAc and cortical regions in either males or females. Numerous studies have reported sex-specific neural transcriptional responses to stress using bulk RNA-seq methods (79), in which different cells are combined during the RNA extraction process. A weakness of these studies, including ours, is that this approach generally does not provide sufficient power to detect differential expression that passes false discovery correction [but see (80)]. Thus, although RRHO analyses identified broad similarities in gene expression signatures in female rodent and human NAc samples, few transcripts met criteria for differential expression (Figure 2A). Despite this weakness, when combined with follow-up analyses of different biological replicates, these approaches have led to the successful identification of numerous transcripts such as *Dusp4*, *Dnmt3a*, and *Emx1* with sex-specific transcriptional responses to stress. Here, we showed that bulk sequencing approaches are effective for hypothesis generation when paired with replication and manipulation of candidate gene function. Our analyses also identified *Slc22A3* as a transcript downregulated by stress in California mice and decreased in samples from women diagnosed with depression. One protein encoded by this transcript is OCT3 (organic cation transporter 3), a low-affinity high-capacity transporter for monoamines (81). Although less is known about OCT3, it enhances place preference responses to cocaine (82) and is sensitive to glucocorticoids (83). Our sequencing data suggests that it is an intriguing target for further study. Moving forward, greater use of strategies that have more statistical power is needed. This could be achieved using larger sample sizes or analyses of more defined cell populations via single-cell analyses of transcription (84). This will increase the utility of comparisons across stress models and enhance our ability to assess the extent to which depression and anxiety disorders are linked to sex-specific molecular signatures.

These studies show that *Rgs2* is a stress-sensitive transcript in the NAc that modulates depression- and anxiety-like behavior. Our results suggest that facilitating *Rgs2* activity could have important therapeutic properties, especially in females. This is important because women are twice as likely to develop MDD, and some underlying mechanisms may be distinct from men. Identifying distinct mechanisms could facilitate sex-specific targets for therapeutic intervention. These studies in California mice highlight the utility of model systems in which social stress can be studied in males and females.

ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by National Institutes of Health Grant Nos. K99/R00 MH115096 (to CJP), R01MH051399 (to EJN), R01NS086444 (to VZ), R01NS086444S1 (to RAS), R01MH121829S1 (to AVW), R01MH121829 (to BCT), and R01MH103322 (to BCT).

We thank Cindy Clayton, Marcus Petagara, and vivarium staff for coordinating animal care; M. Wu and J. Tollkuhn for technical assistance; and Andrew Fox and Jill Silverman for helpful discussions.

The authors report no biomedical financial interests or potential conflicts of interest.

ARTICLE INFORMATION

From the Department of Psychology (AVW, SR-M, AL-M, EO-S, RH, SY, CX, PXL, TD, SB, AMB, BCT); Bioinformatics Core Facility (MB, MS), UC Davis Genome Center; and Department of Public Health Sciences (BD-J), University of California, Davis, California; Nash Family Department of Neuroscience and Friedman Brain Institute (CJP, BL, RAS, AR, VZ, EJN), Icahn School of Medicine at Mount Sinai, New York, New York; Princeton Neuroscience Institute (CJP), Princeton, New Jersey; Department of Psychology (EO-S), Temple University, Philadelphia, Pennsylvania; Gene Delivery Technology Core (RLN), Massachusetts General Hospital, Boston, Massachusetts; and the Department of Psychiatry and Neuroscience (BL), Laval University, Québec, Quebec, Canada.

Address correspondence to Brian C. Trainor, Ph.D., at bctrainor@ucdavis.edu.

Received Mar 21, 2022; revised Jun 21, 2022; accepted Jun 22, 2022.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2022.06.030.

REFERENCES

1. Goel N, Bale TL (2009): Examining the intersection of sex and stress in modelling neuropsychiatric disorders. J Neuroendocrinol 21:415–420.
2. Bale TL, Epperson CN (2015): Sex differences and stress across the lifespan. Nat Neurosci 18:1413–1420.
3. Laman-Maharg A, Trainor BC (2017): Stress, sex, and motivated behaviors. J Neurosci Res 95:83–92.
4. Culpepper L (2010): Why do you need to move beyond first-line therapy for major depression? J Clin Psychiatry 71(suppl 1):4–9.
5. Depression: How effective are antidepressants?. (2017). Cologne, Germany: Institute for Quality and Efficiency in Health Care (IQWiG). Available at: https://www.ncbi.nlm.nih.gov/books/NBK361016/. Accessed March 1, 2019.
6. Enneking V, Krüssel P, Zaremba D, Dohm K, Grotegerd D, Förster K, *et al.* (2019): Social anhedonia in major depressive disorder: A symptom-specific neuroimaging approach. Neuropsychopharmacology 44:883–889.
7. Nauczyciel C, Robic S, Dondaine T, Verin M, Robert G, Drapier D, *et al.* (2013): The nucleus accumbens: A target for deep brain stimulation in resistant major depressive disorder. J Mol Psychiatry 1:17.
8. Delaloye S, Holtzheimer PE (2014): Deep brain stimulation in the treatment of depression. Clin Res 16:83–91.
9. Russo SJ, Nestler EJ (2013): The brain reward circuitry in mood disorders [published correction appears in Nat Rev Neurosci 2013; 14: 736. Nat Rev Neurosci 14:609–625.
10. Carlezon WA Jr, Thomas MJ (2009): Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. Neuropharmacology 56(suppl 1):122–132.
11. Britt JP, Benaliouad F, McDevitt RA, Stuber GD, Wise RA, Bonci A (2012): Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. Neuron 76:790–803.
12. Sesack SR, Grace AA (2010): Cortico-basal ganglia reward network: Microcircuitry. Neuropsychopharmacology 35:27–47.
13. Mogenson GJ, Jones DL, Yim CY (1980): From motivation to action: Functional interface between the limbic system and the motor system. Prog Neurobiol 14:69–97.
14. Roitman MF, Wheeler RA, Carelli RM (2005): Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. Neuron 45:587–597.
15. Humphries MD, Prescott TJ (2010): The ventral basal ganglia, a selection mechanism at the crossroads of space, strategy, and reward. Prog Neurobiol 90:385–417.
16. Parkinson JA, Willoughby PJ, Robbins TW, Everitt BJ (2000): Disconnection of the anterior cingulate cortex and nucleus accumbens core impairs Pavlovian approach behavior: Further evidence for limbic cortical-ventral striatopallidal systems. Behav Neurosci 114:42–63.
17. Stefanik MT, Kupchik YM, Brown RM, Kalivas PW (2013): Optogenetic evidence that pallidal projections, not nigral projections, from the nucleus accumbens core are necessary for reinstating cocaine seeking. J Neurosci 33:13654–13662.
18. Williams AV, Trainor BC (2018): The impact of sex as a biological variable in the search for novel antidepressants. Front Neuroendocrinol 50:107–117.
19. Will TR, Proaño SB, Thomas AM, Kunz LM, Thompson KC, Ginnari LA, *et al.* (2017): Problems and progress regarding sex bias and omission in neuroscience research. eNeuro 4:ENEURO.0278-17.2017.
20. Labonté B, Engmann O, Purushothaman I, Menard C, Wang J, Tan C, *et al.* (2017): Sex-specific transcriptional signatures in human depression [published correction appears in Nat Med 2018; 24:525. Nat Med 23:1102–1111.
21. Seney ML, Huo Z, Cahill K, French L, Puralewski R, Zhang J, *et al.* (2018): Opposite molecular signatures of depression in men and women. Biol Psychiatry 84:18–27.
22. Hodes GE, Pfau ML, Purushothaman I, Ahn HF, Golden SA, Christoffel DJ, *et al.* (2015): Sex Differences in nucleus accumbens transcriptome profiles associated with susceptibility versus resilience to subchronic variable stress. J Neurosci 35:16362–16376.
23. Ordoñes Sanchez E, Bavley CC, Deutschmann AU, Carpenter R, Peterson DR, Karbalaei R, *et al.* (2021): Early life adversity promotes resilience to opioid addiction-related phenotypes in male rats and sex-specific transcriptional changes [published correction appears in Proc Natl Acad Sci U S A 2022; 119:e2204210119]. Proc Natl Acad Sci U S A 118.
24. Bangasser DA, Cuarenta A (2021): Sex differences in anxiety and depression: Circuits and mechanisms. Nat Rev Neurosci 22:674–684.
25. Kuske JX, Trainor BC (2022): Mean girls: Social stress models for female rodents. Curr Top Behav Neurosci 54:95–124.
26. Trainor BC, Pride MC, Villalon Landeros R, Knoblauch NW, Takahashi EY, Silva AL, Crean KK (2011): Sex differences in social interaction behavior following social defeat stress in the monogamous California mouse (Peromyscus californicus). PLoS One 6:e17405.
27. Watson N, Linder ME, Druey KM, Kehrl JH, Blumer KJ (1996): RGS family members: GTPase-activating proteins for heterotrimeric G-protein alpha-subunits. Nature 383:172–175.
28. Butler-Struben HM, Kentner AC, Trainor BC (2022): What's wrong with my experiment?: The impact of hidden variables on neuropsychopharmacology research. Neuropsychopharmacology 47:1285–1291.
29. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, *et al.* (2020): The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18:e3000410.
30. Williams AV, Laman-Maharg A, Armstrong CV, Ramos-Maciel S, Minie VA, Trainor BC (2018): Acute inhibition of kappa opioid receptors before stress blocks depression-like behaviors in California mice. Prog Neuropsychopharmacol Biol Psychiatry 86:166–174.
31. Williams AV, Duque-Wilckens N, Ramos-Maciel S, Campi KL, Bhela SK, Xu CK, *et al.* (2020): Social approach and social vigilance are differentially regulated by oxytocin receptors in the nucleus accumbens. Neuropsychopharmacology 45:1423–1430.
32. Streett DA, Petersen KR, Gerritsen AT, Hunter SS, Settles ML (2015): expHTS: Analysis of high throughput sequence data in an experimental framework. In: Proceedings of the 6th ACM Conference on

Bioinformatics, Computational Biology and Health Informatics, September 9, 523–524.
33. Li H (2013): Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Quant Biol. arXiv http://doi.org/10.48550/arXiv.1303.3997.
34. Robinson MD, McCarthy DJ, Smyth GK (2010): edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26:139–140.
35. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK (2015): limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 43:e47.
36. Alexa A, Rahnenfuhrer J (2020): R package. In: topGO: Enrichment Analysis for Gene Ontology version 2.41.0.
37. Cahill KM, Huo Z, Tseng GC, Logan RW, Seney ML (2018): Improved identification of concordant and discordant gene expression signatures using an updated rank-rank hypergeometric overlap approach. Sci Rep 8:9588.
38. Greenberg GD, Laman-Maharg A, Campi KL, Voigt H, Orr VN, Schaal L, Trainor BC (2014): Sex differences in stress-induced social withdrawal: Role of brain derived neurotrophic factor in the bed nucleus of the stria terminalis. Front Behav Neurosci 7:223.
39. Peña CJ, Smith M, Ramakrishnan A, Cates HM, Bagot RC, Kronman HG, *et al.* (2019): Early life stress alters transcriptomic patterning across reward circuitry in male and female mice. Nat Commun 10:5098.
40. Wu MV, Tollkuhn J (2017): Estrogen receptor alpha is required in GABAergic, but not glutamatergic, neurons to masculinize behavior. Horm Behav 95:3–12.
41. Neve RL, Neve KA, Nestler EJ, Carlezon WA Jr (2005): Use of herpes virus amplicon vectors to study brain disorders. Biotechniques 39:381–391.
42. Peña CJ, Kronman HG, Walker DM, Cates HM, Bagot RC, Purushothaman I, *et al.* (2017): Early life stress confers lifelong stress susceptibility in mice via ventral tegmental area OTX2. Science 356:1185–1188.
43. Keppel G (1991): Design and Analysis: A Researcher's Handbook, 3rd ed. Englewood Cliffs, NJ: Prentice Hall.
44. Trainor BC, Takahashi EY, Campi KL, Florez SA, Greenberg GD, Laman-Maharg A, *et al.* (2013): Sex differences in stress-induced social withdrawal: Independence from adult gonadal hormones and inhibition of female phenotype by corncob bedding. Horm Behav 63:543–550.
45. Walker DM, Zhou X, Ramakrishnan A, Cates HM, Cunningham AM, Peña CJ, *et al.* (2020): Adolescent social isolation reprograms the medial amygdala: Transcriptome and sex differences in reward. bioRxiv. https://doi.org/10.1101/2020.02.18.955187.
46. Walker DM, Cates HM, Loh YHE, Purushothaman I, Ramakrishnan A, Cahill KM, *et al.* (2018): Cocaine self-administration alters transcriptome-wide responses in the brain's reward circuitry. Biol Psychiatry 84:867–880.
47. Ruiz-Ortiz J, Tollkuhn J (2021): Specificity in sociogenomics: Identifying causal relationships between genes and behavior. Horm Behav 127:104882.
48. Wright EC, Hostinar CE, Trainor BC (2020): Anxious to see you: Neuroendocrine mechanisms of social vigilance and anxiety during adolescence. Eur J Neurosci 52:2516–2529.
49. Laredo SA, Steinman MQ, Robles CF, Ferrer E, Ragen BJ, Trainor BC (2015): Effects of defeat stress on behavioral flexibility in males and females: Modulation by the mu-opioid receptor. Eur J Neurosci 41:434–441.
50. Semplicini A, Lenzini L, Sartori M, Papparella I, Calò LA, Pagnin E, *et al.* (2006): Reduced expression of regulator of G-protein signaling 2 (RGS2) in hypertensive patients increases calcium mobilization and ERK1/2 phosphorylation induced by angiotensin II. J Hypertens 24:1115–1124.
51. Asselmann E, Hertel J, Schmidt CO, Homuth G, Nauck M, Beesdo-Baum K, *et al.* (2018): Interplay between RGS2 and childhood adversities in predicting anxiety and depressive disorders: Findings from a general population sample. Depress Anxiety 35:1104–1113.
52. Smoller JW, Paulus MP, Fagerness JA, Purcell S, Yamaki LH, Hirshfeld-Becker D, *et al.* (2008): Influence of RGS2 on anxiety-related temperament, personality, and brain function. Arch Gen Psychiatry 65:298–308.
53. Koenen KC, Amstadter AB, Ruggiero KJ, Acierno R, Galea S, Kilpatrick DG, Gelernter J (2009): RGS2 and generalized anxiety disorder in an epidemiologic sample of hurricane-exposed adults. Depress Anxiety 26:309–315.
54. Stein MB, Keshaviah A, Haddad SA, Van Ameringen M, Simon NM, Pollack MH, Smoller JW (2014): Influence of RGS2 on sertraline treatment for social anxiety disorder. Neuropsychopharmacology 39:1340–1346.
55. Hohoff C, Weber H, Richter J, Domschke K, Zwanzger PM, Ohrmann P, *et al.* (2015): RGS2 ggenetic variation: Association analysis with panic disorder and dimensional as well as intermediate phenotypes of anxiety. Am J Med Genet B Neuropsychiatr Genet 168B:211–222.
56. Otowa T, Shimada T, Kawamura Y, Sugaya N, Yoshida E, Inoue K, *et al.* (2011): Association of RGS2 variants with panic disorder in a Japanese population. Am J Med Genet B Neuropsychiatr Genet 156B:430–434.
57. Amstadter AB, Koenen KC, Ruggiero KJ, Acierno R, Galea S, Kilpatrick DG, Gelernter J (2009): Variant in RGS2 moderates posttraumatic stress symptoms following potentially traumatic event exposure. J Anxiety Disord 23:369–373.
58. Amstadter AB, Koenen KC, Ruggiero KJ, Acierno R, Galea S, Kilpatrick DG, Gelernter J (2009): Variation in RGS2 is associated with suicidal ideation in an epidemiological study of adults exposed to the 2004 Florida hurricanes. Arch Suicide Res 13:349–357.
59. Cui H, Nishiguchi N, Ivleva E, Yanagi M, Fukutake M, Nushida H, *et al.* (2008): Association of RGS2 gene polymorphisms with suicide and increased RGS2 immunoreactivity in the postmortem brain of suicide victims. Neuropsychopharmacology 33:1537–1544.
60. Lifschytz T, Broner EC, Zozulinsky P, Slonimsky A, Eitan R, Greenbaum L, Lerer B (2012): Relationship between Rgs2 gene expression level and anxiety and depression-like behaviour in a mutant mouse model: Serotonergic involvement. Int J Neuropsychopharmacol 15:1307–1318.
61. Oliveira-dos-Santos AJ, Matsumoto G, Snow BE, Bai D, Houston FP, Whishaw IQ, *et al.* (2000): Regulation of T cell activation, anxiety, and male aggression by RGS2. Proc Natl Acad Sci U S A 97:12272–12277.
62. Nakahara T, Hashimoto K, Hirano M, Rajendram R, Martin CR, Ar Preedy VR (2010): Gender differences in the relative abundance of RGS2 mRNA in brain-stem, cortex, cerebellum and midbrain and the effects of chronic alcohol feeding. Proc Nutr Soc 69:E338.
63. Okimoto N, Bosch OJ, Slattery DA, Pflaum K, Matsushita H, Wei FY, *et al.* (2012): RGS2 mediates the anxiolytic effect of oxytocin. Brain Res 1453:26–33.
64. Hepler JR (1999): Emerging roles for RGS proteins in cell signalling. Trends Pharmacol Sci 20:376–382.
65. Heximer SP, Watson N, Linder ME, Blumer KJ, Hepler JR (1997): RGS2/G0S8 is a selective inhibitor of Gqalpha function. Proc Natl Acad Sci U S A 94:14389–14393.
66. Phan HTN, Sjögren B, Neubig RR (2017): Human missense mutations in regulator of G protein signaling 2 affect the protein function through multiple mechanisms. Mol Pharmacol 92:451–458.
67. Siderovski DP, Hessel A, Chung S, Mak TW, Tyers M (1996): A new family of regulators of G-protein-coupled receptors? Curr Biol 6:211–212.
68. Koelle MR, Horvitz HR (1996): EGL-10 regulates G protein signaling in the C. elegans nervous system and shares a conserved domain with many mammalian proteins. Cell 84:115–125.
69. Sakloth F, Polizu C, Bertherat F, Zachariou V (2020): Regulators of G protein signaling in analgesia and addiction. Mol Pharmacol 98:739–750.
70. Ladds G, Goddard A, Hill C, Thornton S, Davey J (2007): Differential effects of RGS proteins on Gαq and Gα11 activity. Cell Signal 19:103–113.

71. Taymans JM, Leysen JE, Langlois X (2003): Striatal gene expression of RGS2 and RGS4 is specifically mediated by dopamine D1 and D2 receptors: Clues for RGS2 and RGS4 functions. J Neurochem 84:1118–1127.
72. Taymans JM, Kia HK, Claes R, Cruz C, Leysen J, Langlois X (2004): Dopamine receptor-mediated regulation of RGS2 and RGS4 mRNA differentially depends on ascending dopamine projections and time. Eur J Neurosci 19:2249–2260.
73. Stanwood GD, Parlaman JP, Levitt P (2006): Genetic or pharmacological inactivation of the dopamine D1 receptor differentially alters the expression of regulator of G-protein signalling (Rgs) transcripts. Eur J Neurosci 24:806–818.
74. Saunders A, Macosko EZ, Wysoker A, Goldman M, Krienen FM, de Rivera H, *et al.* (2018): Molecular diversity and specializations among the cells of the adult mouse brain. Cell 174:1015–1030.e16.
75. Campi KL, Greenberg GD, Kapoor A, Ziegler TE, Trainor BC (2014): Sex differences in effects of dopamine D1 receptors on social withdrawal. Neuropharmacology 77:208–216.
76. Sutton LP, Khalatyan N, Savas JN, Martemyanov KA (2021): Striatal RGS7 regulates depression-related behaviors and stress-induced reinstatement of cocaine conditioned place preference. eNeuro 8: ENEURO.0365-20.2020.
77. Stratinaki M, Varidaki A, Mitsi V, Ghose S, Magida J, Dias C, *et al.* (2013): Regulator of G protein signaling 4 [corrected] is a crucial modulator of antidepressant drug action in depression and neuropathic pain models [published correction appears in Proc Natl Acad Sci U S A 2013; 110:11660]. Proc Natl Acad Sci U S A 110:8254–8259.
78. Paden W, Barko K, Puralewski R, Cahill KM, Huo Z, Shelton MA, *et al.* (2020): Sex differences in adult mood and in stress-induced transcriptional coherence across mesocorticolimbic circuitry. Transl Psychiatry 10:59.
79. Scarpa JR, Fatma M, Loh YHE, Traore SR, Stefan T, Chen TH, *et al.* (2020): Shared transcriptional signatures in major depressive disorder and mouse chronic stress models. Biol Psychiatry 88:159–168.
80. McCann KE, Sinkiewicz DM, Rosenhauer AM, Beach LQ, Huhman KL (2019): Transcriptomic analysis reveals sex-dependent expression patterns in the basolateral amygdala of dominant and subordinate animals after acute social conflict. Mol Neurobiol 56:3768–3779.
81. Gasser PJ (2019): Roles for the uptake$_2$ transporter OCT3 in regulation of dopaminergic neurotransmission and behavior. Neurochem Int 123:46–49.
82. McReynolds JR, Taylor A, Vranjkovic O, Ambrosius T, Derricks O, Nino B, *et al.* (2017): Corticosterone potentiation of cocaine-induced reinstatement of conditioned place preference in mice is mediated by blockade of the organic cation transporter 3. Neuropsychopharmacology 42:757–765.
83. Amphoux A, Vialou V, Drescher E, Brüss M, Mannoury La Cour C, Rochat C, *et al.* (2006): Differential pharmacological in vitro properties of organic cation transporters and regional distribution in rat brain. Neuropharmacology 50:941–952.
84. Gegenhuber B, Wu MV, Bronstein R, Tollkuhn J (2022): Gene regulation by gonadal hormone receptors underlies brain sex differences. Nature 606:153–159.