

**Supplementary Material for:**

**Consummatory, anxiety-related and metabolic adaptations in female rats with  
alternating access to preferred food**

Pietro Cottone, Ph.D.<sup>1,2,3\*</sup>; Valentina Sabino, Ph.D.<sup>1,2\*</sup> Luca Steardo, M.D., Ph.D.<sup>3</sup>; Eric P. Zorrilla, Ph.D.<sup>1,2</sup>

<sup>1</sup>Committee on the Neurobiology of Addictive Disorders (CNAD), <sup>2</sup>Harold L. Dorris Neurological Research Institute, The Scripps Research Institute, 10550 N. Torrey Pines Rd. La Jolla, California, 92037, USA

<sup>3</sup>Department of Human Physiology and Pharmacology, University of Rome La Sapienza, Rome, Italy

\*These authors equally contributed to this work.

**Running title:** Effects of cyclic access to preferred food

**Supplementary materials:** 5 Supplementary Figures and Legends

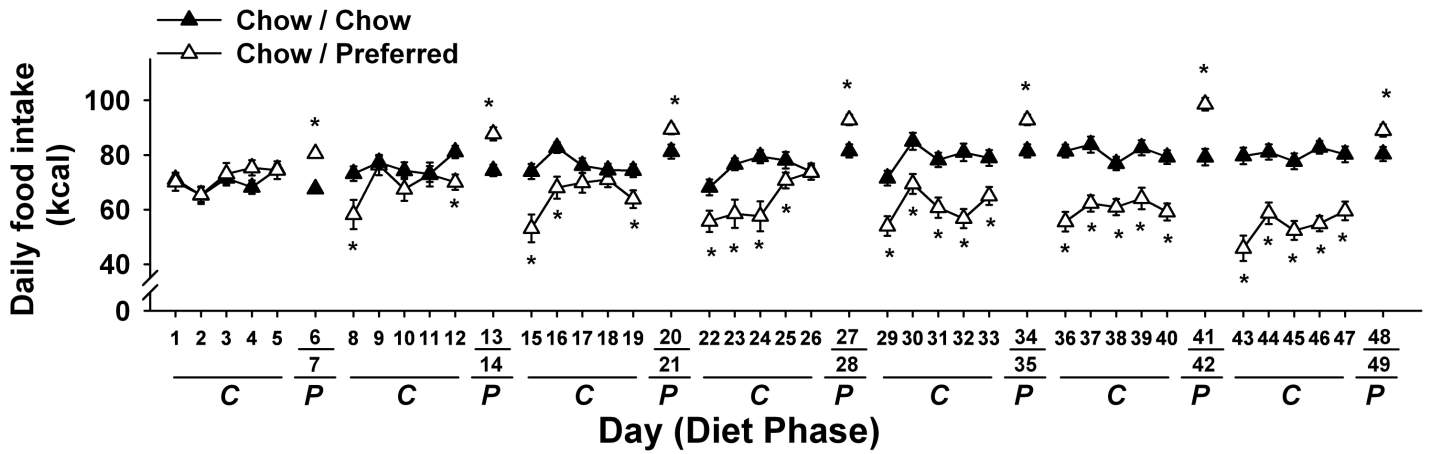
Correspondence and requests for materials should be addressed to:

Pietro Cottone (Email: [cottone@scripps.edu](mailto:cottone@scripps.edu))  
Committee on the Neurobiology of Addictive Disorders, SP30-2400  
The Scripps Research Institute  
10550 N. Torrey Pines Road  
La Jolla, CA 92037 USA  
Phone: 858-784-7464  
Fax: 858-784-7405

or

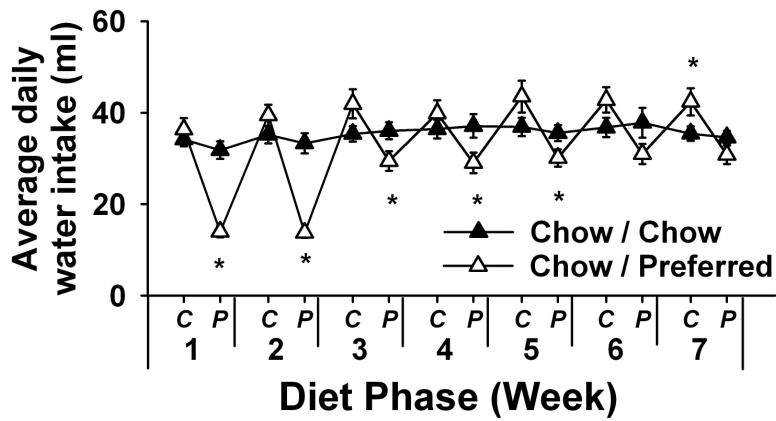
Eric P. Zorrilla (Email: [ezorrilla@scripps.edu](mailto:ezorrilla@scripps.edu))  
Committee on the Neurobiology of Addictive Disorders, SP30-2400  
The Scripps Research Institute  
10550 N. Torrey Pines Road  
La Jolla, CA 92037 USA  
Phone: 858-784-7416  
Fax: 858-784-7405

Supplementary Figure 1



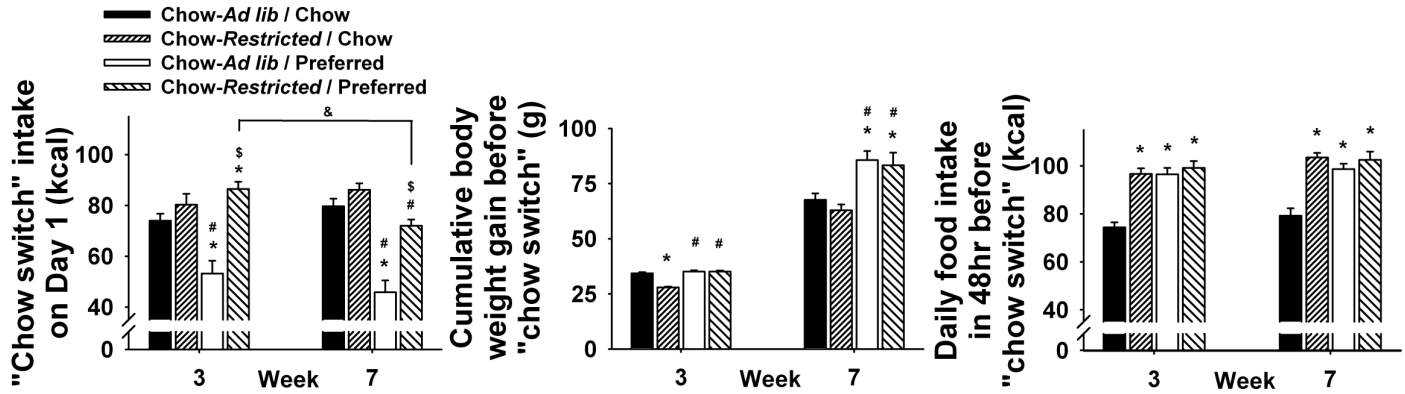
Effects of repeated, alternating 5-day access to chow and 2-day access to either chow (*Chow/Chow*,  $n=16$ ) or highly preferred chocolate-flavored sugary diet (*Chow/Preferred*,  $n=14$ ) in female Wistar rats. Panels represent  $M \pm SEM$ . Daily food intake, average for 2-day access phase. \*differs from *Chow/Chow*  $p < 0.05$ .

Supplementary Figure 2



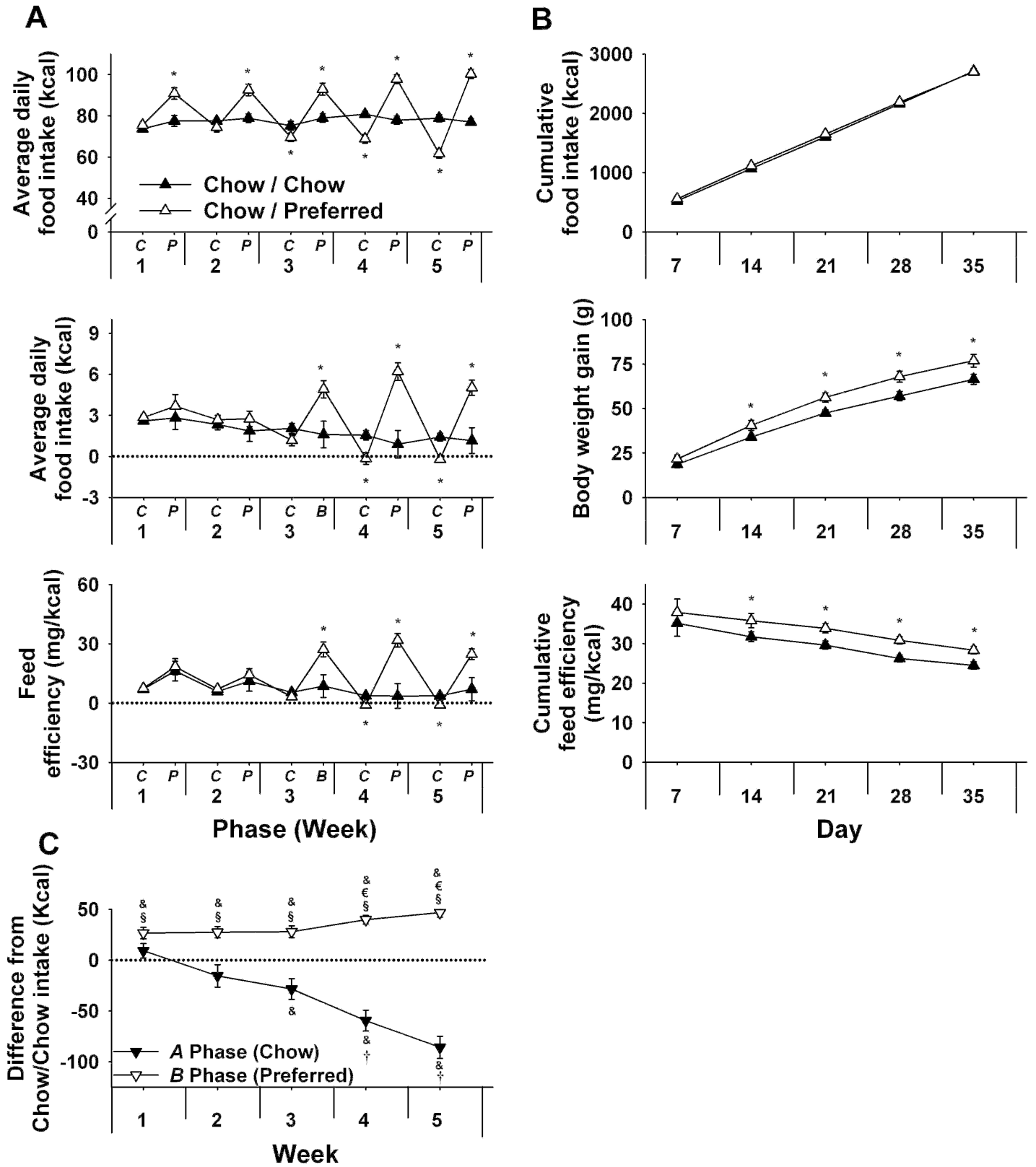
Effects of repeated, alternating 5-day access to chow and 2-day access to either chow (*Chow/Chow*,  $n=16$ ) or highly preferred chocolate-flavored sugary diet (*Chow/Preferred*,  $n=14$ ) in female Wistar rats. Panels represent  $M \pm SEM$ . Average daily water intake during each 5- (*C Phase*) or 2-day (*P Phase*) diet phase of each week. \*differs from *Chow/Chow*  $p < 0.05$ .

### Supplementary Figure 3



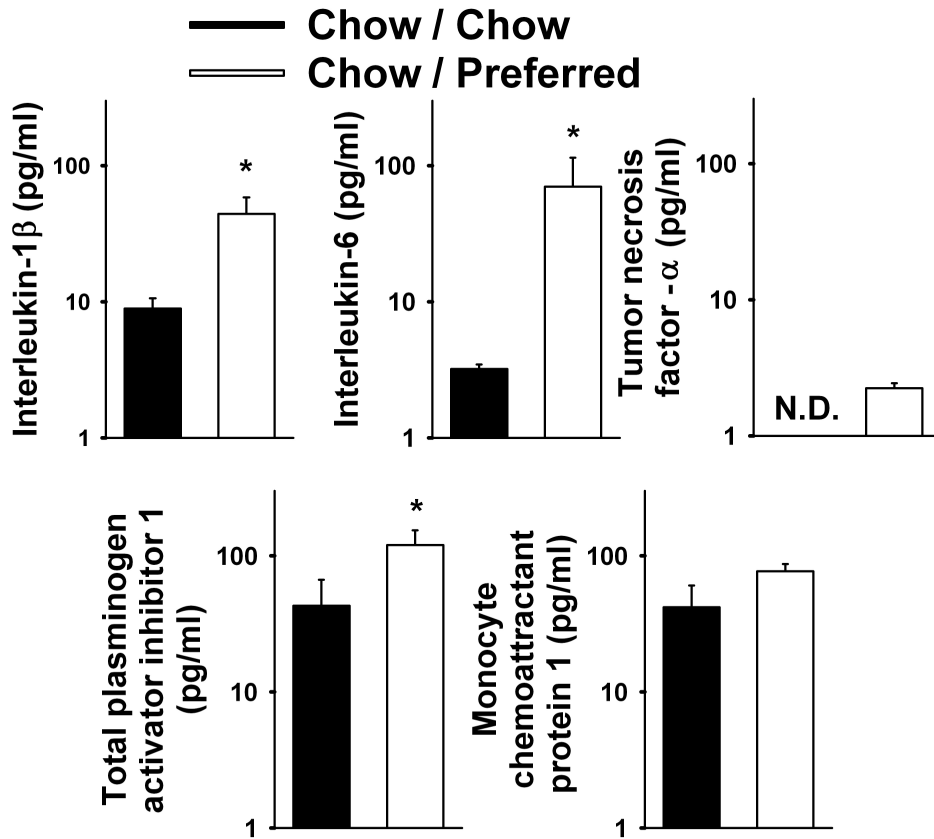
Effects of diet alternation with or without caloric restriction during chow access. Effects of diet and caloric restriction history on intake upon return to chow (*left*) Highlight of Day 1 chow intake in weeks 3 and 7. (*middle*) Cumulative body weight gain or (*right*) preceding daily intake during *P* phases prior to switching from preferred to chow diets at onset of weeks 3 and 7. Panels represent  $M \pm SEM$ . Differs from \**Chow-Ad lib/Chow*  $p < 0.05$ ; #*Chow-Restricted/Chow*  $p < 0.05$ ; \$*Chow-Ad lib/Preferred*  $p < 0.05$ .

Supplementary Figure 4



Effects of repeated, alternating 5-day access to chow and 2-day access to either chow (*Chow/Chow*,  $n=19$ ) or highly preferred chocolate-flavored sugary diet (*Chow/Preferred*,  $n=20$ ) in female Wistar rats. Panels represent  $M \pm \text{SEM}$ . (A) (*Top*) Average daily food intake, (*Middle*) average daily change in body weight, and (*Bottom*) “local” feed efficiency (body weight change/energy intake) during each 5- (*C Phase*) or 2-day (*P Phase*) diet phase of each week. (B) Weekly perspective of cumulative (*Top*) food intake (note that error bars are smaller than symbols), (*Middle*) body weight gain, (*Bottom*) feed efficiency, as calculated at each week’s end. (C) Underacceptance and hyperphagia of food in *Chow/Preferred* rats within *C (Chow)* and *P (Preferred)* phase diets, respectively, in relation to *Chow/Chow* intake. \*differs from *Chow/Chow*  $p < 0.05$ , &differs from 0  $p < 0.05$ , †differs from *A Phase* of Week 2  $p < 0.05$ , €differs from *P Phase* of Week 2  $p < 0.05$ , §differs from *C Phase*  $p < 0.05$ .

Supplementary Figure 5



Long-term effects of diet schedule on circulating adipokine levels. Panel shows ( $M+SEM$ ) plasma levels of proinflammatory adipokines of a random subset of female Wistar rats from Experiment 1 ( $n=15$ ). On Day 92, rats were fed chow diet only for 10 consecutive days to control for acute diet effects. On day 102, overnight fasted rats were decapitated 2-5 hr into the dark cycle, and trunk blood was collected. Data were log-transformed for statistical analysis, and panels therefore depict antilog values on a logarithmic scale. Differs from \**Chow/Chow*  $p<0.05$ .