The long-lasting effects of early life adversities are sex dependent: the signature of miR-34a

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Abstract

Background. Exposure to early life adversities (ELA) can influence a plethora of biological mechanisms leading to stress-related disorders later in life through epigenetic mechanisms, such as microRNAs (miRs). MiR-34 is a critical modulator of stress response and stress-induced pathologies and a link between ELA and miR-34a has been reported.

Methods. Here using our well-established model of ELA (Repeated Cross Fostering) we investigate the behavioral long-term effects of ELA in male and female mice. We also assess basal and ELA-induced miR-34a expression in adult mice and investigate whether ELA affects the later miR-34a response to adult acute stress exposure across brain areas (medial preFrontal Cortex, Dorsal Raphe Nuclei) and peripheral organs (heart, plasma) in animals from both sexes. Finally, based on our previous data demonstrating the critical role of Dorsal Raphe Nuclei miR-34a expression in serotonin (5-HT) transmission, we also investigated prefrontal-accumbal 5-HT outflow induced by acute stress exposure in ELA and Control females by *in vivo* intracerebral microdialysis.

Results. ELA not just induces a depressive-like state as well as inducing changes in miR-34a expression, but also alters miR-34a expression in response to adult acute stress exclusively in females. Finally, altered DRN miR-34a expression is associated with prefrontal-accumbal 5-HT release under acute stress exposure in females.

Limitations. A translational study on humans is necessary to verify the results obtained in our animal models of ELA-induced depression.

Conclusions. This is the first evidence showing long-lasting sex related effects of ELA on brain and peripheral miR-34a expression levels in an animal model of depression-like phenotype.

Introduction

Early life adversities (ELA) include a broad range of negative experiences ranking from psychological or physical abuse to alteration of attachment bond and adverse events in the family setting (Dong et al., 2004; Ports et al., 2020; Berman et al., 2022). ELA has been reliably associated with several health problems across lifespan (Afifi et al., 2017; McCrory and Viding, 2015; Gerin et al., 2019), including heart disease (Nilsson et al., 2008) and cancer (Kelly-Irving et al., 2013). It has also the potential to cause widespread alterations in brain function that persist over the lifetime increasing the vulnerability to the expression of psychopathologies (Babicola et al., 2021; Bick and Nelson, 2016; Gapp et al., 2014; Cattane et al., 2019; Torres-Berrío et al., 2019; Bale, 2014; Luoni and Riva, 2016; Provençal and Binder, 2015; Cattaneo et al., 2020; Carr et al., 2013; Allen and Dwivedi, 2020; Serafini et al., 2021; He et al., 2020), through epigenetic programming (Allen and Dwivedi, 2020; Dwivedi et al., 2021).

Among epigenetic mechanisms, microRNAs (miRs) have recently been implicated in several mental illnesses induced by ELA exposure (Gapp et al., 2014; Cattane et al., 2019; Allen and Dwivedi, 2020; McKibben and Dwivedi 2021; Dwivedi et al., 2021; Serafini et al., 2021; Van Der Auwera et al., 2019). MiRs are small noncoding RNA molecules that regulate the stability and translational efficiency of target messenger RNAs. As

master regulators of gene expression (McKibben and Dwivedi 2021; Issler and Chen, 2015; O'Connor et al., 2016), miRs have the potential to fine-tune different biological pathways favoring (or not) organism's adaptation to stressful events (Lacal and Ventura, 2018; McKibben and Dwivedi 2021; Roy et al., 2020; Zurawek and Turecki, 2021). To note, large subsets of miRs are evolutionarily conserved between rodents and humans (McKibben and Dwivedi 2021), and peripheral miRs levels are currently being investigating as putative biomarkers of illnesses in clinical and preclinical studies (Serafini et al., 2021; Van Der Auwera et al., 2019; Jeyaseelan et al., 2008; Liu et al., 2010; Sorensen et al., 2014; Zurawek and Turecki, 2021; Bocchio-Chiavetto et al., 2013).

Many ELA-related diseases are reported to have a different incidence in females and males (Keller and Roth, 2016; Seney and Logan, 2021; Bale, 2006; Murphy et al., 2017) and, notably, epigenetic modifications (Alyamani and Murgatroyd, 2018) are increasingly being recognized as critical to understand sex differences in brain development and response to early environment (Keller and Roth, 2016). In addition, sex was recently suggested to be a key factor for understanding both miR and gene expression changes induced by ELA in the brain (Morgan and Bale, 2012; Sharma and Eghbali, 2014; Labonté et al., 2017; Barko et al., 2019; Brivio et al., 2020; Parel and Peña, 2022; McKibben and Dwivedi, 2021). However, surprisingly, studies investigating modifications in miRs expression as a result of ELA experience (Allen and Dwivedi, 2020; Mazzelli et al., 2020; Cattaneo et al., 2020) have focused almost exclusively on males, with a few exceptions (McKibben and Dwivedi, 2021; Cattaneo et al., 2020), thus lacking sex differences in the miRs and ELA literature (McKibben and Dwivedi, 2021).

Overall, our understanding of how miRs contributes to sex related changes in diseases induced by ELA is so far extremally limited (McKibben and Dwivedi, 2021).

Members of the miRNA-34 (miR-34) family are critical modulators of stress response and stress-induced psychopathologies (Issler and Chen, 2015; O'Connor et al., 2012; Haramati et al., 2011; Bocchio-Chiavetto et al., 2013; Andolina et al., 2016; 2018; Lo Iacono et al., 2020a; Sun et al., 2016; Zurawek and Turecki, 2021). We have previously unrevealed the role of miR-34a within the prefrontal-amygdala brain circuit in modulating stress-coping behaviors (Andolina et al., 2016, 2018; Lo Iacono et al., 2020a), in vulnerability to depression-like phenotype (Lo Iacono et al., 2020a) and in response to fluoxetine treatment in mice (Lo Iacono et al., 2021a). In addition, our recent paper suggests the involvement of miR-34a in cardiovascular disease induced by stress exposure (Andolina et al., 2021).

Interestingly, a recent study showed that reduced miR-34a peripheral expression correlated with the total amount of adverse childhood experiences in depressed patients (Lo Iacono et al., 2020b), and the link between the amount of adverse childhood experiences and miR-34a has been also reported in human and mouse sperm (Dickson et al., 2018). Overall, although there is overwhelming evidence supporting the role of miR-34a in stress response and stress-related pathologies in adulthood (Haramati et al., 2011, Andolina et al., 2016; 2018), only few studies have investigated its role in the long-lasting consequences of ELA (Dickson et al., 2018; Wang et al., 2021) and so far, no study investigated sex-related differences in peripheral and brain basal miR-34a levels as well as ELA-induced expression changes.

Using our well-established paradigm of ELA (Repeated Cross Fostering (RCF)) to model the depression-like behavior (D'Addario et al., 2021; Di Segni et al., 2016; Lo Iacono et al., 2021b; Luchetti et al., 2021) in DBA/2J, here we assessed both basal and ELA-induced miR-34a expression in adult male and female mice.

Moreover, we also investigate whether ELA affects the later miR-34a expression in response to adult acute stress exposure across brain areas and peripheral organs in animals from both sexes. Specifically, we evaluated miR-34a expression in: (a) medial preFrontal Cortex (mpFC) and Dorsal Raphe Nuclei (DRN), two brain regions playing a main role in stress response as well as in functional alterations associated with stress-related pathologies (Andolina et al., 2016, 2018; Ventura et al., 2006; 2013; Di Segni et al., 2014; 2016); (b) heart, in which miR-34a is highly expressed (Conception et al., 2012) and sensitive to stress exposure (Andolina et al., 2021) and (c) plasma, in order to investigate the possibility to use miR-34a as a peripheral biomarker of ELA effects. In addition, to challenge the interconnection between different system, the levels of miR-34a across different brain areas (mpFC, DRN) and peripheral levels (heart, plasma) were correlated. Finally, based on previous data demonstrating the critical role of DRN miR-34a expression in serotonin (5-HT) transmission (Andolina et al., 2016; 2018, Lo Iacono et al., 2020a), we investigated the stress-induced 5-HT outflow in some projecting areas of DRN mpFC and nucleus accumbes (NAc)) which are known to be involved in the stress response in ELA and Control mice by *in vivo* intracerebral microdialysis.

Methods

Animals

DBA2/J (DBA) male and female mice (purchased when 6/7 weeks old from Charles River, Italy) were mated when 12 weeks old. Mating protocol consisted in housing 2 females with 1 male in transparent polysufone cages (26.7 × 20.7 × 14.0 cm) with water and food available ad libitum. Room temperature (21 ± 1 °C) and a 12:12 h light–dark cycle (lights on at 07.00 PM) was kept constant. After 15 days, males were removed and pregnant females were isolated, left in clean cages, and inspected twice a day for live pups. Adequate measures were taken to minimize pain or discomfort of mice and all experiments were carried out in accordance with Italian national laws (DL 116/92 and DL 26/2014) on the use of animals for research based on the European Communities Council Directives (86/609/EEC and 2010/63/UE). Experimental protocol (no. 769/2017) was approved by Italian Ministry of Health. Mice 10–12 weeks old were used for both behavioral and molecular experiments.

Early Life Adversity (ELA): Repeated Cross Fostering procedure

As previously described (D'Amato et al., 2011; Ventura et al., 2013; D'Addario et al., 2021; Lo Iacono et al., 2021b), litters born on the same day spent the first postnatal day (PND0) with their biological mother. On PND1, litters were randomly assigned to experimental (Repeated Cross Fostering, ELA groups) or control (Cont) condition. From PND1 to PND4 pups of the same litter was moved into the home cage of different dams whose pups had just been removed and moved to a different adoptive mother and pups were left until weaning (PND28) with the last adoptive mother. In the Cont group, pups of the same litter were left together from PND0 until weaning and were only picked up daily and reintroduced in their home cage. At weaning, animals were separated by sex and housed in group of 4 littermates. All experiments were performed on 3-month-old adult mice. Different cohorts were used for behavior, molecular and neurochemistry experiments. To prevent potential estrous cycle group synchronization, experimental subjects for cross-fostered and control female groups were sorted by collecting not > 2 individuals per cage/ litter (Ventura et al., 2013; Di Segni et al., 2016; 2017).

Acute stress protocol

Adult (PND 90) ELA and Cont animals from both sexes were randomly assigned to "basal" or "adult acute stress" (AAS) condition; in the basal condition mice were extracted from their home cage and immediately sacrificed, while in the acute stress condition, mice were sacrificed 1 hour after the end of AAS experience (restraint, 2 hours) (Cabib et al., 1997; Di Segni et al., 2016).

Behavioral test

Saccharin Preference Test (SPT)

Saccharin solution was prepared using water and pure saccharin. Saccharin Preference Test (SPT) consisted of a habituation day and an experimental phase, as previously described (Ventura et al., 2013; Di Segni et al., 2016; D'Addario et al., 2021). During the habituation day, animals were moved into their own test cages with two bottles containing water (100 mL); after 24 hours they were exposed to a double choice drinking test (saccharin solution 0.5% or drinking water) from graduated tubes (100 mL volume) and intake was measured to the nearest 1 mL. The test cage was the same throughout the experiment. Only mice drinking at least 1 mL on day 1 were included in the study. The percentage of saccharin intake (saccharin intake (ml) *100/saccharin + H 20 intake (ml)) was evaluated.

Forced Swimming Test (FST)

As previously described (Ventura et al., 2013; Di Segni et al., 2016), mice were individually placed in a 18 cm diameter glass cylinder (height 40 cm) containing 20 cm water at 28 ± 2°C.

The behavior exhibited by each animal during the experimental procedure was video-recorded for 10 minutes before returning the mice in their home cage by a video camera placed frontally. The duration (s) of immobility (total absence of movement) was manually scored using EthoVision (Noldus, The Nederlands) by a trained observer blind to the animals' treatment.

Tail suspension test (TST)

Each mouse was suspended by the tail at 60 cm above the ground in a white plastic chamber using adhesive tape placed d < 1 cm from the tip of the tail according to Yan et al., (2015). Behavior was recorded for 10 min using a digital camera placed frontally. The duration of immobility was manually scored using the "EthoVision" software (Noldus, The Netherlands) by a trained observer blind to the animals' treatment. Immobility was defined as the period in which the animal stopped struggling for ≥1 s.

Brain, heart and plasma tissue collection

Brain tissue micro-punches of mpFC and DRN were obtained from frozen brain slices (coronal sections; according to the atlas of Paxinos and Franklin (Paxinos and Franklin, 2019) not thicker than 300 µm using stainless-steel tubes of 1.0 mm inner diameter and stored at -80 °C until the day of the assay. Heart tissue micro-punches were obtained from frozen whole heart using stainless-steel tubes of 1.0 mm inner diameter and stored at -80 °C until the day of the assay (Andolina et al., 2021). For plasma collection, blood samples were collected after decapitation and immediately centrifugated (10 minutes, 1500 rcf, 4 °C). The supernatant was taken and stored at -80 °C until the day of the assay.

RNA extraction and Quantitative real-time RT-PCR (qPCR)

qPCR was performed for the evaluation of miR-34a levels in mpFC, DRN, heart and plasma of Cont and ELA female and male DBA mice, both in basal and following AAS exposure (restraint stress).

RNA from punches of mpFC, DRN, and heart as well as from plasma samples was extracted using microcolumn from Total RNA purification Plus Kit (Norgen Biotek, Canada) and RNA quantity was determined by absorbance at 260 nm using a NanoDrop UV-VIS spectrophotometer. Complementary DNA (cDNA) was obtained using the High-Capacity Reverse Transcription kit (Applied Biosystems, USA). cDNA samples (1,66 ng) were amplified by qPCR using the 7900HT thermal cycler apparatus equipped with SDS software version 2.3 (Applied Biosystems, AB) for data collection. Each data point was obtained from 5 to 6 biological replicates. Ct values were normalized to measures of SNO135 for brain and heart punches, and of miR-39 (C. elegans) for plasma samples, to achieve ΔCt values. Relative expression was calculated as 2^-ΔCt. All data were run in triplicate and expressed as means ± SE.

In vivo microdialysis

The microdialysis experiment in mpFC and NAc was carried out as previously described (Di Segni et al., 2016). Animals were anesthetized with Zoletil and Rompun and NAc probe implantation was counterbalanced in either the right or left hemisphere. Vertical concentric dialysis probes were prepared with AN69 fibers (Hospal Dasco, Bologna, Italy) (Andolina et al., 2016). The coordinates from bregma (measured according to the atlas of Paxinos and Franklin (2019) and Mouse Brain Atlases, The Mouse Brain Library, www.nervenet.org) were: mpFC, 2.0 AP, ±0.6 L; NAc, 1 AP; ±0.6 L. Different probe length was used for mpFC and NAc (mpFC: 3 mm shaft length, dialysis membrane length 2 mm o.d. 0.24 mm; NAc: 4.0 mm shaft length, dialysis membrane length 1mm o.d. 0.24 mm). The final depth of the probe was 3 mm for mpFC and 5.0 mm NAc ventral to the skull surface, respectively.

The day before use, the membranes were tested to verify in vitro 5-HT recovery. The UHPLC system consisted of an UltiMate 3000 (Thermo Fisher Scientific S. p.A. Italy) system and a coulometric detector (UltiMate 3000 ECD-3000RS) provided with an analytical cell (6011RS ultra Coulometric Cell). The electrode 1 was set at 100 mV, and the electrode 2 at 250 mV. A C18 column (ACCLRSLC PA2 2.2U2.1X100, Thermo Fisher Scientific S. p.A. Italy) maintained at 35 °C was used coupled with a Sentry Guard pre-column (ACCLAIM, V-2 GUARD). The flow rate was 0.25 ml/min. The mobile phase consisted of 3% methanol in 0.1 M Na phosphate buffer, 1.3 mM Na2 EDTA, 0.34 mM 1-octane sulfonic acid Na salt (Sigma Aldrich, USA), pH 3.5. The detection limit of the assay was 0.1 pg. Experiments were carried out 48 h after probe placement. The mean concentration of the 3 samples collected immediately before treatment (< 10% variation) was taken as baseline concentration. Following, Cont and ELA mice were restrained. Dialysate was collected every 20 min for 120 min. Twenty microliters of the dialysate samples were analyzed by ultra-high-performance liquid chromatography (UHPLC). The remaining 20 µL were kept for possible subsequent analysis. Concentrations (20 pg/µL) were not corrected for probe recovery. Each experimental group was composed by 7-9 animals.

Probe placement

At the end of the experiments, mice were sacrificed. Brains were post-fixed overnight in PB 4% paraformaldehyde solution to check the correct probe placement by visual inspection of the probe tracks on Nissl-stained coronal sections (40 µm). Only mice with correct probe placement in mpFC and NAc were

considered in the results.

Statistics

One sample Student's *t* -test was performed for the time spent in immobility during FST and TST and for percentage of saccharin intake in SPT for each sex.

One sample Student's *t* -test was used for the relative expression of miR-34a in baseline within each brain structures (mpFC and DRN), and peripheral tissue (heart and plasma) for each sex as well as to compare male and female miR-34a expression. Two-factor ANOVA was used to compare miR-34a induced by AAS exposure in ELA and Cont mice (pretreatment, two levels: Cont, ELA; treatment, two levels: unstressed, AAS) within each sex. For microdialysis experiment, percent (%) changes from basal levels were analyzed by repeated measures ANOVAs with one between factor (pretreatment, 2 levels: Cont, ELA) and one within factor (time, 7 levels: 0, 20, 40, 60, 80, 100, 120). Individual between-groups comparisons were performed, when appropriate, by post hoc test (Fisher's LSD).

Principal component analysis (PCA)

To investigate the association among brain structures and peripheral tissues with regards to the miR-34a expression, a Principal Component Analysis (PCA) was applied on the entire dataset. The Bartlett's test of sphericity was used to test whether the correlation matrix deviates from an identity matrix, and thus could be meaningfully subjected to PCA. The Scree test was used to determine the number of components to be extracted.

The extracted components were then varimax rotated to preserve their statistical independence. The estimated factor scores on each of the rotated principal components were estimated through the regression method, and then analyzed in a sex (male vs female) by pre-treatment (control vs RCF) by treatment (baseline vs stress) ANOVA design.

Results

ELA affects behavior in a sex dependent manner

ELA affects the behavior phenotype exclusively in female mice. ELA female mice show a significant increase of immobility in FST ($t(14)=2.378$; $p< 0.05$) and TST ($t(12)=2.992$; $p<0.05$), and a reduced sucrose consumption ($t(12)=4.151$; $p<0.01$) in SPT (Fig. 1 A,C,E) in comparison with Cont females. No significant difference between ELA and Cont was evident in male mice (FST ($t(12)=0.244$; n.s.); TST ($t(10)=0.545$; n.s.); and SPT ($t(12)=0311$; n.s.) (Fig. 1 B,D,F)).

Heart and plasma miR-34a basal expression is higher in females than males

No significant difference in the level of miR-34a expression is found between sexes in mpFC ($t(9)=0.273$; n.s.) (Fig. 2A), and a not significant trend is evident in DRN ($t(9)=1.967$; $p=0.08$) (Fig. 2B). However, miR-34a expression is significantly higher in the heart ($t(8)=2.776$; $p<0.05$) (Fig. 2C) and plasma ($t(8)=2.346$; $p<0.05$) of females than males (Fig. 2D).

ELA affects miR-34a expression exclusively in females

We test whether ELA (pretreatment) and the AAS (treatment) exposure could alter miR-34a expression in a sex-dependent fashion. Concerning female data, two-way ANOVA shows no significant pretreatment x treatment interaction for mpFC ($F(1, 17)=0,6309$; $p=0,4380$). However, a significant effect of pretreatment ($F(1, 17)=6,726$; $p<0.05$) and treatment ($F(1, 17)=7,463$; $p<0.05$) is evident. T-test analysis reveals a significant increase of miR-34a in ELA in comparison with Cont female mice ($t(9)=3.07$; $p<0.05$), and a significant increase of miR-34a in Cont exposed to adult acute stress (Cont+AAS) compared to unstressed Cont females ($t(8)=2.838$; $p<0.05$) (Fug. 3A). A significant pretreatment x treatment interaction is evident for DRN ($F(1,17) =6,755$; $p<0.05$). Post hoc analyses reveals significantly higher miR-34a levels in ELA female mice in comparison with Cont females ($t(17)=2.857$;$p<0.05$) and a significant decrease in stressed ELA (ELA+AAS) in comparison with unstressed ELA females ($t(17)=2.340$;$p<0.05$) (Fig. 3B). No significant pretreatment x treatment interaction is evident for heart ($F(1, 16)=1,196$; $p= 0,29$) (Fig. 3 C). A significant pretreatment x treatment interaction is evident for plasma ($F(1, 16)=12,16$; $p<0.01$). Post hoc analysis reveals significantly higher miR-34a levels in ELA female mice compared to Cont ($t(16)=4.04$;$p<0.001$), a significant reduction of miR-34a in ELA+AAS in comparison with unstressed ELA ($t(16)=2.834$;$p<0.05$), and a not significant trend for increased miR-34a levels in Cont+AAS female mice compared to unstressed Cont ($p=0.052$) (Fig. 3D). Differently from females, miR-34a levels are unaltered by both ELA and AAS exposure in male mice. Two-way ANOVA shows no significant pretreatment x treatment interaction for mpFC ($F(1,18)=0.593$;$p=0.451$) (Fig. 3E), DRN ($F(1,18)=0.334$;$p=0.57$) (Fig. 3F), heart ($F(1,18)=0.433$;$p=0.518$) (Fig. 3G) and plasma ($F(1,17)=0.001$;$p=0.972$) (Fig. 3H).

Prefrontal-accumbal 5-HT release induced by adult acute stress in female mice

We measured 5-HT release in mpFC and NAc during the AAS in control and ELA female mice. Repeated measures ANOVA shows a significant pretreatment × time interaction ($F(6,84)=4.132$; $p<0.01$) for mpFC. Post hoc analysis indicates increased 5-HT outflow in Cont mice at time 20 and 40 min, and decreased 5-HT release in ELA female mice at time 20 min. Moreover, a significant difference between ELA and Cont mice is evident at 20, 40, 100 and 120 min (Fig. 4A). No significant difference was evident for basal levels (Fig. 4B). Repeated measures ANOVA for NAc data shows a significant pretreatment × time interaction ($F(6,72)=2.385$; $p<0.05$). Post hoc analysis indicates a significant increase of 5-HT release in Cont mice at 20, 40, 80 and 100 min in comparison with basal levels. No significant effect is evident in ELA mice. Moreover, a significant difference between ELA and Cont mice is evident at 20 and 40 min (Fig. 4C). No significant difference was evident for basal levels (Fig. 4D).

Principal Component Analysis (PCA)

The Bartlett's test of sphericity suggested that the correlation matrix is significantly different from an identity matrix ($X26=16.245$, $p=.012$). The Scree test (Eigenvalues = 1.780, .961, .772, .487) suggested a two-component solution that, after being varimax-rotated, accounted for the 68.5% of the total variance. The first component, accounting for the 34.4% of the total variance, was significantly saturated by the miR-34 expression in DRN and Plasma (Table 1); the second component, accounting for the 34.1% of the total variance, was significantly saturated by the miR-34a expression in the mpFC and Heart (Table 1).

The ANOVA on the factor scores on the first component (Table 2), reflecting the miR-34 expression in the

DRN and plasma, showed a significant main effect of sex (F1,32=4.574, p=.04, observed power=.546), due to the scores on the first component being higher in the female animals than in the male animals. Also, the pretreatment by treatment (F1,32=7.325, p=.01, observed power=.747) and the sex by pretreatment by treatment (F1,32=5.206, p=.03, observed power=.6) interactions were significant. The second order interaction was due to pretreatment by treatment interaction being different for female and male animals.

For the female animals in the control condition there was no difference between the factor scores on the first component between the baseline and the stress conditions (F1,10=3.361, p=.104), whereas in the RCF condition the factor scores on the first component were significantly smaller in the stress condition compared to the baseline (F1,10=8.5, p=.019) (Fig. 5A).

For male animals in the control condition there was no difference between the factor scores on the first component between the baseline and the stress conditions both in the control condition (F1,10=.348, p=.572), and in the RCF condition (F1,10=.004, p=.95) (Fig. 5B).

The ANOVA on the factor scores on the second component, reflecting the miR 34 expression in the mpFC and heart, showed significant main effects of sex (F1,32=25.924, p<.001, observed power=.99), and treatment (F1,32=6,773, p=.01, observed power=.714). The main effect of sex was due to the scores on the first component being higher in the female animals than in the male animals. The main effect of treatment was due to the scores on the second component being higher in the stress condition than in the baseline condition (Fig. 5B,D). Also, the sex by treatment (F1,32=6.690, p=.01, observed power=.708) interaction was significant. This interaction was due to the female animals in the stress condition showing higher scores than female animals in the baseline condition (F1,18=8,996, p=.007). whereas there was no difference between male animals in the baseline and stress condition (F<1).

Discussion

Using our well validated model of ELA, here we confirm the pro-depression behavioral effects of RCF exclusively in female mice, as previously reported (Di Segni et al., 2016; Lo Iacono et al., 2021b).

Most importantly, our results provide the first evidence of sex related differences of miR-34a expression in basal condition and following ELA exposure across different tissues. ELA exposure not just produces enduring changes in miR-34a expression in a sex-dependent manner, but also alters the modulation of miR-34a expression that occur in response to adult acute stress. Finally, altered miR-34a expression in DRN is functionally associated with prefrontal-accumbal 5-HT release under acute stress exposure in females.

First, we show a differential basal expression of miR-34a through two (DRN, plasma) of the four tissues investigated between males and females, with females showing higher miR-34a expression in plasma and heart and male mice higher expression in DRN. This is, to our knowledge, the first evidence of a different basal sex-related miR-34a expression.

MiRs are important regulatory molecules of several physiological processes, such as cell growth and differentiation, metabolism, morphogenesis, maturation, and apoptosis (Kaurich et al., 2013; Li et al., 2018; Zhang et al., 2009; Giraldez et al., 2005; Poy et al., 2004). Because males and females of inbred strains do not vary in genetic sequences except for the Y chromosome, the transcriptional regulatory action of the miRs could play an important role in sex-specific differences (Sharma and Eghbali, 2014; Morgan and Bale, 2012; Warnefors et al., 2017). Sex hormones and sexual chromosomes-linked genes can have an influence in the biogenesis of miRs. Males and females differ for gonadal steroid production, such as dihydrotestosterone, progesterone, and estradiol. These neurohormones regulate genes expression through nuclear hormone receptors, which are ligand-activated transcription factors (Kininis and Kraus, 2008; Pandey and Picard, 2010; Bethke et al., 2009). This suggests that they can also regulate the expression of miRs, directly by binding their promoter elements, or indirectly regulating downstream target genes that, in turn, can induce or repress miRs expression (Yang and Wang, 2011). In fact, hormones like estradiol can regulate the ribonuclease Drosha and Dicer, that are part of a protein complex regulating miRs biogenesis and maturation (Gregory et al., 2004; Bhat-Nakshatri et al., 2009). Therefore, basal differences in miR-34 expression between sexes may represent an important factor helping to elucidate some mechanisms underlying different neurobiological processes in males and females. Interestingly, sex dimorphism is observed in a wide range of human diseases, such as immune, cardiovascular or neurodegenerative pathologies, as well as in psychopathological conditions like depression (Dorak and Karpuzoglu, 2012; Maas and Appelmen, 2010; Whitacre, 2001; Eid et al., 2019).

Second, here we demonstrate that ELA affects the long lasting miR-34a expression exclusively in female mice. We have previously demonstrated that exposure to an early-life adverse experience interfering with the mother-pups attachment bond development (RCF (ELA)) induces in DBA female mice increased long-term passive coping strategy and depression-like state (Di Segni et al., 2016; Lo Iacono et al., 2021b). Here we confirm these data, showing that ELA impacts adult coping strategy selectively in females, while no effect is evident in males. Interestingly, a parallel effect of ELA is evident on miR-34a expression exclusively in females. Notably, epigenetic modifications (Alyamani and Murgatroyd, 2018) are recognized to be critical factors to understand sex differences in brain development and response to early environment (Keller and Roth, 2016), and sex has been recently suggested to be a key actor for shedding light on both miRs and gene expression changes induced by ELA in the brain (Labonté et al., 2017; Barko et al., 2019; Brivio et al., 2020; Parel and Peña, 2022;

McKibben and Dwivedi, 2021). However, surprisingly, only few studies investigated sex differences in miRs expression (McKibben and Dwivedi, 2021; Cattaneo et al., 2020) in animals precociously exposed to aversive life events (McKibben and Dwivedi, 2021; Cattaneo et al., 2020), thus currently lacking sufficient reports regarding sex differences in the miRs-ELA literature (McKibben and Dwivedi 2021).

MiR-34, and specifically a and c isoforms, are involved in adult stress response and stress-related behaviors (Haramati et al., 2011; Andolina et al., 2018). Moreover, a recent study reported an association between exposure to early life stressful events and sperm levels of different members of miR-34 family, both in animals and humans (Dickson et al., 2018). In addition, peripheral miR-34a expression in depressed adults correlates with the number of negative events (neglect/abuse) experienced in childhood (Lo Iacono et al., 2020b), thus suggesting the possibility that miR-34 represents a biomarker of early adversities. Unfortunately, no study has to date investigated sex related differences in miR-34a following ELA exposure. Here we find, for the first time, increased central (DRN, mpFC) and peripheral (plasma and a not significant trend in hearth) miR-34a levels exclusively in ELA females. This pattern seems to be related to depressive-like phenotype shown by ELA female mice (Di Segni et al., 2016). In support of this hypothesis, we find no alterations in miR-34a expression in ELA males which, accordingly, don't show a depressive-like state (Lo Iacono et al., 2021b and present data). Overall, these data point to the potential role of miR-34a as a sex-related biomarker of depressive-like state induced by ELA.

Third, similarly to ELA, exposure to acute adult stress impacts miR-34a expression exclusively in females DBA mice as demonstrated by higher expression of miR-34a in mpFC and plasma of Control females subjected to restraint stress (stressed) compared to unstressed females. Again, adult stress exposure does not induce alterations in miR-34a levels in males. These results, together with data from ELA experiments, strongly suggest miR-34a as a sex-related factor of stress effects 'vulnerability.

In addition, our data indicate that ELA is also able to modulate the subsequent miR-34a response to adult stress exposure in a sex-related manner. In fact, while ELA females respond to adult stress differently compared to Control females, no effect of ELA on later stress exposure is evident in males. MiR-34a expression is increased in mpFC and plasma of stressed Control in comparison with unstressed Control female animals, while a significant reduction is observed in both DRN and plasma of stressed ELA compared to unstressed ELA females. We can speculate that our early manipulation has dysregulated the miR-34a "system" in female mice making females not able to respond later when subjected to a subsequent aversive experience in adulthood. This observation is further supported by functional data by *in vivo* microdialysis experiment. In fact, while Control females respond to adult acute stress (AAS) with increased prefrontal-accumbal 5-HT release, accordingly to literature concerning stress effects on serotoninergic transmission (Andolina et al., 2016; Tao et al., 2017), no significant augmentation is evident in ELA females. These data indicate that reduced DRN miR-34a expression in ELA females is associated to reduced prefrontal 5-HT release under acute stress, according with previous reports showing that genetic deletion of miR-34 family members prevents 5-HT release in mpFC under stress exposure (Andolina et al., 2016). The DRN, the origin of central 5-HT system, plays a pivotal role in stress-induced 5-HT modulation through the complex network of its forebrain projections, including the mpFC (Waselus et al., 2011; Andolina et al., 2018). Interestingly, among the brain regions mostly involved in the stress-related behaviors, the miR-34a displayed the strongest expression in DRN (Andolina et al., 2018). We have also recently investigated a possible mechanism through which miR-34 could contribute to the stress-

induced 5-HT mpFC release (Andolina et al., 2018). The corticotropin-releasing factor receptors (CRFR 1 and 2) are critical in mediating the mpFC-5-HT response to stress (Andolina et al., 2018; Waselus et al., 2011), and the CRFR1 is a target of miR-34a (Haramati et al., 2011). Thus, in a previous study, we have hypothesized that the miR-34a-dependent modulation of CRFR1 expression may be involved in the DRN-5-HT regulation. In line with this hypothesis, we have reported that genetic deletion of miR-34 family in mice leads to increased DRN CRFR1 that, in turn, inhibits the 5-HT-DRN system and blunts prefrontal 5-HT release (Andolina et al., 2018). Finally, several genes that have been identified as miR-34a targets, such as the 5- HT2c receptor (Lo Iacono et al., 2021a), encode for proteins that contribute to modulate 5- HT release (Lo Iacono et al., 2020a). Interestingly, CRFR1 and 5-HT2C in the DRN are expressed in GABAergic neurons and it has been demonstrated that increased activity of these receptors enhances GABAergic response (Waselus et al., 2011; Spoida et al. 2014). Serotonergic activity is finely controlled by local GABA release and increased DRN-GABAergic activity causes an inhibition of serotonin tone (Hernández-Vázquez et al., 2018). Thus, based on this evidence, it is possible to hypothesize that the reduced expression of miR-34a in the DRN induced by RCF induces an increase in the expression of CRFR1 and 5-HT2C enhancing DRN-GABAergic activity. This mechanism would explain the reduced release of prefrontal 5-HT in RCF mice.

In addition, here we show that reduced miR-34a expression in DRN is also associated with decreased accumbal 5-HT release induced by acute stress. 5-HT-DRN neurons projecting to NAc play an important role in motivation interacting with dopamine transmission and regulating its release (Parsons and Justice, 1993). Damped serotonergic responsivity under stress conditions seems to be involved in depressive phenotype (De La Garza and Mahoney, 2004), and chronic antidepressant treatment normalizes accumbal 5-HT-DA interaction as well as depressive behavior in FST (Zangen et al., 2001). Notably, we have previously demonstrated reduced dopamine release in the NAc of adult ELA females exposed to acute stress (Di Segni et al., 2016). It is possible to speculate that the reduced serotonin and dopamine neurotransmission responsivity to adult aversive experiences in NAc contributes to modulate the vulnerability to depressive-like phenotype shown by adult ELA female mice.

Finally, to investigate the association among brain structures and peripheral tissues with regards to the miR-34a expression, a PCA was performed. This analysis strongly reveals correlations between the brain areas/tissues investigated. Two components were extracted: DRN-plasma (factor 1; 34.4 %) and mpFC-heart (factor 2; 34.1%), overall accounting for 68.5 % of the total variance. The DRN-plasma relationship appears specially interesting, allowing us to speculate that peripheral miR-34a expression could be used as peripheral biomarker of brain expression. However, deeper analysis is needed to investigate this possibility in the future.

Interestingly, over the past few years ELA has been established as the major risk factor for major depression, and epigenetic factors are the main candidates for ELA effects. MiRs are global modulators of gene expression and thanks to their role, they have emerged as a key regulator of neural plasticity shaped by environment. Moreover, circulating miRs are attracting interest because of their potential correlation with brain levels and with the pathogenesis of psychiatric disorders. Our data suggest the possibility to use miR-34a plasma levels as peripheral indicators of DRN miR-34a expression, although future experiments are needed to deeply investigate this chance. A translational study on humans is necessary to verify the results obtained in our animal models of ELA-induced depression and to test the possibility to use miR-34a plasma levels as biomarker.

Several clinical studies have highlighted the relationship between miRs alterations and psychiatric conditions.

Genetic association studies reported chromosome deletions, insertions, duplications or copy number variations in region encoding miRs biogenesis-related proteins, as well as specific miRs, that are associated with susceptibility to psychiatric disorders. Moreover, SNPs in miRs biogenesis-related genes or in specific miR-encoding loci have been associated with increased risk for psychiatric disorders (Geaghan and Cairns, 2014; Hommers et al., 2015; Issler et al., 2014; Lopez et al., 2014). Furthermore, studies on circulating miRs have highlighted the possible relationship between the expression of central and peripheral miRs, thus suggesting the possibility to using peripheral miRs as biomarkers.

(Lopez et al., 2014; Rao et al., 2013; Issler and Chen, 2015). The present data support this correlation also pointing to miRs alterations as potential biomarkers of genetic and/or environmental vulnerability to disease expression.

This kind of approach would allow to monitor the neurobiological mechanisms underlying the development of psychiatric conditions, the adherence, and the efficacy of the treatment. Moreover, patients-specific miRs expression profiles could be a useful tool to assess the appropriate pharmacological treatment for a given patient, as part of the personalized medicine approach. Our data support this point of view, showing how sex differences should be taken in consideration in order build up a more accurate diagnosis and effective pharmacological approach.

Overall, despite some limitations mainly related to animal models, our results provide, to our knowledge, the first evidence of sex related differences in both basal and ELA-induced levels of miR-34a across different brain areas and peripheral levels. In addition, we show that ELA modulates the subsequent response of miR-34a to acute stress in adulthood in a sex-related manner.

Uncovering sex-related miRs characteristics specific to ELA and to identify peripheral biomarkers of disease susceptibility will aid in designing more effective, personalized, and preventive treatments (Allen and Dwivedi, 2020; McKibben and Dwivedi, 2021).

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Rossella Ventura designed the study and wrote the manuscript. She also analyzed and interpreted the data.

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References

Afifi, T.O., Ford, D., Gershoff, E.T., Merrick, M., Grogan-Kaylor, A., Ports, K.A., MacMillan, H. L., Holden, G.W., Taylor, C.A., Lee, S.J., Peters Bennett, R. 2017. Spanking and adult mental health impairment: The case for the designation of spanking as an adverse childhood experience. Child Abuse Negl. 71, 24-31. https://doi.org/10.1016/j.chiabu.2017.01.014.

Allen, L., Dwivedi, Y. 2020. MicroRNA mediators of early life stress vulnerability to depression and suicidal behavior. Mol Psychiatry, 25(2), 308-320. https://doi.org/10.1038/s41380-019-0597-8.

Alyamani, R., Murgatroyd, C. 2018. Epigenetic Programming by Early-Life Stress. Prog Mol Biol Transl Sci. 157, 133-150. https://doi.org/10.1016/bs.pmbts.2018.01.004.

Andolina, D., Di Segni, M., Bisicchia, E., D'Alessandro, F., Cestari, V., Ventura, A., Concepcion, C., Puglisi-Allegra, S., Ventura, R. 2016. Effects of lack of microRNA-34 on the neural circuitry underlying the stress response and anxiety. Neuropharmacology. 107, 305-316. https://doi.org/10.1016/j.neuropharm.2016.03.044.

Andolina, D., Di Segni, M., Accoto, A., Lo Iacono, L., Borrelli, A., Ielpo, D., Berretta, N., Perlas, E., Puglisi-Allegra, S., Ventura, R. 2018. MicroRNA-34 Contributes to the Stress-related Behavior and Affects 5-HT Prefrontal/GABA Amygdalar System through Regulation of Corticotropin-releasing Factor Receptor 1. Mol Neurobiol. 55(9), 7401-7412. https://doi.org/10.1007/s12035-018-0925-z.

Andolina, D., Savi, M., Ielpo, D., Barbetti, M., Bocchi, L., Stilli, D., Ventura, R., Lo Iacono. L., Sgoifo, A, Carnevali, L. 2021. Elevated miR-34a expression and altered transcriptional profile are associated with adverse electromechanical remodeling in the heart of male rats exposed to social stress. Stress. 24(5), 621-634. d10.1080/10253890.2021.1942830.

Babicola L, Ventura R, D'Addario SL, Ielpo D, Andolina D, Di Segni M. Long term effects of early life stress on HPA circuit in rodent models. 2021. Mol. Cell Endocrinol. 5,521:111125. 10.1016/j.mce.2020.111125.

Bale, T.L. 2006. Stress sensitivity and the development of affective disorders. Horm Behav. 50(4), 529-33. 10.1016/j.yhbeh.2006.06.033.

Bale, T.L. 2014. Lifetime stress experience: transgenerational epigenetics and germ cell programming. Dialogues Clin Neurosci. 16(3), 297-305. https://doi.org/10.31887/DCNS.2014.16.3/tbale.

Barko, K., Paden, W., Cahill, K.M., Seney, M. L., Logan, R.W. 2019. Sex-Specific Effects of Stress on Mood-Related Gene Expression. Mol Neuropsychiatry, 5(3), 162-175. https://doi.org/10.1159/000499105.

Berman, I.S., McLaughlin, K.A., Tottenham, N., Godfrey, K., Seeman, T., Loucks, E., Suomi, S., Danese, A.,

Sheridan, M.A. 2022. Measuring early life adversity: A dimensional approach. Dev Psychopathol. 22:1-13. 10.1017/S0954579421001826.

Bethke, A., Fielenbach, N., Wang, Z., Mangelsdorf, D.J., Antebi, A. 2009. Nuclear hormone receptor regulation of microRNAs controls developmental progression. Science. 324(5923), 95-98. https://doi.org/10.1126/science.1164899.

Bhat-Nakshatri, P., Wang, G., Collins, N.R., Thomson, M.J., Geistlinger, T.R., Carroll, J.S., Brown, M., Hammond, S., Srour, E.F., Liu, Y., & Nakshatri, H. 2009. Estradiol-regulated microRNAs control estradiol response in breast cancer cells. Nucleic Acids Res. 37(14), 4850-4861. https://doi.org/10.1093/nar/gkp500.

Bocchio-Chiavetto, L., Maffioletti, E., Bettinsoli, P., Giovannini, C., Bignotti, S., Tardito, D., Corrada, D., Milanesi, L., Gennarelli, M. 2013. Blood microRNA changes in depressed patients during antidepressant treatment. Eur. Neuropsychopharmacol. 23(7), 602–611. https://doi.org/10.1016/j.euroneuro.2012.06.013.

Bick, J., Nelson, C.A. 2016. Early Adverse Experiences and the Developing Brain. Neuropsychopharmacol. 41(1), 177–196. https://doi.org/10.1038/npp.2015.252.

Brivio, E., Lopez, J.P., Chen, A. 2020. Sex differences: Transcriptional signatures of stress exposure in male and female brains. Genes Brain Behav. 19(3), e12643. https://doi.org/10.1111/gbb.12643.

Cabib, S., Oliverio, A., Ventura, R., Lucchese, F., Puglisi-Allegra, S. 1997. Brain dopamine receptor plasticity: testing a diathesis-stress hypothesis in an animal model. Psychopharmacology (Berl). 132(2),153-60. 10.1007/s002130050331.

Carr, C.P., Martins, C.M., Stingel, A.M., Lemgruber, V.B., Juruena, M.F. 2013. The role of early life stress in adult psychiatric disorders: a systematic review according to childhood trauma subtypes. J Nerv Ment Dis. 201(12), 1007-1020. https://doi.org/10.1097/NMD.0000000000000049.

Cattane, N., Mora, C., Lopizzo, N., Borsini, A., Maj, C., Pedrini, L., Rossi, R., Riva, M.A., Pariante, C.M., Cattaneo, A. 2019. Identification of a miRNAs signature associated with exposure to stress early in life and enhanced vulnerability for schizophrenia: new insights for the key role of miR-125b-1-3p in neurodevelopmental processes. Schizophr Res. 205, 63–75. https://doi.org/10.1016/j.schres.2018.07.030.

Cattaneo, A., Suderman, M., Cattane, N., Mazzelli, M., Begni, V., Maj, C., D'Aprile, I., Pariante, C.M., Luoni, A., Berry, A., Wurst, K., Hommers, L., Domschke, K., Cirulli, F., Szyf, M., Menke, A., Riva, M.A. 2020. Long-term effects of stress early in life on microRNA-30a and its network: Preventive effects of lurasidone and potential implications for depression vulnerability. Neurobiol Stress. 23,13:100271. 10.1016/j.ynstr.2020.100271.

Concepcion, C-P., Han, Y.C., Mu, P., Bonetti, C., Yao, E., D'Andrea, A., Vidigal, J.A., Maughan, W.P., Ogrodowski, P., Ventura A. 2012. Intact p53-dependent responses in miR-34-deficient mice. PLoS Genet. 8(7):e1002797. 10.1371/journal.pgen.1002797.

D'Addario, S.L., Di Segni, M., Ledonne, A., Piscitelli, R., Babicola, L., Martini, A., Spoleti, E., Mancini, C., Ielpo, D., D'Amato, F.R., Andolina, D., Ragozzino, D., Mercuri, N.B., Cifani, C., Renzi, M., Guatteo, E., Ventura, R. 2021. Resilience to anhedonia-passive coping induced by early life experience is linked to a long-lasting reduction of Ih current in VTA dopaminergic neurons. Neurobiol Stress. 15;14:100324. 10.1016/j.ynstr.2021.100324.

D'Amato, F.R., Zanettini, C., Lampis, V., Coccurello, R., Pascucci, T., Ventura, R., Puglisi-Allegra, S., Spatola, C.A., Pesenti-Gritti, P., Oddi, D., Moles, A., Battaglia, M. 2011. Unstable maternal environment, separation anxiety, and heightened CO2 sensitivity induced by gene-by-environment interplay. PloS One. 6(4), e18637. https://doi.org/10.1371/journal.pone.0018637.

De La Garza, R., Mahoney, J.J. 2004. A distinct neurochemical profile in WKY rats at baseline and in response to acute stress: implications for animal models of anxiety and depression. Brain Res. 1021(2):209-18. doi:10.1016/j.brainres.2004.06.052.

Dickson, D.A., Paulus, J.K., Mensah, V., Lem, J., Saavedra-Rodriguez, L., Gentry, A., Pagidas, K., Feig, L.A. 2018. Reduced levels of miRNAs 449 and 34 in sperm of mice and men exposed to early life stress. Transl Psychiatry. 8(1), 101. https://doi.org/10.1038/s41398-018-0146-2.

Di Segni, M., Patrono, E., Patella, L., Puglisi-Allegra, S., Ventura, R. 2014. Animal models of compulsive eating behavior. Nutrients. 6(10),459[illegible]-609. 10.3390/nu6104591.

Di Segni, M., Andolina, D., Luchetti, A., Babicola, L., D'Apolito, L.I., Pascucci, T., Conversi, D., Accoto, A., D'Amato, F.R., Ventura, R. 2016. Unstable maternal environment affects stress response in adult mice in a genotype-dependent manner. Cereb Cortex. 26(11). 4370-4380. https://doi.org/10.1093/cercor/bhv204.

Di Segni, M., Andolina, D., Coassin, A., Accoto, A., Luchetti, A., Pascucci, T., Luzi, C., Lizzi, A.R., D'Amato, F.R., Ventura, R., 2017. Sensitivity to cocaine in adult mice is due to interplay between genetic makeup, early environment and later experience. Neuropharmacol. 125, 87–98. https://doi.org/10.1016/j.neuropharm.2017.07.014.

Dong, M., Anda, R.F., Felitti, V.J., Dube, S.R., Williamson, D.F., Thompson, T.J., Loo, C.M., Giles, W.H. 2004. The interrelatedness of multiple forms of childhood abuse, neglect, and household dysfunction. Child Abuse Negl. 28(7):771-84. 10.1016/j.chiabu.2004.01.008.

Dorak, M.T., Karpuzoglu, E. 2012. Gender differences in cancer susceptibility: an inadequately addressed issue.

Front Genet. 3, 268. https://doi.org/10.3389/fgene.2012.00268.

Dwivedi, V.K., Pardo-Pastor, C., Droste, R., Kong, J.N., Tucker, N., Denning, D.P., Rosenblatt, J., Horvitz, H.R. 2021. Replication stress promotes cell elimination by extrusion. Nature. 593(7860), 591–596. https://doi.org/10.1038/s41586-021-03526-y.

Eid, R.S., Gobinath, A.R., Galea, L.A. 2019. Sex differences in depression: Insights from clinical and preclinical studies. Prog Neurobiol. 176, 86-102. https://doi.org/10.1016/j.pneurobio.2019.01.006.

Gapp, K., Jawaid, A., Sarkies, P., Bohacek, J., Pelczar, P., Prados, J., Farinelli, L., Miska, E., Mansuy, I.M. 2014. Implication of sperm RNAs in transgenerational inheritance of the effects of early trauma in mice. Nat Neurosci. 17(5), 667–669. https://doi.org/10.1038/nn.3695.

Geaghan, M., Cairns, M. J. 2014. MicroRNA and posttranscriptional dysregulation in psychiatry. Biol Psychiatry. 78(4), 231–239. http://dx.doi.org/10.1016/j. biopsych.2014.12.009.

Gerin, M.I., Hanson, E., Viding, E., McCrory, E.J. 2019. A review of childhood maltreatment, latent vulnerability and the brain: Implications for clinical practice and prevention. Adoption & Fostering, 43(3), 310-328.

Giraldez, A.J., Cinalli, R.M., Glasner, M.E., Enright, A.J., Thomson, J.M., Baskerville, S., Hammond, S.M., Bartel, D.P., Schier, A.F. 2005. MicroRNAs regulate brain morphogenesis in zebrafish. Science. 308(5723), 833-838. https://doi.org/10.1126/science.1109020.

Gregory, R.I., Yan, K.P., Amuthan, G., Chendrimada, T., Doratotaj, B., Cooch, N., Shiekhattar, R. 2004. The Microprocessor complex mediates the genesis of microRNAs. Nature. 432(7014), 235-240. https://doi.org/10.1038/nature03120.

Haramati, S., Navon, I., Issler, O., Ezra-Nevo, G., Gil, S., Zwang, R., Hornstein, E., Chen, A. 2011. MicroRNA as repressors of stress-induced anxiety: the case of amygdalar miR-34. J Neurosci. 31(40), 14191-14203. https://doi.org/10.1523/JNEUROSCI.1673-11.2011.

He, T., Guo, C., Wang, C., Hu, C., Chen, H. 2020. Effect of early life stress on anxiety and depressive behaviors in adolescent mice. Brain Behav. 10(3):e01526. 10.1002/brb3.1526.

Hernández-Vázquez, F., Garduño, J., Hernández-López, S. 2018. GABAergic modulation of serotonergic neurons in the dorsal raphe nucleus. Rev Neurosci. 30(3):289-303. doi: 10.1515/revneuro-2018-0014.
Hommers, L. G., Domschke, K., Deckert, J. 2015. Heterogeneity and individuality: microRNAs in mental disorders. J Neural Transm. 122, 79–97. doi.org/10.1007/s00702-014-1338-4

Issler, O., Chen, A. 2015. Determining the role of microRNAs in psychiatric disorders. Nat Rev. Neurosci. 16(4), 201–212. https://doi.org/10.1038/nrn3879.

Issler, O., Haramati, S., Paul, E. D., Maeno, H., Navon, I., Zwang, R., Gil, S., Mayberg, H. S., Dunlop, B. W., Menke, A., Awatramani, R., Binder, E. B., Deneris, E. S., Lowry, C. A., Chen, A. 2014. MicroRNA 135 is essential for chronic stress resiliency, antidepressant efficacy, and intact serotonergic activity. Neuron 83(2), 344-360. https://doi.org/10.1016/j.neuron.2014.05.042.

Jeyaseelan, K., Lim, K.Y., Armugam, A. 2008. MicroRNA expression in the blood and brain of rats subjected to transient focal ischemia by middle cerebral artery occlusion. Stroke. 39(3), 959-966. https://doi.org/10.1161/STROKEAHA.107.500736.

Karrich, J.J., Jachimowski, L.C., Libouban, M., Iyer, A., Brandwijk, K., Thanmann-Kueter, E.W., Nagasawa, M., de Jong, E.C., Uittenbogaart, C.H., Blom, B. 2013. MicroRNA-146a regulates survival and maturation of human plasmacytoid dendritic cells. Blood. 122(17), 3001–3009. https://doi.org/10.1182/blood-2012-12.

Keller, S.M., Roth, T.L. 2016. Environmental influences on the female epigenome and behavior. Environ Epigenet. 2(2), dvw007. https://doi.org/10.1093/eep/dvw007.

Kelly-Irving, M., Lepage, B., Dedieu, D., Lacey, R., Cable, N., Bartley, M., Blane, D., Grosclaude, P., Lang, T., Delpierre, C. 2013. Childhood adversity as a risk for cancer: findings from the 1958 British birth cohort study. BMC public health, 13, 767. https://doi.org/10.1186/1471-2458-13-767.

Kininis, M., Kraus, W.L. 2008. A global view of transcriptional regulation by nuclear receptors: gene expression, factor localization, and DNA sequence analysis. Nucl Recept. Sign. 6, e005. https://doi.org/10.1621/nrs.06005.

Labonté, B., Engmann, O., Purushothaman, I., Menard, C., Wang, J., Tan, C., Scarpa, J.R., Moy, G., Loh, Y. E., Cahill, M., Lorsch, Z.S., Hamilton, P.J., Calipari, E.S., Hodes, G.E., Issler, O., Kronman, H., Pfau, M., Obradovic, A., Dong, Y., Neve, R.L., … Nestler, E. J. 2017. Sex-specific transcriptional signatures in human depression. Nat Med. 23(9), 1102–1111. https://doi.org/10.1038/nm.4386.

Lacal, I., Ventura, R. 2018. Epigenetic Inheritance: Concepts, Mechanisms and Perspectives. Front Mol Neurosci. 11, 292. https://doi.org/10.3389/fnmol.2018.00292.

Li, Y., Jiang, J., Liu, W., Wang, H., Zhao, L., Liu, S., Li, P., Zhang, S., Sun, C., Wu, Y., Yu, S., Li, X., Zhang, H., Qian, H., Zhang, D., Guo, F., Zhai, Q., Ding, Q., Wang, L., Ying, H. 2018. microRNA-378 promotes autophagy and inhibits apoptosis in skeletal muscle. Proc Natl Acad Sci USA. 115(46), E10849–E10858. https://doi.org/10.1073/pnas.1803377115.

Liu, D.Z., Tian, Y., Ander, B.P., Xu, H., Stamova, B.S., Zhan, X., Turner, R.J., Jickling, G., Sharp, F.R. 2010. Brain and blood microRNA expression profiling of ischemic stroke, intracerebral hemorrhage, and kainate seizures. J Cereb Blood Flow Metab. 30(1), 92–101. https://doi.org/10.1038/jcbfm.2009.186.

Lo Iacono, L. (a), Ielpo, D., Accoto, A., Di Segni, M., Babicola, L., D'Addario, S.L., Ferlazzo, F., Pascucci, T., Ventura, R., Andolina, D. 2020. MicroRNA-34a Regulates the Depression-like Behavior in Mice by Modulating the Expression of Target Genes in the Dorsal Raphè. Mol Neurobiol. 57(2), 823–836. https://doi.org/10.1007/s12035-019-01750-2.

Lo Iacono, L. (b), Bussone, S., Andolina, D., Tambelli, R., Troisi, A., Carola, V. 2020. Dissecting major depression: The role of blood biomarkers and adverse childhood experiences in distinguishing clinical subgroups. J Affect Disord. 276, 351-360. https://doi.org/10.1016/j.jad.2020.07.034.

Lo Iacono, L. (a), Ielpo, D., Parisi, C., Napoli, G., Accoto, A., Di Segni, M., Babicola, L., D'Addario, S.L., Guzzo, S.M., Pascucci, T., Ventura, R., Andolina, D. 2021. MicroRNA-34a regulates 5-HT2C expression in dorsal raphe and contributes to the anti-depressant-like effect of fluoxetine. Neuropharmacol. 190, 108559. https://doi.org/10.1016/j.neuropharm.2021.108559.

Lo Iacono, L. (b), Mancini, C., Babicola, L., Pietrosanto, M., Di Segni, M., D'Addario, S.L., Municchi, D., Ielpo, D., Pascucci, T., Cabib, S., Ferlazzo, F., D'Amato, F.R., Andolina, D., Helmer-Citterich, M., Cifani, C., Ventura, R. 2021. Early life adversity affecting the attachment bond alters ventral tegmental area transcriptomic patterning and behavior almost exclusively in female mice. Neurobiol Stress. 15, 100406. https://doi.org/10.1016/j.ynstr.2021.100406.

Lopez, J. P., Lim, R., Cruceanu, C., Crapper, L., Fasano, C., Labonte, B., Maussion, G., Yang, J. P., Yerko, V., Vigneault, E., El Mestikawy, S., Mechawar, N., Pavlidis, P., Turecki, G. 2014. miR-1202 is a primate-specific and brain-enriched microRNA involved in major depression and antidepressant treatment. Nat Med. 20(7), 764-768. https://doi.org/10.1038/nm.3582.

Luchetti, A., Di Segni, M., Andolina, D., Ventura, R., Battaglia, M., D'Amato, F.R. 2021. Mouse model of panic disorder: Vulnerability to early environmental instability is strain-dependent. Dev Psychobiol. 63(6):e22135. 10.1002/dev.22135.

Luoni, A., Riva, M.A. 2016. MicroRNAs and psychiatric disorders: From aetiology to treatment. Pharmacol Ther. 167, 13–27. https://doi.org/10.1016/j.pharmthera.2016.07.006.

Maas, A.H., Appelman, Y.E. 2010. Gender differences in coronary heart disease. Heart. 102(14), 1142–1149. https://doi.org/10.1136/heartjnl-2014-306463.

Mazzelli, M., Maj, C., Mariani, N., Mora, C., Begni, V., Pariante, C.M., Riva, M.A., Cattaneo, A., Cattane, N.

2020. The Long-Term Effects of Early Life Stress on the Modulation of miR-19 Levels. Front Psychiatry, 11, 389. https://doi.org/10.3389/fpsyt.2020.00389.

McCrory, E.J., Viding, E. 2015. The theory of latent vulnerability: Reconceptualizing the link between childhood maltreatment and psychiatric disorder. Dev Psychopathol. 27(2), 493–505. https://doi.org/10.1017/S0954579415000115.

McKibben, L.A., Dwivedi, Y. 2021. Early-life stress induces genome-wide sex-dependent miRNA expression and correlation across limbic brain areas in rats. Epigenomics. 13(13), 1031-1056. https://doi.org/10.2217/epi-2021-0037.

Morgan, C.P., Bale, T.L. 2012. Sex differences in microRNA regulation of gene expression: no smoke, just miRs. Biol Sex Differ. 3(1), 1-9. https://doi.org/10.1186/2042-6410-3-22.

Murphy, M.O., Herald, J.B., Wills, C.T., Unfried, S.G., Cohn, D.M., Loria, A.S. 2017. Postnatal treatment with metyrapone attenuates the effects of diet-induced obesity in female rats exposed to early-life stress. Am J Physiol Endocrinol Metab. 1;312(2), E98-E108. 10.1152/ajpendo.00308.2016.

Nilsson, P.M., Lurbe, E., Laurent, S. 2008. The early life origins of vascular ageing and cardiovascular risk: the EVA syndrome. J Hypertens. 26(6), 1049-1057. https://doi.org/10.1097/HJH.0b013e3282f82c3e.

O'Connor, R.M., Dinan, T.G., Cryan, J.F. 2012. Little things on which happiness depends: microRNAs as novel therapeutic targets for the treatment of anxiety and depression. Mol Psychiatry. 17(4):359-76. 10.1038/mp.2011.162.

O'Connor, R M., Gururajan, A., Dinan, T.G., Kenny, P.J., Cryan, J.F. 2016. All Roads Lead to the miRNome: miRNAs Have a Central Role in the Molecular Pathophysiology of Psychiatric Disorders. Trends Pharmacol Sci. 37(12), 1029-1044. https://doi.org/10.1016/j.tips.2016.10.004.

Pandey, D.P., Picard, D. 2010. Multidirectional interplay between nuclear receptors and microRNAs. Curr Opin Pharmacol. 10(6), 637-642. https://doi.org/10.1016/j.coph.2010.08.009.

Parel, S.T., Peña, C.J. 2022. Genome-wide Signatures of Early-Life Stress: Influence of Sex. Biol Psychiatry. 91(1), 36-42. https://doi.org/10.1016/j.biopsych.2020.12.010.

Parsons, L.H., Justice, J.B. 1993. Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area, and dorsal raphe nucleus following repeated cocaine administration. J. Neurochem. 61(5), 1611-1619. https://doi.org/10.1111/j.1471-4159.1993.tb09794.x.

Paxinos, G., Franklin, K.B. 2019. Paxinos and Franklin's the mouse brain in stereotaxic coordinates. Academic

press.

Ports, K.A., Ford, D.C., Merrick, M.T., Guinn, A.S. 2020. ACEs: Definitions, measurement, and prevalence Adverse childhood experiences: Using evidence to advance research, practice, policy, and prevention. Elsevier Academic Press. 17-34.

Poy, M.N., Eliasson, L., Krutzfeldt, J., Kuwajima, S., Ma, X., Macdonald, P.E., Pfeffer, S., Tuschl, T., Rajewsky, N., Rorsman, P., Stoffel, M. 2004. A pancreatic islet-specific microRNA regulates insulin secretion. Nature. 432(7014), 226-230. https://doi.org/10.1038/nature03076.

Provençal, N., Binder, E.B. 2015. The effects of early life stress on the epigenome: From the womb to adulthood and even before. Exp Neurol. 268, 10-20. https://doi.org/10.1016/j.expneurol.2014.09.001.

Rao, P., Benito, E., & Fischer, A., 2013. MicroRNAs as biomarkers for CNS disease. Front Mol Neurosci. 6, 39. https://doi.org/10.3389/fnmol.2013.00039.

Roy, B., Yoshino, Y., Allen, L., Prall, K., Schell, G., Dwivedi, Y. 2020. Exploiting Circulating MicroRNAs as Biomarkers in Psychiatric Disorders. Mol Diagn Ther. 24(3), 279-298. 10.1007/s40291-020-00464-9.

Seney, M.L., Logan, R.W. 2021. Critical roles for developmental hormones and genetic sex in stress-induced transcriptional changes associated with depression. Neuropsychopharmacol. 46(1), 221-222. https://doi.org/10.1038/s41386-020-00792-8.

Serafini, G., Trabucco, A., Corsini, G., Escelsior, A., Amerio, A., Aguglia, A., Nasrallah, H., Amore, M. 2021. The potential of microRNAs as putative biomarkers in major depressive disorder and suicidal behavior. Biomarkers in Neuropsychiatry. 5,100035 https://doi.org/10.1016/j.bionps.2021.100035.

Sharma, S., Eghbali, M. 2014. Influence of sex differences on microRNA gene regulation in disease. Biol Sex Differ. 5(1), 3. https://doi.org/10.1186/2042-6410-5-3.

Sorensen, S.S., Nygaard, A.B., Nielsen, M.Y., Jensen, K., Christensen, T. 2014. miRNA expression profiles in cerebrospinal fluid and blood of patients with acute ischemic stroke. Transl Stroke Res. 5(6), 711-718. https://doi.org/10.1007/s12975-014-0364-8.

Spoida, K., Masseck, O.A., Deneris, E.S., Herlitze, S. 2014. Gq/5-HT2c receptor signals activate a local GABAergic inhibitory feedback circuit to modulate serotonergic firing and anxiety in mice. Proc Natl Acad Sci U.S.A. 111(17):6479-84. doi: 10.1073/pnas.1321576111.

Sun, N., Lei, L., Wang, Y., Yang, C., Liu, Z., Li, X., Zhang, K. 2016. Preliminary comparison of plasma notch-

associated microRNA- 34b and -34c levels in drug naive, first episode depressed patients and healthy controls. J Affect Disord. 194, 109–114. https://doi.org/10.1016/j.jad.2016.01.017.

Tao CS, Dhamija P, Booij L, Menard JL. 2017. Adversity in early adolescence promotes an enduring anxious phenotype and increases serotonergic innervation of the infralimbic medial prefrontal cortex. Neuroscience. 19, 364:15-27. 10.1016/j.neuroscience.2017.09.004.

Torres-Berrío, A., Issler, O., Parise, E. M., Nestler, E.J. 2019. Unraveling the epigenetic landscape of depression: focus on early life stress. Dialogues Clin Neurosci. 21(4), 341-357. https://doi.org/10.31887/DCNS.2019.21.4/enestler.

Van Der Auwera, S., Ameling, S., Wittfeld, K. d'Harcourt Rowold, E., Nauck, M., Völzke, H., Suhre, K., Najafi-Shoushtari, H., Methew, J., Ramachandran, V., Bülow, R., Völker, U., Grabe, H. J. 2019. Association of childhood traumatization and neuropsychiatric outcomes with altered plasma micro RNA-levels. Neuropsychopharmacol. 44(12), 2030-2037. https://doi.org/10.1038/s41386-019-0460-2.

Ventura, R., De Carolis, D., Alcaro, A., Puglisi-Allegra, S. 2006. Ethanol consumption and reward depend on norepinephrine in the prefrontal cortex. Neuroreport. 17(17), 1813-7. 10.1097/01.wnr.0000239964.83566.75.

Ventura, R., Coccurello, R., Andolina, D., Latagliata, E.C., Zanettini, C., Lampis, V., Battaglia, M., D'Amato, F.R., Moles, A. 2013. Postnatal aversive experience impairs sensitivity to natural rewards and increases susceptibility to negative events in adult life. Cereb Cortex, 23(7), 1606-1617. https://doi.org/10.1093/cercor/bhs145.

Wang, H., Ma, Z., Shen, H., Wu, Z., Liu, L., Ren, B., Wong, P., Sethi, G., Tang, F. 2021. Early Life Irradiation-Induced Hypoplasia and Impairment of Neurogenesis in the Dentate Gyrus and Adult Depression Are Mediated by MicroRNA- 34a-5p/T-Cell Intracytoplasmic Antigen-1 Pathway. Cells 10(9), 2476. https://doi.org/10.3390/cells10092476.

Warnefors, M., Mössinger, K., Halbert, J., Studer, T., VandeBerg, J.L., Lindgren, I., Fallahshahroudi, A., Jensen, P., Kaessmann, H. 2017. Sex-biased microRNA expression in mammals and birds reveals underlying regulatory mechanisms and a role in dosage compensation. Genome Res. 27(12), 1961-1973. https://doi.org/10.1101/gr.225391.117.

Waselus, M., Valentino, R.J., Van Bockstaele, E.J. 2011. Collateralized dorsal raphe nucleus projections: a mechanism for the integration of diverse functions during stress. J Chem Neuroanat. 41(4), 266-280. https://doi.org/10.1016/j.jchemneu.2011.05.011.

Whitacre, C.C. 2001. Sex differences in autoimmune disease. Nat Immunol. 2(9), 777–780. https://doi.org/10.1038/ni0901-777.

Yan, S., You, Z. L., Zhao, Q.Y., Peng, C., He, G., Gou, X.J., Lin, B. 2015. Antidepressant-like effects of Sanyuansan in the mouse forced swim test, tail suspension test, and chronic mild stress model. Kaohsiung J Med Sci 31(12), 605-612. https://doi.org/10.1016/j.kjms.2015.10.009.

Yang, Z., Wang, L. 2011. Regulation of microRNA expression and function by nuclear receptor signaling. Cell Biosci. 1(1), 31. https://doi.org/10.1186/2045-3701-1-31.

Zangen, A., Nakash, R., Overstreet, D.H., Yadid, G. 2001. Association between depressive behavior and absence of serotonin-dopamine interaction in the nucleus accumbens. Psychopharmacol. 155(4), 434–439. https://doi.org/10.1007/s002130100746.

Zhang, J., Jima, D.D., Jacobs, C., Fischer, R., Gottwein, E., Huang, G., Lugar, P.L., Lagoo, A.S., Rizzieri, D.A., Friedman, D.R., Weinberg, J.B., Lipsky, P.E., Dave, S.S. 2009. Patterns of microRNA expression characterize stages of human B-cell differentiation. Blood. 113(19), 4586-4594. https://doi.org/10.1182/blood-2008-09-178186.

Żurawek, D., Turecki, G. 2021. The miRNome of Depression. Int J Mol Sci. 22(21), 11312. https://doi.org/10.3390/ijms222111312.

Figure legends

Fig. 1. ELA induces a depressive-like behavior in female mice only

Effects of ELA on immobility in FST and TST and on preference for saccharine (SPT) in female (A,C,E) and male mice (B,D,F).

A. Significant difference in immobility in FST between ELA and Control females (Con7) (Cont n=8, ELA n=8) (*p<0.05) (mean ± SEM) **B.** No significant difference in immobility in FST between ELA and Cont males (Cont n=7, ELA n=7) **C.** Significant difference in immobility in TST between ELA and Control females (Cont n=7, ELA n=7) (*p<0.05) (mean ± SEM) **D.** No significant difference in immobility in TST between ELA and Cont males (Cont n=6, ELA n=6) **E.** Significant difference in increase of percentage of sucrose consumption between ELA and Control females (Cont n=7, ELA n=7) (**p<0.01) (mean ± SEM) **F.** No difference in increase of percentage of sucrose consumption between ELA and Control males (Cont n=7, ELA n=7) .

All data are expressed as mean time spent (in second) ±SEM. *P < 0.05 in comparison with the respective Control (Cont) group.

Fig. 2. miR-34a is differently expressed in male and female mice

Relative expression (fold changes) of miR-34a in mpFC (A), DRN (B), heart (C) and plasma (D) of naïve female and male mice. The relative expression level of miR-34a was evaluated with respect to male mice.

A. No significant difference in miR-34a expression is evident between female and male mice in mpFC (males n=6, females n=5). B. A not significant trend, with miR-34a levels higher in males than females, is evident in DRN (male n=6, female n=5). C. A significantly higher expression of miR-34a is observed in both hearth (*p<0.05) (mean ± SEM) and (D.) plasma (*p<0.05) (mean ± SEM) (males n=5, female n=5) of females than males.

Fig. 3. miR-34a expression is affected by ELA only in female mice

A. Relative expression (fold changes) of miR-34a in mpFC, DRN, heart and plasma of unstressed and stressed (AAS) Control (Cont) and ELA female (A,B,C,D) and male mice (E,F,G,H). The relative expression level of miR-34a was evaluated with respect to the unstressed Cont group within each sex.

ELA affects miR-34a expression in the mpFC of female mice. AAS increases miR-34a expression in Cont females (Cont n=5, ELA n=6; Cont+AAS n=5; ELA+AAS n=6). B. ELA affects miR-34a expression in the DRN of female mice. AAS decreases miR-34a expression in in ELA mice (Cont n=5; ELA n=6; Cont+AAS n=5; ELA+AAS n=5). C. ELA and AAS doesn't affect miR-34a expression in the heart (Cont n=5; ELA n=5; Cont+AAS n=5; ELA+AAS n=5). D. ELA affects miR-34a expression in plasma. AAS increases and decreases miR-34a expression in Cont and ELA, respectively (Cont n=5; ELA n=5; Cont+AAS n=6; ELA+AAS n=5). ELA and AAS do not affect miR-34a expression in the mpFC (E) (Cont n=5; ELA n=5; Cont+AAS n=5; ELA+AAS n=6), DRN (F) (Cont n=5; ELA n=5; Cont+AAS n=5; ELA+AAS n=6), hearth (G) (Cont n=6; ELA n=5; Cont+AAS n=6; ELA+AAS n=5) and plasma (H) (Cont n=5; ELA n=5; Cont+AAS n=6; ELA+AAS n=5) of male mice. *p<0.05; ***p<0.0001

Fig. 4. miR-34a alteration in DRN modulate 5-HT release in mpFC and NAc

A. 5-HT release induced by restraint (2h) in mpFC of Cont (continuous line) and ELA (dashed line) female groups. **B.** Basal 5-HT outflow of Cont (red bar) and ELA (white bar) groups (Cont n=9, RCF n=7). **C.** 5-HT release induced by restraint (2h) in NAc of Cont (continuous line) and ELA (dashed line) groups. **D.** Basal 5-HT outflow in Cont (red bar) and ELA (white bar) groups (Cont n=7, RCF n=7). Animals undergo to adult acute stress (AAS) at time 0. Data are expressed as percentage changes from baseline level of each group (**A,C**) and raw concentration (pg/20µl) (**B,D**) *p < 0.05; **p < 0.01 (between groups), #p < 0.05; ##p < 0.001; ###p < 0.001 (within group, Cont), §p < 0.05 (within group, ELA) (mean ± SEM).

Fig. 5. Principal Component Analisys (PCA)

A. Average Factor scores on the first (DRN and plasma) Principal Component as a function of treatment and pretreatment conditions, in female (left) and male (right) animals. **B.** Average Factor scores on the second (mpFC and heart) Principal Component as a function of treatment and pretreatment conditions, in female (left) and male (right) animals.

Table 1. Varimax rotated factor structure for the entire dataset

	Components	
	1	**2**
DRN	0.779	0.248
mpFC	0.079	0.8
Heart	0.165	0.813
Plasma	0.859	0.022

Table 2. Average scores on the first component and standard error as a function of sex, pretreatment, and treatment.

sex	treat	pretreatment	Mean	Std. Error	95% Confidence Interval	
					Lower Bound	Upper Bound
Females	***Baseline***	Cont	-0.524	0.381	-1.301	0.252
		RCF	1.508	0.381	0.731	2.284
	Acute Stress	Cont	0.414	0.381	-0.363	1.19
		RCF	-0.244	0.381	-1.021	0.533
Males	***Baseline***	Cont	-0.425	0.381	-1.202	0.352
		RCF	-0.303	0.381	-1.08	0.473
	Acute Stress	Cont	-0.159	0.381	-0.935	0.618
		RCF	-0.266	0.381	-1.043	0.511

Highlights

Early life adversity induces a depressive-like state in female mice

Early life adversity affects miR-34a expression only in female mice

Early life adversity modulates miR-34a response to adult acute stress exposure in female mice

MiR-34a expression in DRN correlates with plasmatic miR-34a levels

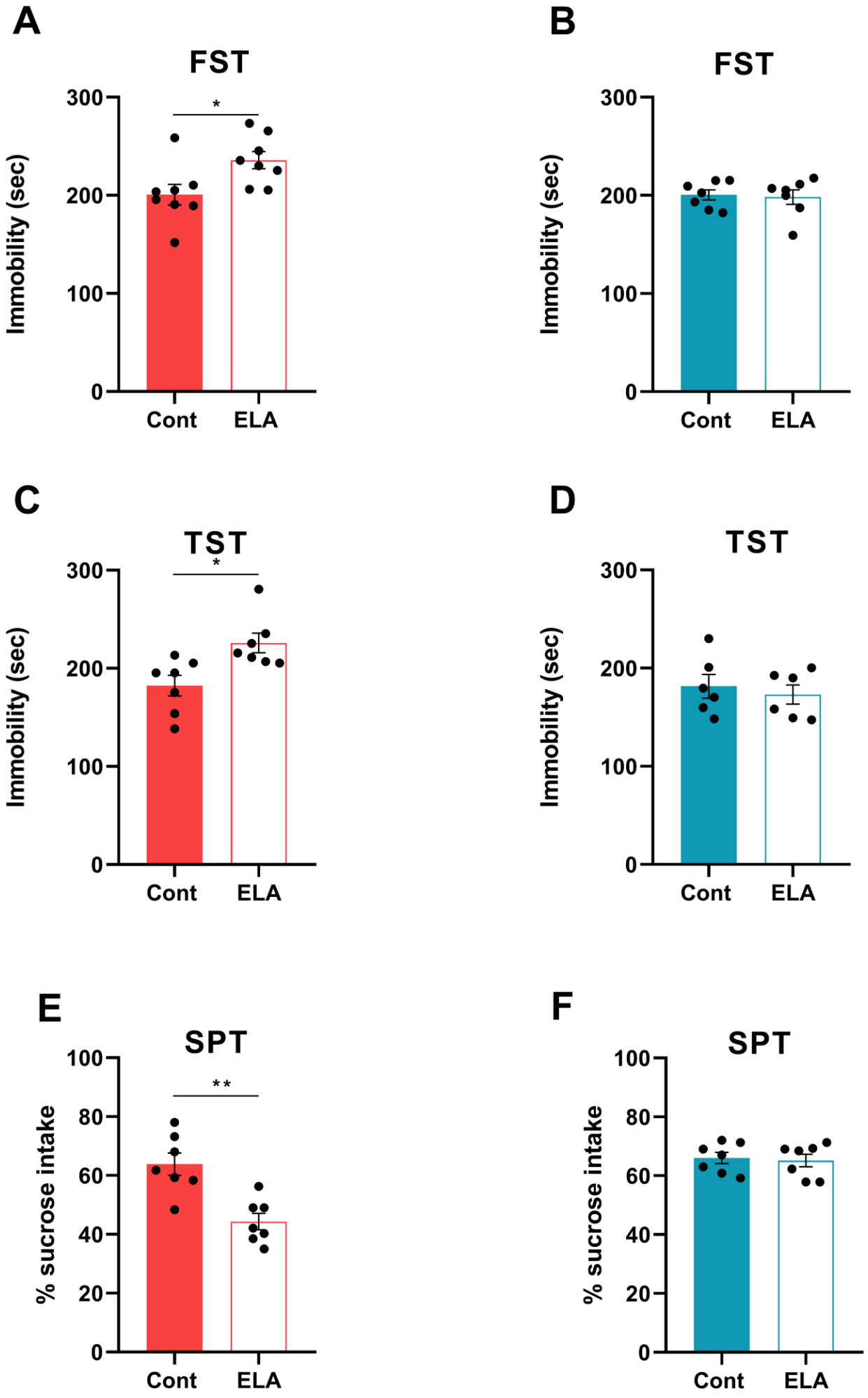

A
FST
Immobility (sec)
300
200
100
0
*
Cont
ELA
B
FST
Immobility (sec)
300
200
100
0
Cont
ELA
C
TST
Immobility (sec)
300
200
100
0
*
Cont
ELA
D
TST
Immobility (sec)
300
200
100
0
Cont
ELA
E
SPT
% sucrose intake
100
80
60
40
20
0
**
Cont
ELA
F
SPT
% sucrose intake
100
80
60
40
20
0
Cont
ELA

Figure 1

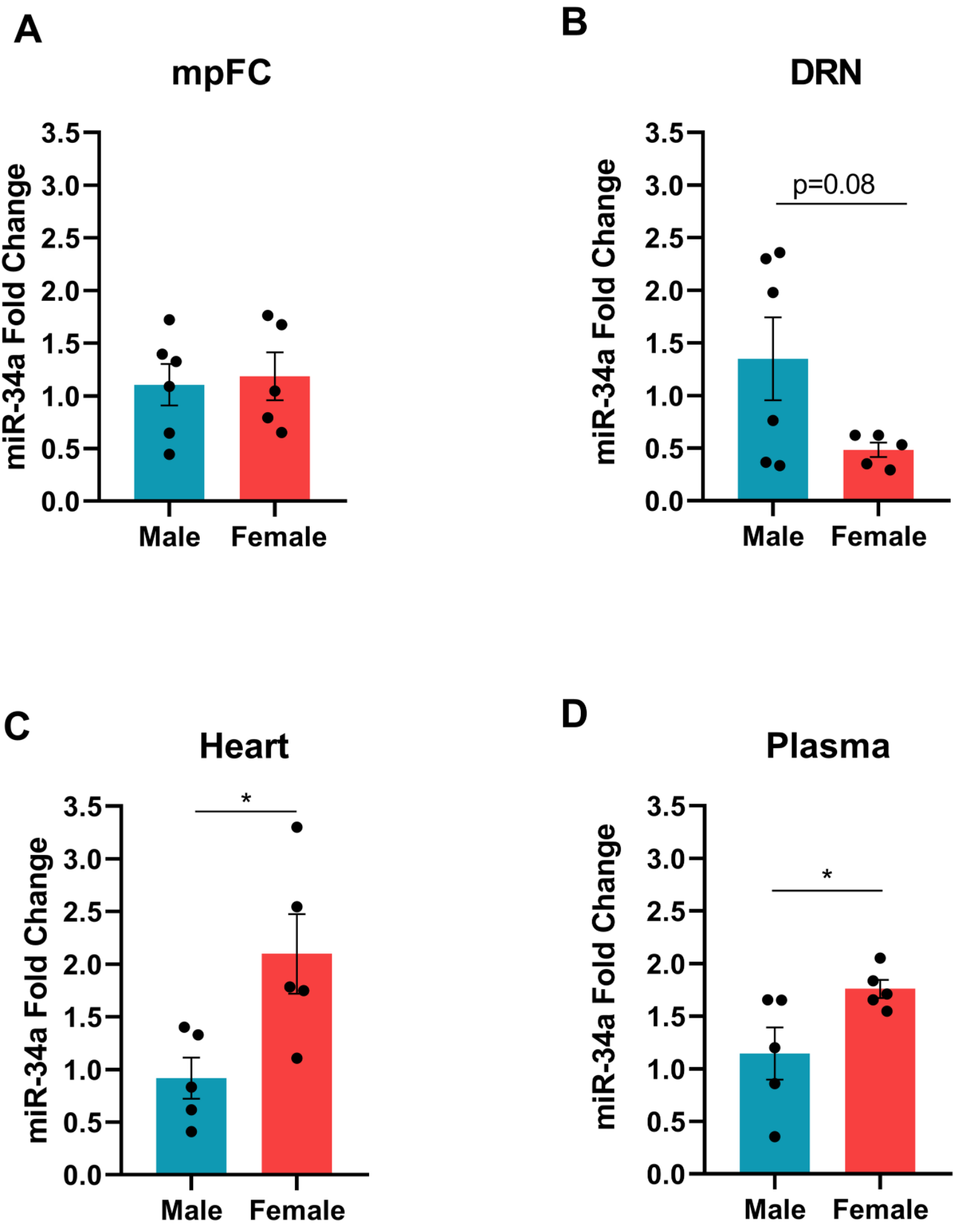

A
mpFC
miR-34a Fold Change
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Male
Female
B
DRN
p=0.08
C
Heart
*
D
Plasma

Figure 2

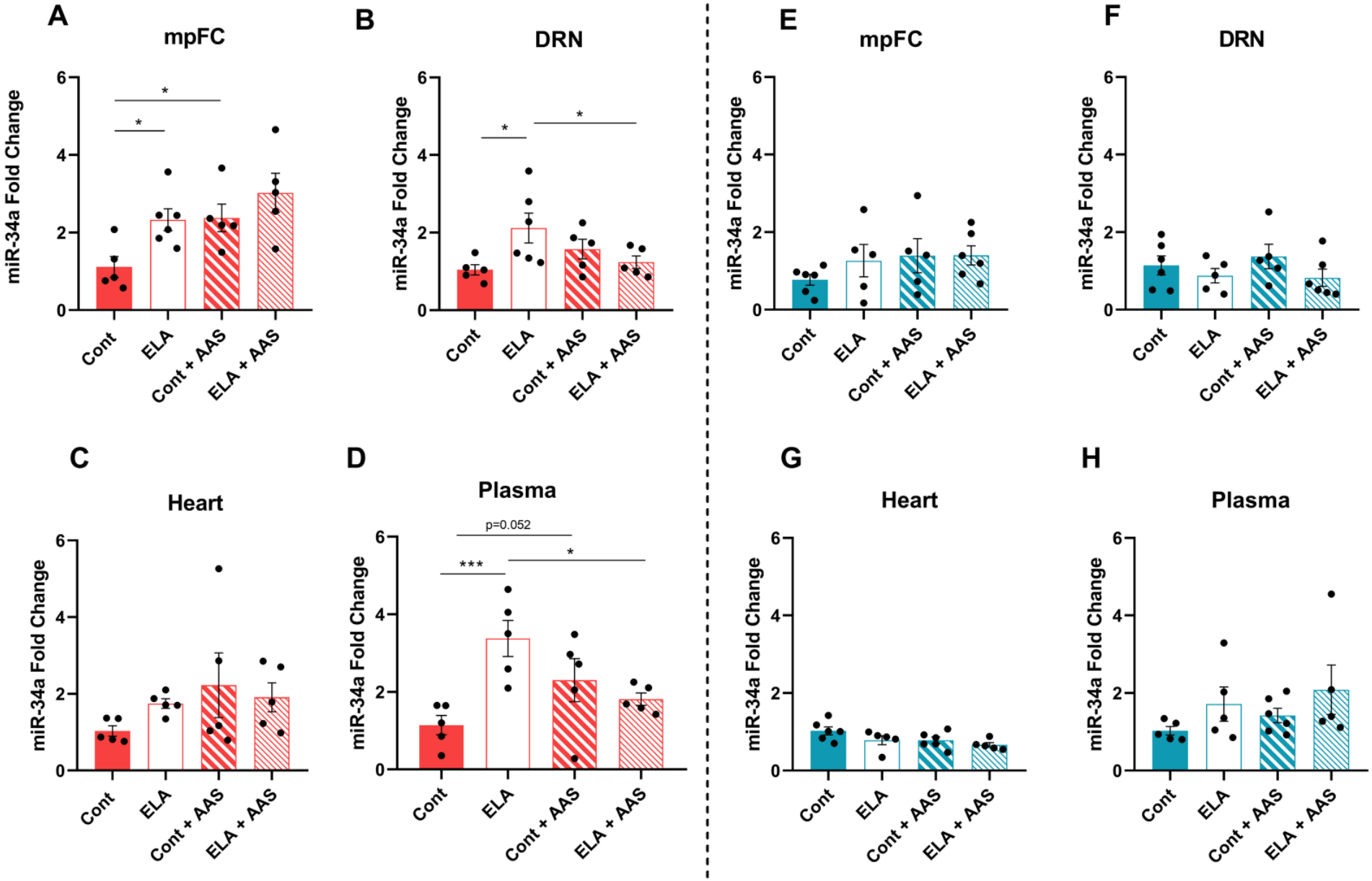
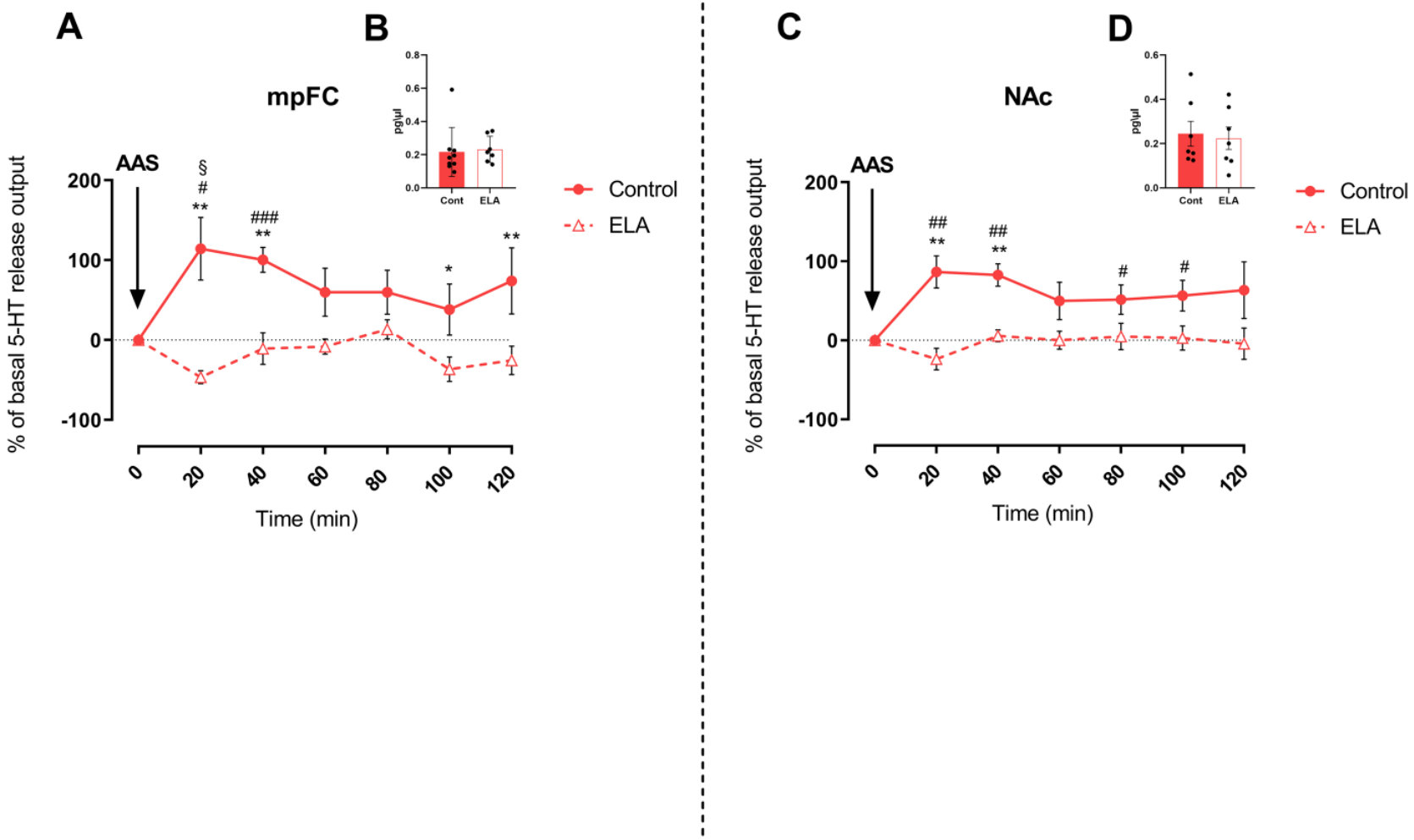

A
mpFC
B
DRN
E
mpFC
F
DRN
C
Heart
D
Plasma
G
Heart
H
Plasma
miR-34a Fold Change
0
2
4
6
*
*
*
*
***
p=0.052
Cont
ELA
Cont + AAS
ELA + AAS

Figure 3


A
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mpFC
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200
100
0
-100
% of basal 5-HT release output
0
20
40
60
80
100
120
Time (min)
Control
ELA
pg/µl
0.8
0.6
0.4
0.2
0.0
Cont
ELA
C
D
NAc
AAS
##
**
##
**
#
#
200
100
0
-100
% of basal 5-HT release output
0
20
40
60
80
100
120
Time (min)
Control
ELA
pg/µl
0.6
0.4
0.2
0.0
Cont
ELA

Figure 4

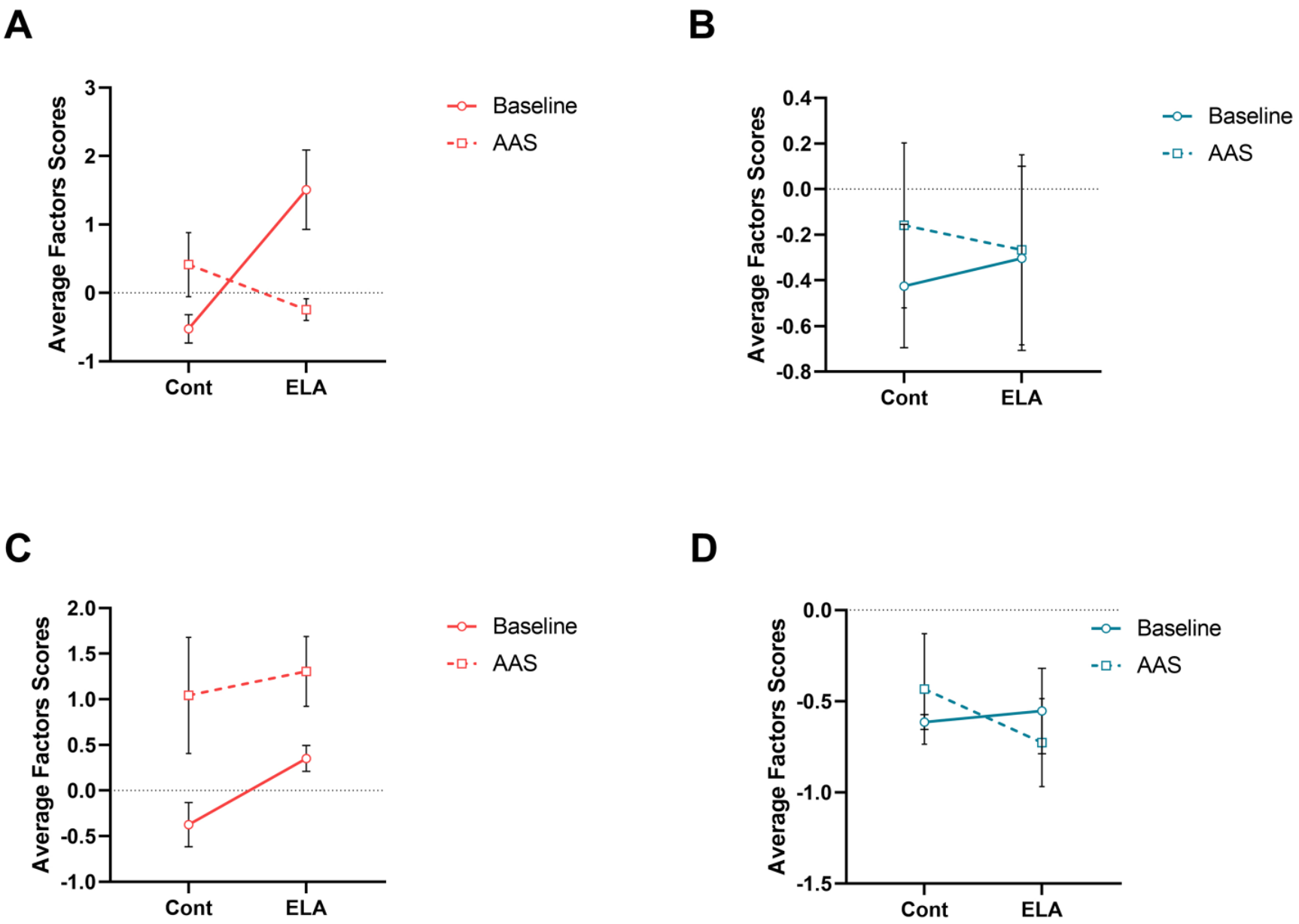
A
Average Factors Scores
3
2
1
0
-1
Cont
ELA
Baseline
AAS
B
Average Factors Scores
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
Cont
ELA
Baseline
AAS
C
Average Factors Scores
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
Cont
ELA
Baseline
AAS
D
Average Factors Scores
0.0
-0.5
-1.0
-1.5
Cont
ELA
Baseline
AAS

Figure 5