Irisin injection into the hippocampus suppresses acute stress-induced memory impairment and anxiety-like behavior in a sex dependent manner

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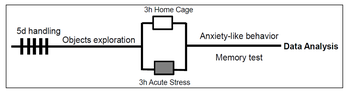
ABSTRACT

Acute stress can disrupt a variety of neural processes, including reducing levels of brain-derived neurotrophic factor (BDNF) in the hippocampus. Exercise, on the other hand, can increase BDNF levels and has overall beneficial effects for health and brain function. Irisin is a myokine that is released into the peripheral blood during aerobic exercise. Although the main known functions of Irisin, both in human and rodents, are browning white adipose tissue and improving glucose homeostasis, recent findings have shown that Irisin mediates the activation of an exercise-induced BDNF-mediated neuroprotective pathway in the hippocampus. Therefore, in this study we tested the hypothesis that Irisin can counteract the deleterious effects of acute stress when directly injected into the hippocampus. To test our hypothesis, we first established a 3h physical restraint stress in adult mice that resulted in sex-dependent increased anxiety-like behaviors and memory impairment in a combined open field/novel object recognition (OF/NOR) test. Moreover, acute stress also reduced skin temperature and body weight in both female and male mice. We then injected Irisin via bilateral stereotaxic injection and repeated the acute stress paradigm and combined OF/NOR test. We found that Irisin partially blocked stress-induced anxiety-like behavior and memory impairment in male mice, while also preventing the reduction in skin temperature and body weight. Interestingly, in female mice Irisin only prevented the skin temperature and body weight reduction but showed no beneficial effects on neurobehaviors. Taken together, our results suggest a novel role for Irisin in counteracting acute stress-induced neurobehavioral and physiological abnormalities. Also, our results support the idea that exercise can be a potentially effective tool to promote the maintenance of healthy neural function.

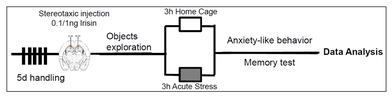
METHODS

EXPERIMENTAL MICE: Adult 8-10 weeks old C57BL/6J mice (Charles River, Wilmington, MA) were group housed (maximum 5 mice per cage) in ventilated cages inside an air-conditioned room. The room was kept at 21-23°C and 40-60% humidity on a 12:12 h light/dark cycle. Mice had ad libitum access to standard laboratory food and water.

ACUTE RESTRAINT STRESS PROTOCOL: For acute restraint stress mice were placed in 50-mL conical plastic tubes for 3h. Each tube was perforated with multiple holes for ventilation and was placed over a workstation surface inside the experimental room. Control mice were kept in their home cages for the same amount of time.



STEREOTAXIC SURGERY: Human recombinant irisin (1ng) or vehicle (sterile PBS) were injected into the hippocampus by bilateral stereotaxic surgery. All injections were performed using the following coordinates: anteroposterior (AP) -2.6 mm, mediolateral (ML) 2.0 mm and dorsoventral (DV) −2.4 mm coordinates.



BEHAVIORAL TESTS:

Open Field Test(OFT): This test was used to assess locomotor activity and anxiety-like behaviors. This test was performed concurrent with the acquisition phase of the NOR test. The mice were individually placed in the periphery area of the arena and allowed to explore for 10 min. The arena was divided in 2 equally sized areas (i.e. center and periphery) and the following parameters were quantified using EthoVision XT Version 10.1 (Noldus Information Technologies, Inc.): time spent in the center and rearing (both supported and unsupported).

Novel Object recognition (NOR): We performed NOR test in two phases: acquisition and recognition test, separated by a 3h interval. In the acquisition phase mice explored two identical objects ("A") placed in an open field arena (45cm × 29.8cm with the 52cm diagonal) for 10min. After 3h one object was replaced with a novel object ("B") and mice were placed back and allowed to explore for 5min. Short-term memory function was measured by quantifying exploratory behavior during the recognition test. Exploration was defined as time in which the mouse was directly interacting with either object (i.e. sniffing, rearing on it, or directing the nose towards the object). We then calculated a preference index (PI) using the formula PI = [Time on B / (Time on B + Time on A) *100].

NON-CONTACT INFRARED TEMPERATURE MEASUREMENT AND BODY WEIGHT MONITORING: A non-contact infrared (IR) thermometer was used to measure surface temperature from the intracapsular brown adipose tissue (iBAT). During the 3h period, temperature was measured starting at the beginning (T0) and then every 60 minutes until the end. Change in surface temperature (ΔT) was calculated using formula [Tn-T0] for each time point. We monitored mouse body weight before acute restraint stress and after by weighing mice using a bench top scale. Then we calculated weight loss percentage by using the formula = [(Weight $_{Beginning}$ – Weight $_{End}$)/Weight $_{Beginning}$)] *100.

STATISTICAL ANALYSIS: All statistical analyses were performed by using Origin software (OriginLab Corporation). We used Student's t-test to determine significant differences between two groups. One-way ANOVA followed by Tukey's post-hoc test was used for statistical significance between more than two groups. P<0.05 was considered statistically significant.

RESULTS

Acute stress induces anxiety-like behavior in both male and female adult mice

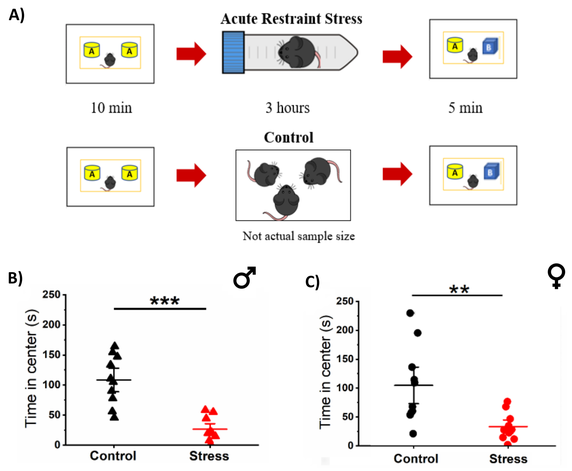


Figure 1. Acute restraint stress induces anxiety like behavior male mice. A) Experimental time course. Spending time in the center in B) male and C) female mice. Statistical significance was determined by unpaired student's t test. (n=10). Data represent mean ± SEM. **P<0.01, ***P< 0.001

Acute stress induces short-term memory impairment in male mice

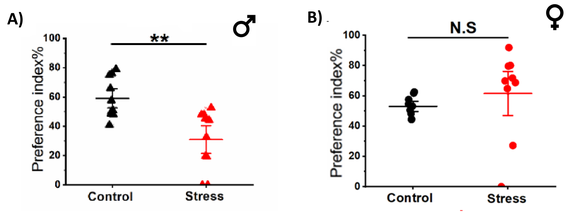


Figure 2. Acute restraint stress induces memory impairment in male mice. Preference index % in A) male and B) female mice. Statistical significance was determined by unpaired student's t test. (n=10). Data represent mean ± SEM. **P<0.01,

Acute stress impacts on exploratory behaviors in both male and female mice

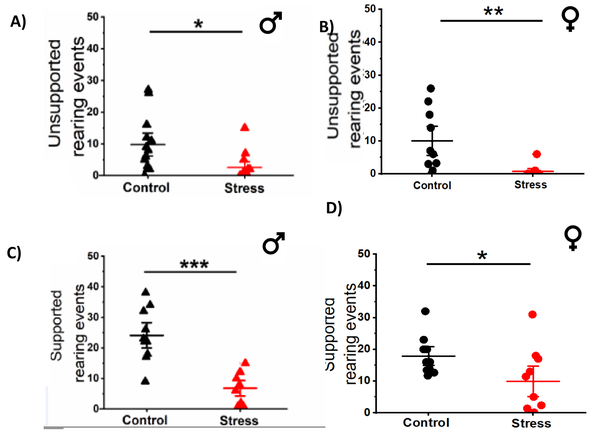


Figure 3. Acute restraint stress impacts on exploratory behaviors in both male and female mice. Unsupported rearing events in A) male and B) female mice. Supported rearing events in C) male and D) female. Statistical significance was determined by unpaired student's t test. (n=10). Data represent mean ± SEM. *P< 0.05, **P<0.01, ***P< 0.001

Acute stress influences skin temperature and weight loss percentage differentially in both male and female mice

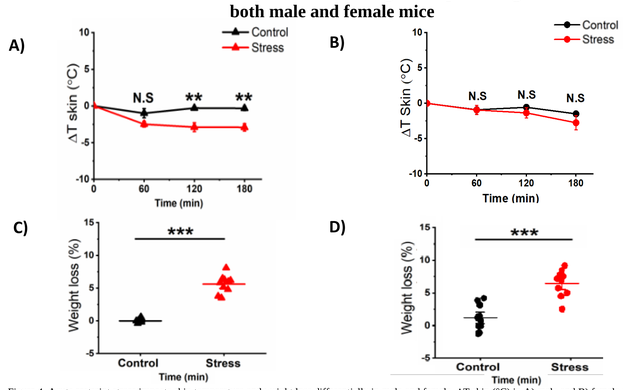


Figure 4. Acute restraint stress impacts skin temperature and weight loss differentially in male and female. ΔT skin (°C) in A) male and B) female mice. Weight loss (%) in C) male and D) female. Statistical significance was determined by unpaired student's t test. (n=10). Data represent mean ± SEM. **P<0.01, ***P< 0.001

Irisin administration into the hippocampus suppresses acute stress-induced anxiety-like behavior and memory impairment in male mice

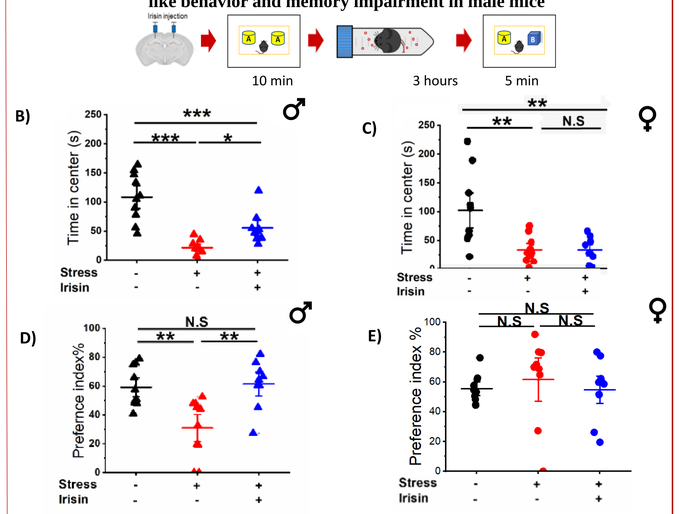


Figure 4. Irisin suppress acute stress-induced anxiety-like behavior and memory impairment in male mice. Spending time in the center in A) male and B) female mice. Preference index % in C) male and D) female. Statistical significance was determined by One-way ANOVA and Tukey test for mean comparison. n=10 mice in each group. Data represent mean ± SEM. *p<0.05,**p<0.01, ***p<0.001, N.S. denotes not significant (p>0.05).

Irisin administration into the hippocampus has no effects on acute stress-induced exploratory behavior impairments

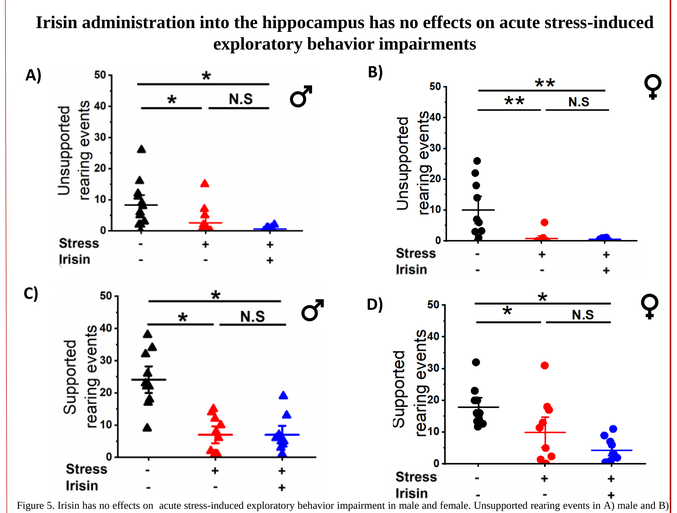


Figure 5. Irisin has no effects on acute stress-induced exploratory behavior impairment in male and female. Unsupported rearing events in A) male and B) female mice. Supported rearing events in C) male and D) female. Statistical significance was determined by One-way ANOVA and Tukey test for mean comparison. n=10 mice in each group. Data represent mean ± SEM. *p<0.05,**p<0.01, N.S. denotes not significant (p>0.05).

Irisin administration into the hippocampus suppresses acute stress-induced skin temperature decline and weight loss in both male and female

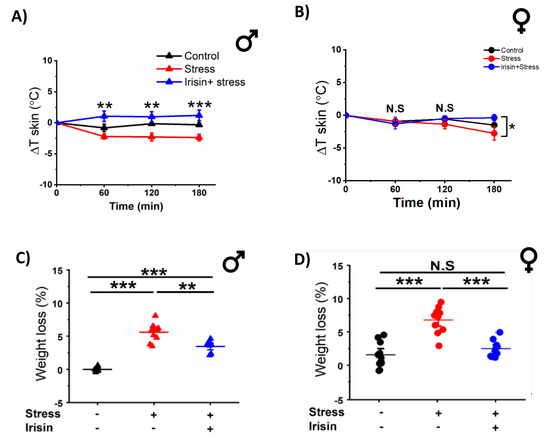


Figure 6. Irisin suppress acute stress-induced skin temperature decline and weight loss in both male and female . Differences (ΔT) in skin temperature in A) male and B) female mice. Weight loss % in C) male and D) female. Statistical significance was determined by One-way ANOVA and Tukey test for mean comparison. n=10 mice in each group. Data represent mean ± SEM. *p<0.05,**p<0.01, ***p<0.001, N.S. denotes not significant (p>0.05).

Irisin administration into the hippocampus with no exposure to acute stress has no effects in male mice

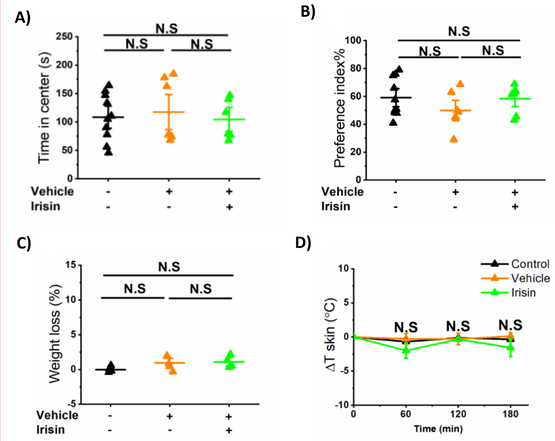


Figure 7. Irisin administration into the hippocampus with no exposure to acute stress has no effects on behavioral and physiological parameters in male mice. A) Spending time in the center B) Preference index % C) weight loss % D) ΔT skin temperature Statistical significance was determined by One-way ANOVA and Tukey test for mean comparison. n=6-10 mice in each group. Data represent mean ± SEM. N.S. denotes not significant (p>0.05). Vehicle is sterile PBS.

CONCLUSION

Acute stress induces neurobehavioral and physiological impairments differentially in male and female mice.

Irisin administration into the hippocampus suppresses acute stress-induced neurobehavioral and physiological impairments in sex dependent manner.

Irisin administration into the hippocampus with no exposure into the acute stress has no effects on neurobehaviors and physiological parameters.

Irisin could be potential therapeutic agent for suppressing acute stress-induced impairments.

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