Comparative transcriptional analyses in the nucleus accumbens identifies RGS2 as a key mediator of depression-related behavior

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Abstract

Background: Major depressive disorder (MDD) is one of the most commonly diagnosed mental illnesses worldwide, with a higher prevalence in women than 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 into 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 post-mortem samples of the NAc from men and women diagnosed with MDD.

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Results: Our cross-species computational analyses identified regulator of G-protein signaling 2 (*Rgs2*) as a transcript downregulated by defeat stress in female California mice and in women with MDD. 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.

Keywords

Peromyscus californicus ; prefrontal cortex; ventral tegmental area; social defeat; nucleus accumbens; major depression; depression; reward

Introduction

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 impacting 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 about 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). As 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 or 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 focus exclusively on males (18). This forms a gap in knowledge as 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 brain regions in men and women with MDD (20,21). Similar findings have been reported in rodents exposed to sub-chronic 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 difficulty in establishing robust protocols in conventional rodents (25). In California mice (*Peromyscus californicus*), 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 defeat stress impacts the transcriptome of reward-related brain regions. We used RNAseq to examine transcriptional responses to social defeat in the NAc of male and female California mice and compared these data to transcriptional profiles from post-mortem samples of the NAc of patients with MDD. Our analyses identified the G-protein regulator *Rgs2* as a transcript down-regulated in samples from stressed female mice and women with depression. This protein facilitates the process of GTP 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 supplementary materials.

Animals and housing conditions

All studies on California mice (*Peromyscus californicus*) were in accordance with the NIH 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 (SDS)

Mice were randomly assigned to control handling or three episodes of social defeat as previously described (30). Behavior tests and brain tissue collection were conducted two 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 two days with two bottles of tap water. The next day water in one bottle was replaced with a 1% sucrose solution for a 24 hr observation period. Percent sucrose preference was calculated as the amount of sucrose solution consumed (mL) over total amount of solution consumed (water and sucrose solution combined, mL).

Social Interaction Test

Social interaction testing was performed as previously described (30,31). We define 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 towards the target mouse while outside the interaction zone.

RNA extraction and RNA-sequencing library preparation

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

RNAseq Data Analysis

Raw reads were processed with expHTS (32) to trim low-quality reads and adapter contamination and to remove 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 GO 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 to those not annotated with a given term (36). Full threshold-free differential expression lists were performed using RRHO (37). Parameters for significant differential expression were set at an uncorrected $p < 0.05$ and a logFC $> |1.15|$ between stress comparisons (42, 44).

In-situ hybridization

In-situ hybridization was performed as previously described (38). Brains were coronally cryosectioned at 60μm, fixed, treated with proteinase K, acetylated, permeabilized, and equilibrated in hybridization solution. A riboprobe (0.3μg/mL) directed against *Rgs2* corresponding to bases 74–550 of cDNA 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; Roche) overnight then developed in nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Roche) at 37°C for 24 hrs.

Western blots

Protein was extracted from NAc punches and then separated with gel electrophoresis and transferred to polyvinylidine fluoride (PVDF) membranes (Bio-Rad, Hercules, CA), rinsed, and blocked. Membranes were incubated overnight in primary rabbit anti-RGS2 (Abcam, Boston, MA; ab155762) 1:1000 at 4°C that was validated using samples from *Rgs2* knockout mice (Supplemental Fig. 1). Membranes were incubated in peroxidase-conjugated anti-rabbit secondary antibody (1:100, Vector, Burlingame, CA). Membranes were washed,

developed, and imaged on a Bio-Rad ChemiDoc. Blots were probed for β-actin as a loading control (Cell Signaling, Danvers, MA, 1:1000), and RGS2 protein bands were normalized to their respective β-actin controls.

Quantitative Real-Time PCR

RNA was extracted from NAc tissue punches from SDS male and female mice (n = 5–8 per group) using an RNAeasy micro kit (Qiagen, 74004) and converted to cDNA using an iScript cDNA synthesis kit (Bio-Rad; 1708891). Real-time qPCR was performed using SybrGreen Fast master mix (Applied Biosystems) on a ViiA 7 Real-Time PCR system and analyzed by the 2– Ct method. For primer sequences see Supplemental Table 1.

Overexpression of *Rgs2* within the NAc by HSV-mediated gene transfer.

To overexpress RGS2, we used a bicistronic p1005 herpes simplex virus (HSV) expressing GFP or GFP and *Rgs2* (Origene). Expression of GFP is driven by a cytomegalovirus (CMV) promoter while the gene of interest is driven by the IE4/5 promoter (39,40). One week following defeat, mice received one bilateral 0.6uL injection of either the RGS2 vector or vector containing GFP alone into the NAc core (A/P: 0.84, M/L: ±1.5, D/V: 6.0). One week later mice were tested for sucrose anhedonia test and social interaction. To confirm that expression was limited to NAc, sections of the NAc were imaged to visualize GFP colocalization with Neurotrace (ThermoFisher). RGS2 overexpression was confirmed via western blot.

Statistical analyses

All statistical analyses were performed using R statistical software. Normality of data was assessed using Shapiro-tests. A Fligner-Killeen test was used to assess homogeneity of variance. Two-way ANOVA (sex and stress) was used to analyze qPCR 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 versus GFP controls (41).

Results

Effects of social defeat on male and female transcriptional responses in the NAc of California mice

We performed behavioral and transcriptional analyses two weeks after social defeat or control manipulations (Fig. 1A). Similar to previous studies of California mice (42,43), stressed females but not males showed a decrease in social interaction ratio when the target was present (Fig. 1B, stress*sex interaction effect, $F_{1,29}$=4.37, p<0.05) and planned comparisons showed an effect of stress in females but not males. There were no differences in the open field phase (Fig. 1C). We performed RNA-seq on samples of the NAc from these mice (Supplemental Table 2). We first set more liberal parameters for identifying alterations in transcription (uncorrected p<0.05, $\log_2$ fold-change (logFC) > |0.38|) to identify broad patterns of transcriptional changes (44,45). Heatmaps plotting normalized transcript expression (RPKMs) show contrasting transcriptional expression patterns in

females, where low abundance transcripts in control females were more abundant in stressed females, and vice versa (Fig. 1D); this pattern is less distinct in males (Fig. 1D). Most of the top ten highly enriched terms following gene ontology analyses of differentially expressed transcripts in females are related to dopamine signaling pathways and G-alpha(q) second messenger signaling cascades (Fig. 1E). When comparing these datasets using RRHO, we found more overlap in overexpressed transcripts for males and females (Supplemental Fig. 2) and less overlap in transcripts that were reduced after defeat. When stricter parameters were used to identify differentially expressed genes (uncorrected p<0.05, logFC > |1.15) (44,46), we found a more robust effect of stress in females, where 320 transcripts were elevated in stressed females versus 36 transcripts in males (Fig. 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 compared to males (Supplemental Fig. 3). Together these results suggest that there may be sex-specific changes in transcriptomic responses in the NAc, with stronger responses occurring in females compared to males. For example, only 11 transcripts that were upregulated in stressed females were also upregulated in stressed males (Fig. 1F; Supplemental Table 3). For 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 (Supplemental Fig. 4) 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 RNAseq 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 RNAseq results to data obtained from the NAc of men and women with MDD (GEO accession number: GSE102556).

Transcriptional patterns related to social defeat and major depressive disorder

Using RRHO we observed that the effects of social defeat stress on transcriptional responses in female California mice were broadly similar to differences observed in samples from women with MDD (Fig. 2A). This overlap was largely absent in samples from male California mice and samples from men with MDD (Fig. 2B). We then identified 17 transcripts present in both stressed female mice and women with MDD that had an uncorrected p<0.05 and log FC>|1.15| (Fig. 2A). One of these transcripts is *Rgs2*. We identified a sex-specific effect of stress on *Rgs2* RPKMs in female California mice (Fig. 2C, sex*stress interaction effect, $F_{1,29}=4.762$, p<0.05), with planned comparisons showing an effect of stress in females but not males. Importantly, *Rgs2* was not identified as a DEG 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 for females (Fig. 2D, Pearson *r*=0.6, p<0.05) but not males (Fig. 2E, *r*=0.098, p>0.05). No effects of stress were observed on *Rgs2* RPKMs in the VTA or PFC (Supplemental Fig. 5). To determine the robustness of social defeat stress on *Rgs2* expression, we measured gene expression in one set of biological replicates and RGS2 protein in a separate set of samples (Fig. 3A).

Social defeat stress reduces *Rgs2* mRNA and RGS2 protein expression in the female NAc

Using *in-situ* hybridization we confirmed *Rgs2* expression in the NAc (Fig. 3B). Social defeat reduced social approach in females but not males when the target was present (Supplemental Fig. 6A, stress*sex, $F_{1,26}=5.995$, p<0.05) but not when it was absent (Supplemental Fig. 6B). In these mice, real-time PCR analyses showed that social defeat reduced *Rgs2* mRNA in the NAc in females but not males (Fig. 3C, stress*sex, $F_{1,26}=4.687$, p<0.05). While RNAseq 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 control males (p<0.05). Similar to analyses of sequencing data, *Rgs2* mRNA was positively correlated with social approach in females (Fig. 3D, Spearman $\rho=0.57$, p=0.03) but not in males ($\rho=-0.27$, p=0.39). In a separate group of biological replicates, social defeat significantly decreased RGS2 protein expression in females (Fig. 3E, $t(7) = 6.9$, p <0.001) and RGS2 protein was positively correlated with social approach (Fig. 3F, $\rho=0.73$, p=0.03).

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 (Fig. 4A, B). Viral expression occurred in neurons and the *Rgs2* virus increased RGS2 protein in the NAc (Fig. 4C). There were no differences in sucrose preference prior to defeat stress (Fig. 4D). After defeat, there were significant differences in sucrose preference (one-way ANOVA $F_{2,14}=36.4$, p<0.001), with females receiving the *Rgs2* virus in the NAc consuming more sucrose than GFP (planned comparison p<0.001) whereas misplaced *Rgs2* viral injections did not differ from GFP. In the social interaction test, there were significant differences in social approach (Fig. 4E, $F_{2,14}=11.66$, p<0.01) and social vigilance (Fig. 4F, $F_{2,14}=36.39$, p<0.01). Mice that received the *Rgs2* virus in the NAc had higher social approach (planned comparison, p<0.01) and lower social vigilance (planned comparison, p<0.05) compared to 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 (Fig. 4G, 4H, both p's >0.05). During the open field phase of the social interaction test, *Rgs2* overexpression had no effects on distance traveled (Fig. 4I, p>0.05) or on time spent in the center of the arena (Fig. 4J, p>0.05).

Discussion

An important question in psychiatry is why rates of depression and anxiety are elevated in women versus men. There is growing evidence that distinct neurobiological responses can be evoked by stress in women and men. Here we demonstrate that social defeat stress in female California mice induces broad patterns of transcriptional changes in the NAc that are correlated with transcriptional patterns reported in postmortem 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 within 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 utilizing 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 a lack of efficacy to 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 versus 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, so global knockout approaches have little precision for identifying the brain circuits mediating *Rgs2* action on social behavior. In addition, behavioral studies focused primarily on males. In our qPCR experiment *Rgs2* expression was higher in control females versus control males, although this difference was not replicated in the RNAseq dataset. There are few data on RGS2 expression in male and female brains, although higher *Rgs2* expression was reported in female rat brainstem versus males (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 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 GPCR responses by several other mechanisms. For example, they may act as effector antagonists for G alpha subunits or as regulators of epigenetic and transcriptional processes (69). RGS2 specifically regulates G-alpha(q) signaling events (70). Given this distinction, an important

question to consider is which receptor signaling pathways within the NAc are being impacted by stress-induced reductions in RGS2. Prior findings indicate that RGS2 may regulate dopamine receptor 1 (D1R)-expressing neurons in the NAc (71–73). Single-cell RNAseq analyses of striatum showed that *Rgs2* gene in the striatum clusters significantly with D1R-expressing neurons but not D2R-expressing neurons (74). Although these data lack anatomical specificity, it suggests that RGS2 could be impacting 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 VTA and PFC detected more overlap in transcripts upregulated by stress than transcripts downregulated by stress. In C57Bl6/J, unpredictable chronic mild stress induced similar transcriptional changes in the NAc and PFC in male but not female mice, while in females similar gene expression profiles were observed in the NAc and BLA (78). In analyses of human post-mortem samples, RRHO analyses detected little overlap in gene expression across NAc and cortical regions in either males or females. Numerous studies have reported sex-specific neural transcriptional responses to stress using bulk RNAseq 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 (Fig. 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 with sex-specific transcriptional responses to stress such as *Dusp4*, *Dnmt3a*, and *Emx1*. 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 down-regulated by stress in California mice and decreased in samples from women diagnosed with depression. One protein encoded by this transcript is organic cation transporter 3 (OCT3), 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 demonstrate 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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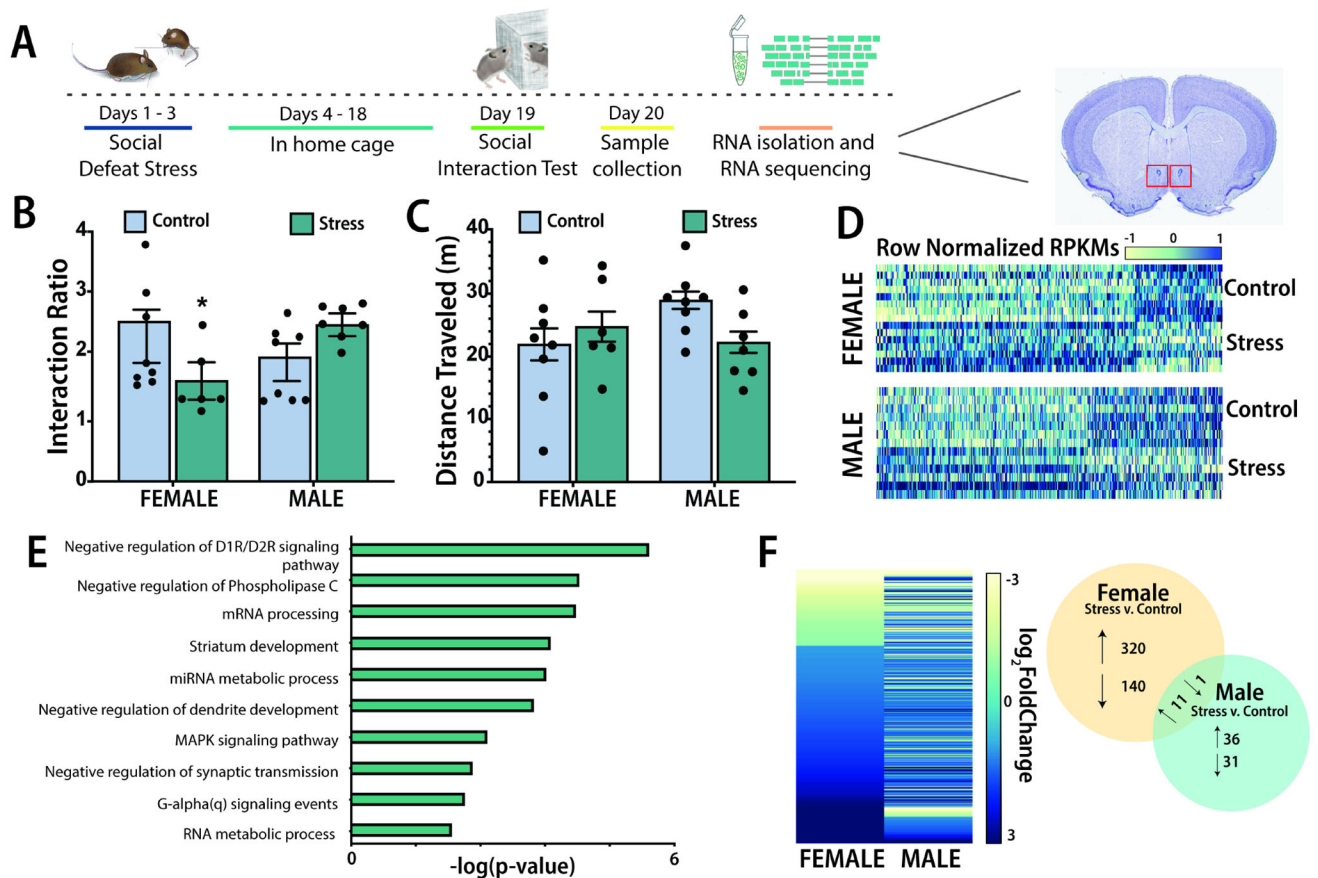**Figure 1. Social defeat stress differentially expresses NAc transcriptional patterns in a sex-specific manner.**

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 NAc were collected for RNAseq (A). Social defeat reduces social approach in females but not males during the social interaction test (B). No differences were seen in distance traveled during the open field phase (C). Stressed female mice have different average transcript expression (RPKM) patterns compared to control females (D). Highly enriched differently expressed GO terms identified from overlapping male and female DEGs (E). Minimal overlap is present in DEGs between males and females (F). *planned comparison p<0.05 v. control. Group N's: males/control: 8, male/stress: 7, female/control: 8, female/stress: 6.
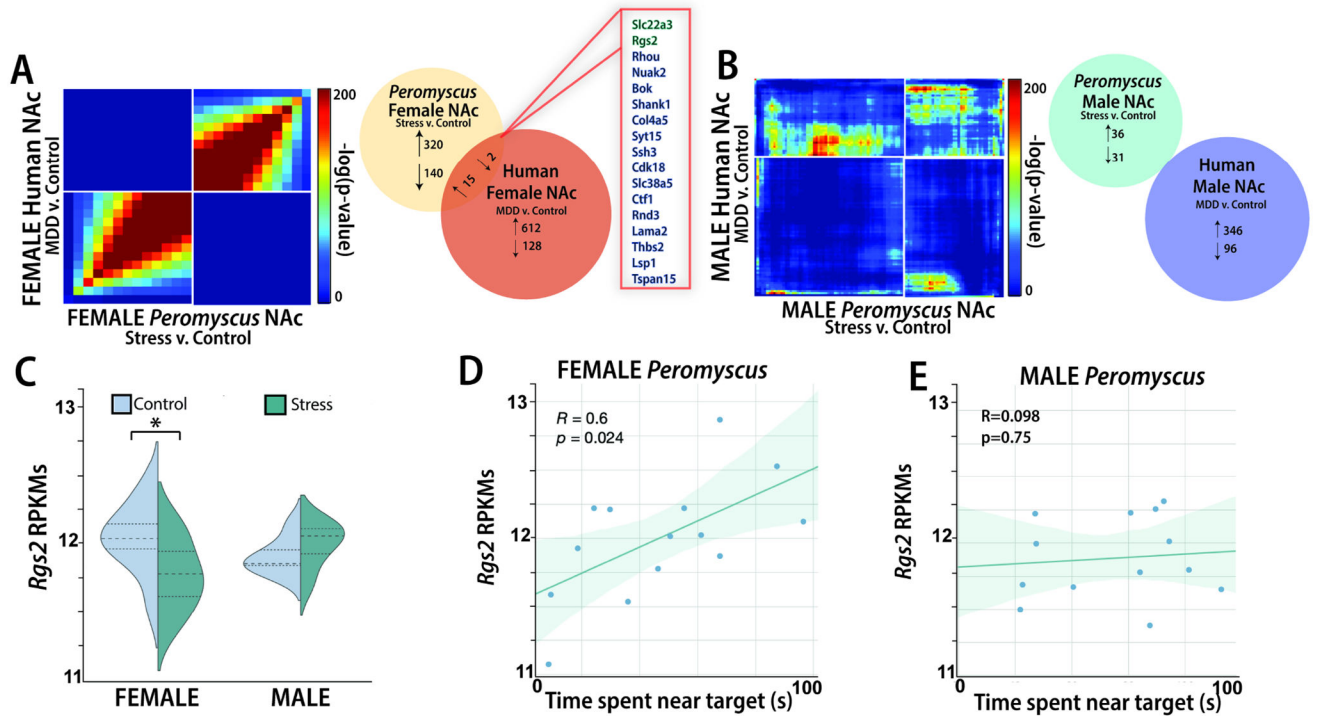
Figure 2. Social defeat stress induces similar gene expression patterns in the NAc in female California mice compared to women with major depression (MDD).

Social defeat stress induces similarities in transcriptional patterns in female California mice compared to transcriptional patterns observed in women with MDD (A); one transcript similarly affected in both data sets is *Rgs2*. No similarities in DEGs were observed in stressed male California mice and men with MDD (B). Stress reduces *Rgs2* average expression in a sex-specific manner (C). *Rgs2* expression is correlated to social avoidance behavior in female (D) but not male (E) California mice. *planned comparison p<0.05 v. control. Group N's: males/control: 8, male/stress: 7, female/control: 8, female/stress: 8, women/MDD: 13, women/control: 9, men/MDD: 13, men/control: 13.
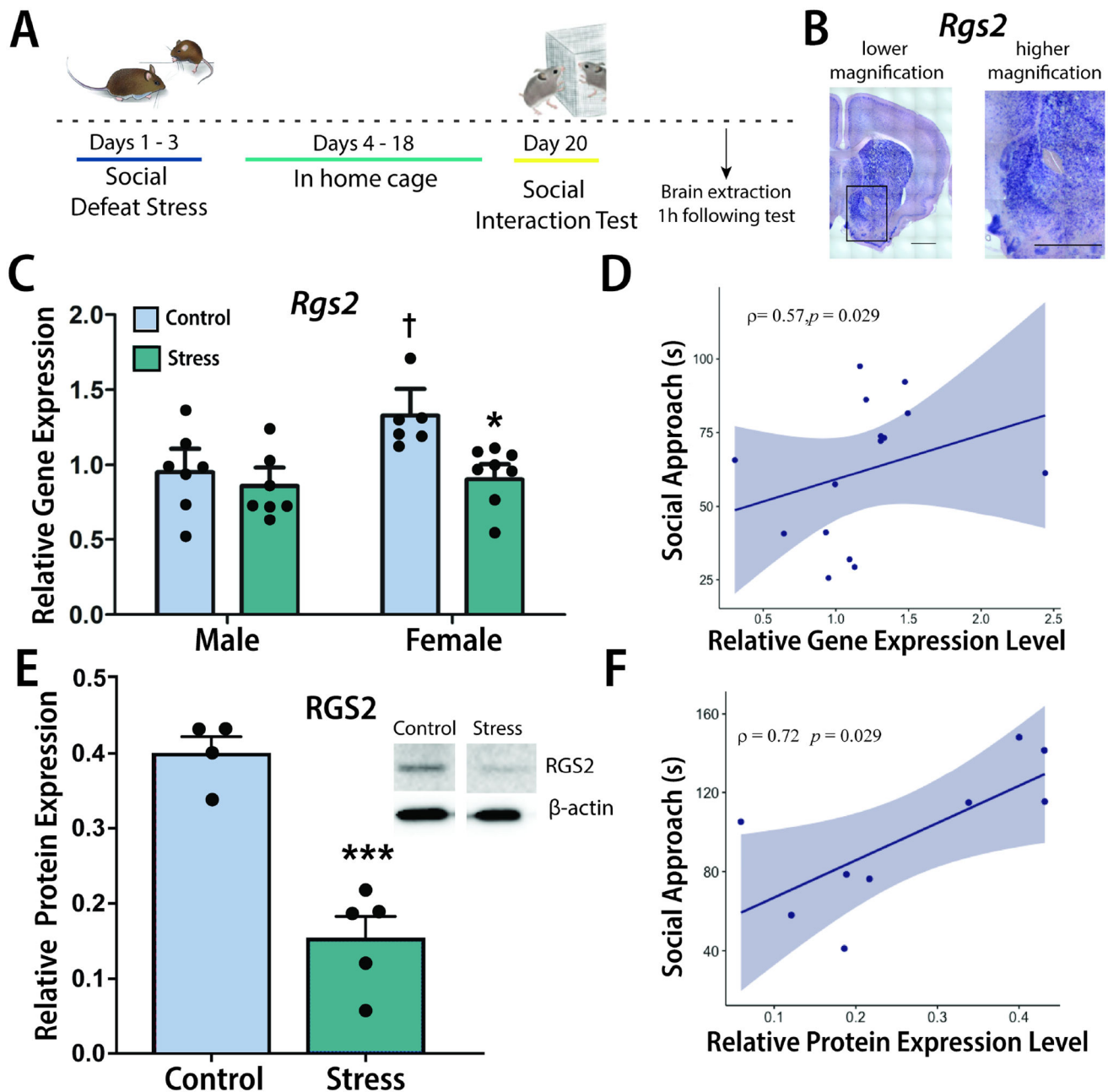
Figure 3. Social defeat stress reduces *Rgs2* mRNA and protein expression in NAc of female California mice.

Timeline for experiment. Male and female California mice were run through control handling or social defeat stress. 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 (A). *Rgs2* expression in the NAc was confirmed using in-situ hybridization (B). Social defeat stress reduced *Rgs2* mRNA in females but not males (C), and Rgs2 mRNA expression levels are positively correlated with social approach behavior (D). Stress reduced RGS2 protein levels in females (E), and these protein levels correlate with social approach (F). *p<0.05 planned comparison vs. female control p<0.05 v. control, ***p<0.001 independent

t-test vs. control, † p<0.05 planned comparison v. male control. qPCR group N's: male/control: 7, male/stress: 7, female/control: 6, female/stress: 8. Western blot group N's control:4, stress:5.
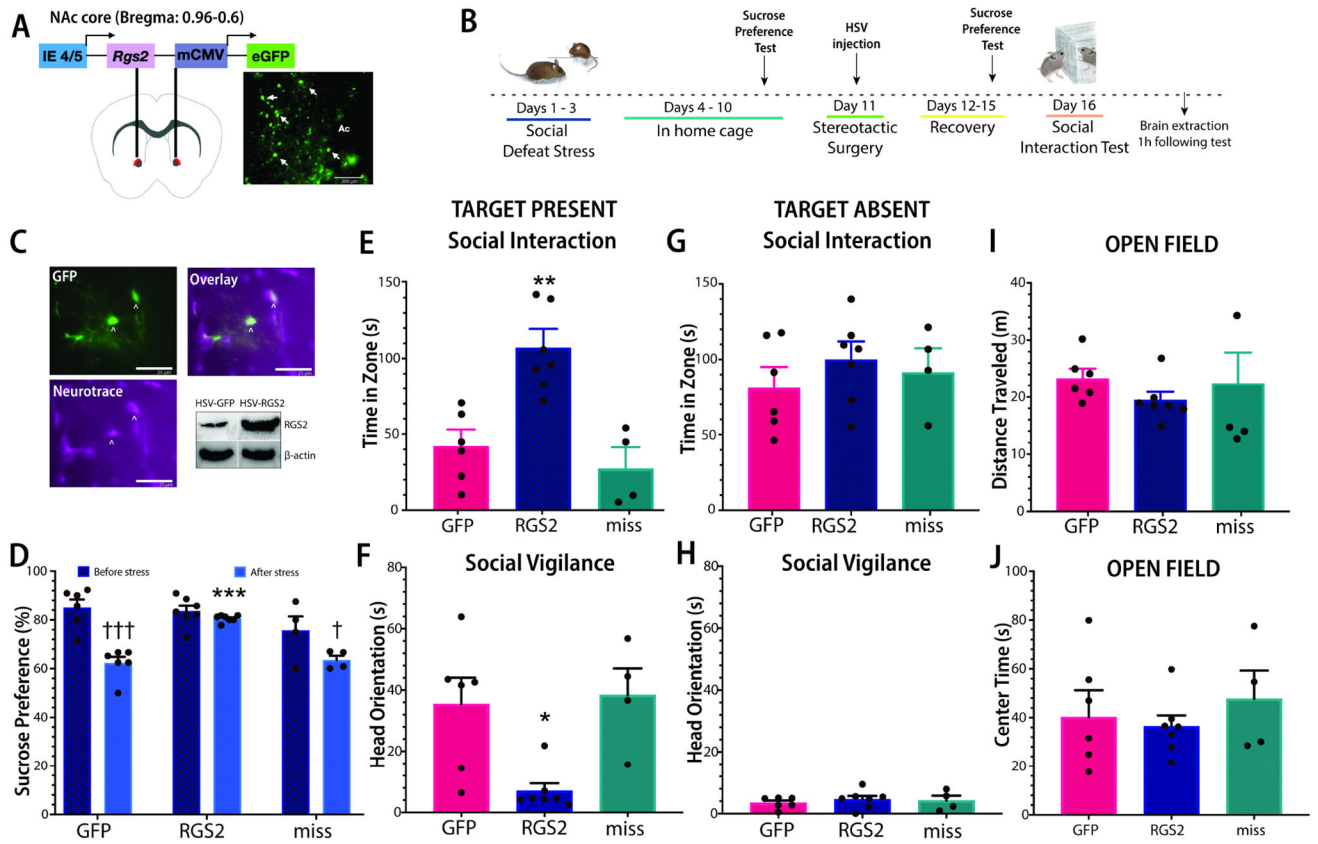
Figure 4. Overexpression of *Rgs2* in the NAc reverses stress-induced depression-like behavior in female California mice.

Schematic of placement site and viral construct and image of viral transfection (GFP in NAc) (A). Timeline of experiment. We took baseline sucrose preference levels from all mice before stress exposure. All females were exposed to social defeat and then one 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 (B). Neurotrace staining shows that GFP expression occurs in neurons and western blot analysis demonstrates that HSV-RGS2 vectors increase RGS2 protein in the NAc (C). *Rgs2* overexpression reversed anhedonia-like phenotypes in the sucrose preference test (D). *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). *Rgs2* overexpression did not alter distance traveled (I) or center time (J) during the open field phase. * $p < 0.05$ planned comparison v. GFP. **$p < 0.01$ planned comparison v. GFP. ***$p < 0.001$ planned comparison v. GFP. †$p < 0.05$ paired t-test with baseline (before stress). †††$p < 0.001$ paired t-test with baseline (before stress). Group N's: RGS2: 7, GFP: 6, miss: 4. Scale bar in 4A=200μM. Scale bar in 4C=25μM.

KEY RESOURCES TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use "this paper" if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources.	Include any additional information or notes if necessary.
Antibody	rabbit anti-RGS2	Abcam	ab155762, RRID:AB_2916033	
Antibody	Goat Anti-Rabbit IgG Antibody (H+L), Peroxidase	Vector Labs	PI-1000-1, RRID:AB_2916034	
Antibody	rabbit anti-b-actin 13E5	Cell Signaling	4970, RRID:AB_2223172	
Deposited Data; Public Database	California mouse RNAseq data	NCBI Bioproject	PRJNA700778	
Deposited Data; Public Database	Human post-mortem brain RNAseq data	NCBI GEO DataSets	GSE102556	
Commercial Assay Or Kit	Neurotrace 530/615	ThermoFisher	N21482	
Primers for RT-qPCR, see Table S1	RGS2 and Hprt primers	This paper, Invitrogen		
Viral vector	HSV-*Rgs2*	Mass General Brigham Gene Delivery Technology Core	CMV promoter for GFP and IE4/5 promoter for *Rgs2*	