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Stress-Induced Despair Behavior Develops Independently of the AHR-ROR γ t Axis in CD4⁺ cells

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Running Title: Depression Develops without Th17 Cells.

Abstract:

Current treatments for major depressive disorder are limited to neuropharmacological approaches and are ineffective for large numbers of patients. Recently, alternative means have been explored to understand the etiology of depression. Specifically, changes in the microbiome and immune system have been observed in both clinical settings and in mouse models. As such, microbial supplements and probiotics have become a target for potential therapeutics. A current hypothesis for the mechanism of action of these supplements is via the aryl hydrocarbon receptor's (AHR) modulation of the T helper 17 cell (Th17) and T regulatory cell axis. As inflammatory ROR γ t⁺ CD4⁺ Th17 T cells and their primary cytokine IL-17 have been implicated in the development of stress-induced depression, the connection between stress, the AHR, Th17s and depression remains critical to disease understanding. Here, we utilize genetic knockouts to examine the role of the microbial sensor AHR in the development of stress induced despair behavior. We observe an AHR-independent increase in gut-associated Th17s in stressed mice, indicating that AHR is not responsible for this communication. Further, we utilized a CD4-specific *Rorc* knockout line to disrupt the production of Th17s. Mice lacking *Rorc* induced IL-17 did not show any differences in behavior from controls before or after stress. Finally, we utilize an unsupervised machine learning system to examine minute differences in behavior that could not be observed in traditional behavioral assays. Our data demonstrate that neither CD4 specific *Ahr* nor *Rorc* are necessary for the development of stress-induced anxiety- or depressive-like behaviors. These data suggest that research approaches should focus on other sources or sites of IL-17 production in stress-induced depression.

Introduction:

In the United States, an estimated 17.3 million adults are diagnosed with major depressive disorder (MDD), with over 60% struggling with severe impairments, making MDD the number one cause of disability in the U.S.¹. Dogma states that depression and other mood disorders are caused by an imbalance in neurotransmitters²⁻⁴. As such, the majority of existing treatments target neurotransmitter uptake (SSRIs, SNRIs, etc)⁵. However, a significant number of patients do not benefit from these therapeutics, suggesting alternate sources for MDD⁵.

The microbiome has emerged as an important contributor to many neurological conditions, ranging from Parkinson's disease to autism spectrum disorder^{6,7}. Thus, it is unsurprising that the microbiome has also been found to play a crucial role in the development and maintenance of MDD. Patients with depression have altered microbiomes when compared to control patients⁸. Microbiome dysbiosis is also present in animal models of MDD⁹⁻¹² and therapeutic administration of probiotics has been found to be beneficial¹². The mechanism by which the microbiome contributes to the etiology of depression remains under investigation. However, one postulated route is through the immune system¹³.

While there are many ways in which inflammation and the microbiome can interact in depression, a microbiome-responsive regulator of the immune system called the Aryl Hydrocarbon Receptor (AHR) has the potential to serve as a lynchpin in this pathway. Indeed, metabolomics, GWAS, and epigenetic studies have indicated that dysfunction of the AHR is associated with both MDD and post traumatic stress disorder^{14,15}. The AHR is a cytoplasmic receptor that can bind microbiome produced metabolites, including tryptophan derived metabolites¹³. AHR activation induces transcription factor activity that can modulate the inflammatory environment of the gut. In particular, AHR has been shown to regulate T helper cell 17 (Th17) and T regulatory cell (Treg) function in response to microbial metabolites¹⁶. This represents an important connection between the microbiome and immune system as IL-17 produced by ROR γ T⁺ Th17s have been shown to contribute to depression- and anxiety-like behaviors^{17,18}. Studies have shown that the number of Th17s in the gut associated lymphoid tissue (GALT) increases in response to stress and correlates with depressive-like behavior¹⁹. Additionally, the transfer of Th17 cells into mice has been found to induce depressive-like behaviors¹⁷. Administration of IL-17A blocking antibodies has also been found to reduce learned helplessness behaviors in mice¹⁷. However, the evidence for the role of IL-17 in human depression remains controversial, with some reports claiming a positive correlation between the inflammatory cytokine and depression and others reporting no differences in IL-17 levels between those with the disease and controls²⁰⁻²⁵. These conflicting results highlight the need for further investigation into the mechanism and role of the AHR and increased inflammatory Th17 cells in depression.

Here, for the first time, we use genetic tools and artificial intelligence behavior analysis to assess the contribution of T cell specific AHR and IL-17 to the development of anxiety- and depressive-like behaviors in a murine model of stress. While our data confirm that stressed mice present with a larger number of Th17 cells in the gut, our behavioral analyses show that the deletion of either AHR or ROR γ T in T cells does not impact anxiety- or depressive-like behaviors in mice. Taken together our data suggest that ROR γ T induced IL-17 in T cells is not necessary for the pathological development of depressive-like behaviors induced by unpredictable chronic mild restraint stress.

Materials and Methods:

All methods were performed in accordance with guidelines and regulations of the University of Virginia and approved by the University of Virginia Animal Care and Use Committee. Experiments involving live animals are reported as described by the ARRIVE Guidelines.

Mice:

*Cd4 Cre*²⁶ (#022071), *Rorc*^{F/F} ¹⁸ (#008771), and *Ahr*^{F/F} ²⁷ mice were purchased from Jackson Laboratories. Mice were bred in house. Mice were kept on a 12 hour light/dark schedule. All behavioral interventions were performed between 8am and 3pm and animals were sacrificed between 7am and 1pm. All mice were housed in cages of up to 5 animals from birth until initiation of the stress protocol. All mice exposed to stress were at least 8 weeks of age and age matched to control animals. Stressed mice were housed individually without enrichment to enhance stress²⁸. Naïve animals were housed in standard cages in groups of 2-5 mice of the same sex. All procedures were approved by the University of Virginia ACUC (protocol #1918).

Experimental Autoimmune Encephalomyelitis:

EAE was induced in 6-8-week old female *Cd4 Cre Ahr*^{F/F} and *Ahr*^{F/F} mice as previously described²⁹. Thirty-minute videos of mice were taken at day 25 post immunization and used for analysis. Healthy controls were age matched females.

Stress Experiments:

In all Unpredictable Chronic Mild Restraint Stress (UCMRS) experiments, mice were exposed to chronic restraint (ventilated 50mL conical vials) for a period of 2 hours daily. Once removed from restraint, an overnight stressor of either cage tilt, wet bedding, or 2x cage change was used. All daily stressors were carried out between 8am and 5pm. Overnight stressors were started upon removal from the daily stressor and remained in place until the next day's daily stressor.

Behavioral Tests:

The forced swim, tail suspension, sucrose preference, open field, elevated plus maze, novel object recognition, marble burying, and three chamber social preference tests we performed as previously described^{7,30-36}. All testing was recorded on a Hero Session 5 GoPro and analyzed with Noldus behavioral analysis software.

DeepLabCut:

Animal pose estimation: Animal pose estimation was performed by using a deep-learning package, DeepLabCut³⁷ (<https://github.com/DeepLabCut/DeepLabCut>). We generated a DeepLabCut convolutional neural network to analyze our open field test videos, which is trained in a supervised manner: 16 manually labeled points were selected as references of transfer learning. 15 randomly selected videos were used for building a training dataset. Finally, the performance of the neural network is evaluated by researchers.

Unsupervised behavior classification: Estimated mouse poses from DeepLabCut were further analyzed by Variational Animal Motion Embedding (VAME)³⁸, which classifies animal behavior in an unsupervised manner (<https://github.com/LINCellularNeuroscience/VAME>). We trained a

unique VAME recursive neural network for each experiment, which classifies each frame of the open field test video into 1 of the 35 behavioral motifs. Then, all behavior motifs were annotated and evaluated by blinded researchers. With annotated frames, we were able to calculate the percentage of time usage of each motif, which is then used for principal component analysis and Kullback-Leibler divergence analysis.

RNA Extraction and Quantitative PCR:

For RNA extraction, cultured cells were pelleted, frozen, lysed, and RNA extracted using the Bioline Isolate II RNA mini kit as per manufacture's protocol (BIO-52073). RNA was quantified with a Biotek Epoch Microplate Spectrophotometer. Normalized RNA was reverse transcribed to cDNA with either the Bioline SensiFast cDNA Synthesis Kit (BIO-65054) or Applied Sciences High-Capacity cDNA Reverse Transcriptase Kit (43-688-13). cDNA was amplified using the Bioline SensiFast NO-ROX kit (BIO-86020), according to manufacturer's instructions. The TaqMan GAPDH probe (Mm99999915_g1) was measured as a normalizer for each sample. The TaqMan probes Cyp1a1 (Mm00487218_m1), Ahr (Mm00478932_m1), Rorc (Mm01261019_g1), and Il17a (Mm00439618_m1) were used to measure transcript levels from the samples. Results were analyzed with the relative quantity ($\Delta\Delta Cq$) method.

CD4 T cells isolation and differentiation:

Naïve CD4 T cells were harvested and skewed as previously described³⁹. After skewing, cells were washed and frozen for qPCR analysis or treated with an AHR antagonist (I_3S -250uM, Sigma-Aldrich I3875-1G), agonist (CH223191-10uM, Tocris Bioscience 301326-22-7), or vehicle control (DMSO-Fisher Scientific D128-1) for 24 hours prior to freezing.

Tissue Harvest and Digestion:

After experimental manipulation, mice were perfused with 0.9% saline plus 5units/mL heparin (Medefil; MIH-3333) and tissues of interest were harvested and processed for flow cytometry as described below.

Small Intestine:

Whole small intestine was collected from the animals, flayed open and rinsed with ice cold HBSS (Gibco, 14175-095). Tissue was cut into ~2cm pieces and stored in 30mL of 5% FBS (R&D systems, S12450H) in HBSS until processing. Small intestine was shaken at 37°C for 20 minutes to remove mucus and debris. Gut pieces were filtered over mosquito net, placed in fresh 30mL of 5% FBS in HBSS, and shaken at 37°C for another 20 minutes. Samples were again filtered over mosquito net. Pieces were cut using a razor blade until fine slurry was created. Slurry was incubated in gut digestion buffer: Collagenase 8 (Sigma, C2139-5G), DNase (Worthington, LS002139) in 5% FBS in HBSS/- for 40 min, shaken at 150rpm at 37°C. Once digested, the solution was filtered through a 70µm filter and washed three times with 5% FBS in HBSS.

Lymph nodes and Peyer's Patches:

Single cell suspension in RPMI was prepared from Peyer's Patches and lymph nodes after fat removal by mechanical dissociation and subsequent filtration using sterile 70µm filters.

Meninges:

Meninges were dissected from skull caps in ice cold RPMI and digested in the digestion buffer: Collagenase 2 (Gibco, 17101-015), collagenase D (Sigma, 11088882001) and DNase (Worthington, LS002139) for 20 minutes at 37°C. Once digested, meninges were physically dissociated with a 1mL pipette and filtered through a sterile 70µm filter.

Flow cytometry

Single cell suspensions were incubated with CD16/32 Fc Block and then stained with a 1:200 antibody dilution (1:100 for transcription factors). For intranuclear staining, the eBioscience FoxP3/Transcription Factor Staining Kit (00-5523-00) was used per manufacturer's instructions. Antibodies used are as follows: 488-conjugated CD8 (53-008182), APCe780-conjugated TCRβ (47-5961-82), e450-conjugated CD4 (48-0042-820, PE-conjugated RORγT (12-6981-82), and PE-Cy7-conjugated FoxP3 (25-5773-82), all purchased from Invitrogen. APC-conjugated CD45.2 (109813) was purchased from BioLegend. A Live/Dead discrimination dye Ghost Dye Violet 510 (Tonbo Biosciences; 13-0870) was used on all samples. OneComp eBeads (Thermo Fisher Scientific, 01-111-42) were used for all color controls except for the viability dye in which cells were used. Flow cytometry was performed using a Beckman Coulter Gallios flow cytometer and data were analyzed with FlowJo software v10.7.1.

Statistical Analysis:

All statistical analyses-except those associated with DeepLabCut-were performed in GraphPad Prism 9. Analyses involving two groups were performed using a two-tailed t-test. If the variances between groups were significantly different, a Welch's correction was applied. Outliers were excluded if they fell more than two standard deviations from the mean. For all analyses, the threshold for significance was at $p < 0.05$. Repeats for each experiment are specified in the figure legend corresponding to the respective panel.

Results:

Absence of AHR in T cells does not Influence Baseline Behaviors:

We and others have previously shown that microbiome dysbiosis induced by unpredictable chronic mild stress (UCMS) is a contributing factor to depressive- and anxiety-like behaviors in mice^{12,40,41}. Furthermore, therapeutic reconstitution with lactobacillus is sufficient to correct behaviors elucidated by UCMS¹². Lactobacillus is known to produce metabolites that engage the AHR receptor, such as tryptophan metabolites which can modify the immune environment⁴². Given the emerging role of T cells and their cytokines in depression^{17,43,44}, we wanted to determine whether T cell AHR sensing of the microbiome leads to immunological and behavioral changes associated with stress. We generated *Cd4 Cre Ahr^{flox/flox}* mice (*Ahr* KO) to examine depressive- and anxiety-like behaviors in the absence of this important receptor in the T cell compartment. As expected, *Ahr* expression was significantly reduced in CD4+ T cells isolated from *Ahr* KO mice by qPCR (Fig. 1A). Furthermore, *in vitro* generated Th17 cells prepared from *Ahr* KO animals failed to upregulate the AHR downstream transcription factor *Cyp1b1* in response to AHR activation using idoxyl-3-sulfate (IS)⁴⁵. *Ahr* KO levels of *Cyp1b1* expression were consistent with those of AHR competent cells treated with an AHR antagonist CH223191. This confirmed a robust

functional deletion of AHR in T cells (Fig. 1B). Next, we explored whether lacking AHR in T cells could influence behaviors at baseline. Both depressive- and anxiety-like behavioral assays were performed on *Ahr* KO mice and age matched controls. No differences were observed in the forced swim, tail suspension, or nestlet shredding tests (Fig. 1C). These results show that mice lacking AHR in T cells do not present with baseline depressive- or anxiety-like behaviors.

UCMRS Drives a Specific Expansion of Th17 cells in the Lamina Propria in an AHR-Independent Manner:

To test the role of the T cell specific AHR in stress response, we subjected *Ahr* KO or age matched controls to 3 weeks of UCMRS (Fig. 2A). Surprisingly, no differences in anxiety- or depressive-like behaviors were observed between groups after stress (Fig. 2B). While classic assays may detect strong phenotypic differences, they may not detect more subtle behavioral changes. To circumvent this limitation, we applied an unsupervised machine learning approach to analyze behaviors in both genotypes using DeepLabCut³⁷. First, we validated this computational approach using a preclinical model of multiple sclerosis (MS) known as experimental autoimmune encephalomyelitis (EAE). EAE produces significant locomotor changes and should produce detectable motor differences between groups to act as a positive control. DeepLabCut was able to detect robust behavioral differences between groups in nearly 40% of behavioral motifs characterized by the software (Sup Fig.1 A-F). However, no differences were found between stressed, *Ahr* KO animals and age matched controls as shown by Kullback-Leibler Divergence-a measure quantifying variances within and between groups-(Fig. 2C and Sup Fig. 2B) and by PCA (Fig. 2D). Similarly, of all behavioral motifs analyzed, only one showed a significant change between groups (2.8% of all behaviors), suggesting the overall behaviors of *Ahr* KO mice are very similar to control animals in response to stress (Sup Fig. 2C-D). Additionally, immunophenotyping revealed no differences in the measured immune compartment between *Ahr* KO and control mice (Sup Fig. 2A). Strikingly, however, both the *Ahr* KO animals and controls had an increase in the number of CD4+ROR γ T+ Th17 cells in the GALT after stress (Fig. 2E). Thus, the microbiome changes and increase in Th17 cells observed in response to stress is not mechanistically linked by AHR activation in CD4+ cells.

Deletion of *Rorc* in T cells does not Induce Spontaneous Anxiety- or Depressive-like Behaviors:

To explore the role Th17 cells in anxiety- and depressive-like behaviors, *Cd4 Cre Rorc^{flox/flox}* mice were generated (*Rorc* KO). Knock down of ROR γ T was confirmed using *in vitro* derived Th17s by examining levels of *Il17* and *Rorc* by qPCR (Fig. 3A) and IL-17 secretion by ELISA quantification (Fig. 3B). Immunophenotyping on spleen revealed the absence of CD4+ROR γ T+ T cells *in vivo* (Sup. Fig. 3A-C). Baseline behaviors were compared between male *Rorc* KO mice and controls. No differences between the *Rorc* KO mice or age matched controls were observed in tasks used to assess depressive- (Fig. 3D) or anxiety- like behaviors (Fig. 3F). As Th17 cells are also known to contribute to autism-like behaviors in male mice¹⁸, we analyzed the marble burying (Fig. 3C), social preference (Fig. 3E) and novel object recognition (Fig. 3G) tests at baseline. No differences between genotypes were observed in these behavior tests. Lastly, to ensure there were no subtle behavior differences between groups that could not be quantified with known behavioral tasks, we again used machine learning to examine unbiased behavioral clusters. No differences were found in the Kullback-Leibler Divergence plot (Fig. 3H and Sup Fig. 3D) and a large overlap in the

PCA plot was observed (Fig. 3I). Additionally, only 3 of 35 behavioral motifs demonstrated significant differences between groups after DeepLabCut analysis (Sup. Fig. 3C-F). Together, these data suggest that at baseline, a lack of *Rorc* from development does not impact autism-, depression-, or anxiety-like behaviors in male mice.

UCMRS Induced Anxiety- or Depressive-like Behaviors are not Affected by the Lack of *Rorc* in Th17 cells:

While no behavioral differences in male mice were observed between groups at baseline, females experience depression at a higher rate than males⁴⁶. Thus, we aimed to examine the impact of *Rorc* in Th17 cells in female mice. Like the male mice, no differences in escape behavior, anhedonia, or anxiety-like behaviors were observed at baseline (Fig. 4A-B). Similarly, our machine learning approach was not able to detect differences in behaviors as shown through the Kullback-Leibler Divergence plot (Fig. 4C and Sup. Fig. 4C), PCA plot (Fig. 4D), and motif usage breakdown plots (Sup. Fig. 4A-B). To examine the impacts of *Rorc* KO in a stressful environment, female mice were exposed to 3 weeks of UCMRS. After exposure to stress, no differences between groups were observed in escape behavior (Fig. 4E). The nestlet shred test revealed that *Rorc* KO mice demonstrate a significant increase in anxiety-like nesting behaviors, whereas the elevated plus maze showed a significant decrease in anxiety-like behaviors, and the open field test showed no difference between groups (Fig. 4F). Ultimately, our unbiased computational approach also did not detect any differences as seen in the Kullback-Leibler Divergence plot (Fig. 4G and Sup. Fig. 4F) and the PCA plot (Fig. 4H). No differences in groups were detected in any of the 35 behavior motifs identified between groups. In summary, although we saw a significant increase in anxiety-like behavior in the nestlet shredding test between *Rorc* KO and control animals, our other behavioral tests did not support this trend and our unbiased behavioral analysis showed no changes between groups. Based on these data, *Rorc* KO in T cells does not significantly impact depressive-like behaviors in female mice before or after UCMRS.

Discussion:

Depression presents a major social, health, and economic concern across the world, representing the number one cause of disability⁴⁷. The root causes of this debilitating disorder have yet to be elucidated. With treatments remaining inconsistent and new options lacking, new research is needed to further our understanding of this disorder. Both the microbiome and T lymphocytes have been gaining support as potential mediators of MDD pathology. Here, we examine the role of T cell specific ROR γ T and AHR in a mouse model of depression. We demonstrate that deletion of the *Ahr* gene does not impact anxiety- or depressive-like behaviors at baseline or after chronic stress exposure. Additionally, while we see an increase in the number of ROR γ T+ cells in the lamina propria of stressed mice, it occurs independently of the AHR. This indicates that there is another mechanism for Th17 cell expansion in response to stress other than environmental sensing through the AHR.

Further, when *Rorc* is deleted from CD4+ T cells, no changes in anxiety or depressive-like behaviors are observed at baseline or after stress exposure. This is supported by previous work showing that increasing Th17s is not sufficient to change the susceptibility of mice to social defeat stress⁴³. Others have suggested that increased Th17s are responsible for stress-induced depressive-like behaviors and have supported this claim by transferring Th17s into mice and

finding increases in depressive-like behaviors. However, this only suggests that an increase in Th17s may be sufficient to drive these behaviors¹⁷, not that it is necessary. Additionally, the origin of IL-17 is not precisely defined. IL-17 can be produced from many cell types and be induced in alternative ways that may contribute to depression outside of Th17s^{48,49}. Supporting this concept, it has been found that IL-17 from $\gamma\delta$ T cells regulates anxiety-like behaviors in mice⁴⁴. Additionally, multiple transcription factors (STAT3, NF- κ B, KLF4, etc) and microRNA can act on IL-17 producing cells to induce IL-17 production⁵⁰. This regulation can act in synergy with ROR γ T or independently of it, as with KLF4⁵⁰, suggesting that IL-17 could be produced without *Rorc* and outside of Th17 cells. These data and our own work suggest that if there is a role of IL-17 in the onset of depression, this cytokine does not solely originate from CD4+ cells or is produced by alternative cell type (ILC3s, $\gamma\delta$ T cells, or others).

The notion that Th17 cells play a critical role in the onset of depression has been growing in popularity but remains controversial. Several human studies have demonstrated that changes in IL-17 are correlated with depression^{21,23,25,51} while others have not^{20,24}. While further work is needed to fully understand the role of IL-17 in the onset of depressive symptoms, our work demonstrates that the AHR is not responsible for the observed stress-induced increase in intestinal Th17s and that these cells are not necessary for the induction of stress-induced depression. Instead, we suggest there are alternative means or sites of IL-17 production in stress-induced depression. If no such mechanisms can be identified, a need to develop novel approaches to identify other potential causes of depression symptoms, such as the microbiome or other inflammatory signals is required.

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Contributions

C.R.N., A.R.M. and A.G. designed the study; C.R.N., A.R.M., A.F.C. and R.M.B. performed experiments; S.L., S.L., J.A.T. and J.B.S performed computational analysis; C.R.N. and A.R.M. analyzed the data and wrote the manuscript; A.G. oversaw the project.

Competing Interests

Authors declare no competing interests.

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Figure 1: Absence of AHR in T cells does not Influence Baseline Behavior. Relative quantity of *Ahr* expression from *in vitro* skewed Th17 cells in *Ahr* KO and *Ahr* competent cells (A). T test, each dot represents an animal. Relative quantity of *Cyp1b1* expression from KO and competent cells skewed *in vitro* to become Th17s and treated with an AHR agonist (I3S), antagonist (CH223191), or control media (DMSO). N=2 (B). One-way ANOVA. Baseline behaviors between *Ahr* KO and littermate controls (C). T tests, each dot represents an animal, N=2-a single representative experiment shown, male mice.

Figure 2: UCMRS Drives Expansion of Th17s in the Lamina Propria in an *Ahr* Independent Manner. Schematic representing timeline for unpredictable chronic mild restraint stress in mice

(A). Measures of learned helplessness and anxiety-like behaviors between *Ahr* KO and littermate controls (B). T tests, $n = 9/\text{group}$. Kullback-Leibler divergence heatmap (C) and PCA plot (D) of DeepLabCut analyzed behaviors between groups. $N=2$ -a single representative experiment shown. Number of CD4⁺ ROR γ T⁺ cells in various immune tissues between stressed and naïve *Ahr* KO and littermate controls(E). One-way ANOVA, each dot represents an animal, $N=1$, male mice.

Figure 3: Depletion of *Rorc* does not Induce Spontaneous Behavioral Changes in Male Mice.

Loss of gene expression of both *Il17* and *Rorc* in *in vitro* skewed Th17s from *Rorc* KO mice and littermate controls by qPCR (A). T test, n=3/group. ELISA representing loss of IL-17 in *in vitro* skewed Th17s from *Rorc* KO animals (B). T test, n= 3 or 4/group. Baseline marble burying (C), depressive-like (D), social preference (E), anxiety-like (F), and novel object recognition (G)

behaviors in *Rorc* KO mice vs littermate. T tests. Kullback-Leibler divergence heatmap (H) and PCA (I) plot of DeepLabCut analyzed behaviors between groups. Each dot represents an animal, N=2, male mice.

Figure 4: Loss of *Rorc* in T cells does not Impact Depressive- or Anxiety- like Behaviors in Female Mice. No differences in depressive-like (A) or anxiety-like (B) behaviors between female *Rorc* KO and littermate controls at baseline. No differences in the Kullback-Leibler divergence heatmap (C) or PCA (D) plot of DeepLabCut analyzed baseline behaviors between groups. Depressive-like (E) and anxiety-like (F) behaviors after 3 weeks of UCMRS between female *Rorc* KO and littermate controls. Kullback-Leibler divergence heatmap (G) and PCA (H) plot of

DeepLabCut analyzed behaviors after 3 weeks of UCMRS between groups. T tests, each dot represents an animal, N=1, female mice.

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