## **Supplemental Material for:**

#### Intermittent preferred food access reduces the reinforcing efficacy of chow in rats

Pietro Cottone<sup>1,2,3,\*</sup>; Valentina Sabino, <sup>1,2,\*</sup>; Luca Steardo<sup>3</sup>; Eric P. Zorrilla<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: Reduced reinforcing efficacy of less preferred food

Supplementary materials: 2 Supplemental Figures, 1 Supplemental Table and Supplemental Legends

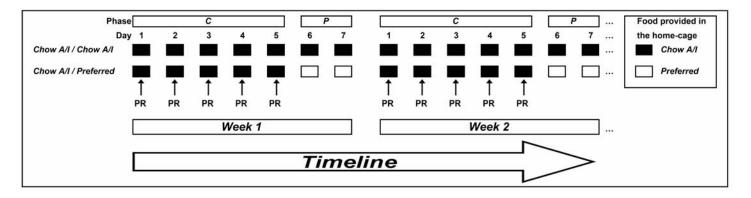
Correspondence and requests for materials should be addressed to:

Pietro Cottone (Email: <u>cottone@scripps.edu</u>) 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

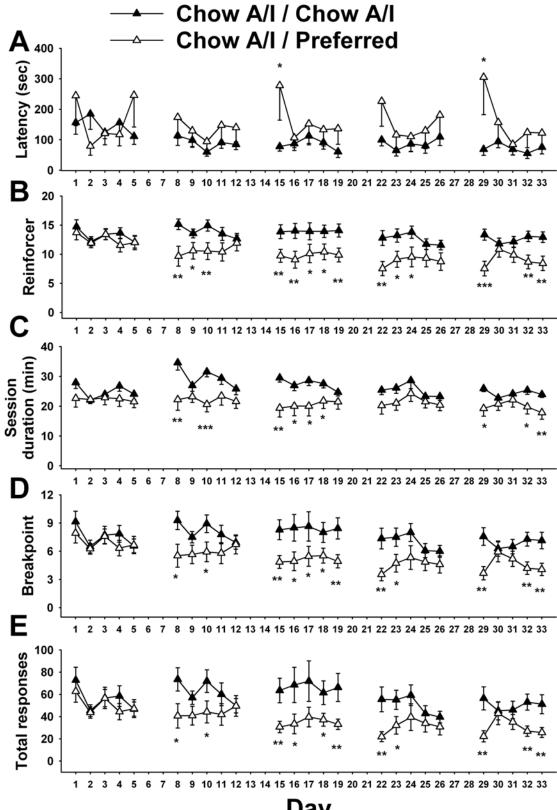
Eric P. Zorrilla (Email: <u>ezorrilla@scripps.edu</u>) Committee on the Neurobiology of Addictive Disorders, SP30-2400 The Scripps Research Institute 10550 N. Torrey Pines Road La Jolla, CA 92037 USA Phone: 858-784-7416 Fax: 858-784-7405

## **Supplemental Figure 1**



Schematic of the experimental design used in this study. Rats were assigned to two groups, matched for food intake, body weight, and baseline progressive ratio performance. One group was provided with chow diet ("Chow A/I") *ad libitum* 7 days per week (*Chow A/I / Chow A/I*), and a second group was provided chow *ad libitum* for 5 days each week followed by 2 days of *ad libitum* access to the highly preferred chocolate flavored, high-sucrose diet ("Preferred") (*Chow A/I / Preferred*). The first 5 days (Chow A/I only) and last 2 days (Chow A/I *or* Preferred according to experimental group) of each week are referred to in all experiments as *C* and *P* phases. Diets were never concurrently available. Progressive ratio (PR) sessions were performed daily during *C* phases.

## **Supplemental Figure 2**



Day

Effects of repeated cycles of 5-day access to Chow A/I alternated by 2-day access to either Chow A/I (*Chow A/I* / *Chow A/I*, n=14) or highly preferred chocolate-flavored sugary diet (*Chow A/I /Preferred*, n=15) on progressive ratio responding for Chow A/I pellets in male Wistar rats. Rats were tested daily at the dark cycle onset during the *C* (Chow A/I) *Phase*. Panels show *M*±SEM (**A**) Latency, the interval from the time the rat was placed into the test chamber until the time it earned its first reinforcer, (**B**) Reinforcers, total number of reinforced responses, (**C**) Session duration, the time between completion of the first ratio and the end of the session, (**D**) Breakpoint, the last ratio completed by a subject prior to the end of the session, (**E**) Total responses, the number of reinforced and non-reinforced responses. Latency and Session duration are shown as backtransformed *M*-SEM to facilitate interpretation. \*differs from *Chow A/I* / *Chow A/I p<*0.05, \*\**p*<0.01, \*\*\**p*<0.001.

## Supplemental Table 1

# Supplemental Table 1. Diet composition and energy density

	Chow	Chow A/I	Preferred	
Manufacturer	Harlan-Teklad	TestDiet	TestDiet	
			5TUL (chocolate-	
Code	LM-485 Diet 7012	5TUM	flavored)	
Macronutrient composition	Kcal%	Kcal%	Kcal%	
Protein	21	24.1	20.5	
Carbohydrate	66	65.5	66.8	
Fat	13	10.4	12.7	
Kcal/g	3.41	3.30	3.48	
Ingredients	Ground corn, dehulled soybean meal, ground oats, wheat middlings, dehydrated alfalfa meal, soybean oil, corn gluten meal, calcium phosphate, brewers dried yeast, iodized salt, choline chloride, magnesium oxide, L-lysine, DL- methionine, vitamin A acetate, vitamin D3 supplement, vitamin E supplement, niacin, calcium pantothenate, riboflavin, thiamine mononitrate, pyridoxine hydrochloride, menadione sodium bisulfite complex (source of vitamin K activity), folic acid, biotin, vitamin B12 supplement, calcium carbonate, manganous oxide, ferrous sulfate, copper sulfate, zinc oxide, calcium iodate, cobalt carbonate, chromium potassium sulfate, locust bean gum, gelatin, kaolin.	LabDiet #5001 Rodent Diet (ground corn, dehulled soybean meal, dried beet pulp, fish meal, ground oats, brewers dried yeast, cane molasses, dehydrated alfalfa meal, dried whey, wheat germ, porcine animal fat preserved with BHA, porcine meat meal, wheat middlings, salt, calcium carbonate, soybean oil, corn gluten meal, calcium carbonate, DL-methionine, choline chloride, cholecalciferol, vitamin A acetate folic acid, menadione sodium bisulfite complex (source of vitamin K), pyridoxine hydrochloride, thiamine mononitrate, nicotinic acid, calcium pantothenate, dl-alpha tocopheryl acetate, cyanocobalamin, riboflavin, ferrous sulfate, manganous oxide, zinc oxide, ferrous carbonate, sodium selenite), corn syrup, silicon dioxide, inert binder, color and/or flavor added where applicable.	Sucrose, casein, maltodextrin, corn starch, corn oil, cellulose, minerals, silicon dioxide, vitamin, magnesium stearate, DL-methionine, color and/or flavor added where applicable.	
Diet specifications a	are available on	line: Harlan	LM-485 (Regular	Che
http://www.teklad.com/stand		5TUM	Diet (Chow	1

http://www.testdiet.com/PDF/5TUM.pdf),

and

5TUL

Chocolate

Diet

(Preferred;

http://www.testdiet.com/PDF/5TUL.pdf).