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# MicroRNA-155 deletion reduces anxiety- and depressive-like behaviors in mice

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ABSTRACT

Depressive disorders have complex and multi-faceted underlying mechanisms, rendering these disorders difficult to treat consistently and effectively. One under-explored therapeutic strategy for alleviating mood disorders is the targeting of microRNAs (miRs). miRs are small non-coding RNAs that cause sequestration/degradation of specific mRNAs, thereby preventing protein translation and downstream functions. miR-155 has validated and predicted neurotrophic factor and inflammatory mRNA targets, which led to our hypothesis that miR-155 deletion would modulate affective behaviors. To evaluate anxiety-like behavior, wildtype (wt) and miR-155 knockout (ko) mice (littermates; both male and female) were assessed in the open field and on an elevated plus maze. In both tests, miR-155 ko mice spent more time in open areas, suggesting they had reduced anxiety-like behavior. Depressive-like behaviors were assessed using the forced swim test. Compared to wt mice, miR-155 ko mice exhibited reduced float duration and increased latency to float. Further, although all mice exhibited a strong preference for a sucrose solution over water, this preference was enhanced in miR-155 ko mice. miR-155 ko mice had no deficiencies in learning and memory (Barnes maze) or social preference/novelty suggesting that changes in mood were specific. Finally, compared to wt hippocampi, miR-155 ko hippocampi had a reduced inflammatory signature (e.g., decreased IL-6, TNF-a) and female miR-155 ko mice increased ciliary neurotrophic factor expression. Together, these data highlight the importance of studying microRNAs in the context of anxiety and depression and identify miR-155 as a novel potential therapeutic target for improving mood disorders.

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## 1. Introduction

The etiology of mood disorders is complex and multi-faceted. Depression arises from widespread alterations in one or more prominent biological pathways: the brain's reward circuitry (Russo and Nestler, 2013), capacity for neuroplasticity (Pittenger and Duman, 2008), and/or neuroinflammatory state (Miller and Raison, 2015). Although various physiological mechanisms underlie depression, the condition has been classified and treated as a single disorder. This has likely contributed to the high failure rate of depression treatments. Indeed, antidepressant have minimal efficacy for <50% of patients and have no significant positive effect for 30–40% of patients (Berton and Nestler, 2006). It has been argued that current antidepressant treatments are not more effective than placebos, which is troublesome given the adverse side effects of these drugs (Kirsch, 2014). Therefore, identifying molecular and cellular mechanisms that contribute to the susceptibility, onset, and heterogeneity of depression may facilitate the development of novel effective therapies for depressive disorders.

Mood disorders likely develop due to complex interactions between environmental factors and genetic predisposition (Krishnan and Nestler, 2008). This suggests that epigenetic processes may be involved. MicroRNAs (miRs) are one type of post-transcriptional regulatory factor that may modulate depression-associated molecular pathways. miRs are 21–23 nucleotide single-stranded RNAs that regulate gene expression by sequestering/degrading complementary mRNA targets (Krol et al., 2010). Individual miRs can target hundreds of mRNAs simultaneously and miRs act as key regulators in myriad cellular processes. In animal models of depression and in the brain and cerebrospinal

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fluid of humans suffering from mood disorders, the expression profile of miRs is dysregulated (Moreau et al., 2011; Wan et al., 2015). By manipulating specific miRs, it may be possible to control signalling pathways in neurons and inflammatory cells that predispose individuals to affective disorders.

Here, we define the behavioral manifestations of deleting miR-155 from mice. miR-155 is considered a “multifunctional miR” that is implicated in numerous biological processes including hematopoiesis, inflammation, and immunity (Faraoni et al., 2009). miR-155 is expressed in the central nervous system (CNS) and has been implicated in several neuroinflammatory disorders/injuries including Alzheimer's disease, amyotrophic lateral sclerosis, stroke, and traumatic brain injury (Butovsky et al., 2015; Caballero-Garrido et al., 2015; Song and Lee, 2015; Wang et al., 2015). A search of predictive targets for miR-155 using PubMed and TargetScan revealed several genes related to inflammation (TNFα, Cebpb, NFκB pathway) and neuroplasticity (BDNF and TGFβ), which are two major pathways implicated in depression (Pittenger and Duman, 2008; Miller and Raison, 2015) (see Supplementary Table 1). Furthermore, miR-155 is upregulated in the CSF, but not the serum of patients suffering from major depressive disorders as compared to non-depressed individuals (Wan et al., 2015). Therefore, we predicted that in miR-155 knockout (KO) mice, depressive-like symptoms would be reduced. An extensive behavioral phenotyping was performed on miR-155 ko mice. Overall, depressive- and anxiety-like behaviors were reduced in both male and female miR-155 ko mice. Sensorimotor skills, learning, memory and social behaviors were unaffected by miR-155 deletion highlighting the specificity of the behavioral alterations regulated by miR-155.

## 2. Methods

### 2.1. Animals

The Ohio State University Institutional Animal Care and Use Committee approved all experimental procedures, and animals were maintained in accordance with the recommendations of the National Institutes of Health and the Guide for the Care and Use of Laboratory Animals. miR-155 heterozygous parents (kind gift from Amy Lovett-Racke; Jackson stock 007745; 129xC57BL/6 background that was backcrossed for >5 generations onto C57BL/6) (Thai et al., 2007) were bred to generate WT and miR-155 KO littermates used for all experiments. Prior to conducting these experiments we validated that miR-155 was not expressed in the hippocampus of miR-155 ko mice with qPCR. Mice were reared with same sex siblings until the start of the experiment at which point they were individually housed. Genotyping was performed via endpoint PCR on ear snips collected between post-natal day 14–21. Mice were maintained in a 12:12 light/dark cycle (lights on at 7A EST) in polypropylene cages (dimensions: $33 \times 19 \times 14$ cm) with corncob bedding at an ambient temperature of $23 \pm 2$ °C and food (Harlan Teklad 8640) and water were provided ad libitum. Male and female mice were housed separately throughout the experiment and behavioral testing was conducted separately with a thorough cleaning of all equipment between testing the sexes. All mice were between 12 and 20 weeks of age at the onset of behavioral testing.

### 2.2. Behavioral testing

Behavioral tests were conducted in the order presented below (see Supplementary Table 2). For behavioral experiments 8 wt males (from 6 breeding pairs), 9 ko males (from 6 breeding pairs), 11 wt females (from 5 breeding pairs), and 7 ko females (from 4 breeding pairs) were used. Group sizes varied because offspring were bred at OSU from heterozygous breeding pairs. Groups were litter-matched when possible (however not every litter had ko and wt male and female offspring). Behavioral tests were only performed once per animal unless otherwise specified (e.g. Barnes maze and rotarod had multiple trials).

#### 2.2.1. Sensorimotor tests

Prior to evaluating more complex behaviors, mice underwent an initial assessment (during the light phase) in which body weight, vibrissae, visual acuity, auditory orientation, and muscle tone were evaluated. For vibrissae assessment, whiskers were scored on a scale of 1–5 with 1 representing a mouse with whiskers present and in good condition (un-barbered) and 5 representing a mouse with no whiskers. Eye appearance was evaluated for discrepancies in size, shape, and condition (0 = normal, 1 = mild abnormalities, 2 = severe abnormalities). Visual placing was evaluated by lowering the mice slowly towards the edge of the table (positive score is given if mouse extends its paws on at least 2 of 3 trials). Muscle tone was evaluated on a similar scale while handling each animal. Auditory orientation was evaluated by measuring a mouse's response to clicker (positive score if mouse turns it head towards the clicker). Grip strength was assessed by suspending each mouse from a wire cage lid 60 cm above a foam pillow. The time until the mouse fell or 90 s passed was recorded; this test was performed 3 times per mouse.

Mice were trained and tested on a rotarod task during their light phase. The mice were habituated to the rod while it slowly rotated (5.0 rotations per minute (RPM)) in three 2-min sessions with 10 s between sessions. If the mouse fell off the rod before 2 min elapsed, then it was replaced onto the rod. Testing consisted of 3 sessions of 3 trials each (1 session directly followed habituation and the other two sessions were performed the next day). Each session had a progressively higher speed of rod rotation (24 RPM, 28 RPM, and 32 RPM). On each of the 3 trials per session, the mouse was placed on the rod until either 60 s elapsed or the mouse fell off. There was a 10 s break between trials, and a 2 h break between sessions. Performance was measured as average time on the rod for the 3-trial session at each speed.

#### 2.2.2. Anxiety-like behaviors

To assess locomotor behavior and anxiety-like responses, mice were placed in a $40 \times 40$ cm clear acrylic chamber lined with clean corncob bedding, inside a ventilated cabinet (Med Associates, St. Albans, VT, USA) during their dark phase. Test chambers were rinsed with 70% ethanol between tests. A frame at the base of the chamber consisting of 32 photobeams in a $16 \times 16$ arrangement, in addition to a row of beams above, detected the location of horizontal movements and rearing, respectively (Open Field Photobeam Activity System, San Diego Instruments, San Diego, CA, USA). Total movement was tracked for 5 min and analyzed for: (1) the percentage of beam breaks in the center (inside $30 \times 30$ cm) of the open field, (2) number of rears and (3) total locomotor behavior.

To further assess anxiety-like responses, mice were next tested in an elevated-plus maze apparatus under dim red illumination during their dark phase. The maze is elevated 1 m above the floor and made of dark-tinted acrylic and consists of two open arms bisected by two arms enclosed by walls. Mice were placed in the central maze area facing a closed arm and recorded for 5 min. The maze was wiped with mild soapy water between tests. An open arm entry was scored when the mouse's entire body entered an open arm. A condition-blind observer using Observer software scored tapes for: (1) total time spent in the open arms and (2) number of open and closed arm transitions.

#### 2.2.3. Social behavior

To assess social behavior mice were tested using a three-chamber social approach task under dim red illumination during

their dark (active) phase [as described in (Nadler et al., 2004)]. In brief, the test mouse was first placed in the three-chamber apparatus and allowed to explore for 10 min. At the end of this period, the test mouse was corralled back into the center chamber of the apparatus and a stimulus mouse was placed in a wire cage in one of the two side chambers (this was randomized). The gates to the two chambers were then lifted and the mouse was again allowed to explore for 10 min. After 10 min, the test mouse was placed back in the center chamber and a novel second stimulus mouse was placed in the other side under a second wire cage (stimulus mouse 1 is left in place). The test mouse then explored the three chambers for a final 10 min period. The test was recorded and scored by a condition blind observer using Observer software (Noldus, Leesburg, VA, USA) for time spent in each of the three chambers. The first trial was used to verify that mice do not have a side preference and allowed them to acclimate to the apparatus. The second trial (with only stimulus mouse 1) is considered a measure of sociability and the final trial (where the mouse can interact with the familiar stimulus mouse 1 or novel stimulus mouse 2) is considered an index of preference for social novelty (Nadler et al., 2004).

#### 2.2.4. Learning and memory

Learning and memory were assessed in the Barnes maze during the light phase as described previously (Fonken et al., 2011). Mice were placed in the center of a brightly lit arena and given 2 min to find an escape hole leading to a dark box. The maze has 18 evenly spaced holes but only one leads to the dark box. On the first day of testing, mice were acclimated to the maze; a bright light and loud fan were turned on and mice were gently guided from the center of the maze to the target hole. After entering the dark box the bright light and fan were turned off and the mouse was left undisturbed for 2 min. Mice received 5 days of training trials, consisting of one daily session of three trials. Each trial lasted 2 min or until the mouse found the escape hole where they were left for 1 min. After each trial the mouse was placed in its home cage for 10 min before beginning the next trial. During the training trials, latency to find the target hole and number of errors were scored.

#### 2.2.5. Depression-like behaviors

To assess depressive-like responses, mice were evaluated in the forced swim task under dim red illumination during their dark phase. Mice were placed in room-temperature ($22 \pm 1\,^{\circ}$C) water ~17 cm deep, within an opaque, cylindrical tank (diameter = 24 cm, height = 53 cm). Swimming behavior was recorded on video for 5 min and scored by a condition-blind observer with the Observer software for: (1) latency to float, (2) total number of floating bouts, and (3) total time spent floating.

Behavioral anhedonia was also evaluated with a sucrose preference task. For this test, mice were first acclimated to novel 15 mL drinking tubes for two nights (one night of water followed by a night of sucrose in both tubes). Mice were then presented with a choice of water versus sucrose (sucrose bottle side was randomized) for 5 h at the onset of the light phase for 3 nights to familiarize them with the task. Finally, mice were presented with the two tube choice overnight (10 h) beginning at the start of the active phase. Bottles were weighed prior to and following placement in the cages during every trial.

### 2.3. qPCR

Hippocampi were isolated from experimentally naïve female and male mice derived from heterozygous pairs (litter-matched when possible; $n$ = 8/group). PCR analyses were conducted in experimentally naïve animals because the stress of behavioral testing could influence the expression of several genes evaluated (Murakami et al., 2005; Garate et al., 2013). Animals under terminal anesthesia were perfused with DEPC-PBS (to inhibit RNases), and then hippocampi were removed and homogenized in Trizol (Life Technologies 15596-018). RNA extraction was performed according to manufacturer's instructions. Reverse-transcriptase (RT) PCR was completed using SuperScript II (Life Technologies). For quantitative (q)PCR, Taqman primers (all Life Technologies: IL-1b: Mm00434228_m1; IL-6: Mm00446190_m1; TNF-a: Mm00443258_m1; Cebp: Mm00843434_s1; Ikbkb: Mm01222247_m1; NFKBIA: Mm00477800_g1; Nos2: Mm00440502_m1; BDNF: Mm04230607_s1; CNTF: Mm04213924_s1; IGF-1: Mm00439560_m1; NGF-b: Mm00443039_m1; TGFBI: Mm01178820_m1) were used with Taqman PCR Master Mix (Life Technologies 4324018). Genes were run in triplicate for each individual sample. Inflammatory or growth factor gene expression was calculated relative to 18S expression. Genes were selected based on previously research and TargetScan prediction of direct interaction with miR-155 (BDNF, TGFB, TNF, Cebpb), as well as on research demonstrating potential indirect associations with miR-155 (see Supplementary Table 1). We specifically focused on the potential for miR-155 to alter both inflammatory pathways and growth factors.

### 2.4. Statistical methods

Behavioral results were analyzed using analysis of variance (ANOVA) with genotype and sex defined as the independent variables. Following a significant F value, post hoc comparisons were made using Tukey's HSD test. For each of the 5 Barnes maze sessions 3 trials were conducted, and variables were averaged per session for each mouse. Repeated-measures ANOVA were then conducted for latency to find the target hole and error rate with genotype and sex as the with-in subject factor and session as the between subject factor. mRNA expression data were analyzed using *t*-tests. Statistical analyses were conducted using StatView (v. 5.0.1., Cary, NC, USA) and Prism software. In all cases, differences between group means were considered statistically significant if $p < 0.05$.

## 3. Results

### 3.1. Anxiety-like responses are reduced in miR-155 ko mice

miR-155 ko mice increased percentage of time spent in the center of the open field compared to wt mice ($F_{1,30} = 7.64$, $p < 0.01$; Fig 1A). There was no effect of genotype or sex on total activity (beam breaks) in the 5 min open field test suggesting that overall changes in locomotor activity did not underlie differences in central tendency. However, there was an interaction between sex and genotype ($F_{1,30} = 5.93$); post hoc analysis revealed that female miR-155 ko mice decreased activity as compared to male miR-155 ko mice (Fig. 1B).

Behaviors evaluated in the elevated plus maze further explored whether miR-155 deletion affects anxiety-like responses. miR-155 ko mice increased the duration of time spent in the open arms of the elevated plus maze ($F_{1,30} = 5.25$, $p < 0.05$; Fig 1C) and increased the percentage of entries into the open arms (open arm entries/total arm transitions × 100) ($F_{1,30} = 7.70$, $p < 0.01$; Fig 1D). There was also a main effect of sex on duration of time spent in the open arms ($F_{1,30} = 31.02$, $p < 0.0001$); female mice spent significantly more time in the open arms than males.

### 3.2. miR-155 knockout mice decrease depressive-like behaviors

Depressive-like behaviors were evaluated in forced swim and sucrose preference tests. The forced swim test is widely used to test for antidepressant efficacy (Porsolt et al., 1977). Although there are limitations to this test (Pollak et al., 2010), floating is

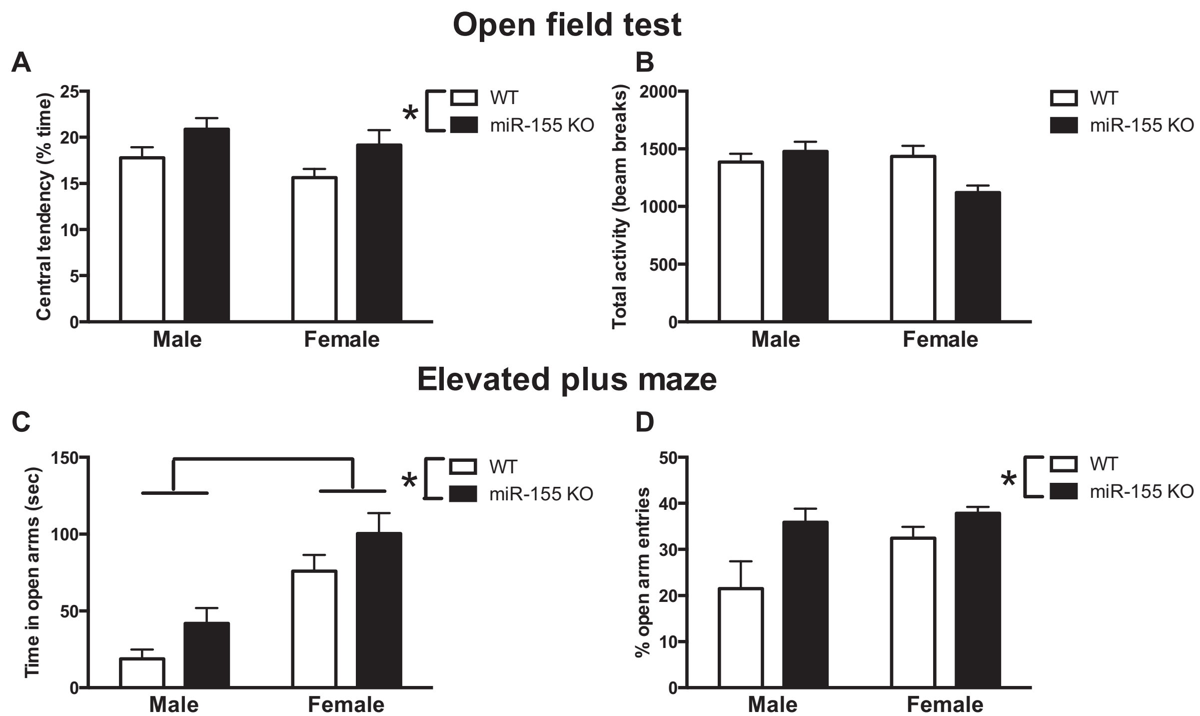


**Fig. 1.** Anxiety-like responses are reduced in miR-155 knockout mice. (A) Central tendency in the open field test; (B) total activity in an a 5 min open field test; (C) time spent in the open arms in an elevated plus maze task; (D) percentage of entries made into the open arms. Data are expressed as mean ± SEM. *$p < 0.05$ main effect of genotype, bar represents $p < 0.05$ main effect of sex.

traditionally considered a resignation behavior that can signify depression. miR-155 ko mice displayed a reduction in time spent floating ($F_{1,29} = 10.60$, $p < 0.005$; Fig 2A). Furthermore, miR-155 ko mice increased latency to first float ($F_{1,29} = 8.33$, $p < 0.01$; Fig 2B). Sex differences were also observed in the forced swim test; female mice reduced floating and increased latency to first float as com-

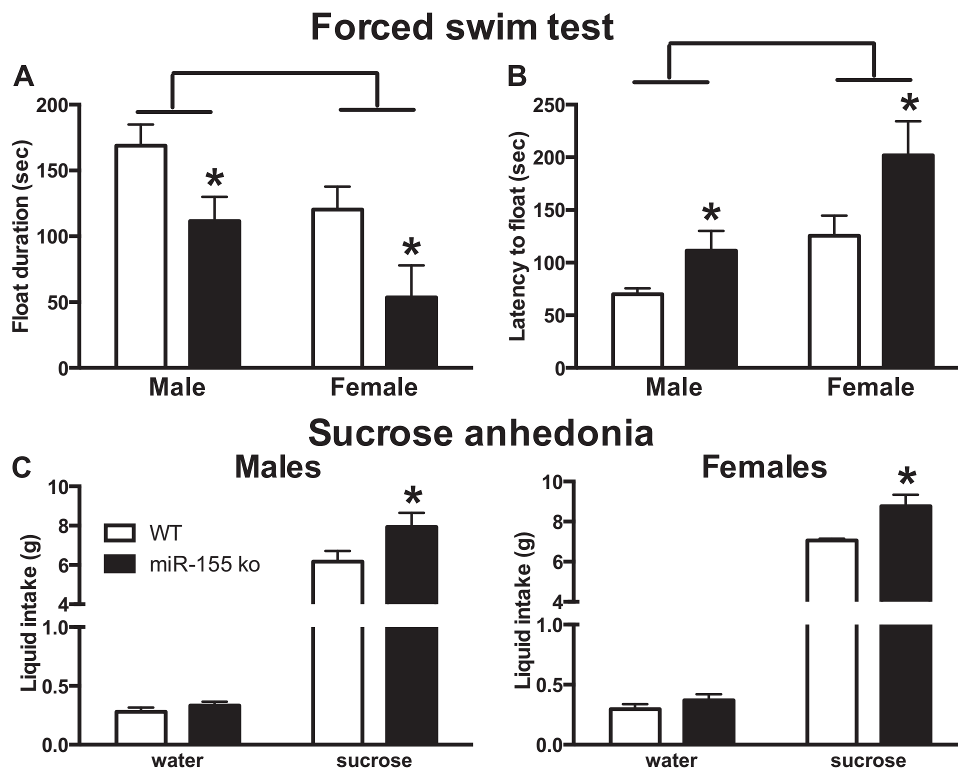


**Fig. 2.** miR-155 ko mice displayed reductions in depressive-like behaviors. (A) Duration of time spent floating in the forced swim test; (B) latency to first float in the forced swim test; (C) water and sucrose intake in 10 h two-choice sucrose preference test. Data are expressed as mean ± SEM, *$p < 0.05$ main effect of genotype, bar represents $p < 0.05$ main effect of sex.

pared to male mice ($F_{1,29}$ = 12.84 and 7.83 respectively, $p < 0.01$; Fig 2).

To explore the response of miR-155 ko mice to reward, sucrose preference was evaluated. All of the mice demonstrated a strong preference for the 2% sucrose solution as compared to water during the 4–5 h habituation sessions. During the final 10 h exposure to the 2 bottle choice test both groups again demonstrated a strong preference for the sucrose solution; however, miR-155 ko mice consumed more sucrose than wt mice ($F_{1,30}$ = 6.68, $p < 0.05$; Fig 2C).

### 3.3. Sensorimotor function and social behavior

Differences in anxiety- and depressive-like behaviors might be explained by gross physiological changes or sensorimotor impairments. Therefore, before behavioral testing mice underwent an initial assessment during which body weight, vibrissae, visual acuity, auditory orientation, and muscle tone were evaluated. All of the mice responded normally to visual acuity and auditory orientation tests. There were no differences between groups on grip strength (every mouse achieved 90 s suspension on at least 1 of the 3 trials). There were no effects of genotype on body mass or vibrissae score ($p > 0.05$ in each case; Supplementary Table 3).

Next, mice were tested on a rotarod task. Female, but not male mice, reduced their latency to fall as the speed of the rotarod was increased ($F_{2,45}$ = 3.49, $p < 0.05$; Supplementary Fig. 1). There was also a main effect of genotype on latency to fall from the rod ($F_{2,45}$ = 4.66, $p < 0.05$); post hoc analysis revealed only female miR-155 KO significantly reduced their latency to fall from the rod at 32RPM as compared to female WT mice ($p < 0.05$). There were no differences among male mice in latency to fall at any of the three speeds tested in the rotatrod task.

There were no differences between groups in the habituation, social preference, or social novelty trials of the social approach task ($p > 0.05$ in each case; Supplementary Fig. 2).

### 3.4. Hippocampal dependent learning behavior is intact in miR-155 ko mice

Mice underwent 5 days of training in a Barnes maze learning and memory test. There were no differences between groups in time to escape the maze or number of errors made during the training trials (Fig 3A and B). Following the 5 days of acquisition, mice were tested in a probe trial. In the probe trial, there were no differences between groups with respect to percentage of time spent in the target quadrant (48.13% versus 47.96%).

### 3.5. Inflammatory gene expression is altered in the hippocampus of miR-155 ko mice

In a separate cohort of male and female mice, hippocampi were removed and used to evaluate expression of inflammatory and neurotrophic-factor related genes. Predicted and/or validated targets for miR-155 were selected based on TargetScan and PubMed searches and include TNFα, Cepbp, BDNF, and TGFβ1. Several additional indirect inflammatory gene targets also were evaluated based on previous research including IL-1β (Li et al., 2013), IL-6 (Cardoso et al., 2012), Nos2 (Cardoso et al., 2012), and NFκB pathway genes [Ikbkb and Nfkbia (Lippai et al., 2013)]. IL-6 and TNF-α (inflammatory cytokines) mRNA expression was reduced in the hippocampus of female and male miR-155 ko mice (female: $t_{13}$ = 2.57 and 3.19; male: $t_{14}$ = 2.50 and 2.22; $p < 0.05$; Fig 4A). Furthermore, NFKBIA (inhibits the NF-κB transcription factor) was upregulated in female ($t_{13}$ = 4.15, $p < 0.05$) but not male ($p > 0.05$) miR-155 ko mice compared to wt mice. Expression of other inflammatory genes including IL-1β, CEBPB, Ikbkb, and NOS2 did not significantly differ between genotypes. We also tested whether miR-155 deletion increased expression of neurotrophic factors in the hippocampus. Although BDNF, IGF-1, NGF-β, and TGFBI expression were unaffected by miR-155 deletion, CNTF was upregulated in the hippocampus of female miR-155 ko mice (Fig. 4B).

## 4. Discussion

To our knowledge, this is the first study demonstrating that experimental manipulation of miR-155 levels affects various mood related behaviors. Specifically, both male and female miR-155 ko mice were found to display reduced anxiety-like responses in both open field and elevated plus maze tasks when compared with wild-type mice. Furthermore, mir-155 ko mice displayed reduced depression-associated behavior in a forced swim task and exhibited an increased affinity for reward as assessed by a sucrose preference test. Changes in affective behaviors were not associated with significant differences in learning and memory. This adds to the growing body of literature implicating microRNAs in the development of mood disorders (Dwivedi, 2015).

The predicted targets for miR-155 include several genes related to inflammation (including TNFα, Cebpb, NFκB pathway) and neuroplasticity (BDNF and TGFβ), two major pathways dysregulated in major depressive disorders (Pittenger and Duman, 2008; Miller and Raison, 2015). This led us to evaluate expression of several inflammatory and neurotropic-related genes. Gene expression was specifically evaluated in the hippocampus because mood disorders are associated with physiological and structural changes in the hippocampus (Bremner et al., 2000) and miR-155 was recently implicated in inflammation-induced hippocampal neurogenic dysfunction (Woodbury et al., 2015). Our results indicate that miR-155 deletion shifts the hippocampus towards a less inflammatory state, with reduced expression of mRNA encoding the inflammatory cytokines IL-6 and TNF-α in male and female miR-155 ko mice. Previous work indicates that miR-155 removal reduces TNF-α and IL-6 levels in the periphery (Bluml et al., 2011; Kurowska-Stolarska et al., 2011; Nazari-Jahantigh et al., 2012). Interestingly, an upregulation of NFKBIA (also called IκBα) mRNA—a negative regulator of the canonical inflammatory transcription factor NFκB was apparent only in female miR-155 ko mice. Female mice may be more susceptible to changes in inflammatory genes in the hippocampus as they typically having significantly more hippocampal microglia than male mice (Schwarz and Bilbo, 2012).

Several key findings implicate hippocampal inflammation in mood disorders (Raison et al., 2006): (1) there are similarities between symptoms of depression and cytokine induced sickness behavior (e.g. social withdrawal, anhedonia, altered sleep, and heightened pain sensitivity), (2) one third of patients undergoing treatment with the cytokines interleukin-2 and interferon-α develop major depressive disorder, (3) patients suffering from chronic inflammatory conditions are more likely to develop depression, and (4) major depressive disorder is associated with increases in cytokines in serum and cerebrospinal fluid, reviewed in (Dantzer et al., 2008).

Hippocampal inflammation may alter mood related behaviors through several mechanisms, including by reducing neurotrophic factors or decreasing neurogenesis. The neurotrophic factors BDNF (Varendi et al., 2014) and TGFβ are potential targets of miR-155. Thus, we also evaluated gene expression of several neurotropic factors in hippocampus. CNTF mRNA (but not other neurotrophic factor RNAs such as BDNF or NGF) was increased in the hippocampus of female miR-155 ko mice. CNTF is a neurotrophic cytokine (in the IL-6 family) that is released by astrocytes (Lee et al., 1997) and is expressed at low levels in the CNS. CNTF promotes neurogenesis in several brain regions (Emsley and Hagg, 2003; Yang et al., 2008), likely via the notch-signaling pathway (Chojnacki et al., 2003). CNTF

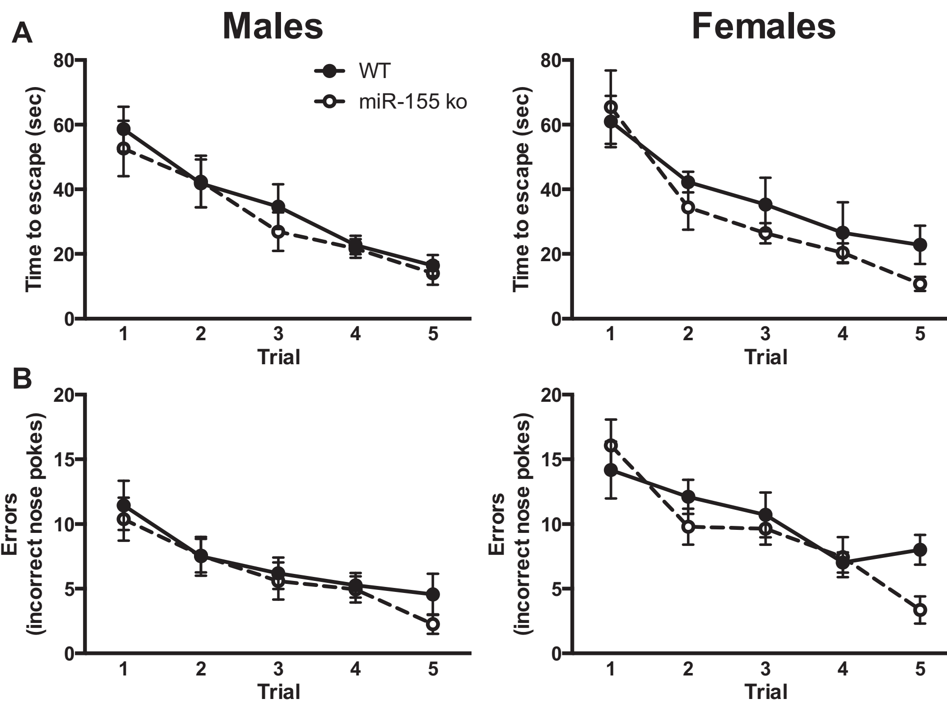


**Fig. 3.** There were no differences in a hippocampal dependent learning and memory test. (A) Time to locate the escape box in the acquisition trials of the Barnes maze; (B) number of errors made in acquisition trials. Data are expressed as mean ± SEM.

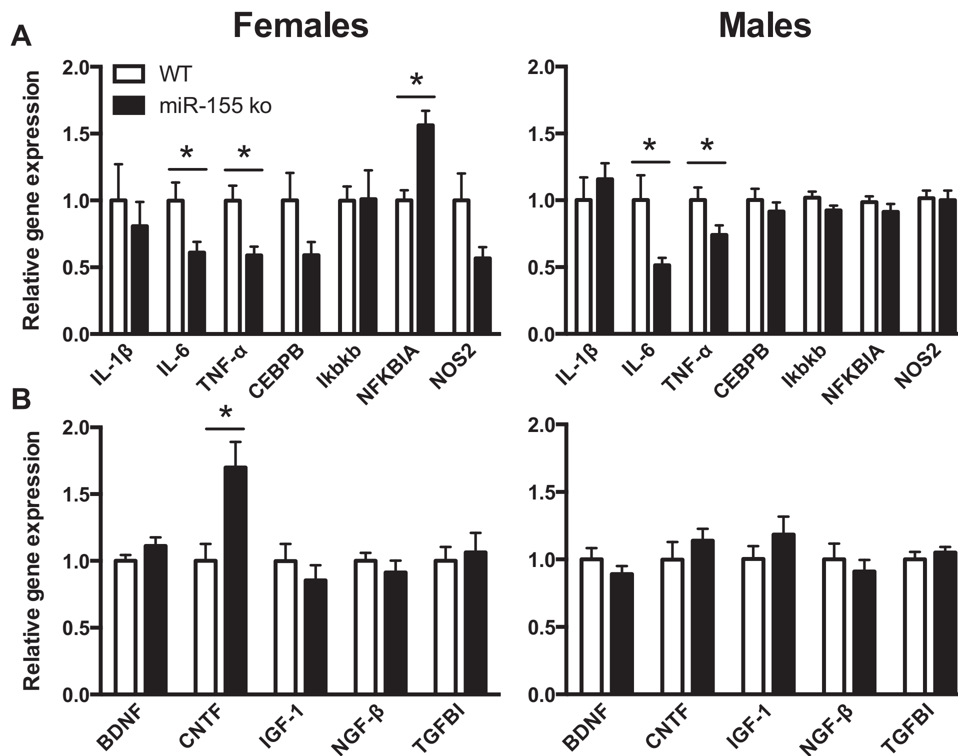


**Fig. 4.** Hippocampal mRNA expression of inflammatory and neurotophic factors. (A) Relative mRNA expression of inflammatory genes; (B) relative mRNA expression of neurotrophic factors. Genes are expressed relative to 18S and data are presented as mean ± SEM, $^{*}p < 0.05$.

increases survival of embryonic rat hippocampal neurons (Ip et al., 1991) and protects several neuron types from cytotoxic or physical insults (Hagg et al., 1992; Masu et al., 1993; Mitsumoto et al., 1994; Anderson et al., 1996; Zala et al., 2004; Pun et al., 2006; Simon et al., 2010; Kang et al., 2012). Further, CNTF ko mice increase depressive-like behavior (Peruga et al., 2012). These published data suggest that increasing CNS CNTF levels may improve neuroprotection and plasticity; however, the efficacy of systemic CNTF is limited by its low blood-brain barrier permeability and adverse side effects (Thoenen and Sendtner, 2002). Our data suggest that

CNTF upregulation may be achieved in female mice by administering miR-155 selective antagonists. Of note, we focused our search on mRNAs related to inflammation and neuroplasticity; miR-155 also affects a number of target genes outside of this context (for example, searching microrna.org for "mmu-miR-155" reveals 4567 transcripts potentially regulated by miR-155). The capacity of miRs to modulate multiple transcripts is both a potential benefit (two pathways can be simultaneously targeted) and limitation (there may be deleterious off target affects) to using miRs therapeutically.

Despite the therapeutic potential of modulating microRNAs, our understanding of how these small molecules may influence mood disorders is in its infancy. A Pubmed search (June 2015) of "microRNA depression" returned only 142 results. Many of these published studies correlate microRNA regulation with depressive-like symptoms, thereby identifying potential microRNA biomarkers of depression. Indeed, Wan and colleagues reported that miR-155 was increased in the cerebrospinal fluid of patients with major depressive disorder (compared to control patients; collected by lumbar puncture) (Wan et al., 2015). These clinical findings parallel our animal study, and provide further evidence that miR-155 may be a valuable target for alleviating symptoms of depression.

Reduced depressive- and anxiety-like behaviors in miR-155 ko mice may suggest a manic state. This has been argued in other knockout mice that display reduced anxiety and depressive behaviors (Roybal et al., 2007; Saul et al., 2012). However, miR-155 ko mice lack several key behaviors that are associated with mania including impairments in learning and memory (Su et al., 2013), hyperlocomotion (Roybal et al., 2007; Kulak et al., 2012; Saul et al., 2012), and altered social behaviors (Kulak et al., 2012). Furthermore, reductions in depressive-like responses have been reported in other models that are not postulated to display manic-like behavior. For example, both environmental enrichment and fluoxetine treatment can reduce depressive-like responses in otherwise unmanipulated rodents (e.g. in the absence of stress, injury, etc) (Nicolas et al., 2015; Olivares-Nazario et al., 2015). Changes in affective responses may also indicate modulation of the hypothalamic–pituitary–adrenal (HPA) axis in miR-155 ko mice. Interestingly, miR-155 has been implicated in regulating circadian rhythms (Curtis et al., 2015); however, it is unknown whether miR-155 affects diurnal rhythms in glucocorticoids or the HPA axis.

Behavioral tests were only performed once per animal so future experiments should focus on replicating (an important concern in animal behavior studies see (Bertuzzi et al., 2012)) and expanding the results. Testing both male and female mice did provide an internal replication in this experiment. While male and female miR-155 ko mice tended to have comparable magnitudes of behavioral change compared to wt mice on tests for affective behaviors (there were no interactions between sex and genotype), sex differences were observed in the forced swim test and elevated plus maze. First, male mice floated more in the forced swim test and reduced latency to first float. These results agree with previously observed sex differences in floating behavior in mice (for example see (Bhatnagar et al., 2004; Workman et al., 2011)), although sex differences in floating behavior may be strain- and species-dependent (female rats tend to show increased floating) (Voikar et al., 2001; Dalla et al., 2010). Second, female mice increased time spent in the open arms of the elevated plus maze but demonstrated a similar percentage of open arm entries compared to male mice. Other groups have reported similar findings in mice (Zimmerberg and Farley, 1993; Voikar et al., 2001; Brydges et al., 2014). Finally, while both male and female mice showed significant levels of 'sociability' in the social preference test, only male mice demonstrated a preference for 'social novelty'. A preference for social novelty is not present in all mouse strains e.g., Moy et al. report 6 out of 10 strains show preference for social novelty (Moy et al., 2007). An earlier study by Moy et al., did report a preference for social novelty in both male and female C57 mice (Moy et al., 2004). It is possible that these contrasting results are due to subtle differences in the design of the test or the background of the miR-155 strain (originally derived from a 129 X C57B6 cross).

Overall, these data implicate miR-155 in the regulation of affective behaviors in mice. Both male and female miR-155 knockout mice decreased anxiety- and depressive-like responses without exhibiting other key symptoms of mania. Future studies should establish whether deleting miR-155 is protective against the development of depressive-like responses in animal models of depression (e.g., following chronic stress). When available, administration of a miR-155 inhibitor should be tested to determine whether it produces similar changes in anxiety- and depression-like responses. Combined with our data, such results could lead to development of novel therapies for depression and other mood disorders.

## Conflict of interest

The authors declare no competing financial interests. All authors concur with the submission of this manuscript and none of the data have been previously reported or are under consideration for publication elsewhere.

## Contributors

L.K.F., A.D.G., R.J.N., and P.G.P. designed experiments. L.K.F., A.D.G., and K.R.G. performed experiments. L.K.F. and A.D.G. analyzed data. All authors contributed to writing and editing the manuscript.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.10.019.

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