

**Online Only Supplemental Section for:
Effects of High-Intensity Interval Training on Physical and Cognitive Function
in Middle-Aged Male Mice**

Justin C. Stephenson^{1*}, Tuan D. Tran², Ted G. Graber^{1,2,3,4,5*}

Affiliations: East Carolina University (ECU) ¹Dept. of Kinesiology, ²Dept. of Psychology, ³Dept. of Physical Therapy, ⁴Dept. of Physiology, ⁵East Carolina Obesity and Diabetes Institute

*Corresponding/Senior Author:

Ted G. Graber, PhD.

ECU College of Allied Health Sciences

Department of Physical Therapy

Health Sciences Building, Rm 2410 | Mail Stop 668

Greenville, NC 27834

Email: grabert19@ecu.edu

Available on FigShare @ doi: 10.6084/m9.figshare.28410149

Table of Contents:

Online Item	Title	Location
Figure S1	Total CFAB Scores and Intervention Assessment Values	Page S2
Figure S2	Open Field Pre- to Post-Training	Page S3
Figure S3	Y-maze Pre- to Post-Training	Page S4
Figure S4	Novel Object Recognition Pre- to Post-Training	Page S5
Figure S5	Puzzle Box Pre- to Post-Training	Page S6
Dataset S1	Physical Function	Excel File
Dataset S2	Body Composition	Excel File
Dataset S3	Muscle Mass	Excel File
Dataset S4	Contractile Function	Excel File
Dataset S5	Cognitive Function	Excel File
Dataset S6	Exercise Intensity/Work	Excel File

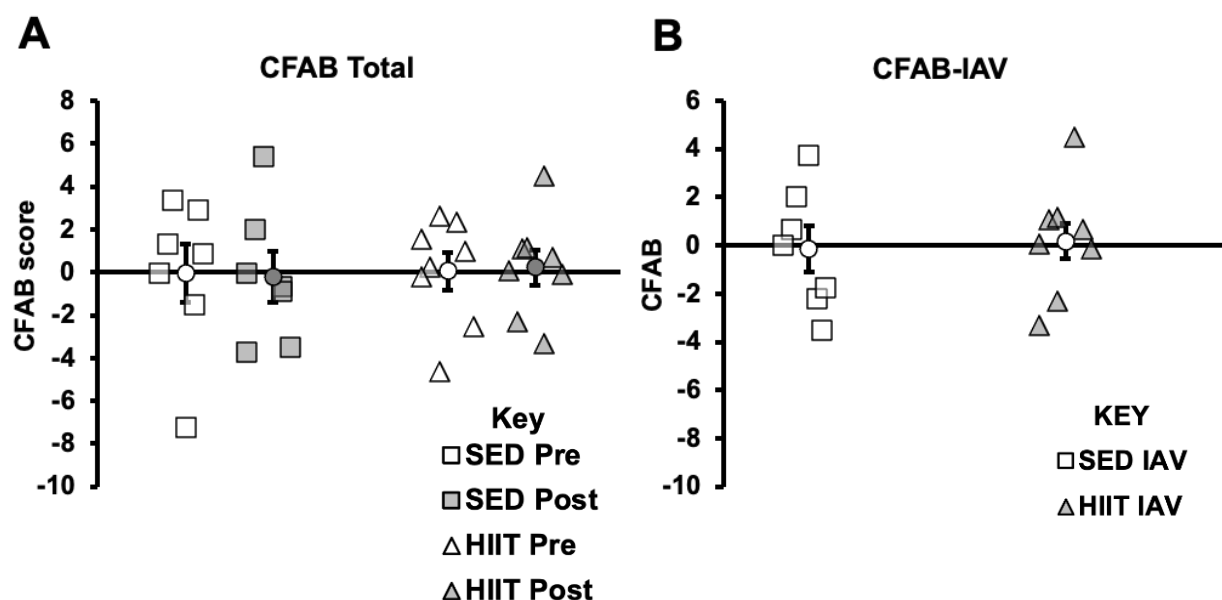


Figure S1: Total CFAB Scores and Intervention Assessment Values. CFAB analysis uses a reference group of 6-month-old mice (mean and standard deviation; SD), where test values are standardized via the distance of each individual mouse's score from the 6m mean. Distance from that mean is measured in units of the reference group's SD. The standardized scores for each functional test are then added together to form the CFAB composite score: a single numeric value representative of overall physical function capacity (A). An intervention assessment value (B) was also used for analysis in this study, in which standardization was achieved by using baseline mean and SD of the entire sample ($n = 15$) before randomization occurred.

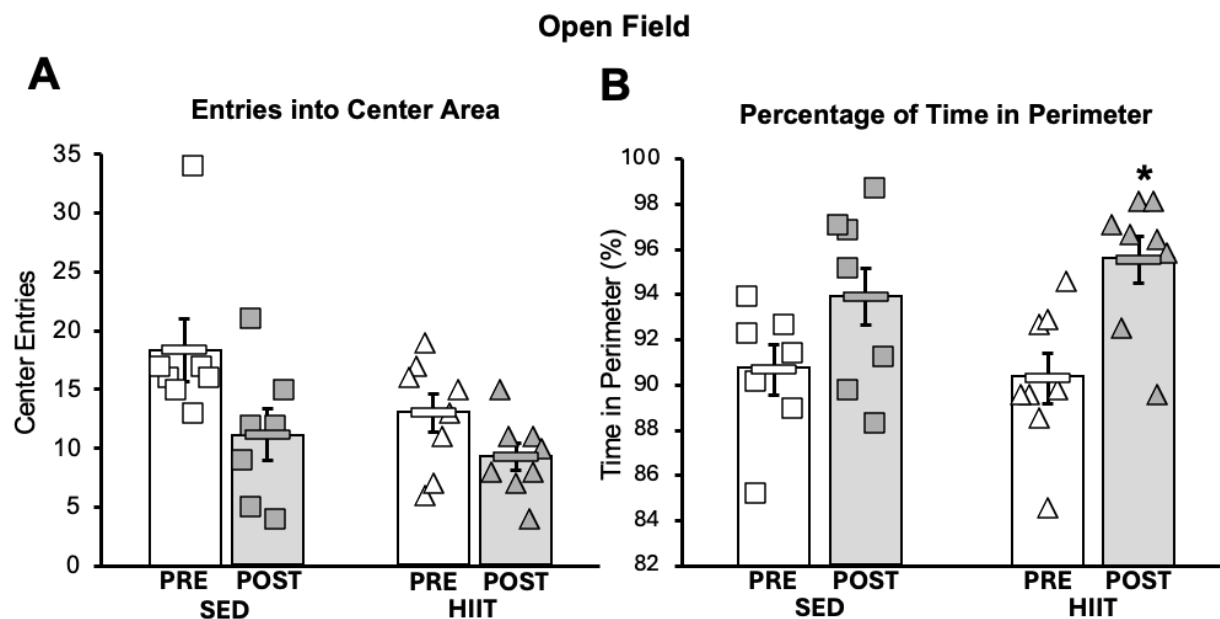


Figure S2: Open Field Pre- to Post-Training. The number of times a mouse entered the center area of the open field box in the allotted time is shown on the left (A). The percentage of their allotted time spent in the perimeter region of the box is shown on the right (B). Naturally, as center entries (A) decrease, the amount of time spent in the perimeter (B) increases. However, this trend of lessened time in the center and added time in the perimeter is indicative of increasing anxiety from pre to post.

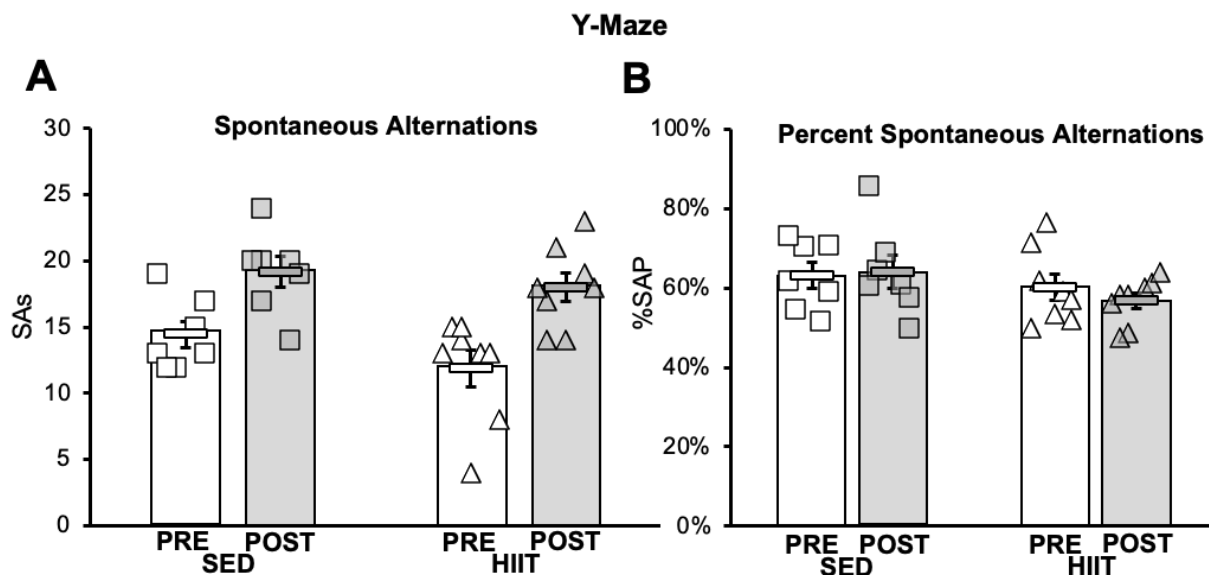


Figure S3: Y-maze Pre- to Post-Training. To assess spatial working memory with Y-maze, spontaneous alternations (SAs) were counted (left). Though the number of SAs increased more for the HIIT group (+49 overall) compared to SED (+33 overall), the percentage of SA performance (right; $\%SAP = SAs / [total\ entries - 2]$) showed no significant differences. %SAP decreased for HIIT (-3.40%) and remained relatively the same for SED (+0.94%).

Novel Object Recognition

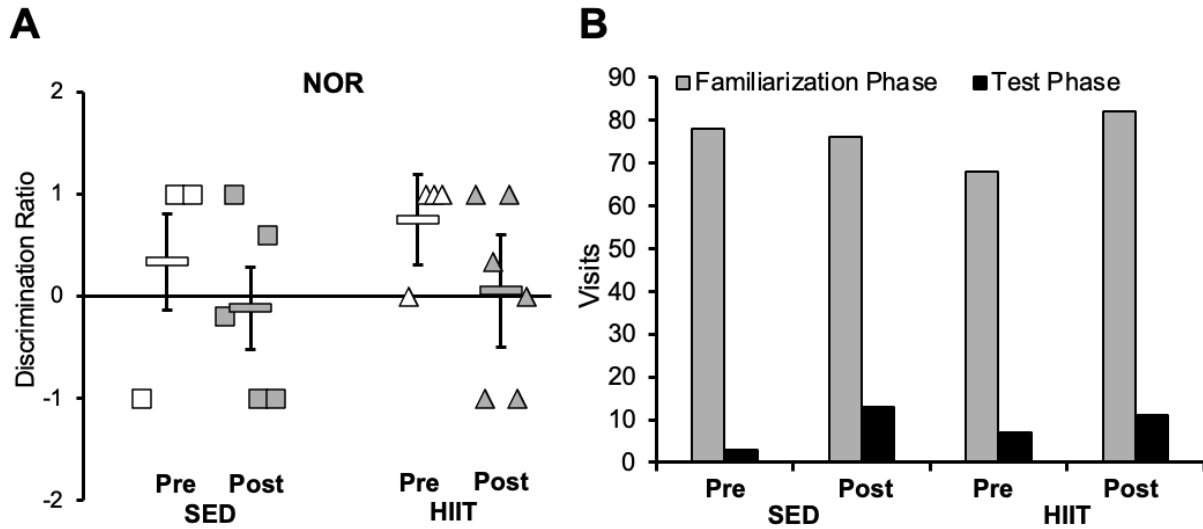


Figure S4: Novel Object Recognition Pre- to Post-Training. The discrimination ratio (DR) is a quantification of the between-object bias observed during the NOR test (A). It is calculated as $DR = (\text{Novel Object visits} - \text{Familiar Object visits}) / \text{total visits}$. The amount of exploration is the number of visits to either object (B). This was significantly reduced across the board between the familiarization phase and the testing phase, indicating the mice may have lost interest in the objects after the familiarization phase, thus, explaining the DR results (A).

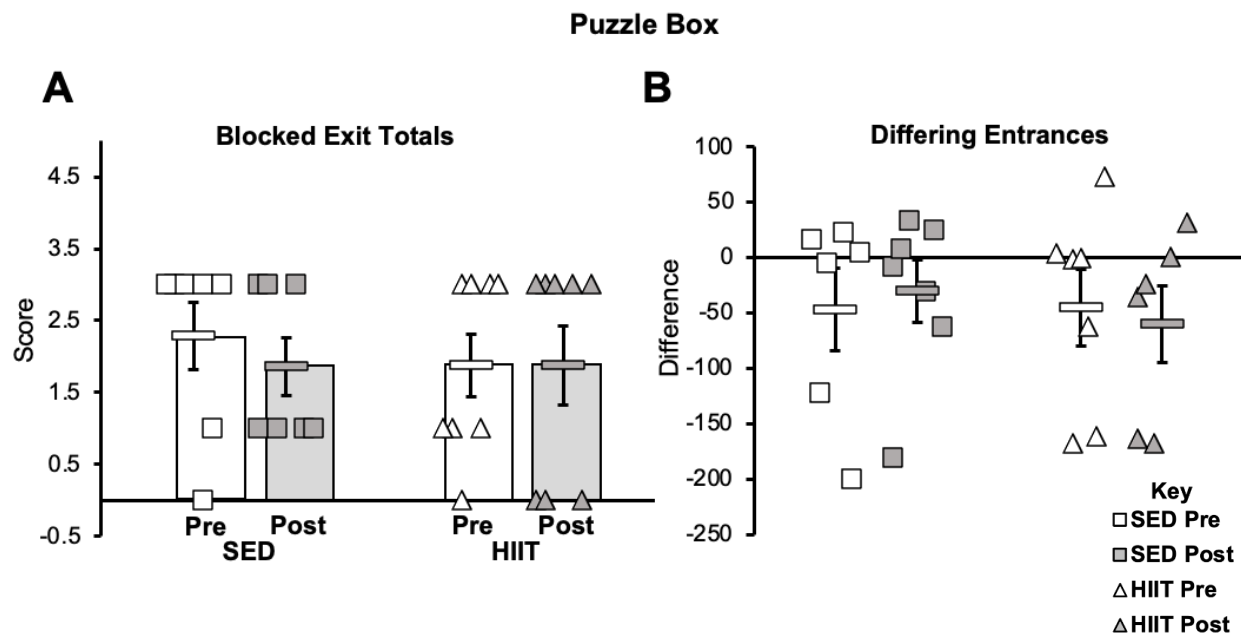


Figure S5: Puzzle Box Pre- to Post-Training. Though no significance was observed, the blocked exit test (A) showed no change in the HIIT group's ability to problem solve, while the SED group did decline in this area. The differing entrances test (B) was analyzed by finding the difference between trial 1 and trial 2, then calculating the difference of those differences from pre to post. Meaning, the more negative from pre to post, the faster the group solved the task.