Supplemental Information

Skeletal muscle thermogenesis induction by exposure to predator odor

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Supplemental figures

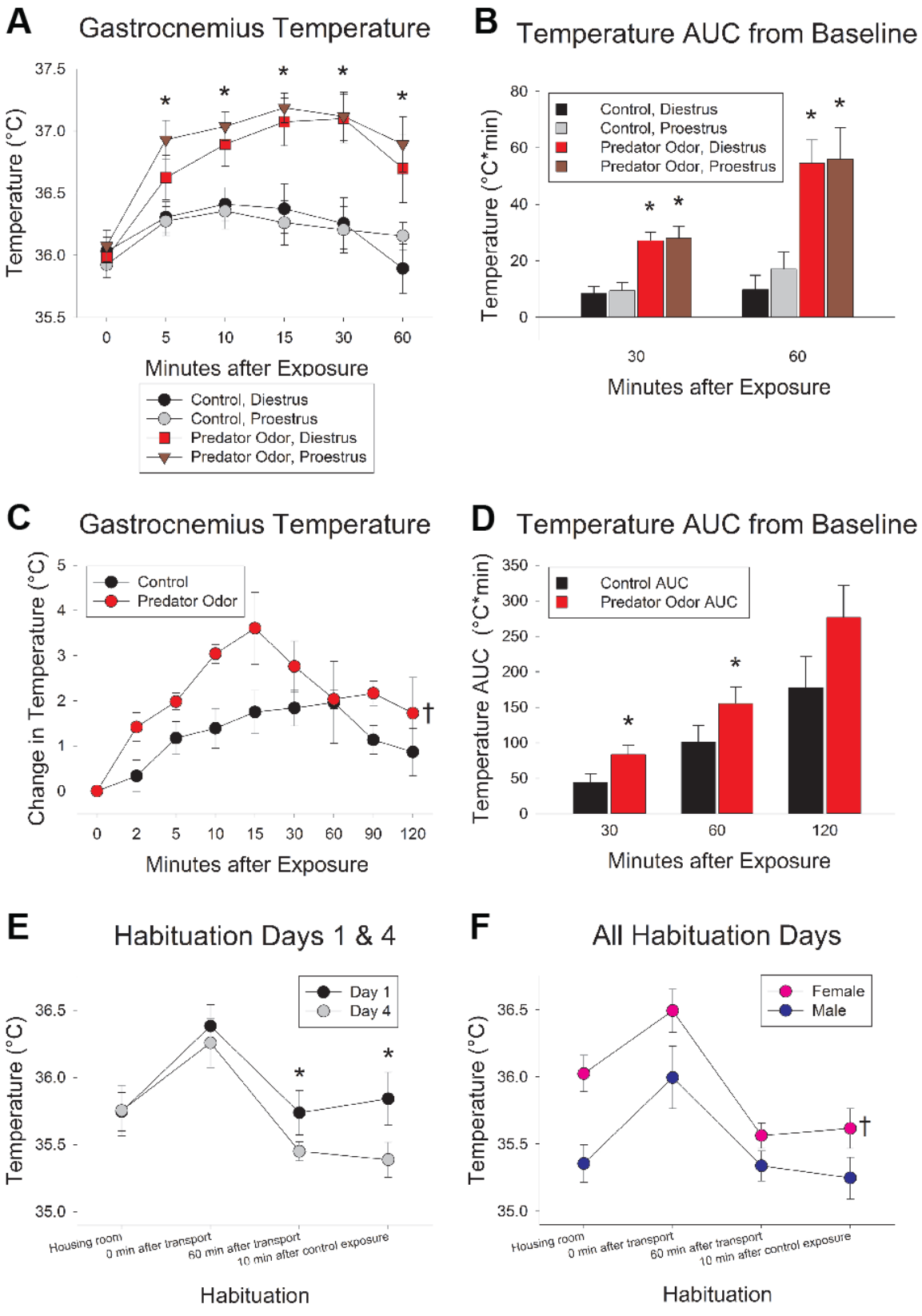

Fig. S1.

(A-B) Experimental replications showing no significant effect of estrous cycle (proestrus vs diestrus) on muscle temperatures in response to control or predator odor exposures; AUC, area under the curve. *predator odor>control

(C-D) Experimental replication showing predator odor-induced change in muscle thermogenesis in male C67/Bl6 mice. (n=3) †main effect of predator odor, *predator odor>control

(E) Experimental replication demonstrating that four days of habituation to the experimental context significantly decreased the thermogenic response to a control stimulus. *Day 1>Day 4.

(F) Average temperatures across four days of habituation, female rats showed higher muscle temperatures during habituation (no sex difference on Day 4). †main effect of sex.

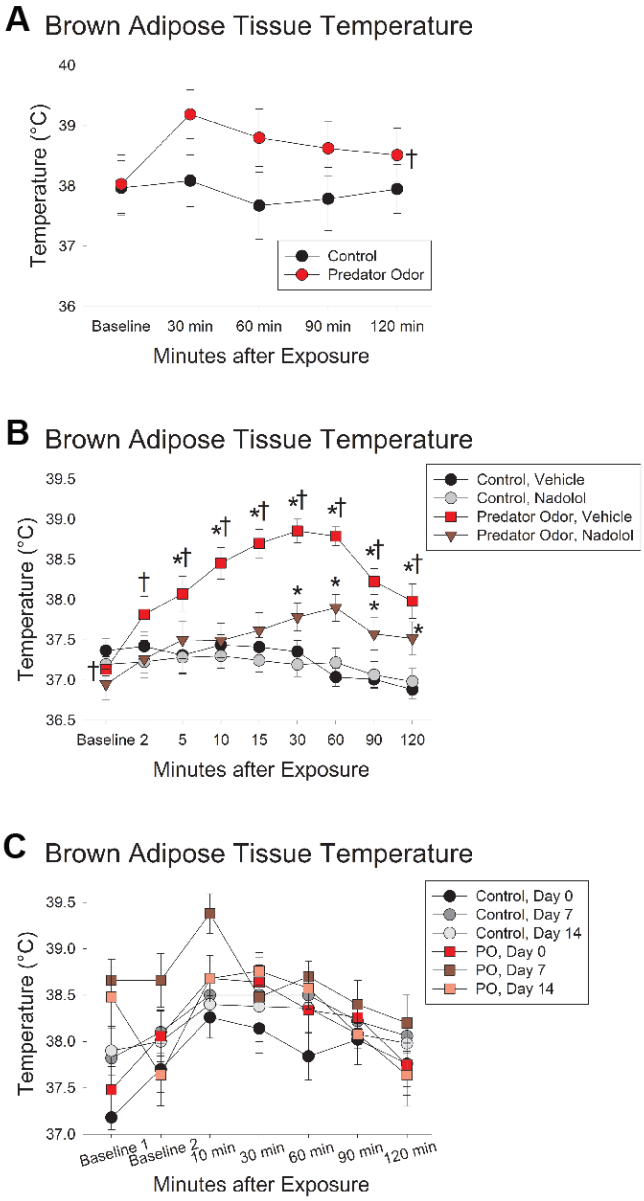

Fig. S2.
Predator odor-induced changes in brown adipose tissue (BAT) temperature.
Exposure to predator odor induced a significant increase in BAT temperature in rats. (n=8) †main effect of odor
(B) The peripherally acting mixed β-adrenergic receptor antagonist nadolol significantly inhibited the ability of predator odor to increase BAT temperature in rats. (n=9) *predator odor>control exposure, †intact>denervated within condition.
(C) Over the course of 15 days of 25% calorie restriction, there was no significant difference between control odor vs predator odor in their ability to alter BAT temperature in rats. (n=5/group)

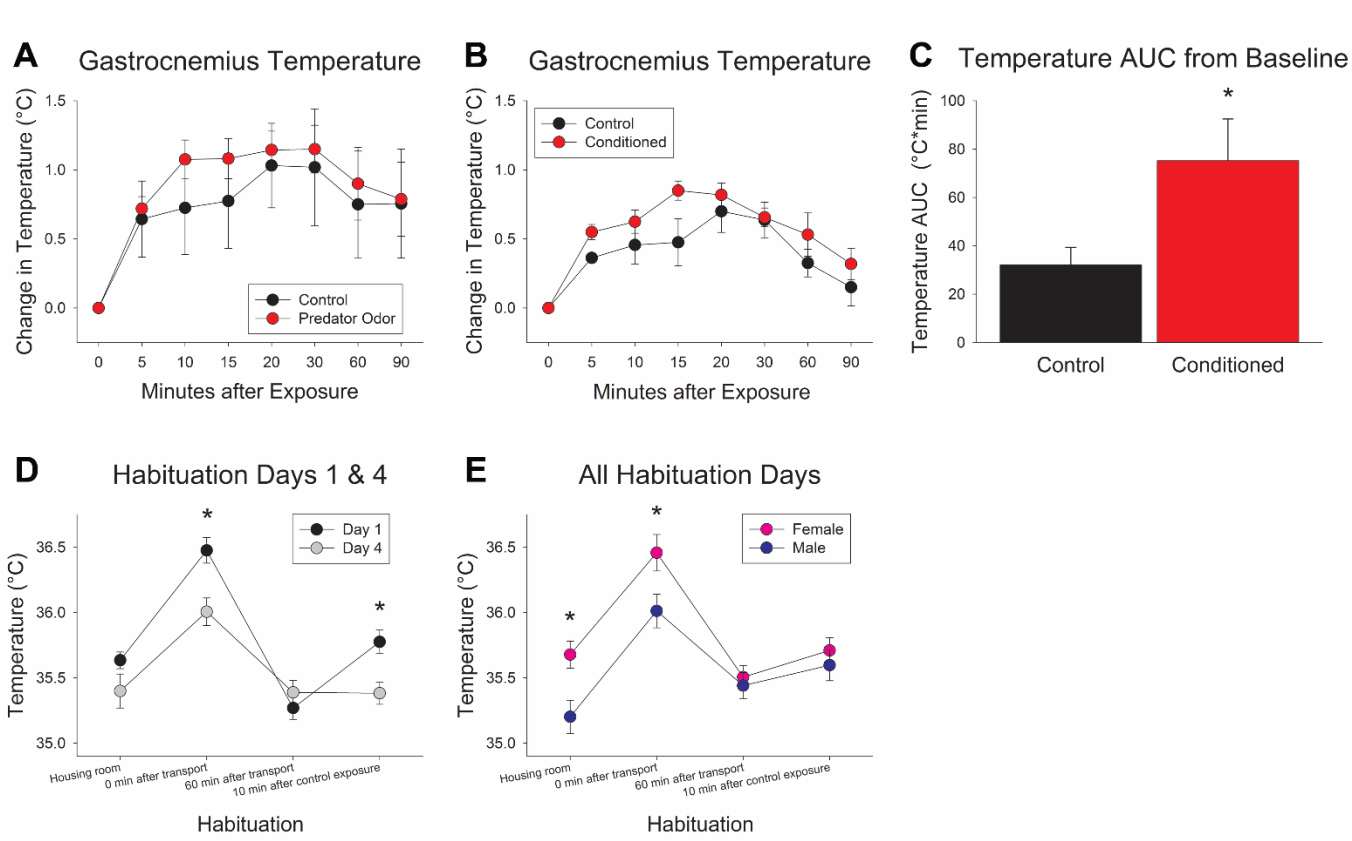

Fig. S3. Predator odor-induced muscle thermogenesis is subject to learned associations. (A) Rats were exposed to either a control stimulus or the same stimulus with the predator (ferret) odor; (B) the following day, all rats were exposed to the control stimulus, which acted as a conditioned stimulus in the rats previously exposed to predator odor. (C) The identical stimulus induced a significantly greater thermogenic response in the rats that had been conditioned to associate the stimulus with the predator odor (AUC, area under the curve, 20 min after exposure; n=8/group). *predator odor>control. (D) Subsequent measurements during habituation of a separate group of rats showed that 4 repetitions of habituation to the procedure were sufficient to significantly decrease rats' thermogenic response to control odor. *Day 1>Day4 (E) Female rats showed higher muscle temperatures than males before, but not after, acclimation to the experimental setting. (n=11 male+12 female) *female>male

Table S1. Changes in physical activity after stimulation by intra-ventromedial hypothalamic melanocortin receptor activation or exposure to predator odor (mean ± standard error of the mean, separate groups of rats, in first 1 hr after treatment).

	Physical activity (beam breaks/min)	Increase in EE (kcal/hr)	Increase muscle temperature in first hour?
MTII	***13.60** ± 2.09	***2.77** ± 0.12	**no**
Vehicle	**5.45** ± 0.88	**2.24** ± 0.10	
Predator odor	***13.50** ± 1.15	***3.41** ± 0.10	**yes**
Control	**2.17** ± 0.34	**2.33** ± 0.05	

Temperature in first hour after microinjection (Gavini et al., 2016), compared to significant induction of muscle thermogenesis detectable within 2 min after exposure to predator odor (see Fig. 1).
EE, energy expenditure; MTII, melanotan II (mixed melanocortin receptor agonist)
*significantly greater than vehicle or control condition, $p<0.05$.

Table S2. Change in kcal food intake and body composition with weight-maintenance food availability during 7 days each of control-odor and predator-odor exposure (mean ± SEM).

		Change in Food Intake (kcal)	**Change in Body Composition** (kcal)
Control Odor Exposure*	Male	**-6.74** ± 3.76	**28.54** ± 16.50
	Female	**-3.46** ± 3.46	**44.56** ± 8.61
Predator Odor Exposure*	Male	**-106.99** ± 18.76	**-225.07** ± 45.85
	Female	**-20.50** ± 5.40	**-51.16** ± 16.97

Change in body composition = (grams of fat loss * 9) + (grams of lean mass loss * 4)
Change in kcal food intake = change in grams food consumed * 3.46 (ProLab RMH 3000)
Change relative to weight-maintenance food intake (over 7 days)
*change in intake ≠ change in body composition, $p<0.01$.
N=12 (7 male, 5 female)

Table S3. Fat and lean mass in rats subjected to 25% calorie restriction during chronic exposure to control or predator odors (mean ± SEM).

	Fat Mass (g)*			Lean Mass (g)*†		
	Day -3	Day 4	Day 15	Day -3	Day 4	Day 15
Control Odor Exposure	**21.93** ± 1.64	**20.52** ± 1.50	**18.61** ± 2.63	**240.23** ± 8.47	**237.97** ± 7.99	**244.08** ± 9.00
Predator Odor Exposure	**23.51** ± 1.09	**20.80** ± 2.69	**18.10** ± 1.92	**238.22** ± 7.71	**227.141** ± 7.12	**231.87** ± 6.05

*significant change over time; †significant interaction; $p<0.05$
N=5/group (male)