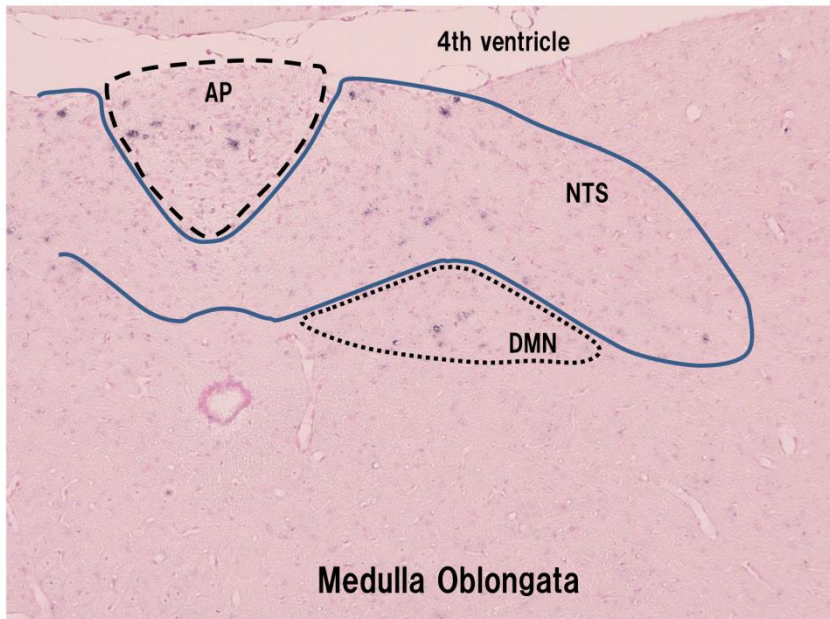


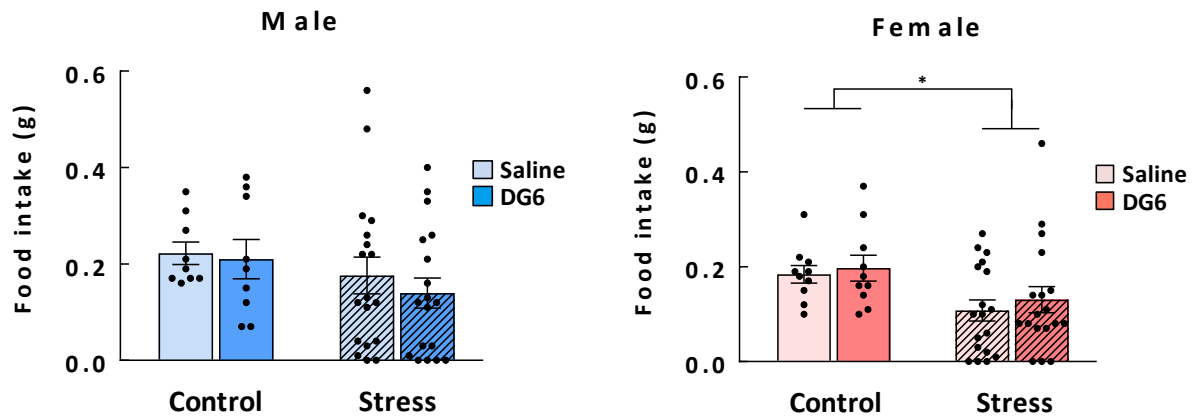
Supplemental figure S1. Experimental design

Vulnerability to psychological stress-induced anorexia in female mice depends on blockade of ghrelin signal in nucleus tractus solitarius  
Yamada C. *et al.*



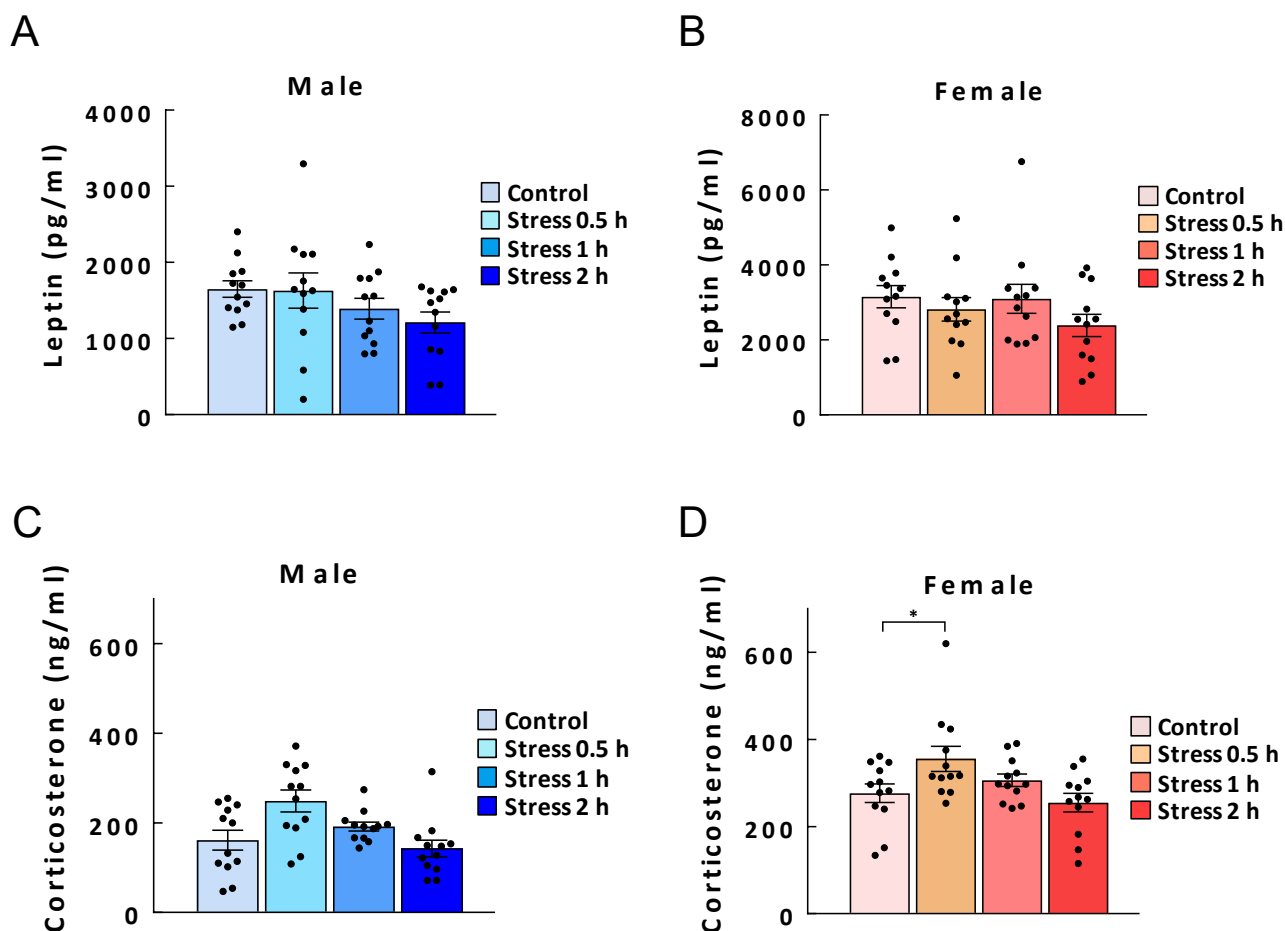
**Supplemental figure S2.**

It shows the typical area of NTS. We counted the number of positive cells in NTS.



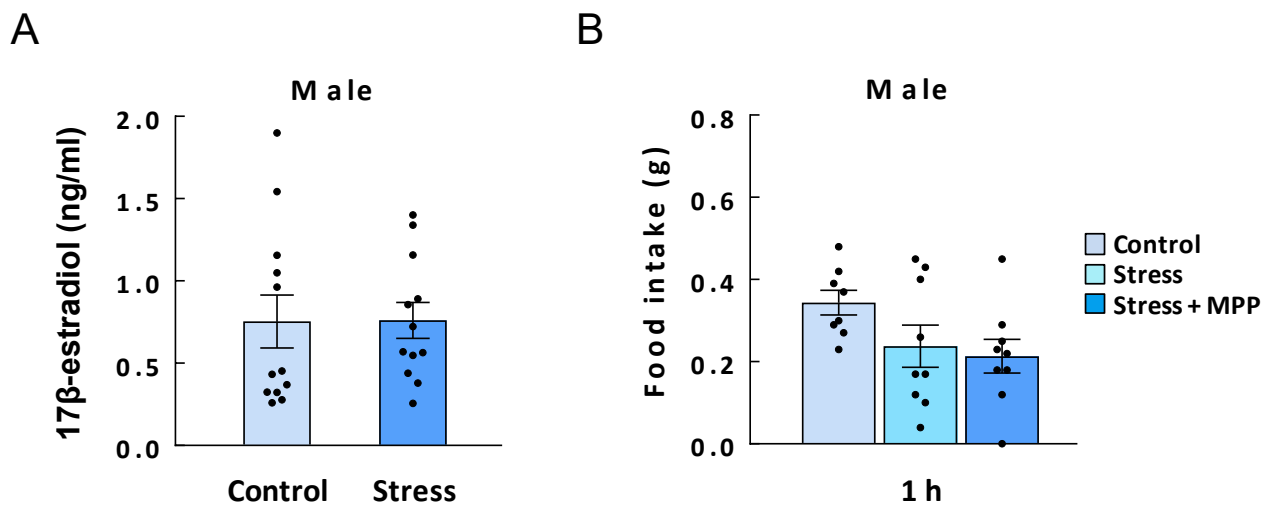
### Supplemental figure S3. IP administration of ghrelin receptor antagonist to stressed mice

[D-Lys<sup>3</sup>]-GHRP-6 (DG6) at 1.5  $\mu\text{mol/kg}$  was administered to mice after stress exposure, and 2-h cumulative food intake was measured after stress. No significant effect was detected in males (stress effect:  $F(1, 50) = 2.259$ , n.s., DG6 effect:  $F(1, 50) = 0.3966$ , n.s., stress  $\times$  DG6 interaction effect is n.s.). Although no significant effect of DG6 was detected in females ( $F(1, 52) = 0.4451$ , n.s., the stress  $\times$  DG6 interaction effect is n.s.), there was a significant effect of stress ( $F(1, 52) = 7.078$ ,  $P < 0.05$ ). \*,  $P < 0.05$  by two-way analysis of variance. Mean  $\pm$  SEM. Male: Control-saline  $n = 9$ , Control-DG6  $n = 9$ , Stress-saline  $n = 18$ , Stress-DG6  $n = 18$ . Female: Control-saline  $n = 10$ , Control-DG6  $n = 10$ , Stress-saline  $n = 18$ , Stress-DG6  $n = 18$ .



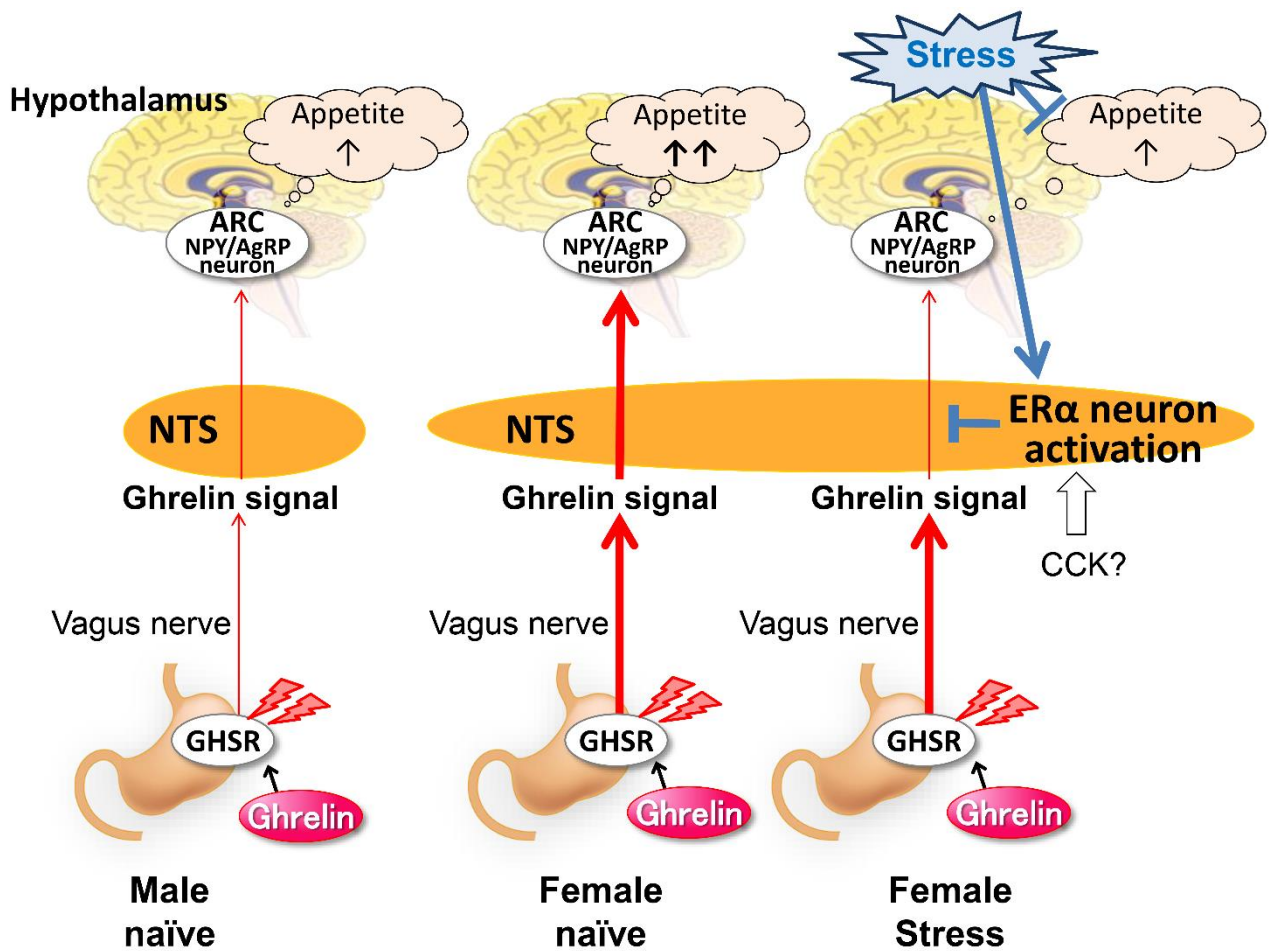
**Supplemental figure S4. Plasma leptin and corticosterone levels under the stress condition**

(A) The plasma leptin levels in male mice. (B) The plasma leptin levels in female mice. (C) The plasma corticosterone levels in male mice. (D) The plasma corticosterone levels in female mice. The blood samples were collected 0.5, 1, 2 h after exposure to novelty stress. \*;  $P < 0.05$  vs. control by Dunnett test. Mean  $\pm$  SEM,  $n = 12$  per group.



**Supplemental figure S5. Plasma estradiol levels and effect of MPP under the stress condition in male mice**

(A) The plasma estradiol levels in male mice under stress condition. The blood samples were collected 0.5 h after exposure to novelty stress. Mean  $\pm$  SEM,  $n = 12$  per group. (B) The food intake at 1 h after exposure to novelty stress in male mice under stress condition. MPP (estrogen  $\alpha$  receptor antagonist, 150  $\mu\text{g}/\text{kg}$ , ip) was administered at 2 h before stress exposure. Mean  $\pm$  SEM,  $n = 8\text{--}9$  per group.



**Supplemental figure S6. Hypothetical schema showing “ghrelin resistance” in stress-loaded female mice.**

In naïve conditions, the affinity, responsiveness of GHS-R1a located at the terminal of the vagus nerve, or ghrelin signal transmission of the vagus nerve in female mice may be higher than that in male mice. Higher ghrelin reactivity of female mice is inhibited at the site of NTS from GHS-R1a, and ghrelin resistance is occurred in stress condition. The ghrelin resistance in female mice may sustain decreased eating behaviors after stress loading.

**Supplemental table S1. Positive cells with c-Fos mRNA at 0.5 h after administration of ghrelin administration/stress exposure in male and female mice**

Male				
	Control		Stress	
	Saline	Ghrelin	Saline	Ghrelin
AP	3.6 ± 2.5	34.8 ± 7.0 *	6.8 ± 5.1	30.6 ± 9.7
VMH	37.2 ± 8.3	23.5 ± 2.7	42.5 ± 5.1	53.9 ± 5.1
Amygdala	26.0 ± 5.0	29.6 ± 2.9	36.1 ± 4.0	47.1 ± 10.1

Female				
	Control		Stress	
	Saline	Ghrelin	Saline	Ghrelin
AP	2.4 ± 1.9	36.2 ± 7.7 *	9.4 ± 6.5	31.8 ± 4.7
VMH	34.8 ± 2.7	32.7 ± 3.8	31.9 ± 5.2	39.0 ± 11.2
Amygdala	29.0 ± 3.3	36.6 ± 3.5	31.1 ± 4.1	47.0 ± 13.0

The mice were administered IP injections of acylated ghrelin at doses of 50 nmol/kg immediately after the stress exposure, and the brain was collected 0.5 h after the onset of the ghrelin administration. The number of c-Fos-positive cells in the AP, VMH, and amygdala. n = 5 per group. \*; P < 0.05 vs. control + saline group, by Tukey–kramer test. Results are shown as means ± SEM.

**Supplemental table S2. Plasma sex hormone levels under the stress condition**

	Control	Male		
		0.5 h	Stress 1 h	2 h
Testosterone (ng/ml)	14.14 ± 5.94	4.32 ± 0.82	6.55 ± 3.54	13.13 ± 7.96
Estradiol (ng/ml)	0.75 ± 0.16	0.76 ± 0.11	0.51 ± 0.08	0.79 ± 0.10

	Control	Female		
		0.5 h	Stress 1 h	2 h
Testosterone (ng/ml)	7.98 ± 1.71	8.49 ± 1.88	8.49 ± 1.18	8.10 ± 1.66
Estradiol (ng/ml)	0.84 ± 0.12	0.64 ± 0.09	0.66 ± 0.09	0.85 ± 0.10

The blood samples were collected 0.5, 1, 2 h after exposure to novelty stress. n = 12 per group.