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## Differential stress induced c-Fos expression and identification of region-specific miRNA-mRNA networks in the dorsal raphe and amygdala of high-responder/low-responder rats

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### Abstract

Chronic stress triggers a variety of physical and mental health problems, and how individuals cope with stress influences risk for emotional disorders. To investigate molecular mechanisms underlying distinct stress coping styles, we utilized rats that were selectively-bred for differences in emotionality and stress reactivity. We show that high novelty responding (HR) rats readily bury a shock probe in the defensive burying test, a measure of proactive stress coping behavior, while low novelty responding (LR) rats exhibit enhanced immobility, a measure of reactive coping. Shock exposure in the defensive burying test elicited greater activation of HR rats' caudal dorsal raphe serotonergic cells compared to LRs, but lead to more pronounced activation throughout LRs' amygdala (lateral, basolateral, central, and basomedial nuclei) compared to HRs. RNA-sequencing revealed 271 mRNA transcripts and 33 microRNA species that were differentially expressed in HR/LR raphe and amygdala. We mapped potential microRNA-mRNA networks by correlating and clustering mRNA and microRNA expression and identified networks that differed in either the HR/LR dorsal raphe or amygdala. A dorsal raphe network linked three microRNAs which were down-regulated in LRs (miR-206-3p, miR-3559-5p, and miR-378a-3p) to repression

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of genes related to microglia and immune response (*Cd74*, *Cyth4*, *Nckap11*, and *Rac2*), the genes themselves were up-regulated in LR dorsal raphe. In the amygdala, another network linked miR-124-5p, miR-146a-5p, miR-3068-3p, miR-380-5p, miR-539-3p, and miR-7a-1-3p with repression of chromatin remodeling-related genes (*Cenpk*, *Cenpq*, *Itgb3bp*, and *Mis18a*). Overall this work highlights potential drivers of gene-networks and downstream molecular pathways within the raphe and amygdala that contribute to individual differences in stress coping styles and stress vulnerabilities.

## Keywords

High Responder/Low Responder; c-Fos; stress coping; microRNA; amygdala; dorsal raphe

## INTRODUCTION

Stress is a well-known environmental risk factor for a variety of mental illnesses [1–5], yet there is a large gap in knowledge explaining why some individuals are susceptible to stress-induced psychopathology while others are resilient. How individuals choose to cope with stress plays an important role in whether they later develop emotional disorders [6–8]. Stress coping styles encompass a range of physiological, psychological, and behavioral responses aimed to avoid or tolerate harm or distress. They are broadly characterized as proactive (a fight-or-flight response to defeat/escape a stressor) or reactive (a withdrawal response to avoid/outlast the stressor)[9]. In humans, proactive vs. reactive coping styles convey risk or resilience to stress-induced psychopathology depending upon the stressor, since each coping style can be adaptive in some circumstances but maladaptive in others [6, 10–12]. Thus, there is a great need to elucidate neural and molecular factors that shape individuals' stress coping styles and stress vulnerabilities.

The present study utilized Sprague-Dawley rats that were selectively bred for differences in behavioral response to novelty and stress reactivity [13] to investigate molecular mechanisms contributing to coping style. Rats bred for high response to novelty (High Responders, HRs) vigorously explore new environments compared to rats bred for low novelty response (Low Responders, LRs). Selective breeding produced rats with differences in anxiety/depression-like behavior, with LR rats displaying high and HR rats displaying low anxiety/depression-like behavior as measured by the forced swim test (FST) [14], resident-intruder test [15], and elevated plus maze [13]. These behavioral differences suggest that HRs display proactive coping across several tests while LR rats display reactive coping. Work in humans and rodents shows that proactive vs. reactive coping styles predict vulnerability to different stressors [16–20]. The HR/LR model recapitulates this phenomenon since LR rats (but not HRs) are vulnerable to chronic mild stress (CMS), which exacerbates their already high levels of anxiety/depressionlike behavior [21], while HRs (but not LR rats) are vulnerable to chronic social defeat (increasing depression-like behavior) [22, 23].

The present experiments utilized the HR/LR rat model to identify neural circuit and molecular differences that potentially drive their proactive vs. reactive stress coping styles as well as disparate stress vulnerabilities. In the first study, we hypothesized that HR rats would

display proactive coping and LR rats would exhibit reactive coping in the defensive burying test, a test of stress coping style where animals may actively cope by shoveling bedding material onto a noxious stimulus (a wall-mounted shock probe), or passively/reactively cope by displaying freezing behavior [24]. We next used c-Fos immunoreactivity to identify circuit differences that may contribute to HR/LR's disparate stress coping styles. Neural activation patterns in two bi-directionally connected regions known for modulating the behavioral response to stress, the dorsal raphe and the amygdala, were measured in HR/LR rats following the defensive burying task. Expression of c-Fos, and other immediate early genes, have previously been used to identify brain-regions and cell types that contribute to stress coping behavior [25–28]. For example, c-Fos mapping identified the A2 noradrenergic cell group as hyporesponsive to stress in Wistar-Kyoto rats, which display high levels of reactive coping and immobility on the forced-swim test. A follow-up experiment showed that lesioning the A2 group increased immobility in Wistar rats [29], demonstrating the potential for c-Fos mapping to identify regions functionally relevant to stress coping style. We focused on the dorsal raphe and the amygdala because previous studies showed HR/LR serotonin (5HT) system differences may contribute to their behavioral phenotypes, including stress coping style. For example, compared to HRs, LR rats exhibit lower tryptophan hydroxylase 2 (*Tph2*) and serotonin reuptake transporter (*Sert*) mRNA levels in the dorsal raphe, median raphe, and B9 cell group [15]. LR rats also show higher *5HT1a* receptor mRNA levels in the cingulate, lateral septum, and CA1 region of the hippocampus, compared to HRs [30], as well as increased *5HT2a* [22], *5HT6* and *5HT7* [31] receptor mRNA in multiple forebrain regions. Pharmacological studies suggest that HR/LR 5HT differences shape aspects of their disparate behavioral phenotypes, including differences in FST performance [14], social interaction [22, 32], aggression [15, 30], cognition [31], reward-processing [33], and anxiety [34].

Our final experiment used next-generation sequencing of mRNAs and microRNAs in the dorsal raphe and amygdala to identify molecular pathways associated with HR/LR stress circuit and behavioral differences. While a variety of stressors engage the dorsal raphe and its targets, the precise molecular mechanisms and behavioral consequences of activation within its different subgroups are not well understood. Transcriptome sequencing is an unbiased approach to identifying potential molecular mechanisms. These technologies also allow for the profiling of coding and non-coding RNA species, which are increasingly becoming recognized for their role in epigenetic regulation [35, 36] and contribution to mental illness [37–39]. MicroRNAs (miRNAs), a type of non-coding RNA, are of particular interest, as each miRNA can potentially regulate several genes, so co-expression of only a few miRNAs powerfully controls large gene networks [40]. miRNAs are short non-coding RNAs that control translation and/or stability of mRNA targets by binding to a 6 to 8 base pair complementary 'seed region' on the 3' UTR of the mRNA [41, 42]. Rodent studies show that acute and chronic stress affect miRNA expression [43, 44], and rats that are vulnerable vs. resilient to developing learned helplessness display miRNA expression differences [45]. Recent findings have demonstrated a causal role for specific miRNAs in mediating stress coping behavior. Mice with miR-17–92 knocked out display increased reactive coping on forced-swim and tail-suspension tests [46], while mice with miR-34 knocked out display resilience to chronic mild stress [47]. However, none of these genetic

manipulations have yet to take into account region-specific miRNA expression. Differences in region-specific miRNA regulated gene networks in HR/LR rats may represent key molecular pathways that potentially regulate stress coping, stress vulnerability, and emotional dysfunction.

## MATERIALS AND METHODS

All experiments were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham and conducted in accordance with National Institutes of Health guidelines on animal care and experimentation.

### Animals

Adult male HR/LR rats were obtained from the 4<sup>th</sup>–6<sup>th</sup> generations of our in-house colony [48]. Rats were pair-housed in a 12:12 light-dark cycle in a temperature- and humidity-controlled environment with food and water available *ad libitum*.

### Behavioral Testing

**Defensive Burying Test**—Adult male HR/LR rats (n=16/phenotype) were evaluated in the defensive burying test. Rats underwent two daily 15-min habituation trials in a Plexiglas chamber (45cm × 45cm × 60cm, Noldus, Wageningen, Netherlands) filled with 3 inches of clean bedding. 24-h after the final habituation, rats returned to the chamber, which contained an electric probe. For half the animals, the electric probe was active and they received a single 4.0mA shock upon interaction with the probe. Behavior was observed for 15-min following the shock. A blinded experimenter measured time spent immobile (reactive coping); and burying, defined as the rat actively pushing or throwing bedding in the direction of the probe with its front paws or head (proactive coping). The arena was divided into front, middle, and back thirds (15cm × 45cm) and time spent near the probe (front) was measured. The other half of animals were placed in the chamber with an *inactive* shock probe; their behavior was measured for 15-min to serve as a control. Videos of all sessions were recorded with Ethovision® XT 8.0 software (Noldus, Wageningen, Netherlands).

**Nociception Testing**—Nociception testing was performed in order to rule out possible differences in sensing or experiencing of electric shock in HR/LRs. One cohort of animals (n=10 HR; n=8 LR) were evaluated on separate days for responsivity to mechanical [49], cold [50], and heat stimuli [51]. And a second cohort of animals (n=10/phenotype) was evaluated on separate days for response to heat stimuli and tail flick [52]. See Supplemental Materials for detailed methods.

### Neuronal activation

**Immunohistochemistry**—Ninety-min after completing the defensive burying task, shock-exposed and non-shocked control HR/LR rats (n=8/group) were deeply anesthetized with sodium pentobarbital (150 mg/kg i.p.) and then transcardially perfused with ~100 ml of physiological saline followed by ~300 ml of 4% paraformaldehyde. Brains were extracted, post-fixed overnight, cryoprotected in 20% sucrose, and processed for immunohistochemistry as described in previous studies [53]. Briefly, brains were sectioned

coronally on a freezing microtome at a thickness of 40  $\mu\text{m}$ . Dual-label immunohistochemistry was performed on free-floating brainstem tissue sections using antibodies against Tph2 (to identify 5HT-containing cells) and c-Fos, an indicator of cellular activation in response to the defensive burying test. Tissue sections were first reacted with rabbit anti-c-Fos antibody (1:3000; Life Sciences, CA#: ABE457 Darmstadt, Germany) for 17–19 h, then incubated 90 min with biotinylated goat anti-rabbit antibody (1:500; Life Sciences, CA#: AP132B). Tissue was then incubated for 90 min in an avidin-biotin-peroxidase complex (1:200; Elite ABC reagent, Cat. No. PK-6100; Vector Laboratories, Burlingame, CA, USA), and finally reacted with 0.01% 3-3'-diaminobenzidine tetrahydrochloride (DAB; Cat. No. D9015, Sigma-Aldrich, St Louis, MO). To identify 5HT-containing cells, sections were next reacted with mouse anti-Tph2 antibody (1:500; Sigma, CA# T0678) for 12–16 h, and then with Cy3-conjugated donkey anti-mouse IgG (1:200; Jackson Laboratories, Bar Harbor, ME; CA#: 715-165-151) for 2 h at room temperature in the dark. Sections were mounted onto glass slides and coverslipped with Permount (Fisher Chemical, Pittsburgh, PA). Forebrain sections underwent the same process but were only single-labelled for c-Fos and visualized with DAB.

**Image analysis**—DAB- and immunofluorescently-labeled tissue was examined using an Olympus BX-UCB microscope (<http://www.olympusamerica.com/>) equipped with a motorized stage (96S100-LE; Ludl Electronic Products, <http://www.ludl.com/>), fluorophore-specific fluorescent filter sets (excitation and emission spectra: Cy3/TRITC – 531/40), and a cooled mono CCD camera (Orca R2; Hamamatsu, <http://hamamatsucameras.com/>). Tissue sections through the dorsal raphe and amygdala were examined at 240  $\mu\text{m}$  intervals. In the dorsal raphe, sections were divided into multiple subregions: caudal dorsomedial dorsal raphe (cDRD), ventrolateral wings of the dorsal raphe (DRVl), dorsomedial dorsal raphe (DRD), ventral dorsal raphe (DRV), rostral dorsomedial dorsal raphe (rDRD), and rostral ventral dorsal raphe (rDRV) [15]. The number of cells dual-labeled with c-Fos and Tph2 were counted in each subregion through the extent of the dorsal raphe (Fig. 2A). In the amygdala, the following subnuclei were examined: lateral amygdala (LA), basolateral amygdala (BLA), central amygdala (CeA), medial amygdala (MeA), and basomedial amygdala (BMA). All c-Fos labeled cells were counted throughout the rostrocaudal extent of each nucleus in 240  $\mu\text{m}$  increments (Fig. 3A). Figures were prepared using Adobe Photoshop CS5 (<http://www.adobe.com/>); brightness and contrast were optimized for presentation purposes.

## Transcriptome Sequencing

Brains were collected from a separate cohort of adult male HR/LR rats for transcriptome sequencing (n=10 per phenotype). These animals were not exposed to behavioral testing since that experience could potentially influence mRNA and/or miRNA expression. Total RNA from dorsal raphe and amygdala was isolated using the miRNeasy Kit (Qiagen, Valencia, CA) and stored at  $-80^{\circ}\text{C}$ . mRNA (n=6/phenotype/region) and miRNA (n=4/phenotype/region) samples were shipped on dry ice to HudsonAlpha (Huntsville, AL) for sequencing on the Illumina HiSeq v4 sequencer (Illumina Inc., San Diego, CA).

**mRNA**—Samples for mRNA analysis underwent poly-A selection prior to 50 base pair paired-end sequencing to an average depth of 20 million reads per sample. Samples were aligned using a previously described pipeline (<https://github.com/HudsonAlpha/aRNAPipe>) [54]. Briefly, reads were trimmed with TrimGalore ([http://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore/](http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/)) prior to alignment with STAR [55] using the rn6 reference genome and ensembl genome browser 84 gene transfer format (GTF) file. Raw count tables were obtained directly from the STAR output. The percentage of uniquely aligned reads was consistent across all samples ranging from 86.64% to 89.94%.

**miRNA**—Samples for miRNA underwent Pipin size selection prior to 50 base pair single-end sequencing to an average read depth of 15 million reads per sample. Raw reads were trimmed of adaptor sequences using cutadapt (version 1.3.1-rc1). Only reads greater than 15 base pairs after trimming were kept. Trimmed reads were aligned to *Rattus norvegicus* miRNA hairpin sequences from miRBase (version 19) using bowtie2 (version 2-2.1.0). Only those reads with two or fewer mismatches were kept. Hairpin alignments were queried for overlap with mature miRNA sequences and counted using BEDTools (v2.14.2).

The percentage of reads mapped to mature miRNAs varied considerably across samples and ranged from 13%-44%. The sample with the lowest percentage of mapped miRNA reads was dropped from each group to reduce the biasing of results by low-quality samples. Differential expression was determined using DESeq2 [56] with default settings, but employing likelihood ratio test (LRT) hypothesis testing. mRNA or miRNA species displaying fold change > 1.2 and a FDR corrected (Benjamini and Hochberg) P value <0.1 were considered differentially expressed. Cell-type and network enrichment analyses were performed using lists with a fold change cut off of 1.2 and uncorrected P value <0.05.

**Cell-type Enrichment**—To assess cell-type specificity, we curated lists (Supplementary Tables 7–11) of the top 300 genes expressed more highly in one cell type than all other cell types (neuron, microglia, astrocyte, oligodendrocyte, endothelial) using brain-based RNA expression data [57] ([http://web.stanford.edu/group/barres\\_lab/brain\\_rnaseq.html](http://web.stanford.edu/group/barres_lab/brain_rnaseq.html)) and used two-sided Fisher's exact test (FDR corrected  $p < 0.1$ ) to determine enrichment.

**mRNA-miRNA Network Creation**—Raw read counts were normalized using DESeq2 variance stabilizing transformation prior to network generation. A spearman correlation matrix was generated containing all miRNA-mRNA correlations and miRNAs were clustered using hierarchical clustering with a Euclidean distance metric based on their respective mRNA correlations. Clusters were defined with the R package dynamicTreeCut [58] using default settings. miRNA-mRNA networks were constructed by selecting the miRNA from each cluster and the mRNA transcripts each was most highly correlated with (absolute value of spearman coefficient >0.9), and plotted using the qgraph package with a “spring” layout.

**Gene Ontology Analysis**—Gene lists were created by selecting networks that displayed enrichment for inversely differentially expressed mRNA and miRNA (i.e. if a network was enriched for down-regulated mRNA transcripts and up-regulated miRNA species or vice versa) and filtering for only differentially expressed mRNA transcripts that were highly



correlated with the differentially expressed miRNA of that network. Lists were evaluated for enrichment of Gene Ontology Terms (GO Terms) with Enrichr (<http://amp.pharm.mssm.edu/Enrichr/>) [59].

**miRNA Target Prediction**—Lists of predicted targets of miRNA species of interest were created using TargetScan 7.1 ([http://www.targetscan.org/vert\\_71/](http://www.targetscan.org/vert_71/)) with default settings [60].

## Statistical Analyses

Data analyses for the miRNA sequencing study are described above. All other data were analyzed with Prism 6.0 software (GraphPad Software, San Diego, CA). For defensive burying behavioral data and c-Fos/Tph2 cell counts, two-way ANOVAs were used with phenotype (HR/LR) and condition (“shock” or “no shock”) as fixed factors. Nociception behaviors were analyzed with one-way ANOVAs. Post-hoc comparisons were evaluated by Fisher’s LSD. Significance was set as  $p < 0.05$ .

## RESULTS

### HR rats display proactive coping while LRs are reactive copers in response to electric shock

**Defensive Burying**—We examined HR/LR behavior in the defensive burying test, a classic test of coping style [24]. HRs displayed more proactive behavior (probe burying) than LRs following shock (effects of phenotype [ $F_{1,28} = 57.52$ ,  $p < 0.0001$ ] and shock [ $F_{1,28} = 48.43$ ,  $p < 0.0001$ ], and a phenotype  $\times$  shock interaction [ $F_{1,28} = 39.86$ ,  $p < 0.0001$ ] on time spent burying). Post hoc analysis showed that shock-exposed HR rats buried the probe more than all other experimental groups (Fig. 1A). LRs displayed more reactive coping (freezing) than HRs (effects of phenotype [ $F_{1,28} = 14.69$ ,  $p < 0.001$ ] and shock [ $F_{1,28} = 108.8$ ,  $p < 0.0001$ ], and a phenotype  $\times$  shock interaction [ $F_{1,28} = 17.36$ ,  $p < 0.001$ ] on immobility time). Post hoc analysis revealed that shock-exposed LR rats spent more time immobile than all other experimental groups. Non-shocked LR rats spent more time immobile than both non-shocked and shock-exposed HRs (Fig. 1B), which is likely due to the well-established HR/LR differences in novelty-induced locomotion.

To control for the possibility that coping behavior may be confounded with fear behavior, we measured time spent in close proximity to the shock probe. We expected that rats that fear the probe would avoid the zone near the probe by remaining in the back of the arena, furthest away from the probe. Shock-exposed HR and LR rats spent less time in close proximity of the probe (i.e. front 15cm of the arena) compared to non-shocked HR/LR rats (effect of shock [ $F_{1,28} = 53.19$ ,  $p < 0.0001$ ]. Non-shocked HRs spent more time near the probe than non-shocked LRs (effects of phenotype [ $F_{1,28} = 8.05$ ,  $p < 0.01$ ]; Fig. 1C), although this was likely due to HR/LR differences in novelty response. Most importantly, HR and LR rats exposed to shock spent similar time in close proximity to the probe, indicating that both groups found the shock probe aversive and were fearful of it (phenotype  $\times$  shock interaction [ $F_{1,28} = 6.38$ ,  $p < .05$ ]). We found no HR/LR differences in response to mechanical, heat, and cold nociceptive stimulation, suggesting that their disparate behavioral responses are likely unrelated to nociception (Supplementary Fig 1).

## Electroshock in the defensive burying task elicits distinct neuronal activation in the dorsal raphe and amygdala of proactive coping (HR) vs. reactive coping (LR) rats

To elucidate 5HT circuit differences that may underlie proactive vs. reactive coping in the defensive burying test, we used c-Fos, a marker widely used to map neuronal activation following stress [61, 62]. Our analysis focused on subregions of the dorsal raphe and the amygdala, a critical limbic targets.

**Dorsal Raphe**—When counting the number of Tph2/c-Fos dual-labelled cells (Fig. 2B), we parsed the dorsal raphe into several subregions (i.e. cDRD, DRD, DRV, rDRV, and rDRD) based on cytoarchitecture [15] (Fig. 2A). The cDRD was the only subregion in which there were HR/LR differences in response to shock (phenotype  $\times$  shock interaction [ $F_{1,26} = 6.20$ ,  $p < .05$ ]). HR rats exposed to shock had more Tph2/c-Fos labelled cells than non-shocked HRs and shocked LR rats (Fig. 2C). There was an effect of phenotype on Tph2 labelled cell activation in the DRV [F<sub>1,26</sub> = 10.03,  $p < .01$ ], rDRD [F<sub>1,24</sub> = 6.37,  $p < .05$ ], and rDRV [F<sub>1,24</sub> = 5.46,  $p < .05$ ]. In the DRV, both shock and non-shock exposed HR rats had more activation of Tph2 labelled cells than their LR counterparts (Fig. 2D). In the rDRD, shock exposed HR rats displayed more activation than shock exposed LR rats, although there was no difference between shock and non-shocked HRs (Fig. 2G). And in the rDRV, non-shock exposed HR rats had more activation than non-shocked LR rats (Fig. 2H). No differences were found in the DRD and DRV (Fig. 2E–F).

**Amygdala**—We counted the number of c-Fos positive cells in multiple nuclei of the amygdala: LA, BLA, CeA, MeA, and BMA (Fig. 3A). Shock exposure elicited c-Fos activation in all amygdalar nuclei (effect of shock on the number of c-Fos positive cells in the LA [F<sub>1,26</sub> = 6.15,  $p < 0.05$ ], BLA [F<sub>1,26</sub> = 9.83,  $p < 0.01$ ], CeA [F<sub>1,25</sub> = 7.76,  $p < .05$ ], BMA [F<sub>1,26</sub> = 11.84,  $p < 0.01$ ], and MeA [F<sub>1,26</sub> = 5.97,  $p < 0.05$ ]). There was an effect of phenotype on activation in the BLA [F<sub>1,26</sub> = 5.03,  $p < 0.05$ ], BMA (F<sub>1,26</sub> = 9.384,  $p < 0.01$ ), and MeA (F<sub>1,26</sub> = 4.33,  $p < 0.05$ ), and a nearly significant effect of phenotype in the LA (F<sub>1,26</sub> = 3.68,  $p = 0.066$ ), CeA (F<sub>1,25</sub> = 3.36,  $p < 0.079$ ). Post hoc analysis revealed that shocked LR rats had a greater number of c-Fos positive cells than all other groups in the LA (Fig. 3B), BLA (Fig. 3C), CeA, (Fig. 3D), and BMA (Fig. 3E). There were no individual group differences in the MeA (Fig. 3F). While c-Fos expression was increased in all amygdala nuclei, except the MeA, in response to shock in LR rats, no differences in c-Fos expression between shocked and non-shocked HRs were observed. Since the cDRD was the only dorsal raphe subregion found to have HR/LR differences in response to shock exposure we examined if there were any correlations between cDRD Tph2 cell activation and amygdalar activation. Tph2 cell activation in shock exposed HR rats was negatively correlated with activation in the CeA ( $r^2 = 0.60$ ,  $p < 0.05$ ; Fig. 3G), while there was no such correlation in shock exposed LR rats. There were no significant correlations between cDRD Tph2 cell activation and activation in any of the other amygdala subregions in either rat strain.



## mRNA and microRNA transcriptome sequencing in dorsal raphe and amygdala tissue reveals region specific differences in gene networks between proactive coping (HR) and reactive coping (LR) rats

**mRNA Expression**—Neural circuit activation differences observed in HR/LR rats' response to defensive burying stress may be due to underlying genetic and epigenetic mechanisms. To begin to address this question, we performed next-generation sequencing of mRNA isolated from the dorsal raphe and amygdala of another cohort of adult male HR/LR rats that were naïve to any behavioral testing or stress exposure ( $n=6$ /phenotype/region; Fig. 4A). We identified 42 genes that were up-regulated in the dorsal raphe of LR rats compared to HRs and 15 genes that were down-regulated (Supplementary Table 1). In the amygdala, we found 56 genes up-regulated in LR rats and 50 genes down-regulated relative to HRs (Supplementary Table 2). Additional analysis of both data sets combined identified *a*) 101 genes up-regulated in LR versus HR rats; *b*) 89 genes down-regulated in LR compared to HRs; and *c*) an interaction effect of phenotype  $\times$  region for 2 genes (Supplementary Table 3). Lists of up-regulated and down-regulated genes from each region were examined for cell-type enrichment (Fig. 4B). Neuronal genes were enriched in the lists of genes up-regulated and down-regulated in the amygdala of LR/HR rats. Astrocytic genes were enriched in the genes down-regulated in the dorsal raphe of LR rats. Oligodendrocytic genes were enriched in the genes down-regulated in the amygdala and up-regulated in the dorsal raphe of LR rats. Microglial genes were enriched in the lists of genes up-regulated in the amygdala and dorsal raphe of LR rats, while endothelial genes were enriched in the lists of genes down-regulated in the amygdala and dorsal raphe of LR rats.

**microRNA Expression**—Since the observed HR/LR differences in gene expression could be related to regulation by miRNAs, we also sequenced miRNA isolated from the dorsal raphe and amygdala of HR/LR rats. Using the same differential expression criteria as the mRNA analysis, we identified differentially expressed miRNAs. In the dorsal raphe, only one miRNA was up-regulated (miR-92a-3p) and one down-regulated (miR-206-3p) in LR versus HR rats. In the amygdala, three miRNA (miR-484, miR-194-5p, and miR-6321) were up-regulated and nine miRNA were down-regulated in LR rats compared to HR rats. In the combined analysis, one miRNA was found to be up-regulated (miR-6324) and two down-regulated (miR-3559-5p and miR-378a-3p) in LR rats compared to HRs. Interestingly, 26 miRNAs displayed an interaction effect between region and phenotype (Fig. 4C, Supplementary Table 4).

Region specific mRNA-miRNA networks were created by correlating all mRNA and miRNA expression data and clustering miRNA species with similar correlation profiles. Networks were created for each miRNA cluster by plotting the miRNA species and the mRNA transcripts each was most highly correlated with (absolute value of spearman coefficient  $> .9$ ). In the dorsal raphe, miRNA species were clustered into 21 networks (Fig. 5A). These networks were tested for enrichment of mRNA transcripts and miRNA species differentially expressed in the dorsal raphe of HR/LR rats. Both lists of differentially expressed mRNA showed enrichment in multiple networks (Fig. 5B). Only two networks were enriched for differentially expressed miRNA, networks 1 and 10 (Fig. 5C). Interestingly, these networks were identified as having reciprocal enrichment of mRNA and

miRNA. Network 1 was enriched for miRNA species down-regulated in LR dorsal raphe and enriched for mRNA transcripts up-regulated in LR dorsal raphe. Network 10 was enriched for miRNA species up-regulated in LR dorsal raphe and down-regulated mRNA transcripts. It is possible that there are direct or indirect functional relationships between the differentially expressed miRNAs and mRNAs in these networks. In the amygdala, miRNA species were clustered into 19 networks (Fig. 5D). Again, many of the networks were enriched for differentially expressed mRNA transcripts (Fig. 5E) and fewer were enriched for miRNA species (Fig. 5F). Networks 7 and 10 were enriched for mRNA transcripts down-regulated in the LR amygdala and for miRNA species that were up-regulated, while the inverse relationship was found for networks 5, 15, and 19.

Figure 6 displays parts of the two dorsal raphe networks (Network 1, Fig. 6A; Network 10, Fig. 6B) that include the differentially expressed miRNAs and the mRNA transcripts they were most highly correlated with. Table 1 shows GO Terms that showed enrichment from a list of mRNAs depicted in dorsal raphe Network 1 that were *a)* highly correlated with the expression of the down-regulated miRNAs; and *b)* up-regulated in LR dorsal raphe. Nearly all of these GO Terms related to immune activation and function. The genes *Cd74*, *Cyth4*, *Nckap1l*, and *Rac2*, which were up-regulated in the LR dorsal raphe, were included in the gene lists of multiple GO Terms and are selectively expressed in microglia, one of the cell types with enrichment in genes up-regulated in the LR dorsal raphe. Of these miRNAs: (1) miR-378a-3p was negatively correlated with expression of all four genes; (2) miR-206-3p was negatively correlated with *Cyth4*, *Nckap1l*, and *Rac2*; and (3) miR-3559-5p was negatively correlated with *Cyth4*. Thus, three miRNAs down-regulated in the dorsal raphe of LR (miR-206-3p, miR-3559-5p, and miR-378a-3p) might drive the observed gene expression differences and may be related to microglia function in that region. miRNA target prediction software did not identify any of these genes as being targets of the mentioned miRNAs [60]. However, it is possible that the miRNAs are involved in their regulation through indirect mechanisms, such as repression of a critical activator. It is also possible that the genes are responsible for regulation of the expression of these miRNA.

We created similar models for the miRNA/mRNA networks in the amygdala dataset (Fig 7 and Supplementary Figures 2–4). Notably, Table 2 shows a list of amygdala Network 7 genes that were down-regulated in LR versus HR amygdala and enriched for numerous GO Terms related to histone, nucleosome, and chromatin function and remodeling. Of the genes included within these lists: (1) *Cenpk* was negatively correlated with miR-124-5p; (2) *Cenpq* was negatively correlated with miR-124-5p, miR-146a-5p, miR-539-3p, and miR-7a-1-3p; (3) *Itgb3bp* was negatively correlated with miR-3068-3p and miR-380-5p; and (4) *Mis18a* was negatively correlated with miR-124-5p, miR-539-3p, and miR-7a-1-3p. miR-7a-1-3p is predicted to target *Mis18a* and *Cenpk* [60]. These noted miRNAs may play a critical role in driving HR/LR amygdalar gene expression differences, which may ultimately contribute to HR/LR differences in neural circuit activation and behavior.

## DISCUSSION

Human stress coping style confers risk or resilience for stress-induced psychopathology [6–8] and the HR/LR rat lines, which exhibit individual differences in stress coping, provide a

novel model organism for studying the cellular and molecular basis of coping style. Our group and others previously reported HR/LR differences in behavioral despair [14], aggression [15], and anxiety-like behavior [13]. These differences suggested that HR rats display a proactive coping style while LR rats display a reactive coping style. The present studies support this idea, as HR rats adopted a proactive coping strategy on the defensive burying task, while LR rats adopted a reactive coping strategy. HRs navigated the shock probe stressor by burying the probe with bedding, while LR rats chose to remain immobile throughout the test. Previous work has also shown differential stress vulnerabilities in HR versus LR rats. For instance, LR rats (but not HRs) are vulnerable to CMS, which exacerbates their already high levels of anxiety/depression-like behavior [21], while HRs (but not LR rats) are vulnerable to chronic social defeat (increasing depression-like behavior) [22, 23]. It would be interesting to test if interventions that alter coping style can shift HR/LR stress vulnerability (i.e. if LR rats were made more proactive would they become resilient to CMS and vulnerable to social defeat), but this will require a greater understanding of neural and molecular factors that may be driving proactive (HR) versus reactive (LR) coping.

Our first experiment in the present study sought to identify brain regions that may contribute to HR/LR coping style differences. Based on previous findings of HR/LR 5HT system differences [15, 30, 31], and work showing that 5HT neurotransmission modulates stress coping style [25, 63, 64], we examined neuronal activation within the dorsal raphe following the defensive burying test. 5HT-synthesizing neurons in the raphe nuclei are located along the midline throughout the rostro-caudal extent of the brainstem [65]. The dorsal raphe is a heterogeneous structure comprised of nine subnuclei differentiated through their cytoarchitecture [66]. In the DRV<sub>L</sub>, rDRD and rDRV, we found greater overall 5HT-cell activation in HR vs. LR rats (effect of phenotype), but no effect of shock stress. These rostral raphe groups project to regions important to dopaminergic signaling, including the substantia nigra, nucleus accumbens, and striatum [67–70]. Therefore, the distinct HR/LR activation within the rostral group could be related to their phenotypic differences in response to novelty and locomotion. Interestingly, shock exposure elicited greater activation of 5HT-cells in the HR versus LR only in the cDRD. The cDRD targets several structures of the limbic system including the septum, hippocampus, and the central amygdala [67, 70–74]. While the central amygdala has been considered a minor target of the cDRD, it is possible that HR/LR differences in 5HTergic activation in the cDRD following shock exposure contribute to their distinct coping styles through modulation of this region.

In addition to our analysis of the dorsal raphe, we also examined shock-induced neuronal activation in the amygdala since it is an efferent target of the dorsal raphe and a key region known to regulate stress responsivity. We found that LR rats displayed greater shock-elicited activation in all the amygdalar nuclei examined (the CeA, LA, BLA, and BMA) except the MeA compared to HRs. There were no differences in c-Fos expression between non-shocked HR/LRs, despite observed differences in mobility. Therefore, while it is likely that the increase in activation across the LR amygdala is related to the increase in immobility following shock, it is possible that amygdala activation is not related baseline differences in locomotion between HR/LR rats. Interestingly, we found that activation of 5HT cells in the cDRD was negatively correlated with activation in the CeA in HR, but not LR rats. Our findings of high dorsal raphe activation/low amygdala activation in proactive coping animals

(and the reverse in reactive coping rats) is congruent with the notion that 5HTergic-limbic circuits govern stress coping behaviors [75]. For example, optogenetic stimulation of glutamatergic neurons in the BLA elicits freezing and anxiety-like behavior [76, 77], while stimulating GABAergic parvalbumin cells in the BLA attenuates conditioned freezing [78]. 5HTergic outflow from the dorsal raphe activates GABAergic interneurons in the amygdala and reduces excitatory output [79]. Thus, increased stress-elicited 5HT cell activation in HR rats may lead to increased GABAergic tone in the amygdala and ultimately proactive stress coping. In LRs, reduced stress-induced dorsal raphe activation may cause disinhibition in the amygdala, leading to increased glutamatergic output and the emergence of reactive coping behavior. However, it is important to note that there is significant bidirectional flow of information between dorsal raphe and amygdala. For example, it was recently demonstrated that GABAergic neurons from the CeA disinhibit output neurons in the DRVl/ventrolateral periaqueductal grey causing freezing and defensive behaviors [80]. Thus it is possible that the differences in amygdalar activation caused the dorsal raphe differences. Future work will determine the directionality of the dorsal raphe and amygdala circuit in regards to HR/LR defensive burying behavior.

To begin to interrogate potential molecular pathways that contribute to HR/LR differences in defensive burying behavior and shock-induced neuronal activation in the dorsal raphe and amygdala, our final experiment utilized next-generation sequencing to assess mRNA and miRNA expression in these brain regions. Our analysis identified numerous mRNA and miRNA species that were differentially expressed in HR/LR dorsal raphe and amygdala. One of the challenges of interpreting gene expression data from whole brain tissue samples is determining the cell types responsible for observed differences. Using lists of genes most uniquely expressed in several cell types (neurons, astrocytes, oligodendrocytes, microglia, and endothelial cells) [57] we checked for enrichment of differentially expressed mRNA transcripts. Neuron-specific genes were enriched in both amygdala lists, while genes that were down-regulated in LR dorsal raphe were enriched for astrocyte-specific genes. Microglia-specific genes were enriched in genes up-regulated in both the LR amygdala and dorsal raphe, while endothelial genes were enriched in genes down-regulated in the LR amygdala and dorsal raphe.

In order to determine potential molecular networks contributing to the HR/LR phenotypes, we created miRNA-mRNA networks by correlating and clustering mRNA and miRNA expression data for each brain region. We then tested these networks for enrichment of differentially expressed miRNAs and mRNAs, focusing on networks that showed reciprocal enrichment of differentially expressed miRNAs and mRNAs (i.e. networks that displayed enrichment for miRNAs up-regulated and mRNAs down-regulated in LRs in that particular region, or vice versa). Two networks were identified in the dorsal raphe, with dorsal raphe Network 1 showing enrichment for several GO Terms related to immune activation and function among genes up-regulated in LRs. These genes (*Cd74*, *Rac2*, *Nckap1l*, and *Cyth4*) were also identified as genes most specific to microglia, a cell type that displayed enrichment for genes up-regulated in the LR dorsal raphe. Several miRNAs that were down-regulated in LR dorsal raphe (e.g., miR-206-3p, miR-3559-5p, and miR-378-3p) were found to be negatively correlated with expression of these immune-related genes. While none of these noted miRNAs are predicted to directly target these genes [60], it is possible that the

miRNAs indirectly regulate their expression through repression of an activator or through other epigenetic processes. It is also possible that the relationship is reversed and that these microglia-related genes lead to repression of the miRNA species. Future work will determine the functional relationship between these genes and miRNAs and whether they contribute to LR/HR phenotype. Of note, an allele that disrupts the ability of miR-206 to bind to target genes was shown in a recent GWAS analysis to be protective for risk of developing schizophrenia [81].

In the amygdala, several mRNA/miRNA networks displayed reciprocal enrichment of miRNAs and mRNAs differentially expressed in HR/LR rats, however only amygdala Network 7 showed enrichment for GO Terms. Genes that were down-regulated in LR amygdala (*Cenpk*, *Cenpq*, *Itgb3bp*, and *Mis18a*) were enriched for GO Terms relating to chromatin and nucleosome assembly and remodeling. These genes were negatively correlated with expression of multiple miRNAs: miR-124-5p, miR-146a-5p, miR-3068-3p, miR-380-5p, miR-539-3p, and miR-7a-1-3p. Here, miR-7a-1-3p is predicted to directly target *Mis18a* and *Cenpk* [60], although the relationship between the other genes and miRNAs may be indirect. Again, future work will examine the relationship between these genes and miRNAs to interrogate their potential roles in shaping the HR/LR phenotypes.

While the scope the data presented herein has been entirely descriptive, we can begin to speculate on how differences in microRNA, mRNA, and c-Fos expression mediate behavioral responses in the defensive burying task and stress coping behavior in general. microRNA-mRNA networks likely contribute to overall responsivity and functionality in each region. Inflammation and activation of microglia in the dorsal raphe reduces Tph2 expression and causes serotonergic hypofunction [82, 83]. Thus, we can hypothesize that the unresponsiveness of the LR dorsal raphe to electric shock stress is related to down-regulation of microRNAs miR-206-3p, miR-3559-5p, and/or miR-378-3p, up-regulation of the associated microglia genes *Cd74*, *Rac2*, *Nckap1l*, and *Cyth4*, and increased microglia activation. It would be interesting to test if increasing expression of the microRNAs and/or decreasing expression of the microglia genes results in increased responsiveness to shock in the LR dorsal raphe, and if such interventions would reduce immobility or increase burying behavior in the defensive burying task. Similar hypotheses can be made regarding the other identified microRNA-mRNA networks in each region. For example, in the amygdala network previously discussed differences in microRNA and genes relating to epigenetic regulation were identified. It is possible that these microRNA and genes are responsible for programs that contribute to neural responsiveness in the amygdala subregions. By manipulating the expression of these targets could LR amygdalar c-Fos expression be reduced or HR c-Fos expression increased? Would such effects alter HR/LR stress coping styles? While these questions cannot be answered at this time, future work will use this data to generate and rigorously test novel hypotheses.

In summary, HR and LR rats display proactive and reactive stress coping styles, respectively. Reactive coping in LR rats is associated with decreased 5HT cell activation in the cDRD subregion of the dorsal raphe and increased activation in amygdalar nuclei LA, BLA, CeA, and BMA. Transcriptome analyses of mRNA and miRNA expression in the dorsal raphe and amygdala identified region-specific miRNA-mRNA networks. Several of these networks



were enriched for miRNAs and mRNAs that were differentially expressed in HR and LR rats. Future work will interrogate these networks further and seek to establish a functional link between specific miRNAs, mRNAs, and HR/LR behavior.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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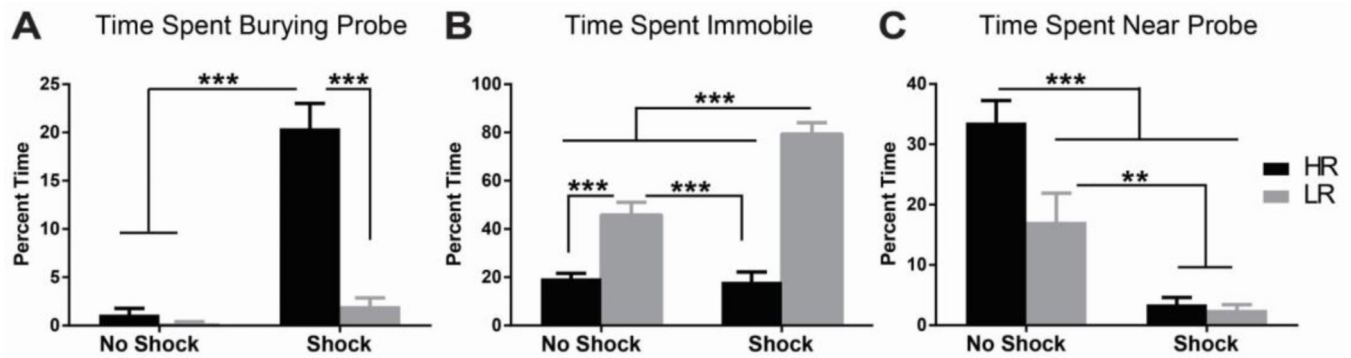
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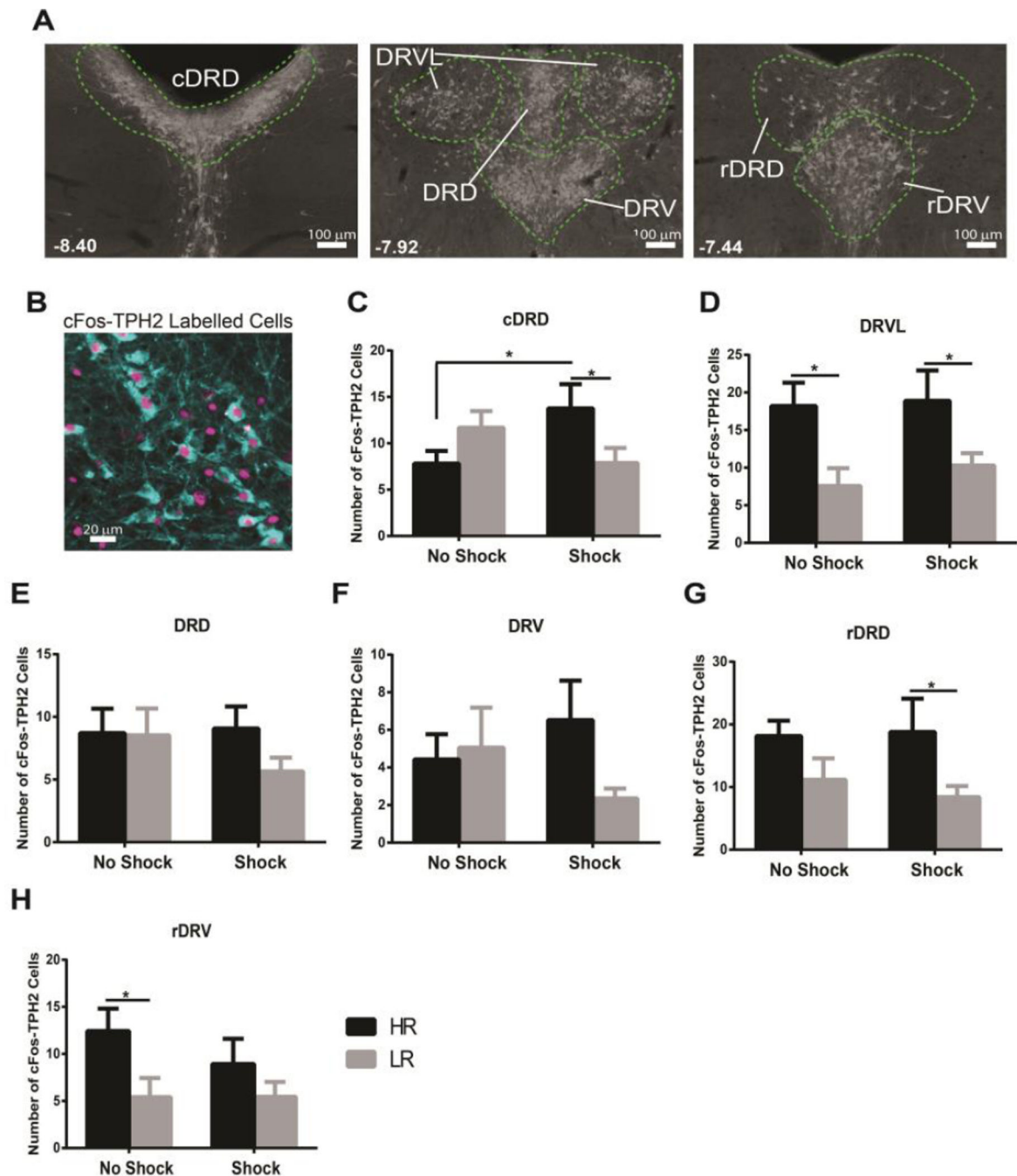
**Highlights**

- HR/LR rats display proactive and reactive coping respectively.
- HR rats display greater shock-induced activation of 5HT-neurons in the cDRD.
- LR rats display greater shock-induced activation across amygdala nuclei.
- Region-specific mRNA-miRNA networks may contribute to HR/LR phenotype.





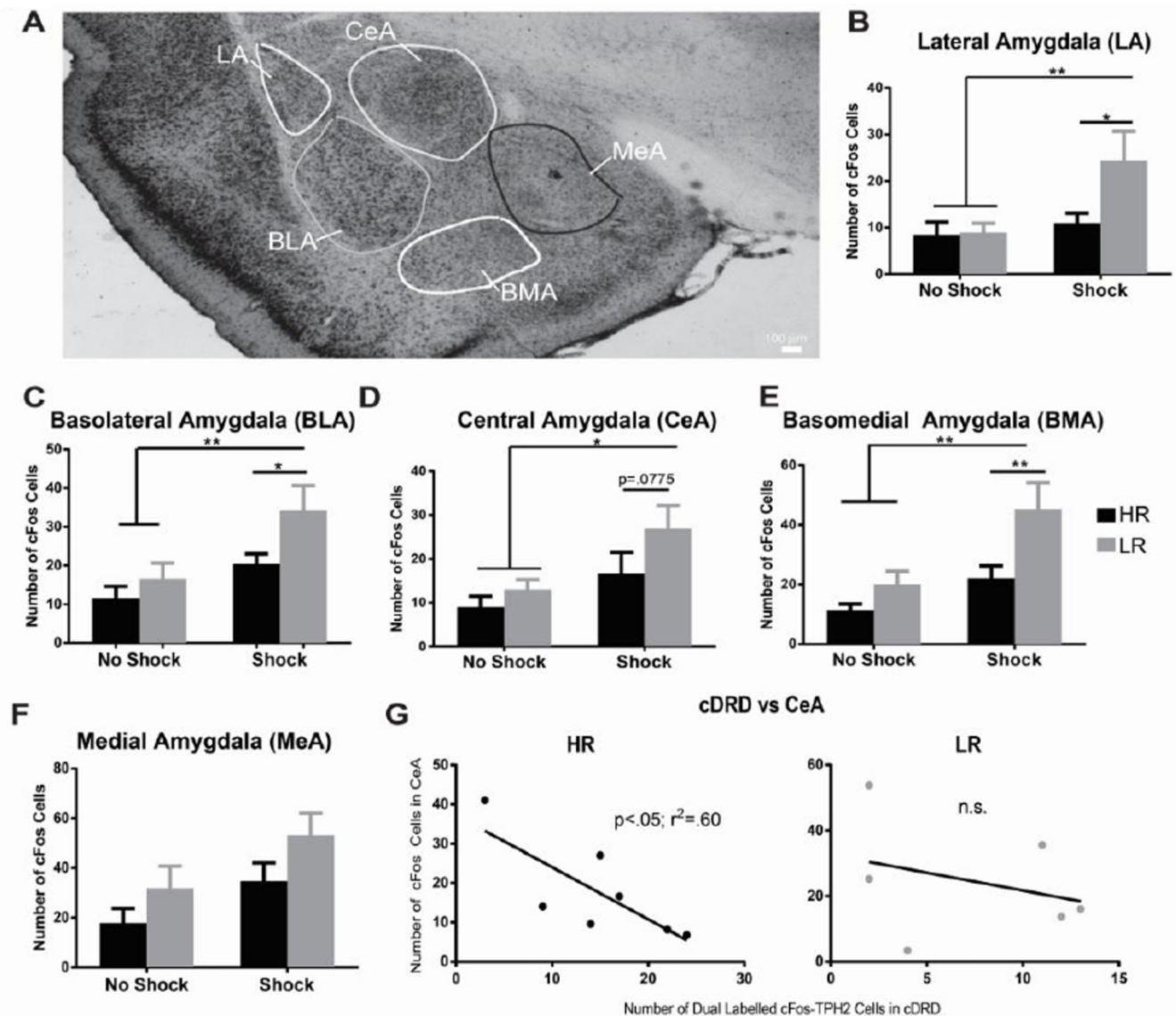
**Figure 1. High novelty responder (HR) and low novelty responding (LR) rats adopt proactive and reactive coping strategies, respectively, on the defensive burying test**  
**(a)** Shocked HR rats spend more time burying the probe than all other groups ( $p < 0.001$ ). **(b)** Shocked LR rats spend more time immobile than all other groups ( $p < 0.001$ ). Non-shocked LR rats spend more time immobile than shocked and non-shocked HRs ( $p < 0.001$ ). **(c)** Non-shocked HR rats spend more time near the probe than all other groups ( $p < 0.001$ ). Non-shocked LR rats spend more time near the probe than shocked HRs and LR rats ( $p < 0.01$ ). \*\* $p < 0.01$ , \*\*\* $p < 0.001$



**Figure 2. HR rats display a greater number of c-Fos positive 5HTergic neurons following shock in the defensive burying test compared to LRs**

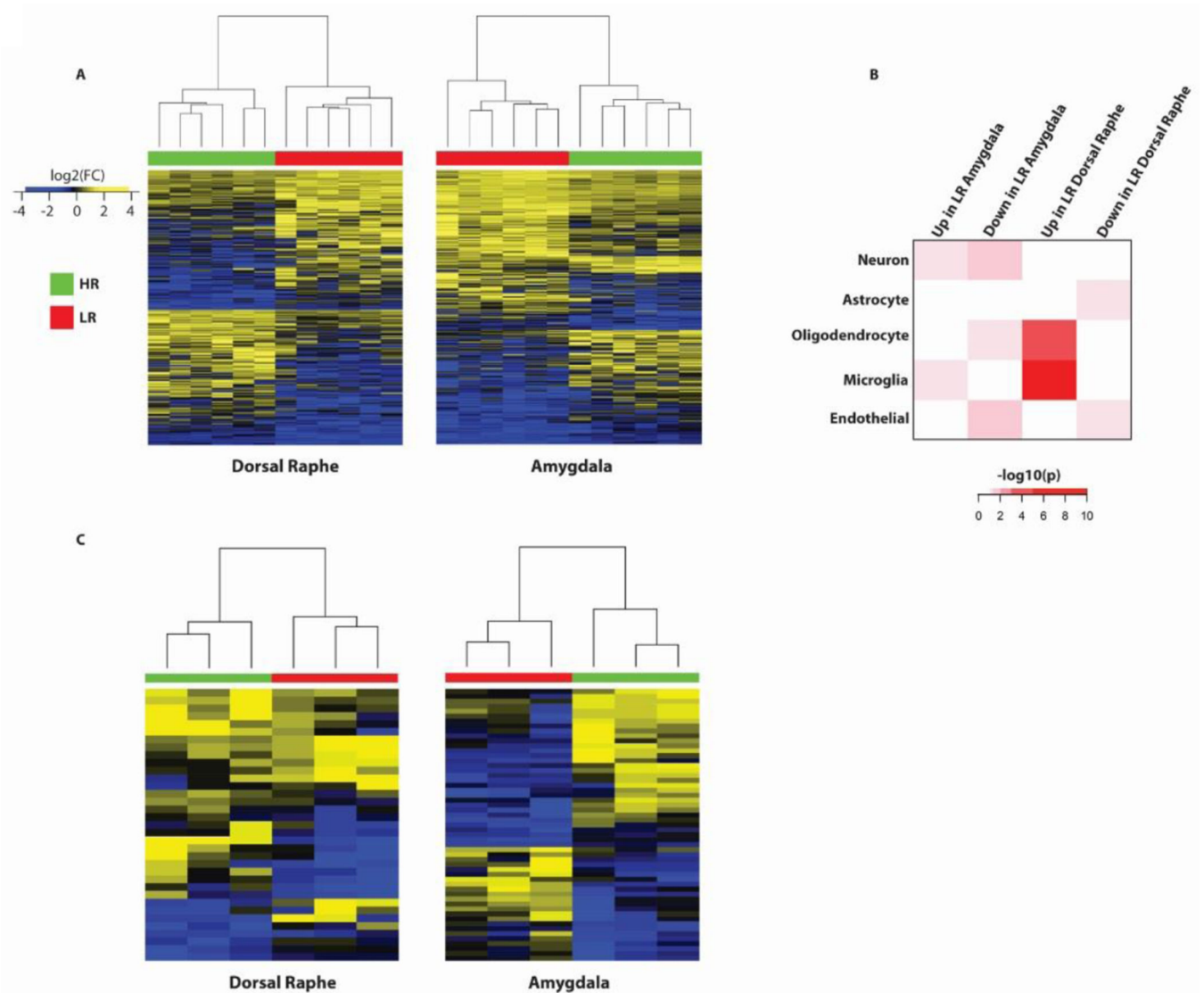
(a) Tph2-immunoreactive neurons in the dorsal raphe. Green dashed line highlights the caudal dorsomedial dorsal raphe (cDRD), dorsomedial dorsal raphe (DRD), ventral dorsal raphe (DRV), ventrolateral dorsal raphe (DRVL), rostral dorsomedial dorsal raphe (rDRD), and rostral ventral dorsal raphe (rDRV). (b) Pseudo-colored Image of Tph2 (cyan) and c-Fos (purple) immunoreactivity. Co-localization of the two signals (arrows) indicates activation of 5HT-containing neurons. (c) In the cDRD, shocked HRs display a greater number of c-Fos/

Tph2 positive cells compared to non-shocked HRs and shocked LRs ( $p<0.05$ ). **(d)** In the DRV, non-shocked and shocked HRs display a greater number of c-Fos/Tph2 positive cells compared to non-shocked and shocked LRs respectively ( $p<0.05$ ). **(e)** In the DRD, there are no differences in the number of c-Fos/Tph2 positive cells between any of the groups. **(f)** In the DRV, there are no differences in the number of c-Fos/Tph2 positive cells between any of the groups. **(g)** In the rDRD, shocked HRs display a greater number of c-Fos/Tph2 positive cells compared to shocked LRs ( $p<0.05$ ). **(h)** In the rDRV, non-shocked HRs display a greater number of c-Fos/Tph2 positive cells compared to non-shocked LRs ( $p<0.05$ ).  
\* $p<0.05$

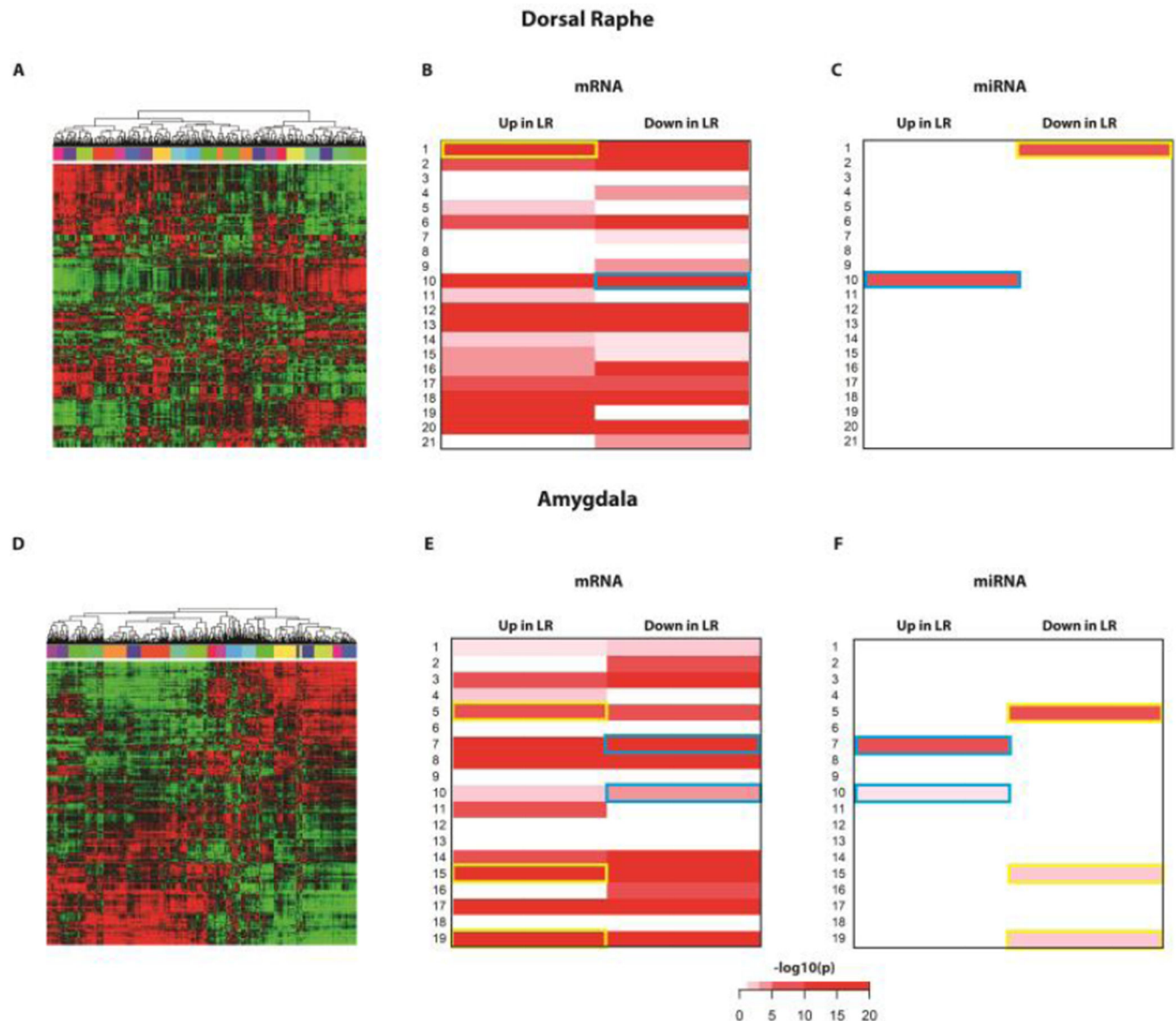


**Figure 3. LR rats display a greater number of c-Fos cells in the amygdala following shock in the defensive burying test compared to HRs**

(a) Regions of the amygdala (neutral red stain). (b) In the lateral amygdala, shocked LR rats display a greater number of c-Fos positive cells than shocked HRs ( $p < 0.05$ ) and non-shocked HRs and LR rats ( $p < 0.01$ ). (c) In the basolateral amygdala, shocked LR rats display a greater number of c-Fos positive cells than shocked HRs ( $p < 0.05$ ) and non-shocked HRs and LR rats ( $p < 0.01$ ). (d) In the central amygdala, shocked LR rats display a greater number of c-Fos positive cells than shocked HRs ( $p = 0.0775$ ) and non-shocked HRs and LR rats ( $p < 0.05$ ). (e) In the basomedial amygdala, shocked LR rats display a greater number of c-Fos positive cells than all other groups ( $p < 0.01$ ). (f) There are no differences in the number of c-Fos positive cells in the medial amygdala. (g) There is a negative correlation between the number of c-Fos/Tph2 positive cells in the cDRD and number of c-Fos positive cells in the central amygdala in HR ( $p < 0.05$ ,  $r^2 = 0.60$ ), but not LR rats. \* $p < 0.05$ ; \*\* $p < 0.01$



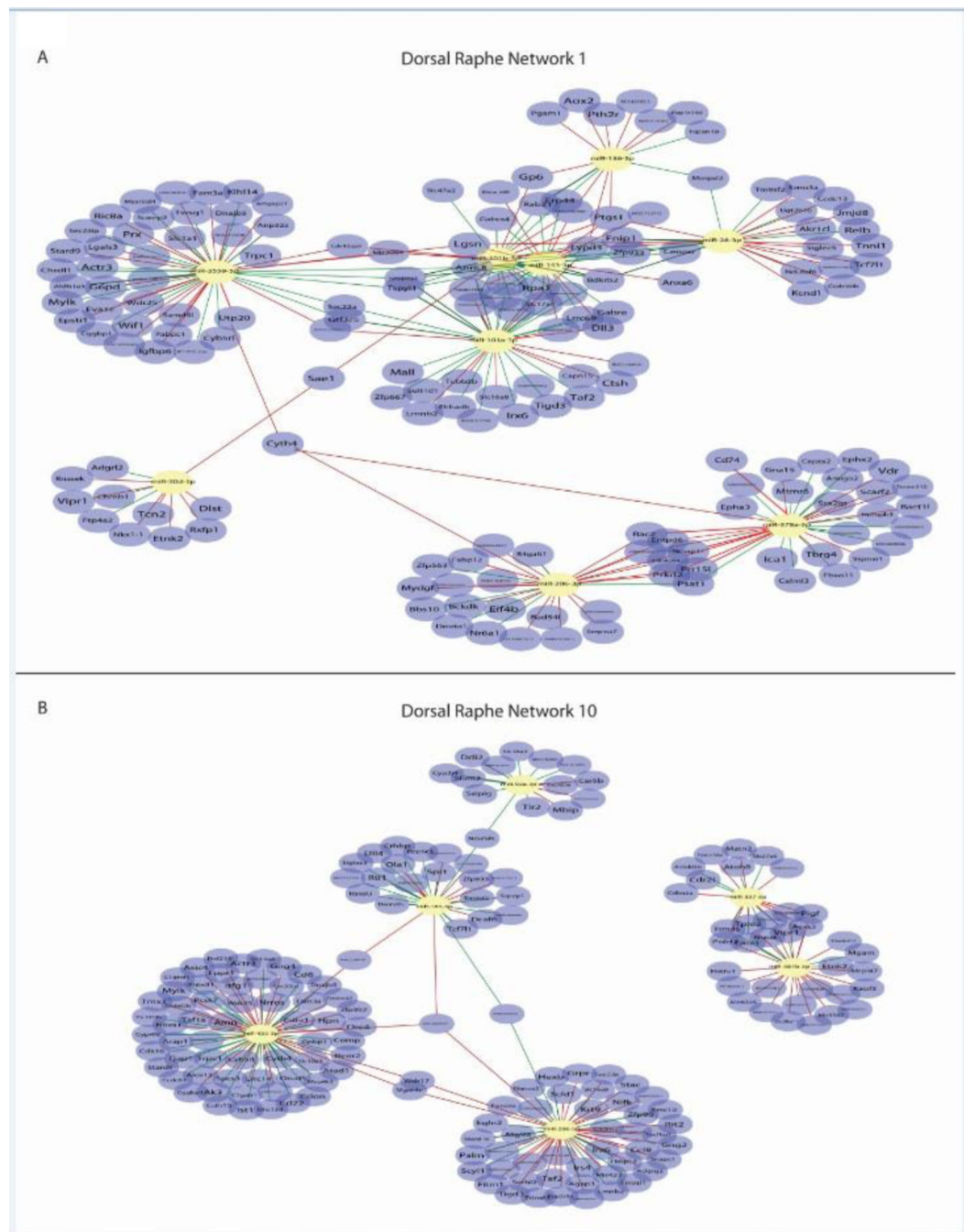
**Figure 4. HR and LR rats display region specific differences in mRNA and miRNA expression**  
**(a)** Heatmaps of mRNA transcripts differentially expressed in HR/LR dorsal raphe and amygdala. **(b)** Heatmap of enrichment for differentially expressed mRNA transcripts in cell-type specific gene lists. **(c)** Heatmaps of miRNA species differentially expressed in HR/LR dorsal raphe and amygdala.



**Figure 5. Region specific mRNA-miRNA networks**

(a) Clustering of mRNA-miRNA networks based on correlations of mRNA-miRNA expression in the dorsal raphe (b) Heatmap of enrichment for differentially expressed mRNA within each network. (c) Heatmap of enrichment for differentially expressed miRNA within each network. (d) Clustering of mRNA-miRNA networks based on correlations of mRNA-miRNA expression in the amygdala (e) Heatmap of enrichment for differentially expressed mRNA within each network. (f) Heatmap of enrichment for differentially expressed miRNA within each network. Yellow outlined boxes represent networks that display enrichment for mRNA transcripts up-regulated and miRNA species down-regulated in LR dorsal raphe or amygdala. Blue outlined boxes represent networks that display enrichment for the inverse relationship.





**Figure 6. Dorsal raphe mRNA-miRNA networks 1 and 10**

(a) Dorsal raphe mRNA-miRNA network 1 was enriched for down-regulated miRNA and up-regulated mRNA in LR dorsal raphe. Yellow nodes represent the differentially expressed miRNA in this network: miR-101a-3p, miR-101b-3p, miR-136-5p, miR-153-3p, miR-206-3p, miR-28-3p, miR-30d-5p, miR-3559-5p, and miR-378a-3p. (b) Dorsal raphe mRNA-miRNA network 10 was enriched for up-regulated miRNA and down-regulated mRNA in LR dorsal raphe. Yellow nodes represent the differentially expressed miRNA in this network: miR-185-3p, miR-296-5p, miR-337-5p, miR-423-3p, miR-487b-3p, and

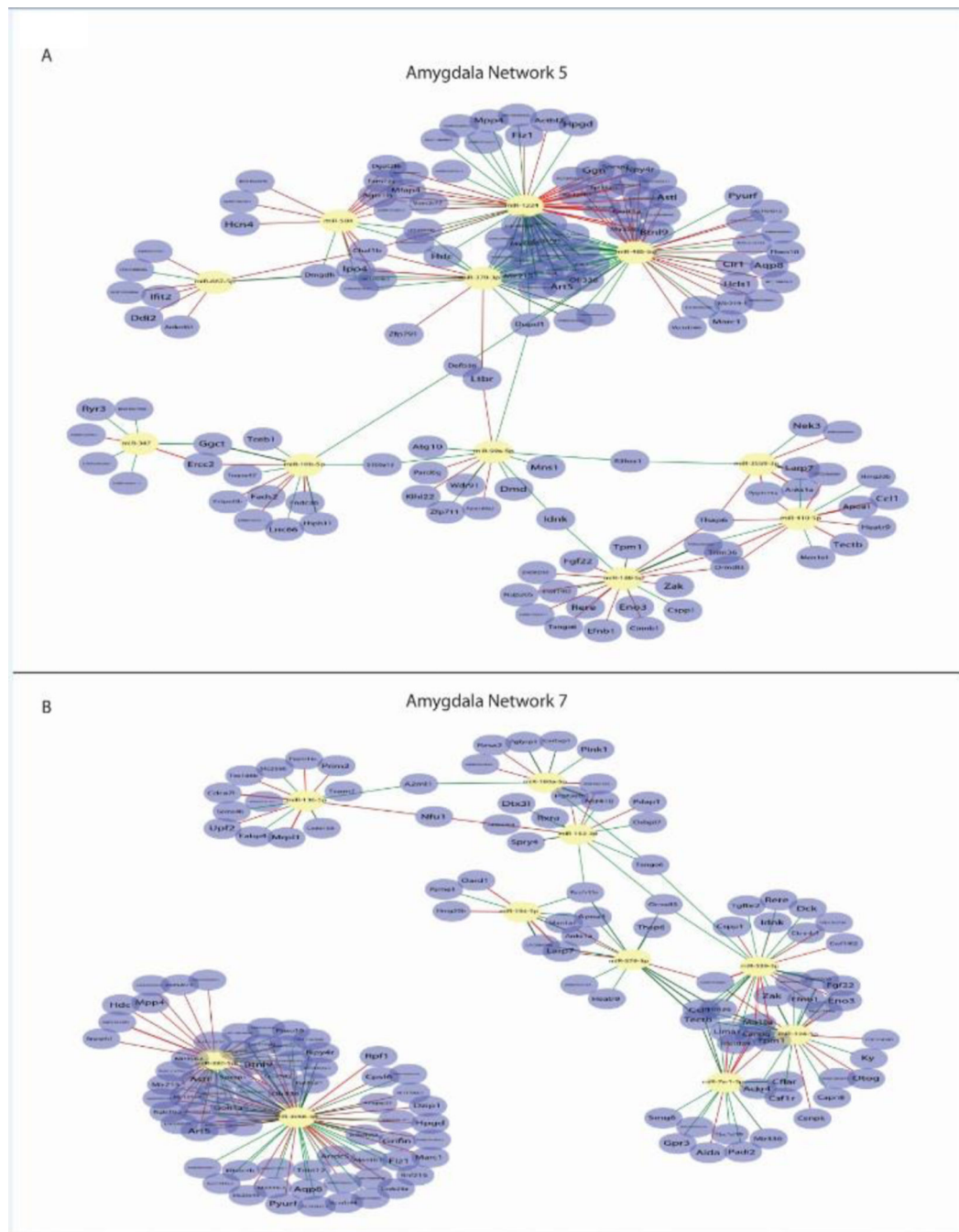
miR-92a-3p. Blue nodes are genes that displayed an absolute correlation  $> 0.9$  with any of the listed miRNAs. Green lines represent positive correlation, red lines negative correlation.

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**Figure 7. Amygdala mRNA-miRNA networks 5 and 7**

(a) Amygdala mRNA-miRNA network 5 was enriched for down-regulated miRNA and up-regulated mRNA in LR amygdala. Yellow nodes represent the differentially expressed miRNA in this network: miR-10b-5p, miR-1224, miR-188-5p, miR-347, miR-3559-5p, miR-370-3p, miR-410-5p, miR-485-5p, miR-504, miR-667-5p, and miR-99a-5p. (b) Amygdala mRNA-miRNA network 7 was enriched for up-regulated miRNA and down-regulated mRNA in LR dorsal raphe. Yellow nodes represent the differentially expressed miRNA in this network: miR-124-5p, miR-136-5p, miR-153-3p, miR-190a-5p, miR-194-5p,

miR-3068-3p, miR-379-3p, miR-380-5p, miR-539-3p, and miR-7a-1-3p. Blue nodes are genes that displayed an absolute correlation  $> 0.9$  with any of the listed miRNAs. Green lines represent positive correlation, red lines negative correlation.

Table 1

Dorsal raphe network 1 GO terms

GO Term	Description	Overlap	P-value	Adjusted P-value	Genes
GO:0032103	positive regulation of response to external stimulus	8/201	1.06E-05	1.61E-02	CD74; DHX58; RAC2; NCKAP1L; PRKD2; FCGR1A; TLR2; VEGFA
GO:0031065	positive regulation of histone deacetylation	3/12	5.51E-05	1.64E-02	SREBF1; PRKD2; VEGFA
GO:1902624	positive regulation of neutrophil migration	3/18	1.58E-04	1.64E-02	CD74; RAC2; NCKAP1L
GO:0090023	positive regulation of neutrophil chemotaxis	3/18	1.58E-04	1.64E-02	CD74; RAC2; NCKAP1L
GO:0051272	positive regulation of cellular component movement	8/296	1.51E-04	1.64E-02	CD74; RAC2; CTSH; PRKD2; ALOX12; NCKAP1L; VEGFA; TLR2
GO:0030335	positive regulation of cell migration	8/280	1.04E-04	1.64E-02	CD74; RAC2; CTSH; NCKAP1L; PRKD2; ALOX12; VEGFA; TLR2
GO:0030155	regulation of cell adhesion	9/336	6.10E-05	1.64E-02	CYTH4; SYK; FES; RAC2; PRKD2; NCKAP1L; ALOX12; FBLN2; VEGFA
GO:0002699	positive regulation of immune effector process	6/133	7.44E-05	1.64E-02	CD74; SYK; FES; DHX58; FCGR1A; TLR2
GO:0002685	regulation of leukocyte migration	5/110	2.96E-04	1.64E-02	CD74; RAC2; NCKAP1L; TLR2; VEGFA
GO:0002250	adaptive immune response	5/91	1.27E-04	1.64E-02	CD74; SYK; CTSH; FCGR1A; VEGFA

Table 2

Amygdala network 7 GO terms

GO Term	Description	Overlap	P-value	Adjusted P-value	Genes
GO:0006336	DNA replication-independent nucleosome assembly	4/33	5.23E-06	9.18E-04	MIS18A; CENPK; CENPQ; ITGB3BP
GO:0043486	histone exchange	4/31	4.16E-06	9.18E-04	MIS18A; CENPK; CENPQ; ITGB3BP
GO:0065004	protein-DNA complex assembly	4/113	4.88E-04	4.76E-02	MIS18A; CENPK; CENPQ; ITGB3BP
GO:0006338	chromatin remodeling	4/118	5.72E-04	4.85E-02	MIS18A; CENPK; CENPQ; ITGB3BP