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Highlights:

-An animal model of emotional contagion that develops across time is described.

-Emotional contagion occurs when stressed female rats cohabitate with healthy male rats.

-Among stress-exposed rats, voluntary exercise reduces some aspects of negative emotional contagion.

-Effects on body weight, depression- and anxiety-like behaviors and BDNF-signaling are examined.

A Model of Negative Emotional Contagion Between Male-Female Rat Dyads: Effects of Voluntary Exercise on Stress-Induced Behavior and BDNF-TrkB Signaling

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Abstract

Emotional contagion refers to the sharing of emotional states between individuals and can cause depressive behaviors in healthy persons who live with depressed individuals. Negative emotional contagion has been observed in animal models, but the vast majority of studies are short-term and bear little resemblance to long-term human relationships. Thus, the first aim of this study was to establish an animal model of stress-induced negative emotional contagion that develops across time and between pairs. To accomplish this, we tested the hypothesis that sedentary male rats that cohabitate for five weeks with a stress-exposed female will exhibit a depression-like phenotype that is observable on behavioral and physiological measures. In addition, drawing from a comprehensive literature that describes the beneficial effects of prior exercise on stress-related behavior, we tested our second hypothesis that in males that were paired with a stressed female, prior voluntary exercise will blunt the impact of negative emotional contagion. We found that pair housing a healthy male with a stressed female led to emotional contagion; males gained less body weight, were anhedonic, demonstrated heightened anxiety-like behavior, had lower serum brain derived neurotrophic factor (BDNF) levels, had decreased hippocampal BDNF-stimulated tyrosine receptor kinase B (TrkB) signaling and increased pro-inflammatory cytokine expression in the hippocampus. For the most part, the five-week exercise window that occurred prior to pair housing had few effects in non-stress paired rats, but had partial, yet substantial protective effects in rats that were pair-housed with a stressed female. Specifically, among stress-paired, exercised rats showed less depressive-like behavior, had partially preserved hippocampal BDNF-stimulated TrkB signaling, had normalized serum BDNF concentration, and had hippocampal cytokine and immediate early gene levels that were equivalent to controls. These preclinical findings introduce a new model of negative emotional contagion between dyads of male-female rats and support the view that inclusion of exercise programs would be beneficial for persons that may, in the future, be susceptible to negative emotional contagion.

1. Introduction

Between 2013 and 2016, 8.1% of Americans were diagnosed with depression, a disorder that includes symptoms of anhedonia, anxiety, fatigue, diminished ability to concentrate, dysregulation of body weight, alterations in circulating hormone levels, and/or recurring suicidal ideation [1-3]. Although the etiology of depression involves heritable factors, there is abundant evidence that environmental influences, including repeated exposure to stressors, lead to depression in humans or depression-like behaviors in rodents [4-12]. Furthermore, exposure to multiple stressors is correlated with reductions in hippocampal health and neurogenesis [13-17] as well as compromised immune function, including elevated pro-inflammatory cytokines [18-21].

Depression affects not only the individual, but also those who are closely associated, such as intimate partners, roommates and care providers [4, 5]. Indeed, the sharing of emotional states between individuals is referred to as emotional contagion, a phenomenon that is proposed to have both evolutionary and social value and has been observed across a myriad of different pairings [22-27]. For example, cohabitating partners of depressed individuals show symptoms of depression and use negative non-verbal communication at significantly greater rates compared to partners of non-depressed individuals [28-31]. Furthermore, Sanislow et al [32] demonstrated that roommates of depressed individuals were significantly more depressed than roommates in dyads with no depression. Thus evidence indicates that when a depressed person lives with a healthy individual, the healthy member of the dyad is at risk of developing depressive symptoms, a finding that remains significant even after controlling for relationship satisfaction [33].

Emotional contagion, particularly for negative emotions, has been characterized in animal models in which both the stress exposure and the social pairings are short-term (for a comprehensive review see [34]; and see [35-46]). In contrast, there are relatively few preclinical studies that examine emotional contagion resulting from extended cohabitation, which more closely models long-term intimate human relationships. In one notable example, Boyko et al. [47] demonstrated that when male rats were exposed to five weeks of chronic unpredictable stress and then re-housed for five weeks in triads of two stressed and one non-stressed male rat, depression- and anxiety-like behaviors were observed in all rats. Key features captured by this model include the presence of repeated environmental stressors and longer-term cohabitation, which strengthens its face validity as an animal model of stress-induced negative emotional contagion. Building upon these findings, a goal of the present study was to develop an animal model of negative emotional contagion involving repeated stress and male-female dyads.

Although some depressed persons are provided with symptom relief from antidepressants, a large percentage of patients are treatment-resistant, leaving considerable room for improvement. Data suggest that exercise may not only be an effective treatment for depression, but it may also be preventative. For example, exercise has been shown to reduce depressive symptomology in college students [48] and aerobic exercise reduces negative affect [49-52]. In addition, Blumenthal et al. [53] found exercise to be as effective as antidepressant medication for patients with major depressive disorder. There are also many accounts that describe the impact of preventative exercise. These include a meta-analysis of 25 studies which revealed that physical activity is negatively associated with the risk of developing depression [54]. Specifically, using a longitudinal design, Camacho et al. [55] found that among subjects who were not depressed at baseline, those who reported a low activity level during an eight-year follow-up survey were at significantly greater risk for depression than were those who reported high levels of activity. Animal models have also been used to evaluate the effects of prior exercise on depression-like and anxiety-like behaviors. Several reports describe the beneficial effects of exercise in male rodents that were not exposed to stress [56-62], whereas others evaluate exercise effects in the context of stress [63-69]. Of central importance for the present study, no reports examine the effects of prior voluntary exercise on negative emotional contagion between male-female rodent dyads.

Clinical and preclinical studies reveal that repeated stress negatively impacts peripheral and central physiology whereas exercise enhances it [70-75]. Included are effects on brain derived neurotropic factor (BDNF) and its cognate receptor, tyrosine receptor kinase B (TrkB). For example, levels of BDNF in the brain and/or serum are negatively correlated with depression in humans [76, 77] and are decreased in hippocampal tissue from stressed rats [78-81, but see 82]. Yet, two weeks of concurrent voluntary exercise has been shown to completely ameliorate stress-induced decreases in BDNF [75]. At present, the impact of negative emotional contagion on peripheral and central BDNF levels is unknown.

TrkB signaling cascades are multifaceted and initiated by BDNF binding [83]. Once ligand-bound, full length TrkB receptors ($TrkB_{145}$) dimerize and auto-phosphorylate at tyrosine residues. Phosphorylated $TrkB_{145}$ ($pY\text{-}TrkB_{145}$) recruits other signaling proteins (e.g., neuronal Src homology and collagen adaptor protein (N-Shc), and phospholipase C-γ1 (PLC), which, in turn, influence cellular activity, including regulation of transcription. In addition, $TrkB_{145}$ activation can also lead to phosphorylation of other membrane-bound proteins including subunits of glutamatergic NMDA receptors (e.g., NR1 and NR2 subunits)[84-86], perhaps through $TrkB_{145}$ -NMDA receptor linkage [87-89]. In the hippocampus, this association has been shown to

influence the expression of plasticity related proteins, (such activity-related cytoskeletal-associated protein (Arc)), which promote neuronal stability and are involved in synaptic plasticity [90-92]. Of particular relevance to the present study, it has been shown that stress and/or exercise substantially alter hippocampal $TrkB_{145}$ mRNA [93, 94] and protein [95], phosphorylated $TrkB_{145}$ [96, 97], and several BDNF-TrkB intracellular signaling molecules [74, 97, 98]. These reports describe changes that depend on the intensity and timing, as well as the recovery from stress exposure. Drawing from this literature, additional efforts have been made to measure dynamic changes (e.g., *ex vivo* stimulation) in BDNF-TrkB signaling in response to stress and/or exercise [84, 87, 99], but never in the context of emotional contagion.

As described above, emotional contagion following short-term exposure to stress has been well documented in human and animal studies, yet few have examined emotional contagion in which stress induction and co-habitation occur over relatively longer periods of time. In addition, a large body of literature indicates that voluntary exercise provides physiological and psychological benefits. Furthermore, in rats, anxiolytic effects of exercise are particularly consistent in the context of evoked stress [68]. Collectively, these reports led us to test two hypotheses in the current study: 1) sedentary male rats that cohabitate for five weeks with a stress-exposed, non-familiar female will exhibit a depression-like phenotype that is observable on behavioral and physiological measures and 2) in male rats that are paired with a stressed female, prior voluntary exercise will blunt the impact of negative emotional contagion. To induce a depression-like phenotype in female rats, a modified version of a chronic unpredictable stress paradigm [11, 12, 47, 100] was used. The sucrose preference test was used to measure depression-like behaviors in male rats [101] and the open field and elevated plus mazes were used to measure anxiety-like behavior. Peripheral measures included assessment of body weight and quantification of serum BDNF levels. In addition, to assess BDNF-TrkB receptor signaling, hippocampal tissue was incubated *ex vivo* with BDNF [87, 89], immunoprecipitated with antibodies to TrkB and subject to immunoblot assays using antibodies against TrkB-associated proteins (i.e., TrkB, pY-TrkB, N-Shc, PLC and NR1). Lastly, to more fully characterize this putative model of negative emotional contagion, exercise- and/or stress-related hippocampal protein expression of across five subgroups (myokines, pro-inflammatory cytokines, immediate early genes, neurotrophic ligands, and TrkB-associated proteins) was quantified.

2. Materials and Methods

2.1. Subjects: Thirty-two female and 32 male Long-Evans rats (Envigo, East Millstone, NJ, USA) were maintained under a 12:12 hour light/dark cycle with food and water available *ad libitum*. All rats were handled for 2 min per day for three days prior to the start of the experiment. Females (125 - 150g upon arrival) were ovariectomized, permitted to recover for one week and were randomly assigned to either the NoStress$^{♀}$ (n = 16) or the Stress$^{♀}$ (n = 16) condition. Males (200 - 225 g upon arrival) were randomly assigned to the Sedentary$^{♂}$ (n = 16) or the Exercise$^{♂}$ (n = 16) condition. Separate rooms in the vivarium were used to house stressed and non-stressed rats and to house males and females before cohabitation. Female body weights were collected before and after stress induction and male body weights were collected before and after voluntary exercise, and after cohabitation. All efforts were made to minimize animal suffering and reduce the number of animals used. All procedures were approved by the Hamilton College Institutional Animal Care and Use Committee.

2.2. Stress Induction: Female rats in the Stress$^{♀}$ condition underwent a chronic unpredictable stress paradigm that consisted of exposure to six stressors that were distributed intermittently across five weeks, for a total of 40 exposures. For each stress session, female rats were removed from their home cage and transported to one of three different locations. **Restraint stress**: Rats were placed in opaque, ventilated restraint tubes for 1 h. **Predator scent**: Rats were placed into individual cages that contained wooden-shaving bedding mixed with a predator scent (2mL of 100% fox urine; Code Blue, Inc. Birmingham, AL, USA) for 2 h. **Footshocks**: Rats were placed into standard operant conditioning chambers (Med Associates, Inc., St. Albans, VT, USA) that consisted of aluminum front and back walls, clear acrylic sides and top, and grid floors. The chambers were housed within sound-attenuating cabinets equipped with a fan. Three 1-sec, 1-mA footshocks were delivered through a grid floor across 7 min. **Audio-visual exposure**: Rats were placed into a dark room that was illuminated by a flashing strobe light. In addition, 36 1000-Hz, 80-dB intermittent tones were presented randomly throughout the 20-min session. **Food or water deprivation:** Either the water bottle or the food hopper was removed from the home cage for 23.5 hours. The food deprivation and water deprivation sessions were separated from one another by a minimum of 72 hr.

2.3. Voluntary exercise: Beginning five weeks prior to the formation of male-female dyads, male rats in the Exercise$^{♂}$ condition (n = 16) were pair-housed in polycarbonate cages attached to activity wheels (Harvard Apparatus, Boston, MA, USA). A magnetic system on the wheels

coupled with a LE 3806 multicounter was used to detect and record each full wheel revolution. Male rats lived in the wheel cages in pairs for three weeks. During a fourth week, the pairs of male rats were housed in a cage without a wheel and left undisturbed. Lastly, to measure if each rat engaged in voluntary exercise, during a fifth week, individual male rats were removed from their home cage and given 24-hr access to the wheels every other day for eight days (i.e., there were four days of solo wheel cage access for each rat). During these eight days, rats in the sedentary groups were also individually housed and switched into a new cage every day.

2.4. Contagion pairing: After stress exposure and the elevated plus maze test for female rats and after the last exercise session for male rats, male-female dyads were formed by pair housing an exercised or a sedentary male with a stressed or a non-stressed female for six weeks. Thus, the following four groups (n = 8/group) were formed: the Sedentary♂/NoStress♀ condition; the Exercise♂/NoStress♀ condition; the Sedentary♂/Stress♀ condition; and the Exercise♂/Stress♀ condition.

2.5. Behavioral testing

2.5.1. Sucrose preference test: Male rats underwent a total of four sucrose preference tests, with three conducted prior to and one conducted after five weeks of male-female pair housing. Male rats were water-restricted for 23.5 h prior to testing. On the day of the test males were transported to a testing room and placed for 30 min into a polycarbonate cage containing two dispensing tubes (Bio-Serv, Flemington, NJ, USA) that were secured to a wooden platform and were counterbalanced for location (right and left) across tests. One tube contained tap water and one tube contained a 1%-sucrose solution. After each 30-min sucrose preference test, rats were placed into individual holding cages for 2 h without access to food or water and were then returned to the pair housing cage. The total volumes consumed were recorded and the percentage of sucrose/water intake per rat was determined. Baseline sucrose preference was calculated by averaging the three sucrose preference scores per rat.

2.5.2. Elevated plus maze: The elevated plus maze was constructed of four black acrylic arms that were raised 76 cm from the floor. Two opposing closed arms had 30 cm-high black walls on three sides whereas the open arms did not. The intersection of the open and the closed arms was referred to as the neutral area and time spent there was not counted as time spent in the arms.

Approximately 24 h after the last stress induction session (but prior to the formation of dyads), female rats were individually transported to a dimly lit behavioral testing room. At the start of the 10-min test, rats were individually placed in the center platform facing an open arm and released. Exploratory behavior was recorded using AnyMaze Video Tracking equipment (Wood Dale, IL, USA). The test occurred during the dark cycle and the apparatus was cleaned with 70% ethanol between each subject. For each rat, the time in the open arms and the number of entries into the open and closed arms were recorded.

2.5.3. Open field test: After six weeks of male-female cohabitation, male rats were individually transported to a dimly lit behavioral testing room and placed into the center of an open field apparatus with a matte black base. Rat exploratory behavior was recorded for 10 min using AnyMaze. The test occurred during the dark cycle and the apparatus was cleaned with 70% ethanol between each subject. For each rat, the total time in the center, the number of entries into the center and the total distance traveled was recorded.

2.6. BDNF assays

2.6.1. Serum BDNF assay: At the completion of the experiment, males were made unconscious via carbon dioxide inhalation and were rapidly decapitated. Trunk blood was collected, coagulated for 1 hr at room temperature, and then centrifuged at 2000 g for 10 min at 4º C. Serum BDNF levels were measured using an enzyme-linked immunosorbent assay (ELISA) kit [102] (Biosensis, BEK-2211-1P, Thebarton, Australia). Samples were diluted 1:20 and assayed in duplicate with a BDNF detection antibody and streptavidin-horseradish peroxidase according to the kit protocol. Total BDNF in serum was converted to concentrations based on a sigmoidal fitted curve and reported in pg/ul.

2.6.2. Hippocampal tissue preparation: Immediately after trunk blood collection, hippocampi were rapidly dissected from the brain, frozen with dry ice and stored at – 80 °C. Hippocampi were sliced using a chilled McIlwain tissue chopper and 5 mg brain slices were suspended in ice-cold oxygenated low-magnesium Krebs'– Ringer (LMKR) that contained 25 mM HEPES, pH 7.4, 118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 0.3 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, 100 μM ascorbic acid, 50 μg/ml leupeptin, 0.2 mM PMSF, 25 μg/ml pepstatin A,

and 0.01 U/ml soybean trypsin inhibitor. Brain slices were centrifuged briefly, washed twice more and suspended in LMKR.

2.6.3. Ex-vivo incubation of hippocampal tissue with rhBDNF: As described in prior reports from our lab [87, 89], brain slices were incubated for 30 min at 37°C in LMKR with or without 50 ng/ml recombinant human BDNF (rhBDNF, abbreviated as BDNF from here on). Every 10 min during incubation, the mixture was aerated with 95% O_2/5% CO_2 for 1 min. BDNF stimulation was terminated by adding ice-cold calcium-free LMKR containing 0.5 mM EGTA/0.1 mM EDTA and phosphatase inhibitors, followed by centrifugation. Samples were homogenized in ice-cold immunoprecipitation buffer, centrifuged at 1000 *g* for 5 min (4°C), and the resultant supernatant (postmitochondrial fraction) was sonicated for 10 s. Protein concentrations were measured by the Bradford method. Briefly, 200 mg of post-mitochondrial fractions were solubilized in 0.5% digitonin/0.2% sodium cholate/0.5% NP-40 for 60 min (4°C) with end-to-end rotation. Lysates were cleared by centrifugation at 50,000 *g* for 5 min and diluted with immunoprecipitation buffer.

2.6.4. Immunoprecipitation and immunoblotting: To measure BDNF-stimulated TrkB signaling, 200 µg of tissue lysates were immunoprecipitated overnight (4°C) onto covalently conjugated protein A/G-agarose beads using 1 µg of immobilized anti-TrkB. Subsequently, anti-TrkB immunoprecipitates were incubated with antigen elution buffer and 2% SDS for 2 min, centrifuged, and neutralized with 1.5 Tris buffer (pH8.8) and 6X sample preparation buffer. The immunoprecipitates were solubilized by boiling for 5 min in SDS-PAGE sample buffer. Fifty µl of the eluates were then size fractionated on 7.5 or 10% SDS-PAGE. Proteins were transferred via electrophoresis to nitrocellulose membranes for immunoblotting to quantify the levels of *TrkB, pY-TrkB, PLC, N-Shc* and the *NR1* subunit of the NMDA receptor. Using additional hippocampal tissue from the same rats, exercise- and/or stress-related protein expression (without immunoprecipitation) was also assessed with immunoblot assays. The proteins were: a **myokine** (*FNDC-5/irisin*); **pro-inflammatory cytokines** (*tumor-necrosis factor alpha (TNFα), Interleukin-6 (IL-6), Interleukin-1β (IL-1β)*); an **immediate early gene** (*activity-regulated cytoskeletal-associated protein (arc*)); **neurotrophin ligands** (*proBDNF, BDNF, Neurotrophin 3 (NT3), Neurotrophin 4 (NT4)*); and **TrkB-associated proteins** (*$TrkB_{95}$, $TrkB_{145}$, PLC, N-Shc, NR1*).

During immunoblotting assays, membranes were washed with 0.1% Tween 20-containing PBS (PBST) and blocked overnight (4°C) with 10% milk in PBST. Following three 5-min PBST washes, the membranes were incubated at 25°C for 2 h with 1:500 to 1:1000 dilutions of selected

antibodies. After three PBST washes, membranes were incubated for 1 h with anti-species IgG-HRP (1:7500 dilution) and washed 3x. The blots were stripped and re-probed with anti-TrkB or with anti-β-actin to assess immunoprecipitation efficiency and/or loading efficiency. Immunoreactivity was detected by reacting with a chemiluminescent reagent for 5 min and visualized by exposure to x-ray film. Specific bands were quantified by densitometric scanning (GS-800 calibrated densitometer, Bio-Rad, Hercules, CA, USA). For the *ex vivo* BDNF stimulation experiment (with immunoprecipitation and immunoblot) each protein (*$TrkB_{95}$, pY-$TrkB_{95}$, $TrkB_{145}$, pY-$TrkB_{145}$, PLC, N-Shc, NR1)* was normalized to TrkB density. For the immunoblot-only experiment each protein (*FNDC-5/irisin, TNFα, IL-6, IL-1β, arc, proBDNF, BDNF, NT3, NT4, TrkB, PLC, N-Shc, NR1)* was normalized to β-actin density. In addition, for the *ex vivo* BDNF-stimulation data, percent BDNF-stimulation values were calculated for each protein from each rat using the formula: (BDNF-stimulated density – non-stimulated density)/non-stimulated density (Table 1).

2.6.5. Materials and Chemicals: BDNF, leupeptin, aprotinin, phenyl-methylsulfonyl fluoride, pepstatin A, soybean trypsin inhibitor, NaF, sodium vanadate, glycerophosphate, 2-mercaptoethanol, NMDA, glycine, Tween 20, NP-40, and Histopaque-1077 were from Sigma. Anti-TrkB (SC-8316), -pY-Trk (SC-8058), -phospholipase C-γ1 (SC-7290), -N-Shc (SC-365598), -NR1 (SC-9058), -TNFα (SC-52746), -IL-6 (SC-57315), -IL-1β (SC-57315), -Arc (SC-365736), -BDNF/proBDNF (SC-2098), –NT3 (SC-547), -NT4 (SC-545), -β-actin (SC-47778) were from Santa Cruz Biotechnology. FNDC-5/irisin (36-335) was from ProSci (Poway, CA, USA). Seize-X immunoprecipitation kit, antigen elution buffer, Bind NeutrAvidin, high binding capacity coated 96-well plates, and West Pico chemiluminescent reagents were from Pierce-Endogen (Rockford, IL, USA). Bradford reagent, SDS-PAGE reagents, and pre-stained molecular weight markers were from Bio-Rad. Protease inhibitors (EDTA-free) and protein phosphatase inhibitor tablets were from Roche (Basel, Switzerland). BDNF was reconstituted according to the manufacturer's instruction. To avoid freezing damage, 10% glycerol was added for a final concentration of 10 ng/µl BDNF and stored in 80°C until use. All other test agents were made fresh according to the manufacturer's recommendation. The DMSO concentration in the incubation medium was 1%.

2.7. Data analysis: The effects of preventative treatment (exercise or sedentary) and exposure (pair housing with a stressed or a non-stressed female) were analyzed using 2x2 ANOVAs followed, when appropriate, with Bonferroni-corrected pairwise comparisons. Dependent variables were grouped by: **depressive-like behaviors** *(baseline sucrose preference and post-pairing sucrose preference)*; **anxiety-like behaviors** (*open field: time in center, number of center entries, total distance traveled*); **peripheral indicators** (*body weight gained, serum BDNF*); ***ex vivo* BDNF-stimulated TrkB-associated proteins** (*$TrkB_{95}$, $pY\text{-}TrkB_{95}$, $TrkB_{145}$, $pY\text{-}TrkB_{145}$, PLC, N-Shc, NR1*); and exercise- and/or stress-associated protein expression including a **myokine** (FNDC-5/irisin); **pro-inflammatory cytokines** (*TNFα, IL-6, IL-1β*); an **immediate early gene** (*arc)*; **neurotrophin ligands** (*proBDNF, BDNF, NT3, NT4)*; and **TrkB-associated proteins** (*$TrkB_{95}$, $TrkB_{145}$, PLC, N-Shc, NR1*).

The main objective of the present study was to test the hypotheses that: 1) sedentary male rats that cohabitate for five weeks with a stress-exposed, non-familiar female will exhibit a depression-like phenotype that is observable on behavioral and physiological measures and 2) in male rats paired with a stressed female, prior voluntary exercise will blunt these emotional contagion effects. Pending statistically significant results from 2x2 ANOVAs, four pairwise comparisons were made. Specifically, hypothesis 1 was tested by comparing the behaviors of male rats that were pair-housed with either a stressed or a non-stressed female (Sedentary$^{\male}$/NoStress$^{\female}$ vs. Sedentary$^{\male}$/Stress$^{\female}$). Hypothesis 2 was tested by comparing the behaviors of male rats that either did or did not exercise and were subsequently pair-housed with a stressed female (Sedentary$^{\male}$/Stress$^{\female}$ vs. Exercise$^{\male}$/Stress$^{\female}$). In addition, comparisons were made between the exercised groups (Exercise$^{\male}$/NoStress$^{\female}$ vs. Exercise$^{\male}$/Stress$^{\female}$). Lastly, comparisons were made between groups that were paired with a non-stressed female (Sedentary$^{\male}$/NoStress$^{\female}$ vs. Exercise$^{\male}$/NoStress$^{\female}$). One-sample or independent-samples *t*-tests were used to assess female behavior, wheel rotations, and sucrose preference. All data are reported as mean ± standard error of the mean (SEM) and data points that were more than two standard deviations (±) from the mean were excluded from the analyses. An alpha level of 0.05 was used for all analyses.

3. Results

Figure 1 depicts the experimental timeline. To assess the effectiveness of stress induction on female physiology and behavior, body weight gain **(Figure 2A)** and anxiety-like behaviors **(Figure 2B-D)** were quantified. An independent samples *t*-test revealed that after the five-week chronic unpredictable stress paradigm, stressed females gained significantly less body weight

compared to non-stressed females (t_{14} = 6.5, p < 0.001). In addition, stressed females spent significantly less time (t_{14} = 2.4, p < 0.05) and made significantly fewer entries (t_{14} = 2.5, p < 0.05) into the open arms of the elevated plus maze compared to non-stressed females. No statistically significant difference was observed between stressed and non-stressed females in the number of closed arm entries, suggesting that locomotor impairments did not account for the anxiety-like behavior displayed by stressed female rats.

Quantification of the last eight days of exercise data in which individual male rats were given 24-h access to the wheel cages every other day revealed that rats in both groups completed an equivalent amount of wheel rotations (**Figure 3A**, mean = 1780 (+/- 270) revolutions/24 h interval, which is approximately 2.0 km). At the conclusion of the five-week exercise window, body weights between sedentary and exercised males did not differ (**Figure 3B**, t_{30} = 0.948, p = 0.351).

Overall, data from behavioral, peripheral and neuronal assays on male rats revealed that pair housing a healthy male with a stressed female produced a robust emotional contagion effect, and that exercise partially or fully countered some of these deficits. Sucrose preference testing was conducted to assess the hedonic state of male rats (**Figure 4**). Prior to male-female pair housing, male rats in all groups showed significant preference for a sucrose solution compared to water (one-sample *t*-tests; ts$_7$ = 12.56 - 17.7; ps < 0.001, **Figure 4,** solid bars, 'pre') that was unaffected by exercise (t_{30} = -0.18, p = 0.859). In contrast in support of Hypothesis 1, after five weeks of male-female pair housing (**Figure 4**, dotted bars), sedentary male rats that were paired with a stressed female (Sedentary♂/Stress♀) displayed anhedonia with no preference for sucrose [51.6% (+/-6.4); one-sample, t_7 = 0.20, p = 0.848]. In contrast, rats in all other groups, including the Exercise♂/Stress♀ group, maintained a preference for sucrose (ts$_7$ = 9.4 – 13.9; ps < 0.001), which supports hypothesis 2. Furthermore, to compare hedonic states between groups, a 2x2 ANOVA on the post-pairing sucrose preference test was conducted and revealed main effects of Stress ($F_{1,28}$ = 46.10, p < 0.001) and Exercise ($F_{1,28}$ = 7.54, p < 0.01) and a Stress x Exercise interaction ($F_{1,28}$ = 4.96, p < 0.05). *Post hoc* pairwise comparisons revealed that rats in the Sedentary♂/Stress♀ group differed significantly from rats in the Sedentary♂/NoStress♀ group (mean difference = 34.98, p < 0.05; Hypothesis 1) and differed significantly from rats in the Exercise♂/Stress♀ group (mean difference = 19.28, p < 0.05; Hypothesis 2). In addition, rats in the Exercise♂/NoStress♀ group also differed from rats in the Exercise♂/Stress♀ group (mean difference = 23.57, p < 0.001). Groups of rats that were pair-housed with a non-stressed female (Sedentary♂/NoStress♀ vs. Exercise♂/NoStress♀) did not differ (mean difference = 2.01, p = 0.324). Thus a stair-step pattern

of hedonic state across groups was observed with Sedentary♂/NoStress♀ ≈ Exercise♂/NoStress♀ > Exercise♂/Stress♀ > Sedentary♂/Stress♀ **(Figure 4, dotted bars)**. Collectively, these data indicate that pair housing a healthy male with a stressed female leads to a significant decrease in hedonic state that is partially attenuated by prior voluntary exercise.

An open field task was conducted to assess anxiety-like behavior in male rats after they were pair-housed with a female (**Figure 5**). The 2x2 ANOVAs conducted on the open field measures revealed a main effect of Stress for the time spent in the center of the maze (**Figure 5A**, $F_{1,28} = 5.51$, $p < 0.05$) and for the number of center entries (**Figure 5B**, $F_{1,28} = 6.62$, $p < 0.05$), but neither main effects of Exercise nor Stress x Exercise interactions. A main effect of Stress ($F_{1,28} = 9.55$, $p < 0.05$) and a Stress x Exercise interaction ($F_{1,28} = 6.00$, $p < 0.05$), was observed in the total distance traveled (**Figure 5C**). Overall the open field data indicate that cohabitation with a stressed female induced pronounced anxiety-like behaviors that were minimally influenced by prior exercise.

Two dependent variables (body weight gain and serum BDNF concentration) served as peripheral indicators of the impact of cohabitation with a stressed female and/or exercise; these measures were collected at the conclusion of pair housing and behavioral testing. 2x2 ANOVAs that assessed body weight gain (**Figure 6A**) or serum BDNF concentration (**Figure 6B**) revealed main effects of Stress (BW: $F_{1,28} = 26.54$, $p < 0.001$; BDNF: $F_{1,28} = 10.87$, $p < 0.01$). Rats paired with a stressed female gained significantly less body weight and had less serum BDNF compared to rats that were paired with a non-stressed female. In addition, main effects of Exercise (BW: $F_{1,28} = 12.26$, $p < 0.01$; BDNF: $F_{1,28} = 5.41$, $p < 0.05$) were observed. Exercised rats gained more body weight and had higher concentrations of serum BDNF compared to sedentary rats. No statistically significant interactions were observed (BW: $F_{1,28} = 1.33$, $p = 0.258$; BDNF: $F_{1,28} = 1.54$, $p = 0.225$). Collectively, these data indicate that pair housing a healthy male with a stressed female negatively impacts both body weight and serum BDNF concentration and that exercise influences these peripheral measures in the opposite direction.

To assess the impact of stress and exercise on TrkB signaling, BDNF was applied *ex vivo* to hippocampal tissue from male rats **(Figure 7)**. Overall, the analyses revealed that exposure to a stressed female substantially decreased BDNF-stimulated hippocampal TrkB signaling (Hypothesis 1), whereas among male rats that were paired with a stressed female, prior exercise partially attenuated this effect (Hypothesis 2). To describe these data more fully, included is a diagram of TrkB signaling depicting the intracellular events that ensue after BDNF binding (**Figure 7A**). As depicted, ligand binding induces auto-phosphorylation of tyrosine residues of

TrkB (i.e., pY-TrkB). Subsequently, PLC and the adaptor protein, N-Shc, are recruited to pY-TrkB. Also, coupling between TrkB and the NR1 subunit of the NMDA receptor is denoted with a black arrow. To investigate changes in these signaling events in the context of emotional contagion and exercise, hippocampal tissue was incubated *ex vivo* with either a vehicle solution (unstimulated samples, -) or with BDNF (stimulated samples, +). Subsequently anti-TrkB antibody was used to immunoprecipitate TrkB and TrkB-associated proteins. Finally, immunoblot assays with antibodies to TrkB and to associated proteins (i.e., pY-TrkB, PLC, N-Shc and NR1, **Figure 7B**), were conducted on the precipitates to enable densitometric quantification. As listed in **Table 1**, and apparent through visual inspection of **Figure 7B**, for all TrkB-associated proteins, across all rat groups, incubation of hippocampal tissue with BDNF (+) produced robust increases in protein density compared to incubation with vehicle solution (-), but to different extents. Densitometric values from these plots were used to calculate percent BDNF stimulation values (**Table 1 and Figure 7C**) as described in methods and in [87, 89]. The 2x2 ANOVAs conducted on the percent BDNF stimulation data for pY-TrkB, PLC, N-Shc, and NR1 revealed main effects of Stress and Exercise and Stress x Exercise interactions. Post hoc analyses of the statistically significant interactions produced outcomes that supported Hypothesis 1 and Hypothesis 2 (**Figure 7C**, and see **Table 2** for a summary of the 2x2 ANOVAs and pairwise comparisons). Specifically, similar to the pattern observed with hedonic state, a stair-step pattern of percent BDNF stimulation was observed with Sedentary$^{\male}$/NoStress$^{\female}$ ≈ Exercise$^{\male}$/NoStress$^{\female}$ > Exercise$^{\male}$/Stress$^{\female}$ > Sedentary$^{\male}$/Stress$^{\female}$ (with averages of: 430% ≈ 425% > 320% , 200% BDNF-stimulated increases, respectively, compared to incubation with vehicle solution).

Additional immunoblot assays (without immunoprecipitation) were conducted to quantify the expression of hippocampal proteins that have been shown to be altered by stress and/or exercise, and are influenced by BDNF signaling. Proteins were categorized into: **myokines** (Figure 8AB), **pro-inflammatory cytokines** (Figure 8CD), **immediate early genes** (Figure 8EF), **neurotrophic ligands** (Figure 9AB) and **TrkB-associated proteins** (Figure 9CD). **Table 3** summarizes the results of 2x2 ANOVAs and pairwise comparisons. Statistically significant main effects, interactions and pairwise comparisons were observed in three of the five subgroups of proteins. First, FNDC-5/irisin, which is a myokine that is upregulated during exercise, was significantly and substantially elevated in the hippocampi of both exercise groups (main effect of exercise, $F_{1,28} = 16.48$, $p < 0.001$) and was lower in rats that were paired with a stressed female compared to rats paired with a non-stressed female (main effect of Stress, $F_{1,28} = 191.72$, $p < 0.001$, **Figure 8AB, Table 3**). Second, expression of pro-inflammatory cytokines (TNFα, IL-6

and IL-1β) was highest in sedentary rats that were paired with a stressed female (**Figure 8CD, Table 3**). Specifically, *post hoc* pairwise comparisons revealed that cytokine levels in the hippocampi of rats in the Sedentary♂/Stress♀ group were elevated compared to levels in the Sedentary♂/NoStress♀ group (Hypothesis 1) and were also significantly elevated compared to hippocampal cytokine levels in the Exercise♂/Stress♀ group (Hypothesis 2). In contrast, cytokine levels in the Exercise♂/NoStress♀ group did not differ from rats in the Exercise♂/Stress♀ group. In addition, no difference was observed between groups that were pair-housed with a non-stressed female (Sedentary♂/NoStress♀ vs. Exercise♂/NoStress♀)**.** These data indicate that prior exercise fully attenuated the stress-pairing induced increase in pro-inflammatory cytokine levels in the hippocampus. Third, expression of the immediate early gene, arc, showed the same pattern as observed with the cytokine analyses with significantly higher expression in sedentary rats that were paired with a stressed female compared to rats paired with a non-stressed female and compared to stress-paired rats that exercised (**Figure 8EF, Table 3**). No other significant differences across groups were observed for the neurotrophin ligands, TrkB or TrkB-associated proteins (**Figure 9A-D, Table 3**).

4. Discussion

Negative emotional contagion occurs when healthy male rats are paired with stressed females.

The present study describes a new animal model in which negative emotional contagion developed in male rats that cohabitated with stressed female rats. The model we report here is substantiated by behavioral and physiological measures that are consistent with symptoms of human depression [11]. Our analyses began with a manipulation check on female rats after stress induction and revealed that stressed females gained significantly less body weight and exhibited significantly heightened anxiety-like behavior on the elevated plus maze compared to controls. These results are consistent with a comprehensive literature describing the occurrence of depression-like and anxiety-like behaviors in rodents following stress exposure [9, 11, 100, 103-105] and confirm the effectiveness of the five-week stress-induction paradigm.

In assessing male responses, depression-like behavior was observed in healthy male rats that were pair-housed with stressed female rats. Specifically, prior to male-female pair housing, healthy male rats had a robust preference (>75%) for a sucrose solution compared to water whereas after five weeks of pair housing with a stressed female, male rats not only showed a decrease from their prior preference, but also displayed complete anhedonia, with no preference for sucrose compared to water (~50%). These results are consistent with those of Boyko et al.,

[47] in which triads of stressed-exposed and control male rats were co-housed for a similar length of time resulting in significant decreases in sucrose preference in all rats in the triad.

Robust anxiety-like behaviors were also observed in males that were pair-housed with a stressed female; a finding that is more pronounced than with male triads [47]. A possible explanation of the enhanced effects in female-male dyads compared to all-male triads is that male rats may be more susceptible to emotional contagion from female rats. This notion is consistent with a meta-analysis by Joiner & Katz [25] in which they conclude that while contagion of depressed mood is observed across all combinations, men may be particularly vulnerable to women's depression. An alternative interpretation is that the social conditions of the triad housing (e.g., group housing) in Boyko et al [47], were partially protective against the acquisition of anxiety-like behaviors in control rats. Support for the view that housing conditions may influence stress susceptibility is provided by the findings of Liu et al., [106] in which, unlike single-housed mice, group-housed mice did not display anxiety-like behaviors after chronic restraint stress.

In addition to behavioral effects, we also observed that male rats that were paired with a stressed female gained significantly less body weight compared to males paired with a non-stressed female. Thus, the present data from female and male rats demonstrate that effects on body weight can occur via direct exposure to stressors [107-109] or through negative emotional contagion. In the present experiment it is possible that the observed reduction in body weight in males that were pair housed with a stressed female was secondary to a reduction in food intake. This possibility is supported in part by reports demonstrating that acute or chronic stress exposure can lead to both decreased food intake and reductions in body weight (Jeong et al 2013; Krahn et al., 1986 Melhorn et al., 2010, Tamashiro et al., 2007; Rybkin et al., 1997). Future studies that evaluate food intake in the context of emotional contagion are needed to characterize the underlying causes of body weight changes that result from vicarious stress exposure. An alternative explanation is that a dominant-submissive relationship could have been established, with males as the submissive partner. The experience of being in a submissive social role has been shown to negatively influence body weight and meal size/frequency in other accounts of male-male relationships [110, 111]. Future studies of emotional contagion that include assessment of chronic dominance-subordinate relationships [112], particularly between female and male subjects, would be informative. It is also possible that the stress experienced by the males in the present study was sufficient in strength to trigger a metabolic deficit [113, 114], perhaps through alarm pheromone-triggered induction of hyperthermia [115, 116]. A limitation of this interpretation is that prior reports of metabolic effects are based on rats that directly experienced stress, and are

most robust when intermittent stress-free periods occur during stress-induction paradigms [111], neither of which were experienced by male rats in the present study.

The present data are the first to demonstrate that serum BDNF concentration and BDNF-stimulated TrkB signaling in the hippocampus are diminished in male rats that cohabitate with a stress-exposed female. As anticipated, in tissue from rats paired with non-stressed females, *ex vivo* BDNF stimulation increased the amount of phosphorylated (activated) full length $TrkB_{145}$. In addition, after BDNF stimulation, there was a substantial increase in the amount of protein that was recruited to activated $TrkB_{145}$ (e.g., pY-$TrkB_{145}$). These include PLC and N-Shc, which in turn interact with additional downstream signaling proteins (e.g., ERK, Akt, mTOR). BDNF stimulation also resulted in increased linkage between TrkB and glutamatergic signaling as demonstrated by an increase in the amount of NR1 detected in the anti-TrkB immunoprecipitate. In contrast, in rats that were paired with a stressed female, BDNF-stimulated TrkB signaling was substantially blunted. This effect was observed for all proteins measured in the anti-TrkB immunoprecipitates, except $TrkB_{95}$, the truncated form of TrkB which lacks kinase activity. These deficits in BDNF-related events have the potential to negatively impact neuronal health, neurogenesis, synaptic stability and synaptic plasticity.

It is noteworthy that basal expression of BDNF, TrkB and TrkB-associated proteins that were measured by immunoblot (without immunoprecipitation), did not differ across groups, suggesting that the experience of being housed with a stressed female affected the *affinity* of BDNF for TrkB and/or TrkB *efficacy*, rather than total protein levels. This interpretation is further supported by the observation that regardless of subcategory (e.g.., subunit of NMDA receptor, adaptor protein, etc.), all pY-$TrkB_{145}$-associated proteins were impacted by stress pairing to a similar extent, suggesting a top-down effect on signaling cascades. The present findings specific to protein expression complement and augment the multitude of reports that describe stress-induced changes in BDNF and TrkB mRNA [79, 80, 93-97, 99].

The experimental design used in the present study differed in several ways from prior studies centered on emotional contagion. Specifically, rats were not familiar with each other at the time of pairing, suggesting that, contrary to many observations [34, 41, 117-119], emotional contagion can occur in the absence of familiarity. In addition, in the present study, male rats did not directly observe female stress induction sessions, nor were they housed in the same room as female rats during the five weeks of chronic unpredictable stress. These findings demonstrate that, like fear responses that are learned vicariously [35, 42, 120], the impact of chronic unpredictable stress can also be shared vicariously.

Voluntary exercise partially attenuates the impact of negative emotional contagion.

A second key finding of the present study is that among rats that were paired with a stressed female, prior voluntary exercise reduced the occurrence of some measures of emotional contagion. For example, analysis of behavioral data revealed that male rats that exercised prior to pair housing with a stressed female maintained a preference for sucrose, whereas sedentary, stress-paired rats did not. These data lend support for the view that not only can exercise serve as a treatment for depression in humans [49, 50, 53] and for depression-like behaviors in rodents [72, 75] but that, as in humans [54], exercise can also serve as a prophylactic measure. Collectively, prior reports and the present preclinical findings argue for the inclusion of exercise programs for persons that may in the future be susceptible to negative emotional contagion. Furthermore, future studies that examine the synergistic effects of voluntary exercise with subthreshold doses of antidepressant treatments may provide a therapeutic avenue for persons that experience negative emotional contagion.

In contrast to our findings pertaining to hedonic state, the degree of anxiety-like behavior did not differ significantly between sedentary and exercised male rats that were paired with a stressed female. However, while a statistically significant interaction between stress and exercise was not observed, visual inspection of the open field data revealed that sedentary males that were paired with a stressed female trended toward heightened anxiety-like behavior compared to exercised, stress-paired males. Nevertheless, the present results differ from several reports that demonstrate a pronounced effect of prior exercise on stress-induced anxiety-like behaviors [63, 65-69]. These differences in findings may be due to methodological differences across studies (e.g., whether the stress-induction paradigm is directly experienced or is vicarious; the duration and intensity of stress-exposure; the methods used to quantify anxiety, etc.). Future studies that use additional tests (e.g., elevated plus maze; light-dark tests; social interaction; learned helplessness tests) will continue to inform how exercise impacts anxiety-like behaviors in the context of negative emotional contagion.

The combination of voluntary exercise and pair housing with a stressed or non-stressed female also markedly impacted BDNF-stimulated TrkB signaling in the hippocampus. Overall, in rats paired with a non-stressed female, incubation of hippocampal tissue with BDNF increased TrkB activation and signaling by an average of over 400% compared to incubation with a vehicle solution. In contrast, rats in both stress-paired groups has a blunted response to BDNF, but importantly, the deficit in sedentary rats was substantially greater than rats that exercised (i.e., only

~200% BDNF-induced stimulation vs. ~330% BDNF-induced stimulation). The present findings are consistent with those of Zheng et al. [75], which examined the effects of stress and voluntary exercise on BDNF mRNA in the hippocampus. In that study, rats lived in cages equipped with an exercise wheel for a total of seven weeks. After one week, rats experienced a four-week chronic unpredictable stress paradigm and underwent behavioral testing after stress exposure. They found that concurrent exercise attenuated stress-induced decreases in BDNF mRNA (and also influenced hedonic state). Collectively, Zheng et al. [75] and the present findings demonstrate that whether protein, mRNA or signaling is measured, whether stress exposure is direct or vicarious, and whether voluntary exercise is concurrent or prior to stress exposure, there is a protective effect of exercise on stress-induced BDNF- and TrkB-related deficits. The present findings indicate that among rats that were paired with a stressed female, exercise positively influences many neural events that are associated with mood and cognition, including neurogenesis, neurohormone production, neuronal health and synaptic plasticity.

The present data also provide a view of the relationships between stress, exercise, immune function and neuronal health. Using immunoblot (without immunoprecipitation), we quantified the expression of proteins that are known to be altered by stress and/or exercise, and that are influenced by BDNF-TrkB signaling. In hippocampal tissue, pro-inflammatory cytokines (i.e., TNFα, IL-6, IL-1β) were elevated in sedentary rats that were paired with a stressed female, yet exercise fully reversed this response. These results are consistent with Chennaoui et al. [121] in which seven weeks of prior exercise fully prevented a sleep deprivation-induced surge in pro-inflammatory cytokines in rodent hippocampal tissue, and with reports that describe exercise-induced decreases in pro-inflammatory cytokines in rats and humans [122-124]. Given the negative impact of pro-inflammatory cytokines on BDNF and/or TrkB levels [19, 125, 126], one explanation of our findings is that exercise partially preserved TrkB signaling by counteracting a stress-induced surge of pro-inflammatory cytokines. We also examined expression of the myokine, FNDC-5/irisin, which is released by muscles during exercise, and is produced in neural tissue [98]. As anticipated, FNDC-5/irisin expression was significantly and substantially elevated in rats that exercised, and was decreased by stress. Given evidence that exercise increases hippocampal BDNF through a metabolic-FNDC-5/irisin signaling pathway [98, 99], a second, but not mutually exclusive possibility is that exercise preserved TrkB signaling by increasing hippocampal FNDC-5/irisin levels. Lastly, exercise prevented a stress-induced increase in expression of the immediate early gene, arc, which is regulated by synaptic activity and is influenced by BDNF-mediated signaling [90-92]. Faulty regulation of arc may have implications

for synaptic stability, efficacy and plasticity in hippocampal tissue. While many components remain unknown, our preliminary analyses of protein expression in hippocampal tissue indicate that exercise partially counteracts stress-pairing induced deficits that impact critical cellular functions including neuronal health, neurogenesis and plasticity, and that are ultimately linked to mood and cognition. Future studies that examine BDNF signaling in additional brain regions such as the prefrontal cortex, anterior cingulate cortex and amygdala [34, 39, 42, 44, 87, 127] will further contribute to our understanding of the relationships between stress, voluntary exercise and emotional contagion.

In summary, this report describes a novel animal model in which stress-induced negative emotional contagion develops across time and between pairs. Our first hypothesis is supported by the finding that pair housing a healthy male with a stressed female produced a robust emotional contagion effect; males gained less body weight, were anhedonic, demonstrated anxiety-like behavior, had lower serum BDNF concentrations, had diminished BDNF-stimulated TrkB signaling, and an elevation of pro-inflammatory cytokines. Our second hypothesis is supported by the finding that prior exercise was beneficial on some measures of emotional contagion; exercised males paired with a stressed female showed less depressive-like behavior, had partially preserved hippocampal BDNF-stimulated TrkB signaling, had normalized serum BDNF concentration, and had hippocampal cytokine and immediate early gene levels that were equivalent to controls. The data support the view that the inclusion of exercise programs could be beneficial for persons that may, in the future, be susceptible to negative emotional contagion and also highlight potential molecular targets for therapeutic intervention.

5. References

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Figure Legends

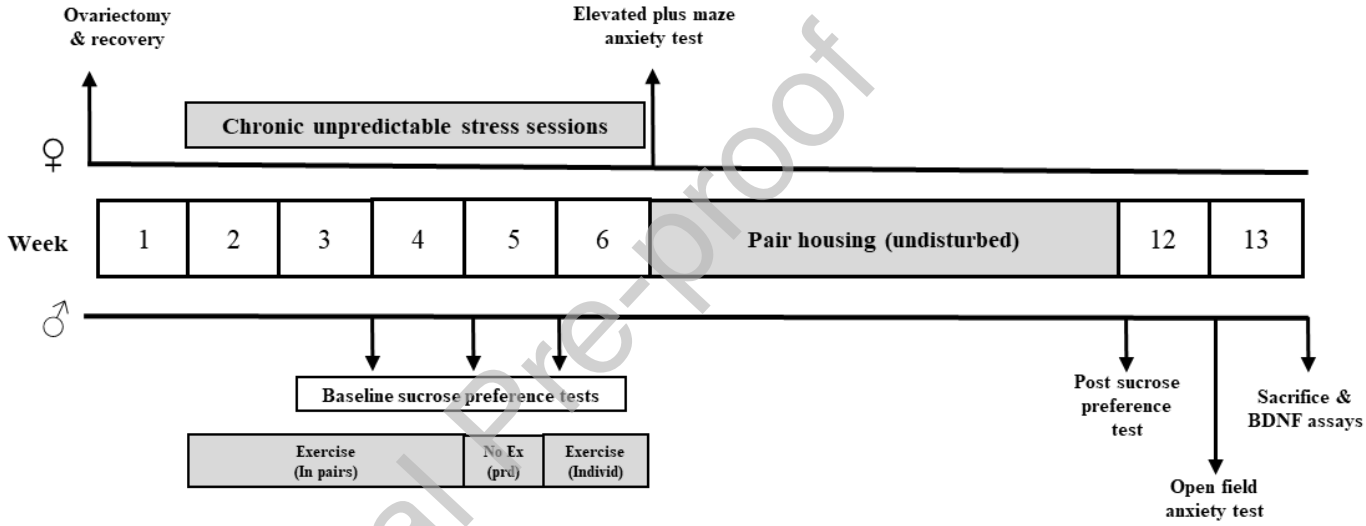

Figure 1. depicts the experimental timeline.

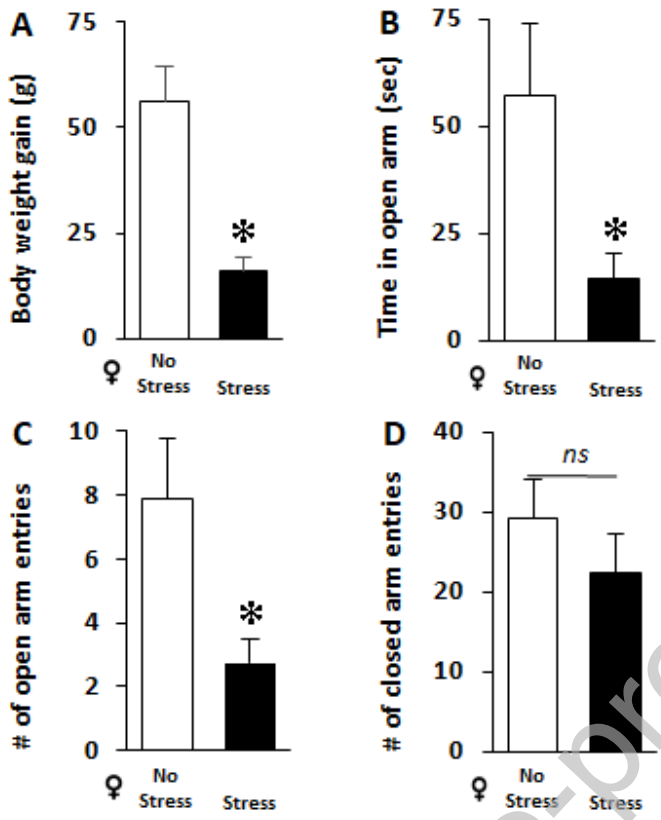

Figure 2. Effects of chronic unpredictable stress on female rats. A. Female body weight gain was significantly reduced after five weeks of stress exposure. **B&C.** Stressed females demonstrated significantly increased anxiety-like behaviors as demonstrated by less time spent in open arm and fewer entries into the open arms during the elevated plus maze test (independent-samples *t*-tests, *ps* < 0.05). **D.** No significant difference in the number of closed arm entries was observed. * indicates significant difference from non-stressed female rats. *ns* = no significant difference.

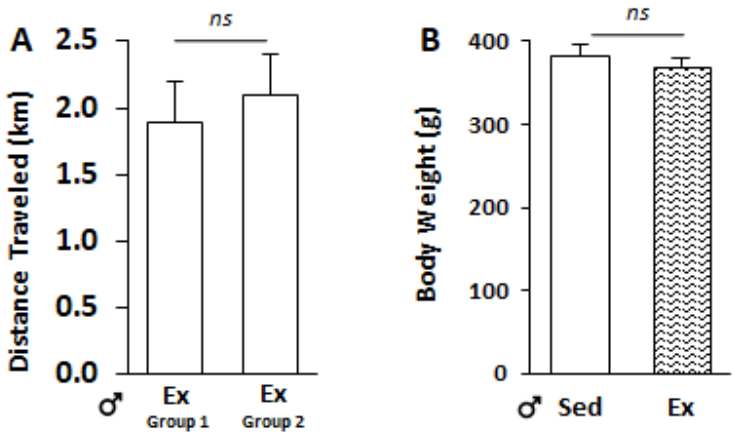

Figure 3. Measures of male behavior and physiology across groups are similar prior to pair housing. A. Average distance traveled (wheel revolutions converted to km) by individual male rats from groups 1 and 2 during the fifth week of exercise. Data reflect the average distance traveled per 24 h interval. **B.** Average body weight of sedentary and exercised rats after the five-week exercise window.

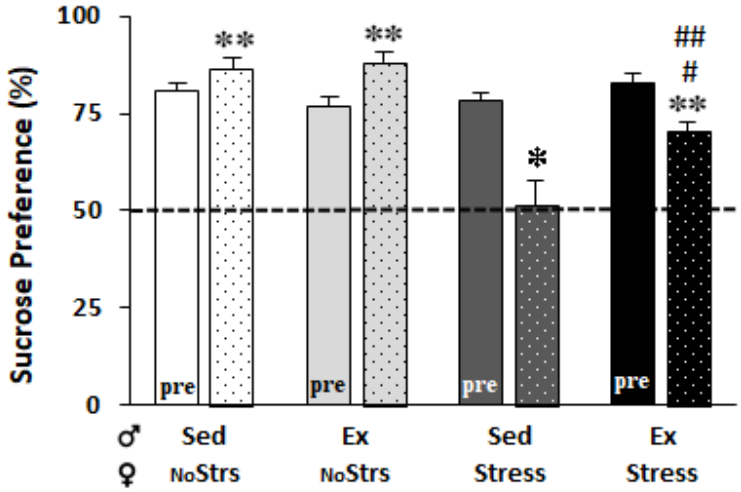

Figure 4. Pair housing with a stressed female causes anhedonia, an effect that is partially attenuated by prior exercise. Prior to pair housing (solid bars, pre), males in all conditions demonstrated preference for a sucrose solution compared to water. After five weeks of pair housing (dotted bars) sedentary males paired with a stressed female displayed anhedonia (no preference for sucrose) whereas rats in all other groups showed a significant preference for sucrose. ** reflect the results of the one-sample *t*-tests that were conducted on the post pair housing data and indicate that animals in these groups maintained a strong preference for sucrose over water. 2x2 ANOVA conducted on the post pairing data (dotted bars) revealed main effects of Stress and Exercise and a Stress x Exercise interaction. Posthoc pairwise comparisons revealed that sedentary rats paired with a stressed female demonstrate anhedonia, whereas exercise partly attenuated this deficit. * indicates significant difference from Sedentary♂/NoStress♀ and # indicates significant difference from Sedentary♂/Stress♀ during the post pair housing sucrose preference test.

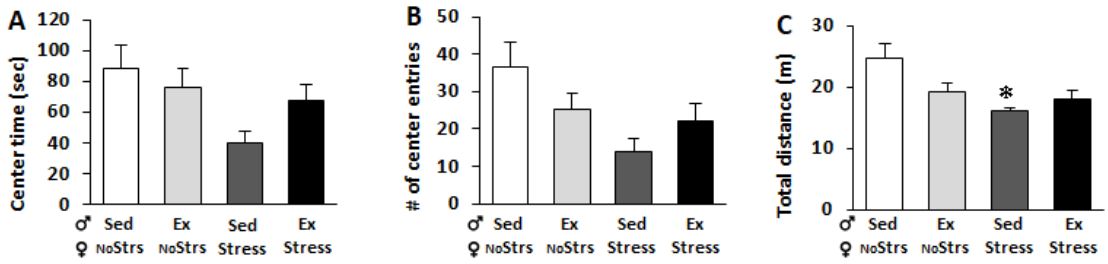

Figure 5. Pair housing with a stressed female heightens anxiety-like behavior in males. Time in center (**A)** and number of entries into the center of the open field **(B)** were significantly decreased in male rats that were paired with a stressed female regardless of whether male rats were sedentary or exercised (2x2 ANOVA, main effects of stress, *p*s < 0.05). **C.** Among sedentary rats, pair housing with a stressed female decreased the total distance traveled in the open field, whereas an effect of stress was not observed among rats that exercised prior to pair housing (2x2 ANOVA, Exercise x Stress interaction, $p < 0.05$). * indicates significant difference from the Sedentary♂/NoStress♀group (pairwise comparison, $p < 0.05$).

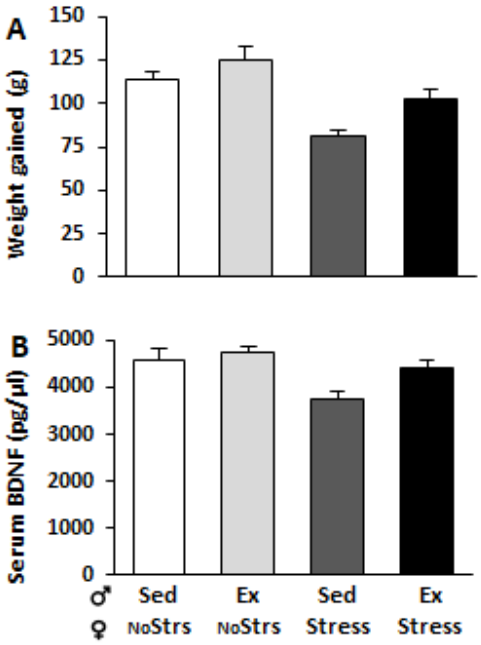

Figure 6. Pair housing with a stressed female, and exercise, influence peripheral measures. **A.** Males that were paired with a stressed female gained significantly less weight compared to males that were paired with a non-stressed female (2x2 ANOVA, main effect of Stress, $p < 0.001$). In addition, exercise significantly increased body weight gain regardless of pair housing condition (2x2 ANOVA, main effect of Exercise, $p < 0.01$). **B.** Males that were paired with a stressed female had significantly less serum BDNF compared to males that were paired with a non-stressed female (2x2 ANOVA, main effect of Stress, $p < 0.01$). In addition, exercise significantly increased serum BDNF regardless of pair housing condition (2x2 ANOVA, main effect of Exercise, $p < 0.05$).

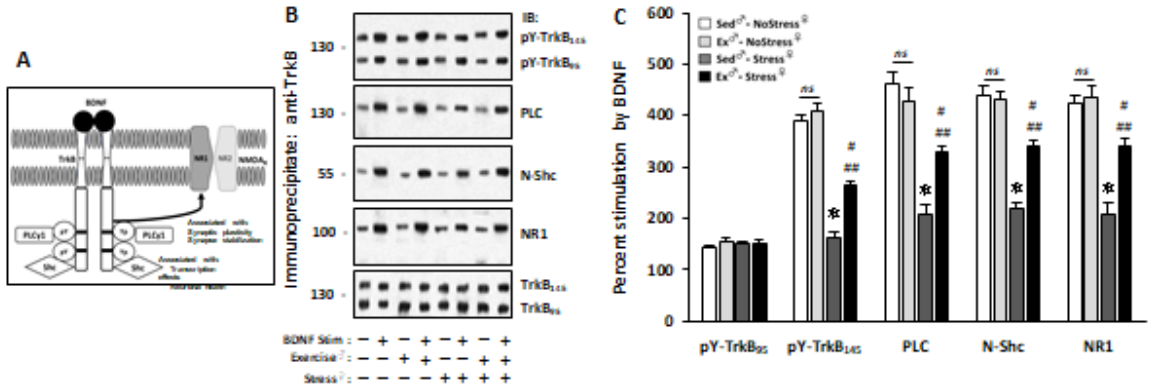

Figure 7. Pair housing with a stressed female diminishes hippocampal BDNF-stimulated TrkB signaling and, among stress-paired rats, prior exercise is beneficial. **A.** Schematic diagram of BDNF-mediated activation of tyrosine receptor kinase B (TrkB). BDNF binding leads to auto-phosphorylation of tyrosine residues (pY) on TrkB. Subsequently, signaling proteins (e.g., PLC and N-Shc) involved in cellular cascades that influence transcription and other events, are recruited to pY-TrkB. In addition, coupling between TrkB and the NR1 subunit of the NMDA receptor, which has been shown to influence plasticity, synapse stability and neuronal health, is depicted (black arrow). **B.** Representative blots depicting optical density bands resulting from *ex vivo* incubation of hippocampal tissue with (+) or without (-) BDNF. Immunoprecipitation with TrkB antibody and immunoblot (IB) assays with antibodies to TrkB-associated proteins (pY-TrkB, PLC, N-Shc, NR1) were conducted after *ex vivo* stimulation (means ± SEMs are reported in Table 1). **C.** Results of 2x2 ANOVAs that assessed the percent signaling induced by *ex vivo* application of BDNF. Analyses revealed statistically significant main effects of Stress and Exercise as well as Stress x Exercise interactions for each protein shown in C (also see Table 1 for means/SEMs and Table 2 for main effects, interactions and pairwise comparisons). Percent BDNF stimulation reflects the amount of protein density observed after incubation with vehicle (unstimulated) relative to the amount of protein density observed after incubation with BDNF (stimulated). Protein densitometric measures are relative to TrkB (**B**, bottom panel). * indicates significant difference from Sedentary♂/NoStress♀; # indicates significant difference from Sedentary♂/Stress♀; ## indicates significant difference from Exercise♂/NoStress♀. Symbols represent pairwise comparisons following statistically significant interactions. Abbreviations: BDNF = Brain-derived neurotrophic factor; NR1 = the NR1 subunit of the glutamatergic

ionotropic NMDA receptor; N-Shc = Neuronal Src homology and collagen adaptor protein; PLC = phospholipase C-ɣ1; $TrkB_{95}$ = truncated form of tyrosine receptor kinase B; pY-$TrkB_{95}$ = phosphorylated $TrkB_{95;}$ $TrkB_{145}$ = full length form of tyrosine receptor kinase B; pY-$TrkB_{145}$ = phosphorylated (activated) $TrkB_{145}$.

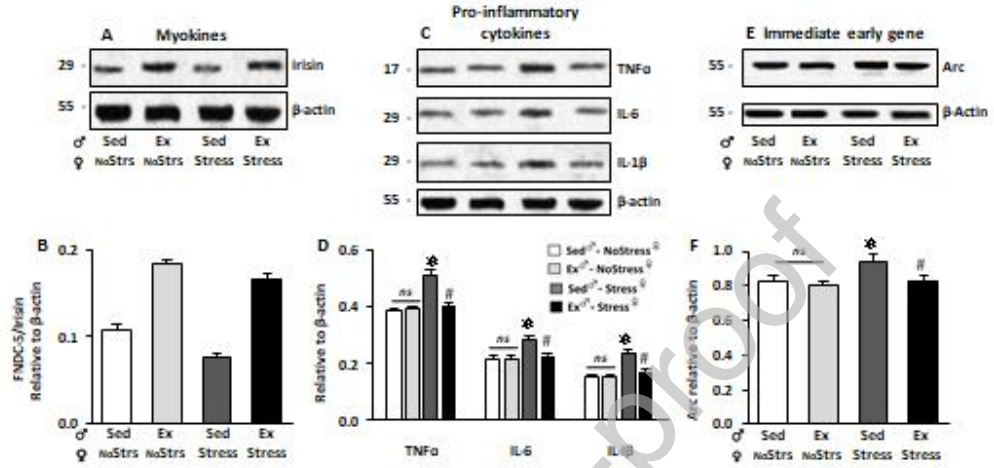

Figure 8. Myokine, pro-inflammatory cytokine and immediate early gene protein expression in the hippocampus are sensitive to stress and/or exercise. A. Representative immunoblots depicting optical density bands of FNDC-5/irisin expression relative to *β*-actin. **B.** Densitometric quantification revealed that exercise significantly increased FNDC-5/irisin expression regardless of pair housing condition (2x2 ANOVA, main effect of Exercise, $p < 0.001$). In addition, pair housing with a stressed female diminished FNDC-5/irisin expression (2x2 ANOVA, main effect of Stress, $p < 0.001$). **C.** Representative immunoblots and **(D)** densitometric quantification of pro-inflammatory cytokine expression revealed that prior exercise prevented a stress-pairing induced increase in hippocampal cytokines including TNFa, Il-6 and Il-1β (2x2 ANOVAs, Stress x Exercise interactions, *p*s < 0.05 – 0.001). **E.** Representative immunoblots and **(F)** densitometric quantification of arc expression revealed an identical pattern as observed with the cytokines. * indicates significant difference from Sedentary♂/NoStress♀ and # indicates significant difference from Sedentary♂/Stress♀. Symbols represent post hoc pairwise comparisons following statistically significant interactions. Abbreviations: FNDC-5 = Fibronectin type III domain-containing protein 5, a precursor for the irisin protein; Irisin = a myokine that is cleaved from FNDC-5; TNFα =

tumor necrosis factor alpha; IL-6 = interleukin 6; IL-1β = interleukin 1-beta; arc = activity-regulated cytoskeletal-associated protein.

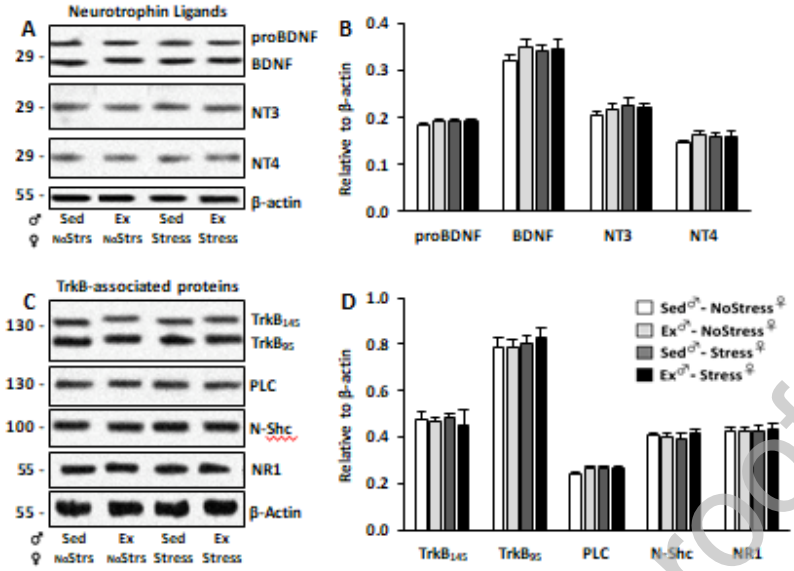

Figure 9. Neurotrophin ligand expression and basal TrkB-associated protein expression are not influenced by exercise or by pair housing with a stressed female. A. Representative blots depicting optical density bands and **(B)** densitometric quantification of neurotrophin ligand protein expression relative to β-actin. **C.** Representative blots depicting optical density bands and **(D)** densitometric quantification of basal expression of TrkB-associated proteins relative to β-actin. 2x2 ANOVAs revealed neither main effects nor interactions for any proteins in **B** and **D**. Abbreviations: BDNF = brain-derived neurotrophic factor; NR1 = the NR1 subunit of the glutamatergic ionotropic NMDA receptor; N-Shc = Neuronal Src homology and collagen adaptor protein; NT3 = neurotrophin 3; NT4 – neurotrophin 4; PLC = phospholipase C-ɣ1; proBDNF = precursor protein for BDNF; $TrkB_{95}$ = truncated form of tyrosine receptor kinase B; $TrkB_{145}$ = full length form of tyrosine receptor kinase B.

Table 1. Protein density following *ex vivo* BDNF stimulation (IP & immunoblot)

			Sedentary♂ NoStress♀		Exercise♂ NoStress♀		Sedentary♂ Stress♀		Exercise♂ Stress♀	
Incubation	Ratio to:	Protein	mean	sem	mean	sem	mean	sem	mean	sem
Basal (K-Ringers)	$TrkB_{95}$	pY-$TrkB_{95}$	.260	*.008*	.284	*.010*	.280	*.007*	.279	*.011*
	$TrkB_{145}$	pY-$TrkB_{145}$	.247	*.007*	.266	*.009*	.267	*.012*	.273	*.011*
		PLC	.155	*.007*	.188	*.012*	.194	*.015*	.196	*.012*
		N-Shc	.156	*.007*	.164	*.007*	.182	*.011*	.165	*.011*
		NR1	.178	*.008*	.186	*.004*	.199	*.006*	.183	*.004*
Stimulated (BDNF)	$TrkB_{95}$	pY-$TrkB_{95}$	.630	*.017*	.727	*.037*	.696	*.014*	.698	*.029*
	$TrkB_{145}$	pY-$TrkB_{145}$	1.208	*.025*	1.347	*.060*	.695	*.030*	.995	*.050*
		PLC	.862	*.027*	.982	*.059*	.592	*.060*	.839	*.050*
		N-Shc	.835	*.023*	.868	*.044*	.581	*.044*	.721	*.045*
		NR1	.927	*.024*	.995	*.036*	.610	*.048*	.804	*.032*
Percent stimulation by BDNF		pY-$TrkB_{95}$	142.6	*4.0*	155.7	*6.8*	149.0	*5.7*	150.8	*6.5*
		pY-$TrkB_{145}$	390.6	*11.2*	406.9	*17.9*	161.6	*10.0*	264.1	*8.9*
		PLC	462.3	*22.3*	427.1	*25.6*	205.6	*18.9*	330.0	*10.8*
		N-Shc	439.4	*18.9*	430.9	*13.7*	217.3	*12.8*	339.9	*12.6*
		NR1	425.3	*13.8*	435.9	*21.0*	207.1	*23.1*	339.4	*17.6*

Table 2. Statistical analyses of *ex vivo* BDNF stimulation (immunoprecipitation plus immunoblot)

	2x2 ANOVA						*post hoc* pairwise comparisons							
	Main effect: Stress		**Main effect:** Exercise		**Interaction:** Stress x Ex		Sed-NoStress vs. Sed-Stress		Sed-Stress vs. Ex-Stress		Ex-NoStress vs. Ex-Stress		Sed-NoStress vs. Ex-NoStress	
Protein	*F*	*p*	*F*	*p*	*F*	*p*	mean diff	*p*	mean diff	*p*	mean diff	*p*	mean diff	*p*
pY-$TrkB_{95}$	0.02	0.901	1.60	0.216	0.93	0.343	6.4	0.447	1.8	0.833	4.9	0.558	13.0	0.126
pY-$TrkB_{145}$	221.52	**<0.001**	22.65	**0.035**	11.90	**<0.001**	229.0	**<0.001**	102.5	**<0.001**	142.8	**<0.001**	16.5	0.362
PLC	77.08	**<0.001**	4.89	**<0.001**	15.69	**<0.001**	256.7	**<0.001**	124.4	**<0.001**	97.1	**0.002**	35.5	0.226
N-Shc	112.96	**<0.001**	15.00	**<0.001**	19.80	**<0.001**	222.1	**<0.001**	122.6	**<0.001**	97.1	**<0.001**	8.5	0.686
NR1	67.27	**<0.001**	13.88	**<0.001**	10.05	**<0.001**	218.2	**<0.001**	132.3	**<0.001**	96.6	**<0.001**	10.6	0.698

Table 3. Statistical analyses of hippocampal protein expression (immunoblot)

		2x2 ANOVA						*post hoc* pairwise comparisons							
		Main effect: Stress		**Main effect:** Exercise		**Interaction:** Stress x Ex		Sed-NoStress vs. Sed-Stress		Sed-Stress vs. Ex-Stress		Ex-NoStress vs. Ex-Stress		Sed-NoStress vs. Ex-NoStress	
Category	Protein	*F*	*p*	*F*	*p*	*F*	*p*	mean diff	*p*	mean diff	*p*	mean diff	*p*	mean diff	*p*
Myokine	FNDC5/Irisin	191.72	**<0.001**	16.45	**<0.001**	1.44	0.240	0.032	**0.001**	0.091	**<0.001**	0.017	0.053	0.076	**0.001**
Pro inflammatory cytokines	TNFα	23.06	**<0.001**	12.16	**0.002**	16.14	**<0.001**	0.127	**<0.001**	0.108	**<0.001**	0.011	0.584	0.008	0.710
	IL-6	9.94	**0.004**	5.03	**0.033**	4.61	**0.040**	0.065	**0.001**	0.054	**0.004**	0.012	0.486	0.001	0.500
	IL-1β	22.05	**<0.001**	12.07	**0.002**	9.08	**0.005**	0.076	**<0.001**	0.064	**<0.001**	0.017	0.244	0.005	0.747
IEG	arc	4.695	**0.039**	5.04	**0.033**	1.89	0.180	0.114	**0.018**	0.116	**0.016**	0.026	0.580	0.028	0.544
Neurotrophic ligands	proBDNF	1.193	0.284	0.452	0.507	1.028	0.319	NS		NS		NS		NS	
	BDNF	0.202	0.657	1.138	0.295	0.689	0.413								
	NT3	1.203	0.282	0.090	0.766	0.765	0.389								
	NT4	0.503	0.484	1.425	0.243	0.873	0.358								
TrkB-associated proteins	$TrkB_{95}$	0.005	0.945	0.100	0.754	0.131	0.720	NS		NS		NS		NS	
	$TrkB_{145}$	0.379	0.543	0.308	0.583	0.102	0.752								
	PLC	0.013	0.909	0.789	0.382	0.480	0.395								
	N-Shc	0.128	0.723	0.196	0.661	0.543	0.467								
	NR1	0.131	0.720	0.122	0.730	0.037	0.849								