1 **Differential stress induced c-Fos expression and identification of**

2 **region-specific miRNA-mRNA networks in the dorsal raphe and**

3 **amygdala of high-responder/low-responder rats.**

4 Running title: stress coping and miRNA-RNA networks

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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 of genes related to microglia and immune response (*Cd74*, *Cyth4*, *Nckap1l*, 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

1 **INTRODUCTION**

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

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

like behavior [21], while HRs (but not LRs) 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 LRs 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 or 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]. LRs 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

1 aspects of their disparate behavioral phenotypes, including differences in FST performance [14],
2 social interaction [22, 32], aggression [15, 30], cognition [31], reward-processing [33], and
3 anxiety [34].

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

26

1 **MATERIALS AND METHODS**

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

5 ***Animals***

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

9

10 ***Behavioral Testing***

11 *Defensive Burying Test.* Adult male HR/LR rats (n=16/phenotype) were evaluated in the
12 defensive burying test. Rats underwent two daily 15-min habituation trials in a Plexiglas
13 chamber (45cm × 45cm × 60cm, Noldus, Wageningen, Netherlands) filled with 3 inches of clean
14 bedding. 24-h after the final habituation, rats returned to the chamber, which contained an
15 electric probe. For half the animals, the electric probe was active and they received a single
16 4.0mA shock upon interaction with the probe. Behavior was observed for 15-min following the
17 shock. A blinded experimenter measured time spent immobile (reactive coping); and burying,
18 defined as the rat actively pushing or throwing bedding in the direction of the probe with its front
19 paws or head (proactive coping). The arena was divided into front, middle, and back thirds
20 (15cm × 45cm) and time spent near the probe (front) was measured. The other half of animals
21 were placed in the chamber with an *inactive* shock probe; their behavior was measured for 15-
22 min to serve as a control. Videos of all sessions were recorded with Ethovision® XT 8.0
23 software (Noldus, Wageningen, Netherlands).

24

25 *Nociception Testing*: Nociception testing was performed in order to rule out possible differences
26 in sensing or experiencing of electric shock in HR/LRs. One cohort of animals (n=10 HR; n=8

1 LR) were evaluated on separate days for responsivity to mechanical [49], cold [50], and heat
2 stimuli [51]. And a second cohort of animals (n=10/phenotype) was evaluated on separate days
3 for response to heat stimuli and tail flick [52]. See **Supplemental Materials** for detailed
4 methods.
5
6 ***Neuronal activation***
7 *Immunohistochemistry.* Ninety-min after completing the defensive burying task, shock-exposed
8 and non-shocked control HR/LR rats (n=8/group) were deeply anesthetized with sodium
9 pentobarbital (150 mg/kg i.p.) and then transcardially perfused with ~100 ml of physiological
10 saline followed by ~300 ml of 4% paraformaldehyde. Brains were extracted, post-fixed
11 overnight, cryoprotected in 20% sucrose, and processed for immunohistochemistry as
12 described in previous studies [53]. Briefly, brains were sectioned coronally on a freezing
13 microtome at a thickness of 40 μm. Dual-label immunohistochemistry was performed on free-
14 floating brainstem tissue sections using antibodies against Tph2 (to identify 5HT-containing
15 cells) and c-Fos, an indicator of cellular activation in response to the defensive burying test.
16 Tissue sections were first reacted with rabbit anti-c-Fos antibody (1:3000; Life Sciences, CA#:
17 ABE457 Darmstadt, Germany) for 17-19 h, then incubated 90 min with biotinylated goat anti-
18 rabbit antibody (1:500; Life Sciences, CA#: AP132B). Tissue was then incubated for 90 min in
19 an avidin-biotin-peroxidase complex (1:200; Elite ABC reagent, Cat. No. PK-6100; Vector
20 Laboratories, Burlingame, CA, USA), and finally reacted with 0.01% 3-3'-diaminobenzidine
21 tetrahydrochloride (DAB; Cat. No. D9015, Sigma-Aldrich, St Louis, MO). To identify 5HT-
22 containing cells, sections were next reacted with mouse anti-Tph2 antibody (1:500; Sigma, CA#
23 T0678) for 12-16 h, and then with Cy3-conjugated donkey anti-mouse IgG (1:200; Jackson
24 Laboratories, Bar Harbor, ME; CA#: 715-165-151) for 2 h at room temperature in the dark.
25 Sections were mounted onto glass slides and coverslipped with Permount (Fisher Chemical,
26 Pittsburgh, PA). Forebrain sections underwent the same process but were only single-labelled

1 for c-Fos and visualized with DAB.

2

3 *Image analysis.* DAB- and immunofluorescently-labeled tissue was examined using an Olympus
4 BX-UCB microscope (http:// www.olympusamerica.com/) equipped with a motorized stage
5 (96S100-LE; Ludl Electronic Products, http://www.ludl.com/), fluorophore-specific fluorescent
6 filter sets (excitation and emission spectra: Cy3/TRITC – 531/40), and a cooled mono CCD
7 camera (Orca R2; Hamamtsu, http://hamamatsucameras.com/). Tissue sections through the
8 dorsal raphe and amygdala were examined at 240 μm intervals. In the dorsal raphe, sections
9 were divided into multiple subregions: caudal dorsomedial dorsal raphe (cDRD), ventrolateral
10 wings of the dorsal raphe (DRVL), dorsomedial dorsal raphe (DRD), ventral dorsal raphe (DRV),
11 rostral dorsomedial dorsal raphe (rDRD), and rostral ventral dorsal raphe (rDRV) [15]. The
12 number of cells dual-labeled with c-Fos and Tph2 were counted in each subregion through the
13 extent of the dorsal raphe (**Fig. 2A**). In the amygdala, the following subnuclei were examined:
14 lateral amygdala (LA), basolateral amygdala (BLA), central amygdala (CeA), medial amygdala
15 (MeA), and basomedial amygdala (BMA). All c-Fos labeled cells were counted throughout the
16 rostrocaudal extent of each nucleus in 240 μm increments (**Fig. 3A**). Figures were prepared
17 using Adobe Photoshop CS5 (http://www.adobe.com/); brightness and contrast were optimized
18 for presentation purposes.

19

20 ***Transcriptome Sequencing***

21 Brains were collected from a separate cohort of adult male HR/LR rats for transcriptome
22 sequencing (n=10 per phenotype). These animals were not exposed to behavioral testing since
23 that experience could potentially influence mRNA and/or miRNA expression. Total RNA from
24 dorsal raphe and amygdala was isolated using the miRNeasy Kit (Qiagen, Valencia, CA) and
25 stored at -80ºC. mRNA (n=6/phenotype/region) and miRNA (n=4/phenotype/region) samples
26 were shipped on dry ice to HudsonAlpha (Huntsville, AL) for sequencing on the Illumina HiSeq

1 v4 sequencer (Illumina Inc., San Diego, CA).

2

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

11

12 *miRNA*: Samples for miRNA underwent Pipin size selection prior to 50 base pair single-end
13 sequencing to an average read depth of 15 million reads per sample. Raw reads were trimmed
14 of adaptor sequences using cutadapt (version 1.3.1-rc1). Only reads greater than 15 base pairs
15 after trimming were kept. Trimmed reads were aligned to *Rattus norvegicus* miRNA hairpin
16 sequences from miRBase (version 19) using bowtie2 (version 2-2.1.0). Only those reads with
17 two or fewer mismatches were kept. Hairpin alignments were queried for overlap with mature
18 miRNA sequences and counted using BEDTools (v2.14.2).
19 The percentage of reads mapped to mature miRNAs varied considerably across
20 samples and ranged from 13%-44%. The sample with the lowest percentage of mapped miRNA
21 reads was dropped from each group to reduce the biasing of results by low-quality samples.
22 Differential expression was determined using DeSeq2 [56] with default settings, but employing
23 likelihood ratio test (LRT) hypothesis testing. mRNA or miRNA species displaying fold change >
24 1.2 and a FDR corrected (Benjamini and Hochberg) P value <0.1 were considered differentially
25 expressed. Cell-type and network enrichment analyses were performed using lists with a fold
26 change cut off of 1.2 and uncorrected P value <0.05.

1

2 *Cell-type Enrichment*: To assess cell-type specificity, we curated lists (**Supplementary Tables**
3 **7-11**) of the top 300 genes expressed more highly in one cell type than all other cell types
4 (neuron, microglia, astrocyte, oligodendrocyte, endothelial) using brain-based RNA expression
5 data [57] (http://web.stanford.edu/group/barres_lab/brain_rnaseq.html) and used two-sided
6 Fisher's exact test (FDR corrected $p<0.1$) to determine enrichment.

7

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

16

17 *Gene Ontology Analysis*: Gene lists were created by selecting networks that displayed
18 enrichment for inversely differentially expressed mRNA and miRNA (i.e. if a network was
19 enriched for down-regulated mRNA transcripts and up-regulated miRNA species or vice versa)
20 and filtering for only differentially expressed mRNA transcripts that were highly correlated with
21 the differentially expressed miRNA of that network. Lists were evaluated for enrichment of Gene
22 Ontology Terms (GO Terms) with Enrichr (http://amp.pharm.mssm.edu/Enrichr/) [59].

23

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

26

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 × 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 × 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 × 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, DRVL, DRV, rDRD, and rDRV) based on cytoarchitecture [15] (**Fig. 2A**). The cDRD was the only subregion in which there were HR/LR differences in response to shock (phenotype x 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 LRs (**Fig. 2C**). There was an effect of phenotype on Tph2 labelled cell activation in the DRVL [$F_{1,26}$= 10.03, p<.01], rDRD [$F_{1,24}$= 6.37, p<.05], and rDRV [$F_{1,24}$= 5.46, p<.05]. In the DRVL, 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 HRs rats displayed more

activation than shock exposed LRs, 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 LRs (**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 [$F_{1,26}$= 6.15 p<0.05], BLA [$F_{1,26}$= 9.83, p<0.01], CeA [$F_{1,25}$= 7.76, p<.05], BMA [$F_{1,26}$= 11.84 p<0.01], and MeA [$F_{1,26}$= 5.97, p<0.05]. There was an effect of phenotype on activation in the BLA [$F_{1,26}$= 5.03, p<0.05], BMA ($F_{1,26}$= 9.384, p<0.01), and MeA ($F_{1,26}$= 4.33, p<0.05), and a nearly significant effect of phenotype in the LA ($F_{1,26}$= 3.68, p=0.066), CeA ($F_{1,25}$= 3.36, p<0.079). Post hoc analysis revealed that shocked LRs 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

1 *mRNA Expression:* Neural circuit activation differences observed in HR/LR rats' response to
2 defensive burying stress may be due to underlying genetic and epigenetic mechanisms. To
3 begin to address this question, we performed next-generation sequencing of mRNA isolated
4 from the dorsal raphe and amygdala of another cohort of adult male HR/LR rats that were naïve
5 to any behavioral testing or stress exposure (n=6/phenotype/region; **Fig. 4A**). We identified 42
6 genes that were up-regulated in the dorsal raphe of LR rats compared to HRs and 15 genes that
7 were down-regulated (**Supplementary Table 1**). In the amygdala, we found 56 genes up-
8 regulated in LR rats and 50 genes down-regulated relative to HRs (**Supplementary Table 2**).
9 Additional analysis of both data sets combined identified *a)* 101 genes up-regulated in LR
10 versus HR rats; b*)* 89 genes down-regulated in LRs compared to HRs; and *c)* an interaction
11 effect of phenotype x region for 2 genes (**Supplementary Table 3**). Lists of up-regulated and
12 down-regulated genes from each region were examined for cell-type enrichment (**Fig. 4B**).
13 Neuronal genes were enriched in the lists of genes up-regulated and down-regulated in the
14 amygdala of LR/HR rats. Astrocytic genes were enriched in the genes down-regulated in the
15 dorsal raphe of LRs. Oligodendrocytic genes were enriched in the genes down-regulated in the
16 amygdala and up-regulated in the dorsal raphe of LRs. Microglial genes were enriched in the
17 lists of genes up-regulated in the amygdala and dorsal raphe or LRs, while endothelial genes
18 were enriched in the lists of genes down-regulated in the amygdala and dorsal raphe of LR rats.
19
20 *microRNA Expression*: Since the observed HR/LR differences in gene expression could be
21 related to regulation by miRNAs, we also sequenced miRNA isolated from the dorsal raphe and
22 amygdala of HR/LR rats. Using the same differential expression criteria as the mRNA analysis,
23 we identified differentially expressed miRNAs. In the dorsal raphe, only one miRNA was up-
24 regulated (miR-92a-3p) and one down-regulated (miR-206-3p) in LR versus HR rats. In the
25 amygdala, three miRNA (miR-484, miR-194-5p, and miR-6321) were up-regulated and nine
26 miRNA were down-regulated in LR rats compared to HR rats. In the combined analysis, one

1 miRNA was found to be up-regulated (miR-6324) and two down-regulated (miR-3559-5p and
2 miR-378a-3p) in LRs compared to HRs. Interestingly, 26 miRNAs displayed an interaction effect
3 between region and phenotype (**Fig. 4C**, **Supplementary Table 4**).

4 Region specific mRNA-miRNA networks were created by correlating all mRNA and
5 miRNA expression data and clustering miRNA species with similar correlation profiles. Networks
6 were created for each miRNA cluster by plotting the miRNA species and the mRNA transcripts
7 each was most highly correlated with (absolute value of spearman coefficient > .9). In the dorsal
8 raphe, miRNA species were clustered into 21 networks (**Fig. 5A**). These networks were tested
9 for enrichment of mRNA transcripts and miRNA species differentially expressed in the dorsal
10 raphe of HR/LR rats. Both lists of differentially expressed mRNA showed enrichment in multiple
11 networks (**Fig. 5B**). Only two networks were enriched for differentially expressed miRNA,
12 networks 1 and 10 (**Fig. 5C**). Interestingly, these networks were identified as having reciprocal
13 enrichment of mRNA and miRNA. Network 1 was enriched for miRNA species down-regulated
14 in LR dorsal raphe and enriched for mRNA transcripts up-regulated in LR dorsal raphe. Network
15 10 was enriched for miRNA species up-regulated in LR dorsal raphe and down-regulated mRNA
16 transcripts. It is possible that there are direct or indirect functional relationships between the
17 differentially expressed miRNAs and mRNAs in these networks. In the amygdala, miRNA
18 species were clustered into 19 networks (**Fig. 5D**). Again, many of the networks were enriched
19 for differentially expressed mRNA transcripts (**Fig. 5E**) and fewer were enriched for miRNA
20 species (**Fig. 5F**). Networks 7 and 10 were enriched for mRNA transcripts down-regulated in the
21 LR amygdala and for miRNA species that were up-regulated, while the inverse relationship was
22 found for networks 5, 15, and 19.

23 **Figure 6** displays parts of the two dorsal raphe networks (Network 1, **Fig. 6A**; Network
24 10, **Fig. 6B**) that include the differentially expressed miRNAs and the mRNA transcripts they
25 were most highly correlated with. **Table 1** shows GO Terms that showed enrichment from a list
26 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 LRs (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 LRs 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 LRs adopted a reactive coping strategy. HRs navigated the shock probe stressor by burying the probe with bedding, while LRs 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 LRs) 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 LRs 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 DRVL, 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 amaygdalar 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

1 examine the relationship between these genes and miRNAs to interrogate their potential roles in
2 shaping the HR/LR phenotypes.
3 While the scope the data presented herein has been entirely descriptive, we can begin to
4 speculate on how differences in microRNA, mRNA, and c-Fos expression mediate behavioral
5 responses in the defensive burying task and stress coping behavior in general. microRNA-
6 mRNA networks likely contribute to overall responsivity and functionality in each region.
7 Inflammation and activation of microglia in the dorsal raphe reduces Tph2 expression and
8 causes serotonergic hypofunction [82, 83]. Thus, we can hypothesize that the
9 unresponsiveness of the LR dorsal raphe to electric shock stress is related to down-regulation
10 of microRNAs miR-206-3p, miR-3559-5p, and/or miR-378-3p, up-regulation of the associated
11 microglia genes *Cd74*, *Rac2*, *Nckap1l*, and *Cyth4, and increased microglia activation*. It would
12 be interesting to test if increasing expression of the microRNAs and/or decreasing expression of
13 the microglia genes results in increased responsiveness to shock in the LR dorsal raphe, and if
14 such interventions would reduce immobility or increase burying behavior in the defensive
15 burying task. Similar hypotheses can be made regarding the other identified microRNA-mRNA
16 networks in each region. For example, in the amygdala network previously discussed
17 differences in microRNA and genes relating to epigenetic regulation were identified. It is
18 possible that these microRNA and genes are responsible for programs that contribute to neural
19 responsiveness in the amygdala subregions. By manipulating the expression of these targets
20 could LR amygdalar c-Fos expression be reduced or HR c-Fos expression increased? Would
21 such effects alter HR/LR stress coping styles? While these questions cannot be answered at
22 this time, future work will use this data to generate and rigorously test novel hypotheses.
23 In summary, HR and LR rats display proactive and reactive stress coping styles,
24 respectively. Reactive coping in LR rats is associated with decreased 5HT cell activation in the
25 cDRD subregion of the dorsal raphe and increased activation in amygdalar nuclei LA, BLA,
26 CeA, and BMA. Transcriptome analyses of mRNA and miRNA expression in the dorsal raphe

1 and amygdala identified region-specific miRNA-mRNA networks. Several of these networks
2 were enriched for miRNAs and mRNAs that were differentially expressed in HR and LR rats.
3 Future work will interrogate these networks further and seek to establish a functional link
4 between specific miRNAs, mRNAs, and HR/LR behavior.
5

6 **Accession Numbers**

7 Raw and processed RNA and microRNA-seq expression data are available via the Gene
8 Expression Omnibus database, GEO: GSE86936
9

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15

FIGURE LEGENDS

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 LRs spend more time immobile than all other groups ($p<0.001$). Non-shocked LRs 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 LRs spend more time near the probe than shocked HRs and LRs ($p<0.01$). **$p<0.01$, ***$p<0.001$

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-shcoked HRs and shocked LRs ($p<0.05$). **(d)** In the DRVL, 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 ($p<0.05$). *$p<0.05$

<u>Figure 3.</u> 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 LRs ($p<0.01$). **(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 LRs ($p<0.01$). **(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 LRs ($p<0.05$). **(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. *$p<0.05$; **$p<0.01$

<u>Figure 4.</u> 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.

<u>Figure 5.</u> 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

1 networks based on correlations of mRNA-miRNA expression in the amygdala **(b)** Heatmap of
2 enrichment for differentially expressed mRNA within each network. **(c)** Heatmap of enrichment
3 for differentially expressed miRNA within each network. Yellow outlined boxes represent
4 networks that display enrichment for mRNA transcripts up-regulated and miRNA species down-
5 regulated in LR dorsal raphe or amygdala. Blue outlined boxes represent networks that display
6 enrichment for the inverse relationship.

7

8 **Figure 6. Dorsal raphe mRNA-miRNA networks 1 and 10. (a)** Dorsal raphe mRNA-miRNA
9 network 1 was enriched for down-regulated miRNA and up-regulated mRNA in LR dorsal raphe.
10 Yellow nodes represent the differentially expressed miRNA in this network: miR-101a-3p, miR-
11 101b-3p, miR-136-5p, miR-153-3p, miR-206-3p, miR-28-3p, miR-30d-5p, miR-3559-5p, and
12 miR-378a-3p. **(b)** Dorsal raphe mRNA-miRNA network 10 was enriched for up-regulated miRNA
13 and down-regulated mRNA in LR dorsal raphe. Yellow nodes represent the differentially
14 expressed miRNA in this network: miR-185-3p, miR-296-5p, miR-337-5p, miR-423-3p, miR-
15 487b-3p, and miR-92a-3p. Blue nodes are genes that displayed an absolute correlation > 0.9
16 with any of the listed miRNAs. Green lines represent positive correlation, red lines negative
17 correlation.

18

19 **Figure 7. Amygdala mRNA-miRNA networks 5 and 7. (a)** Amygdala mRNA-miRNA network 5
20 was enriched for down-regulated miRNA and up-regulated mRNA in LR amygdala. Yellow
21 nodes represent the differentially expressed miRNA in this network: miR-10b-5p, miR-1224,
22 miR-188-5p, miR-347, miR-3559-5p, miR-370-3p, miR-410-5p, miR-485-5p, miR-504, miR-667-
23 5p, and miR-99a-5p. **(b)** Amygdala mRNA-miRNA network 7 was enriched for up-regulated
24 miRNA and down-regulated mRNA in LR dorsal raphe. Yellow nodes represent the differentially
25 expressed miRNA in this network: miR-124-5p, miR-136-5p, miR-153-3p, miR-190a-5p, miR-

1 194-5p, miR-3068-3p, miR-379-3p miR-380-5p, miR-539-3p, and miR-7a-1-3p. Blue nodes are
2 genes that displayed an absolute correlation > 0.9 with any of the listed miRNAs. Green lines
3 represent positive correlation, red lines negative correlation.
4
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References

[1] C. Faravelli, C. Lo Sauro, L. Lelli, F. Pietrini, L. Lazzeretti, L. Godini, L. Benni, G. Fioravanti, G.A. Talamba, G. Castellini, V. Ricca, The role of life events and HPA axis in anxiety disorders: a review, Current pharmaceutical design 18(35) (2012) 5663-74.
[2] A. Caspi, K. Sugden, T.E. Moffitt, A. Taylor, I.W. Craig, H. Harrington, J. McClay, J. Mill, J. Martin, A. Braithwaite, R. Poulton, Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene, Science 301(5631) (2003) 386-9.
[3] I.T. Petersen, J.E. Bates, J.A. Goodnight, K.A. Dodge, J.E. Lansford, G.S. Pettit, S.J. Latendresse, D.M. Dick, Interaction between serotonin transporter polymorphism (5-HTTLPR) and stressful life events in adolescents' trajectories of anxious/depressed symptoms, Developmental psychology 48(5) (2012) 1463-75.
[4] E. Leppink, B.L. Odlaug, K. Lust, G. Christenson, K. Derbyshire, J.E. Grant, Losing control: assaultive behavior as a predictor of impulse control disorders in young adults, Comprehensive psychiatry 55(8) (2014) 1831-6.
[5] G.P. Lee, C.L. Storr, N.S. Ialongo, S.S. Martins, Association between adverse life events and addictive behaviors among male and female adolescents, The American journal on addictions / American Academy of Psychiatrists in Alcoholism and Addictions 21(6) (2012) 516-23.
[6] J.K. Connor-Smith, B.E. Compas, M.E. Wadsworth, A.H. Thomsen, H. Saltzman, Responses to stress in adolescence: measurement of coping and involuntary stress responses, Journal of consulting and clinical psychology 68(6) (2000) 976-92.
[7] B.E. Compas, J.E. Champion, R. Forehand, D.A. Cole, K.L. Reeslund, J. Fear, E.J. Hardcastle, G. Keller, A. Rakow, E. Garai, M.J. Merchant, L. Roberts, Coping and parenting: Mediators of 12-month outcomes of a family group cognitive-behavioral preventive intervention with families of depressed parents, Journal of consulting and clinical psychology 78(5) (2010) 623-34.
[8] G. Creasy, N. Mittis, S. Cantanzaro, Associations among daily hassles, coping, and behavior problems in nonreferred kindergarteners, Journal of Clinical Child Psychology 24 (1995) 311-319.
[9] J.M. Koolhaas, S.M. Korte, S.F. De Boer, B.J. Van Der Vegt, C.G. Van Reenen, H. Hopster, I.C. De Jong, M.A. Ruis, H.J. Blokhuis, Coping styles in animals: current status in behavior and stress-physiology, Neuroscience and biobehavioral reviews 23(7) (1999) 925-35.
[10] M. O'Brien, G. Margolin, R.S. John, Relation among marital conflict, child coping, and child adjustment, Journal of Clinical Child Psychology 24 (1995) 346-361.
[11] L.S. Walker, C.A. Smith, J. Garber, D.A. Van Slyke, Development and validation of the Pain Response Inventory for Children, Psychological Assessment 9 (1997) 392-405.
[12] J.P. Dunbar, L. McKee, A. Rakow, K.H. Watson, R. Forehand, B.E. Compas, Coping, Negative Cognitive Style and Depressive Symptoms in Children of Depressed Parents, Cognitive therapy and research 37(1) (2013).
[13] J.D. Stead, S. Clinton, C. Neal, J. Schneider, A. Jama, S. Miller, D.M. Vazquez, S.J. Watson, H. Akil, Selective breeding for divergence in novelty-seeking traits: heritability and enrichment in spontaneous anxiety-related behaviors, Behavior genetics 36(5) (2006) 697-712.
[14] M.E. Glover, P.C. Pugh, N.L. Jackson, J.L. Cohen, A.D. Fant, H. Akil, S.M. Clinton, Early-life exposure to the SSRI paroxetine exacerbates depression-like behavior in anxiety/depression-prone rats, Neuroscience 284 (2015) 775-97.
[15] I.A. Kerman, S.M. Clinton, T.A. Bedrosian, A.D. Abraham, D.T. Rosenthal, H. Akil, S.J. Watson, High novelty-seeking predicts aggression and gene expression differences within defined serotonergic cell groups, Brain Research 1419 (2011) 34-45.
[16] S. Diaz-Moran, M. Palencia, C. Mont-Cardona, T. Canete, G. Blazquez, E. Martinez-Membrives, R. Lopez-Aumatell, A. Tobena, A. Fernandez-Teruel, Coping style and stress hormone responses in

genetically heterogeneous rats: comparison with the Roman rat strains, Behavioural brain research 228(1) (2012) 203-10.
[17] E. Frank, P. Salchner, J.M. Aldag, N. Salome, N. Singewald, R. Landgraf, A. Wigger, Genetic predisposition to anxiety-related behavior determines coping style, neuroendocrine responses, and neuronal activation during social defeat, Behavioral neuroscience 120(1) (2006) 60-71.
[18] N. Salome, R. Landgraf, O. Viltart, Confinement to the open arm of the elevated-plus maze as anxiety paradigm: behavioral validation, Behavioral neuroscience 120(3) (2006) 719-23.
[19] A.H. Veenema, I.D. Neumann, Neurobiological mechanisms of aggression and stress coping: a comparative study in mouse and rat selection lines, Brain, behavior and evolution 70(4) (2007) 274-85.
[20] Y.C. Yen, E. Anderzhanova, M. Bunck, J. Schuller, R. Landgraf, C.T. Wotjak, Co-segregation of hyperactivity, active coping styles, and cognitive dysfunction in mice selectively bred for low levels of anxiety, Frontiers in behavioral neuroscience 7 (2013) 103.
[21] K.A. Stedenfeld, S.M. Clinton, I.A. Kerman, H. Akil, S.J. Watson, A.F. Sved, Novelty-seeking behavior predicts vulnerability in a rodent model of depression, Physiology & behavior 103(2) (2011) 210-6.
[22] N. Calvo, M. Cecchi, M. Kabbaj, S.J. Watson, H. Akil, Differential effects of social defeat in rats with high and low locomotor response to novelty, Neuroscience 183 (2011) 81-9.
[23] F. Duclot, F. Hollis, M.J. Darcy, M. Kabbaj, Individual differences in novelty-seeking behavior in rats as a model for psychosocial stress-related mood disorders, Physiology & behavior 104(2) (2011) 296-305.
[24] S.F. De Boer, J.M. Koolhaas, Defensive burying in rodents: ethology, neurobiology and psychopharmacology, European journal of pharmacology 463(1-3) (2003) 145-61.
[25] K.L. Gardner, K.V. Thrivikraman, S.L. Lightman, P.M. Plotsky, C.A. Lowry, Early life experience alters behavior during social defeat: focus on serotonergic systems, Neuroscience 136(1) (2005) 181-91.
[26] E.D. Paul, M.W. Hale, J.L. Lukkes, M.J. Valentine, D.M. Sarchet, C.A. Lowry, Repeated social defeat increases reactive emotional coping behavior and alters functional responses in serotonergic neurons in the rat dorsal raphe nucleus, Physiology & behavior 104(2) (2011) 272-82.
[27] M.K. Lobo, S. Zaman, D.M. Damez-Werno, J.W. Koo, R.C. Bagot, J.A. DiNieri, A. Nugent, E. Finkel, D. Chaudhury, R. Chandra, E. Riberio, J. Rabkin, E. Mouzon, R. Cachope, J.F. Cheer, M.H. Han, D.M. Dietz, D.W. Self, Y.L. Hurd, V. Vialou, E.J. Nestler, DeltaFosB induction in striatal medium spiny neuron subtypes in response to chronic pharmacological, emotional, and optogenetic stimuli, The Journal of neuroscience : the official journal of the Society for Neuroscience 33(47) (2013) 18381-95.
[28] A. Adhikari, T.N. Lerner, J. Finkelstein, S. Pak, J.H. Jennings, T.J. Davidson, E. Ferenczi, L.A. Gunaydin, J.J. Mirzabekov, L. Ye, S.Y. Kim, A. Lei, K. Deisseroth, Basomedial amygdala mediates top-down control of anxiety and fear, Nature 527(7577) (2015) 179-85.
[29] H. Nam, I.A. Kerman, A2 noradrenergic neurons regulate forced swim test immobility, Physiology & behavior 165 (2016) 339-49.
[30] S.M. Clinton, I.A. Kerman, H.R. Orr, T.A. Bedrosian, A.D. Abraham, D.N. Simpson, S.J. Watson, H. Akil, Pattern of forebrain activation in high novelty-seeking rats following aggressive encounter, Brain Res 1422 (2011) 20-31.
[31] S.J. Ballaz, H. Akil, S.J. Watson, Analysis of 5-HT6 and 5-HT7 receptor gene expression in rats showing differences in novelty-seeking behavior, Neuroscience 147(2) (2007) 428-38.
[32] J.A. Cummings, S.M. Clinton, A.N. Perry, H. Akil, J.B. Becker, Male rats that differ in novelty exploration demonstrate distinct patterns of sexual behavior, Behavioral neuroscience 127(1) (2013) 47-58.
[33] N. Capriles, S. Watson, Jr., H. Akil, Individual differences in the improvement of cocaine-induced place preference response by the 5-HT2C receptor antagonist SB242084 in rats, Psychopharmacology 220(4) (2012) 731-40.

[34] M.M. Verheij, J.V. Veenvliet, T. Groot Kormelink, M. Steenhof, A.R. Cools, Individual differences in the sensitivity to serotonergic drugs: a pharmacobehavioural approach using rats selected on the basis of their response to novelty, Psychopharmacology 205(3) (2009) 441-55.
[35] K.S. Kosik, The neuronal microRNA system, Nature reviews. Neuroscience 7(12) (2006) 911-20.
[36] D. O'Carroll, A. Schaefer, General principals of miRNA biogenesis and regulation in the brain, Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 38(1) (2013) 39-54.
[37] N.R. Smalheiser, G. Lugli, H.S. Rizavi, V.I. Torvik, G. Turecki, Y. Dwivedi, MicroRNA expression is down-regulated and reorganized in prefrontal cortex of depressed suicide subjects, PloS one 7(3) (2012) e33201.
[38] R.M. O'Connor, S. Grenham, T.G. Dinan, J.F. Cryan, microRNAs as novel antidepressant targets: converging effects of ketamine and electroconvulsive shock therapy in the rat hippocampus, The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 16(8) (2013) 1885-92.
[39] C. Dias, J. Feng, H. Sun, N.Y. Shao, M.S. Mazei-Robison, D. Damez-Werno, K. Scobie, R. Bagot, B. LaBonte, E. Ribeiro, X. Liu, P. Kennedy, V. Vialou, D. Ferguson, C. Pena, E.S. Calipari, J.W. Koo, E. Mouzon, S. Ghose, C. Tamminga, R. Neve, L. Shen, E.J. Nestler, beta-catenin mediates stress resilience through Dicer1/microRNA regulation, Nature 516(7529) (2014) 51-5.
[40] J. Xu, C.X. Li, Y.S. Li, J.Y. Lv, Y. Ma, T.T. Shao, L.D. Xu, Y.Y. Wang, L. Du, Y.P. Zhang, W. Jiang, C.Q. Li, Y. Xiao, X. Li, MiRNA-miRNA synergistic network: construction via co-regulating functional modules and disease miRNA topological features, Nucleic acids research 39(3) (2011) 825-36.
[41] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116(2) (2004) 281-97.
[42] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell 120(1) (2005) 15-20.
[43] A. Rinaldi, S. Vincenti, F. De Vito, I. Bozzoni, A. Oliverio, C. Presutti, P. Fragapane, A. Mele, Stress induces region specific alterations in microRNAs expression in mice, Behavioural brain research 208(1) (2010) 265-9.
[44] A. Meerson, L. Cacheaux, K.A. Goosens, R.M. Sapolsky, H. Soreq, D. Kaufer, Changes in brain MicroRNAs contribute to cholinergic stress reactions, Journal of molecular neuroscience : MN 40(1-2) (2010) 47-55.
[45] N.R. Smalheiser, G. Lugli, H.S. Rizavi, H. Zhang, V.I. Torvik, G.N. Pandey, J.M. Davis, Y. Dwivedi, MicroRNA expression in rat brain exposed to repeated inescapable shock: differential alterations in learned helplessness vs. non-learned helplessness, The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 14(10) (2011) 1315-25.
[46] J. Jin, S.N. Kim, X. Liu, H. Zhang, C. Zhang, J.S. Seo, Y. Kim, T. Sun, miR-17-92 Cluster Regulates Adult Hippocampal Neurogenesis, Anxiety, and Depression, Cell reports 16(6) (2016) 1653-63.
[47] D. Andolina, M. Di Segni, E. Bisicchia, F. D'Alessandro, V. Cestari, A. Ventura, C. Concepcion, S. Puglisi-Allegra, R. Ventura, Effects of lack of microRNA-34 on the neural circuitry underlying the stress response and anxiety, Neuropharmacology 107 (2016) 305-16.
[48] C.R. McCoy, S.R. Golf, M. Melendez-Ferro, E. Perez-Costas, M.E. Glover, N.L. Jackson, S.A. Stringfellow, P.C. Pugh, A.D. Fant, S.M. Clinton, Altered metabolic activity in the developing brain of rats predisposed to high versus low depression-like behavior, Neuroscience 324 (2016) 469-84.
[49] R.B. Griggs, R.R. Donahue, J. Morgenweck, P.M. Grace, A. Sutton, L.R. Watkins, B.K. Taylor, Pioglitazone rapidly reduces neuropathic pain through astrocyte and nongenomic PPARgamma mechanisms, Pain 156(3) (2015) 469-82.
[50] E.J. Rahn, L. Deng, G.A. Thakur, K. Vemuri, A.M. Zvonok, Y.Y. Lai, A. Makriyannis, A.G. Hohmann, Prophylactic cannabinoid administration blocks the development of paclitaxel-induced neuropathic

1 nociception during analgesic treatment and following cessation of drug delivery, Molecular pain 10
2 (2014) 27.
3 [51] K. Hargreaves, R. Dubner, F. Brown, C. Flores, J. Joris, A new and sensitive method for measuring
4 thermal nociception in cutaneous hyperalgesia, Pain 32(1) (1988) 77-88.
5 [52] F.E. D'Amour, D.L. Smith, A method for determining loss of pain sensation, The journal of
6 pharmacology and experimental therapeutics 72 (1941) 74-9.
7 [53] I.A. Kerman, C. Shabrang, L. Taylor, H. Akil, S.J. Watson, Relationship of presympathetic-premotor
8 neurons to the serotonergic transmitter system in the rat brainstem, The Journal of comparative
9 neurology 499(6) (2006) 882-96.
10 [54] A. Alonso, B.N. Lasseigne, K. Williams, J. Nielsen, R.C. Ramaker, A.A. Hardigan, B. Johnston, B.S.
11 Roberts, S.J. Cooper, S. Marsal, R.M. Myers, aRNApipe: A balanced, efficient and distributed pipeline for
12 processing RNA-seq data in high performance computing environments, bioRxiv (2016).
13 [55] A. Dobin, C.A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, T.R.
14 Gingeras, STAR: ultrafast universal RNA-seq aligner, Bioinformatics 29(1) (2013) 15-21.
15 [56] M.I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq
16 data with DESeq2, Genome biology 15(12) (2014) 550.
17 [57] Y. Zhang, K. Chen, S.A. Sloan, M.L. Bennett, A.R. Scholze, S. O'Keeffe, H.P. Phatnani, P. Guarnieri, C.
18 Caneda, N. Ruderisch, S. Deng, S.A. Liddelow, C. Zhang, R. Daneman, T. Maniatis, B.A. Barres, J.Q. Wu,
19 An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the
20 cerebral cortex, The Journal of neuroscience : the official journal of the Society for Neuroscience 34(36)
21 (2014) 11929-47.
22 [58] P. Langfelder, B. Zhang, S. Horvath, Defining clusters from a hierarchical cluster tree: the Dynamic
23 Tree Cut package for R, Bioinformatics 24(5) (2008) 719-20.
24 [59] E.Y. Chen, C.M. Tan, Y. Kou, Q. Duan, Z. Wang, G.V. Meirelles, N.R. Clark, A. Ma'ayan, Enrichr:
25 interactive and collaborative HTML5 gene list enrichment analysis tool, BMC bioinformatics 14 (2013)
26 128.
27 [60] V. Agarwal, G.W. Bell, J.W. Nam, D.P. Bartel, Predicting effective microRNA target sites in
28 mammalian mRNAs, eLife 4 (2015).
29 [61] R.K. Chan, E.R. Brown, A. Ericsson, K.J. Kovacs, P.E. Sawchenko, A comparison of two immediate-
30 early genes, c-fos and NGFI-B, as markers for functional activation in stress-related neuroendocrine
31 circuitry, The Journal of neuroscience : the official journal of the Society for Neuroscience 13(12) (1993)
32 5126-38.
33 [62] W.E. Cullinan, J.P. Herman, D.F. Battaglia, H. Akil, S.J. Watson, Pattern and time course of
34 immediate early gene expression in rat brain following acute stress, Neuroscience 64(2) (1995) 477-505.
35 [63] K.K. Chung, M. Martinez, J. Herbert, c-fos expression, behavioural, endocrine and autonomic
36 responses to acute social stress in male rats after chronic restraint: modulation by serotonin,
37 Neuroscience 95(2) (2000) 453-63.
38 [64] C.A. Lowry, P.L. Johnson, A. Hay-Schmidt, J. Mikkelsen, A. Shekhar, Modulation of anxiety circuits by
39 serotonergic systems, Stress 8(4) (2005) 233-46.
40 [65] B.L. Jacobs, E.C. Azmitia, Structure and function of the brain serotonin system, Physiological reviews
41 72(1) (1992) 165-229.
42 [66] M.W. Hale, C.A. Lowry, Functional topography of midbrain and pontine serotonergic systems:
43 implications for synaptic regulation of serotonergic circuits, Psychopharmacology 213(2-3) (2011) 243-
44 64.
45 [67] K.G. Commons, Two Major Network Domains within the Dorsal Raphe Nucleus, The Journal of
46 comparative neurology (2015).

[68] D. van der Kooy, T. Hattori, Dorsal raphe cells with collateral projections to the caudate-putamen and substantia nigra: a fluorescent retrograde double labeling study in the rat, Brain Res 186(1) (1980) 1-7.
[69] T.R. Stratford, D. Wirtshafter, Ascending dopaminergic projections from the dorsal raphe nucleus in the rat, Brain Res 511(1) (1990) 173-6.
[70] K.G. Commons, Ascending serotonin neuron diversity under two umbrellas, Brain structure & function 221(7) (2016) 3347-60.
[71] J.K. Abrams, P.L. Johnson, A. Hay-Schmidt, J.D. Mikkelsen, A. Shekhar, C.A. Lowry, Serotonergic systems associated with arousal and vigilance behaviors following administration of anxiogenic drugs, Neuroscience 133(4) (2005) 983-97.
[72] B.D. Waterhouse, G.A. Mihailoff, J.C. Baack, D.J. Woodward, Topographical distribution of dorsal and median raphe neurons projecting to motor, sensorimotor, and visual cortical areas in the rat, The Journal of comparative neurology 249(4) (1986) 460-76, 478-81.
[73] H.W. Steinbusch, D. van der Kooy, A.A. Verhofstad, A. Pellegrino, Serotonergic and non-serotonergic projections from the nucleus raphe dorsalis to the caudate-putamen complex in the rat, studied by a combined immunofluorescence and fluorescent retrograde axonal labeling technique, Neuroscience letters 19(2) (1980) 137-42.
[74] K.G. Commons, K.R. Connolley, R.J. Valentino, A neurochemically distinct dorsal raphe-limbic circuit with a potential role in affective disorders, Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 28(2) (2003) 206-15.
[75] S. Puglisi-Allegra, D. Andolina, Serotonin and stress coping, Behavioural brain research 277 (2015) 58-67.
[76] A.C. Felix-Ortiz, A. Beyeler, C. Seo, C.A. Leppla, C.P. Wildes, K.M. Tye, BLA to vHPC inputs modulate anxiety-related behaviors, Neuron 79(4) (2013) 658-64.
[77] A.P. Yiu, V. Mercaldo, C. Yan, B. Richards, A.J. Rashid, H.L. Hsiang, J. Pressey, V. Mahadevan, M.M. Tran, S.A. Kushner, M.A. Woodin, P.W. Frankland, S.A. Josselyn, Neurons are recruited to a memory trace based on relative neuronal excitability immediately before training, Neuron 83(3) (2014) 722-35.
[78] S.B. Wolff, J. Grundemann, P. Tovote, S. Krabbe, G.A. Jacobson, C. Muller, C. Herry, I. Ehrlich, R.W. Friedrich, J.J. Letzkus, A. Luthi, Amygdala interneuron subtypes control fear learning through disinhibition, Nature 509(7501) (2014) 453-8.
[79] D.G. Rainnie, Serotonergic modulation of neurotransmission in the rat basolateral amygdala, Journal of neurophysiology 82(1) (1999) 69-85.
[80] P. Tovote, M.S. Esposito, P. Botta, F. Chaudun, J.P. Fadok, M. Markovic, S.B. Wolff, C. Ramakrishnan, L. Fenno, K. Deisseroth, C. Herry, S. Arber, A. Luthi, Midbrain circuits for defensive behaviour, Nature 534(7606) (2016) 206-12.
[81] M.E. Hauberg, M.H. Holm-Nielsen, M. Mattheisen, A.L. Askou, J. Grove, A.D. Borglum, T.J. Corydon, Schizophrenia risk variants affecting microRNA function and site-specific regulation of NT5C2 by miR-206, European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology (2016).
[82] R.P. Vetreno, Y. Patel, U. Patel, T. Jordan Walter, F.T. Crews, Adolescent intermittent ethanol reduces serotonin expression in the adult raphe nucleus and upregulates innate immune expression that is prevented by exercise, Brain, behavior, and immunity (2016).
[83] A.R. Howerton, A.V. Roland, T.L. Bale, Dorsal raphe neuroinflammation promotes dramatic behavioral stress dysregulation, The Journal of neuroscience : the official journal of the Society for Neuroscience 34(21) (2014) 7113-23.

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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