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Supplementary Materials for

Acupuncture attenuates alcohol dependence through activation of endorphinergic input to the nucleus accumbens from the arcuate nucleus

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Supplementary Figures

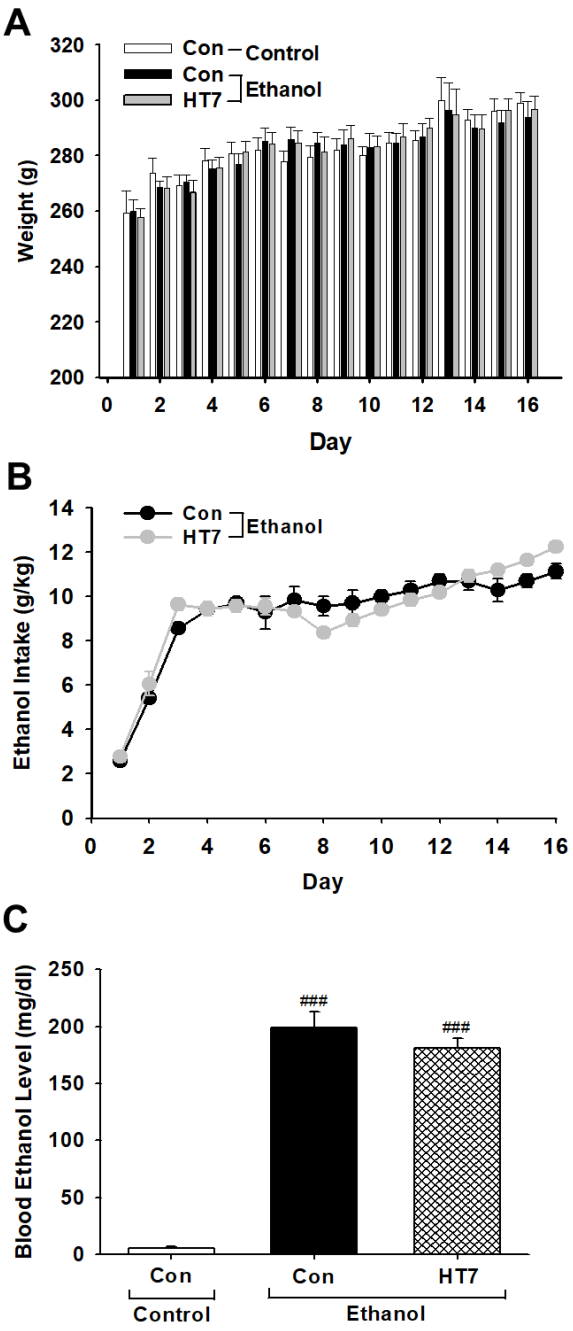

Fig. S1. Effects of chronic ethanol consumption on growth rate and BEC. Rats on the chronic ethanol diet (ethanol group) received an ethanol-containing diet and their pair-fed rats (control group) received an isocaloric diet with dextran substituted for ethanol. Changes in

growth rates, ethanol consumption rates, and blood ethanol levels were measured during and after chronic consumption of ethanol liquid diet, respectively. **A**: Growth rates. **B**: Amount of ethanol intake. **C**: Blood ethanol concentration. Graphs represent mean ± SEM (n=7 per each group). $^{###}$ P<0.05 vs. control group.

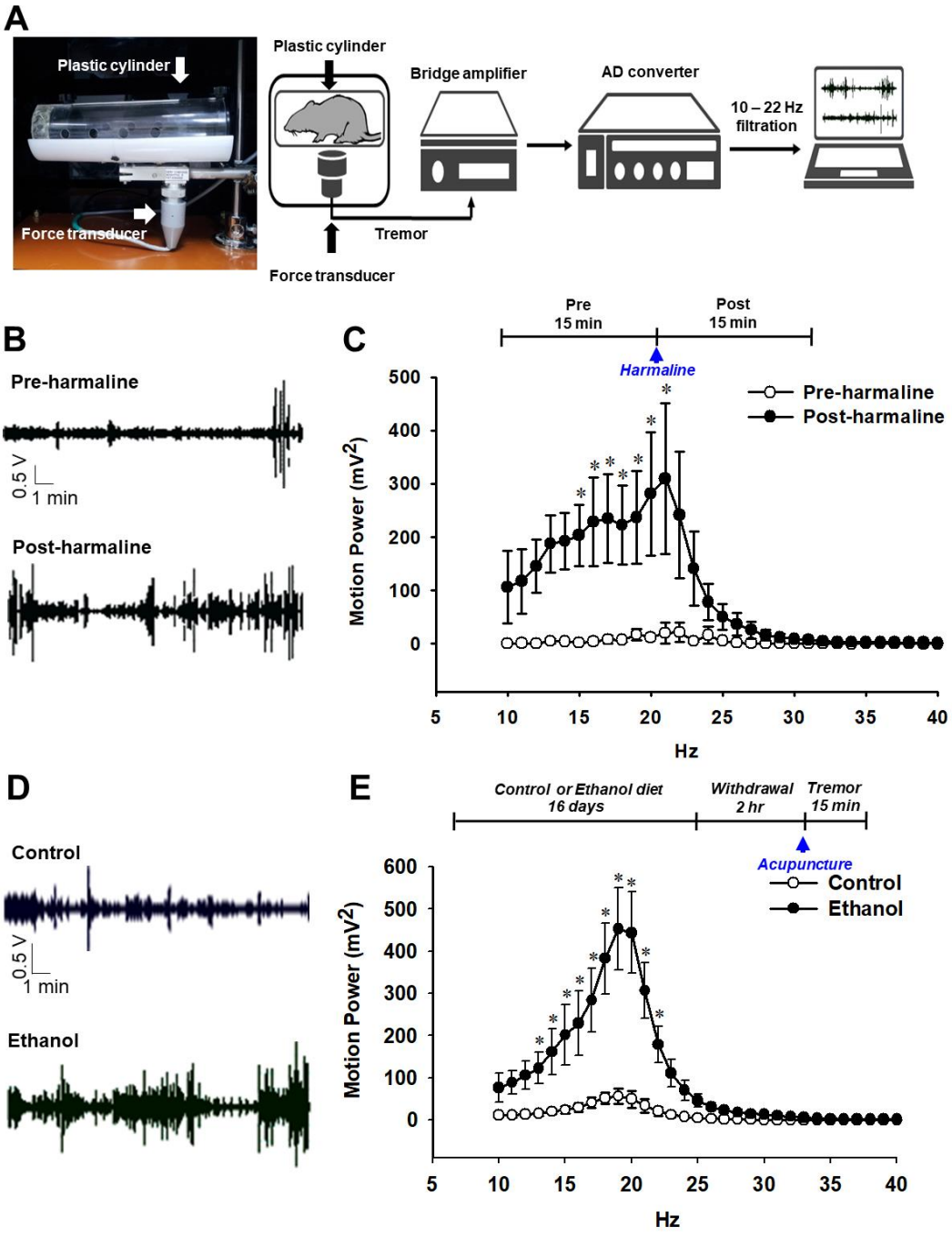

Fig. S2. Measurement of ethanol withdrawal tremor. Tremor was quantified in a real-time manner using ATAMS (fig. S2A). Power spectra for tremor were estimated by testing a tremorogenic compound, harmaline. The 15 min test procedure was performed 5 min after ethanol-naive rats were given a subcutaneous injection of harmaline (10 mg/kg). The second 5 min recording was performed for data. **A**: A custom-made automatic tremor activity monitoring system. **B, C**: Tremor responses to harmaline. Representative force signals before

(upper: Pre-harmaline) and after an injection of harmaline (lower: Post-harmaline) (**B**) and power spectrum (**C**) (*P<0.05 vs. Pre-harmaline, n=4). **D, E**: Tremor activities after the 2 hr ethanol withdrawal. Rats on the chronic ethanol diet received an ethanol-containing diet and their pair-fed rats received an isocaloric diet with dextran substituted for ethanol. Based on the results from validation of ATAMS using harmaline, ethanol withdrawal tremor was measured between 10 and 22 Hz and expressed as motion-power. Representative force signals from control diet rats (upper; control group, n=4) and ethanol diet rats (lower; ethanol group, n=4) (**D**) and their power spectrum (**E**) for 5 min after the 2 hr withdrawal (*P<0.05 vs. control group). Graphs represent mean ± SEM.

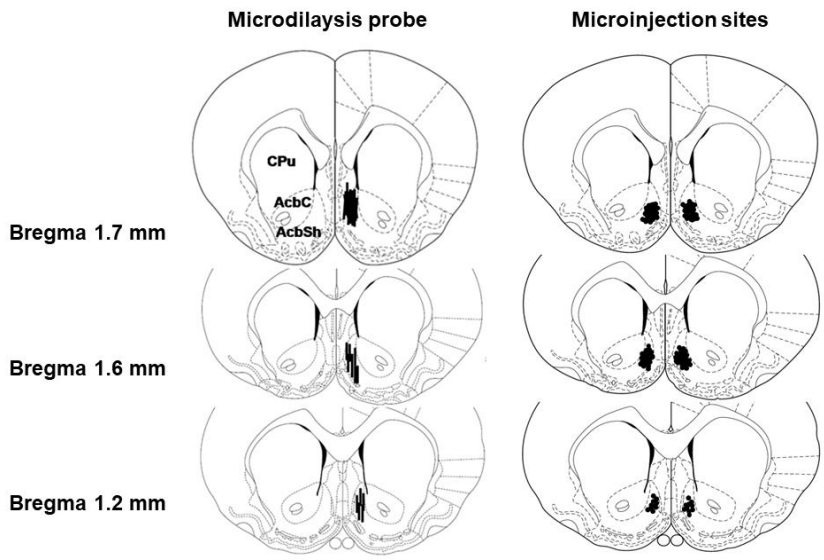

Fig. S3. Schematic localization of microdialysis probes and infusion sites.

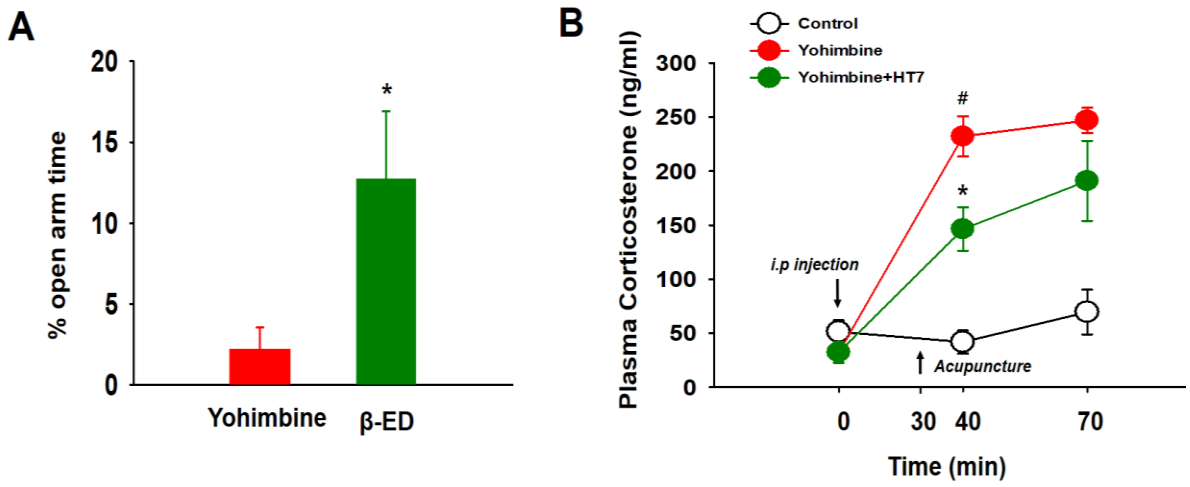

Fig. S4. Effects of intra-NAc infusions of β-endorphin on anxiety-like behavior in the elevated plus maze and acupuncture at HT7 on plasma corticosterone levels in yohimbine-treated rats. A: Effect of intra-NAc infusions of β-endorphin on the time spent in open arms in yohimbine-treated rats. The percentage of time spent in the open arms was measured in the rats given intra-NAc infusions of β-endorphin (0.25 μg/side) 30 min after injection of yohimbine (5 mg/kg, i.p.). Infusions of β-endorphin into the NAc (β-ED group, *n*=7) significantly increased the time spent in the open arms in yohimbine-treated rats, compared to infusions of saline into the NAc (Yohimbine group, *n*=6; $^{*}P<0.05$ vs. Yohimbine group). **B**: Effect of acupuncture on plasma corticosterone levels yohimbine-treated rats. Rats injected with yohimbine (Yohimbine group; *n*=7) showed increases in plasma corticosterone levels, compared to control rats (Control group, saline-injected rats; *n*=8). However, rats receiving acupuncture at HT7 (Yohimbine+HT7 group, *n*=8) had significantly lower plasma corticosterone levels than rats receiving yohimbine ($^{*,\#}P<0.05$ vs. Control).

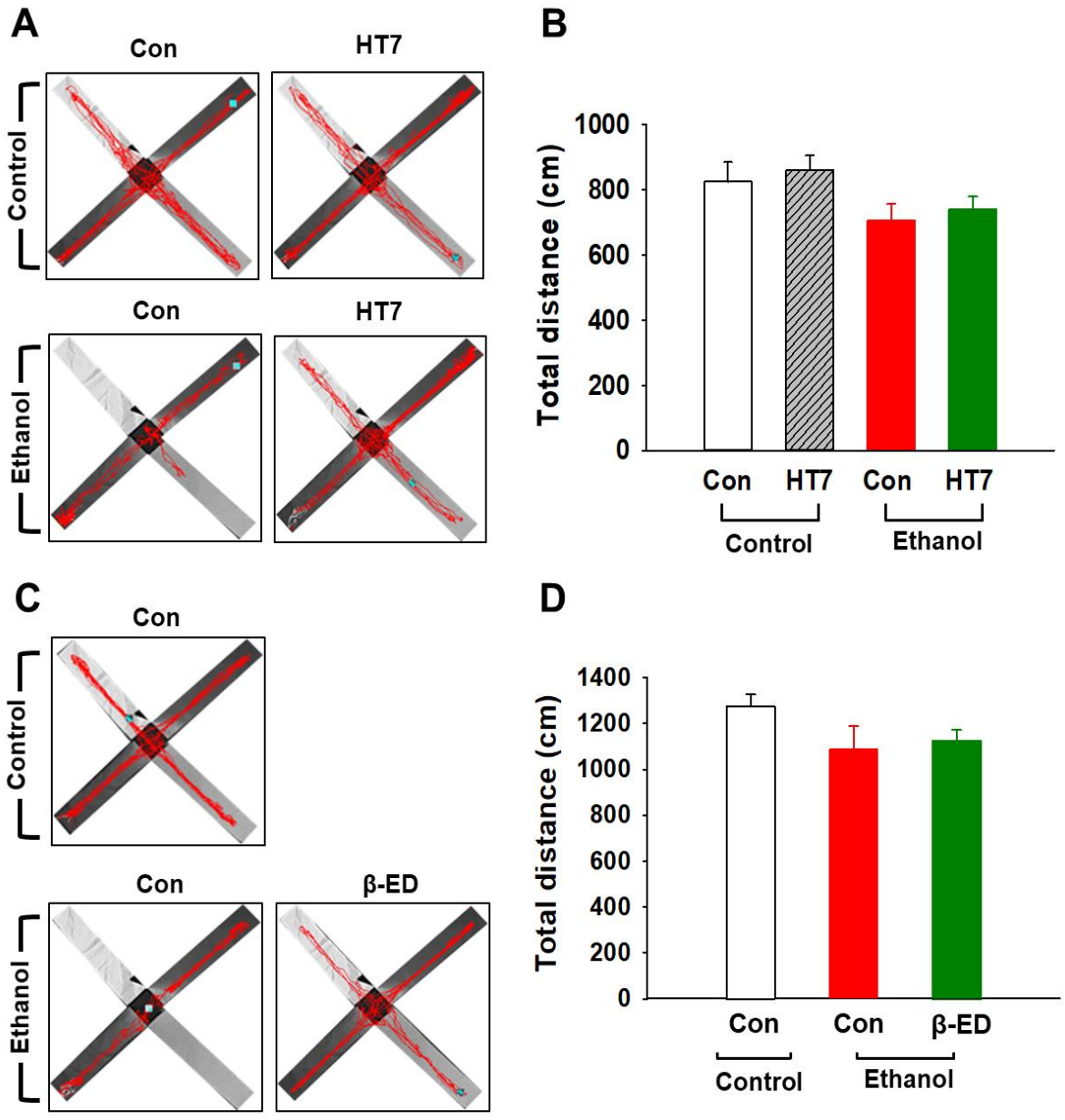

Fig. S5. Effects of HT7 acupuncture and intra-NAc infusions of β-endorphin on locomotor activity in the elevated plus maze. A, B: Effect of acupuncture at HT7 on the locomotor activity in the elevated plus maze in ethanol-dependent rats. Representative activity patterns (**A**) and the total distance of movement in the maze (**B**) following HT7 stimulation in control or ethanol diet groups. Con-control group (*n*=9), HT7-control group (*n*=9), Con-ethanol group (*n*=9), HT7-ethanol group (*n*=8). The total distance of movement

did not differ among groups. C, D: Effect of intra-NAc infusions of β-endorphin on the locomotor activity in the elevated plus maze in ethanol-dependent rats. Representative activity patterns (**C**) and the total distance of movement in the maze (**D**) following intra-NAc infusions of β-endorphin (0.25 μg/side) in ethanol diet groups, *n*=7 per group. The total distance of movement did not differ among groups.

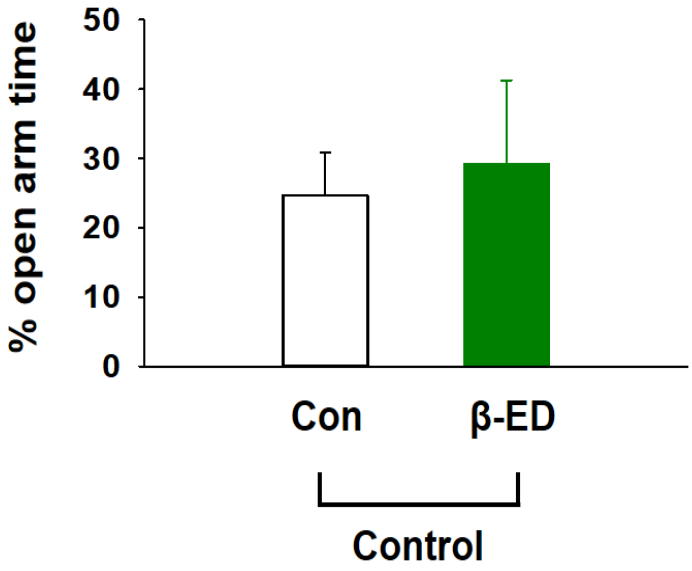

Fig. S6. Effects of intra-NAc infusions of β-endorphin on anxiety-like behavior in the elevated plus maze in control diet–fed rats. The percentage of time spent in the open arms was measured 10 min after intra-NAc infusions of β-endorphin (β-ED group) or saline (Con group) in control diet-fed rats. β-Endorphin did not affect percentage of time spent in the open arms, compared to saline. There were not statistically significant differences (P=0.735) between Con (24.68 ± 6.15, n=6) and β-ED (29.35 ± 11.92, n=6) groups in the percentage of time spent in the open arms.

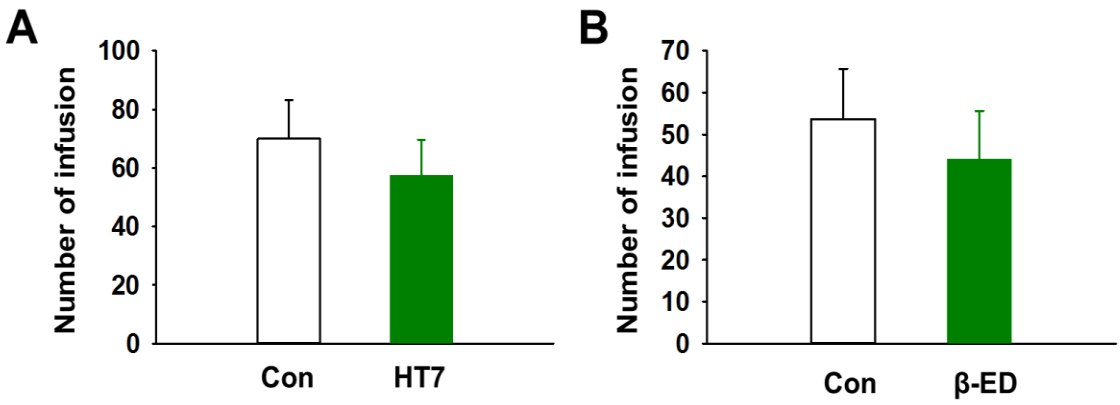

Fig. S7. Effects of HT7 acupuncture and intra-NAc infusions of β-endorphin on water self-administration. A. Water self-administration was measured in rats given acupuncture at HT7. Rats receiving HT7 acupuncture (HT7 group; 57.50 ± 12.10, *n*=6) did not show a significant difference in number of water infusions (*P*=0.501) from rats without insertion of needles (Con group; 70.00 ± 13.17, *n*=6). **B.** Water self-administration was measured after intra-NAc infusions of β-endorphin or saline. Rats receiving β-endorphin (β-ED group; 44.17 ± 11.44, *n*=6) did not show a significant difference in number of water infusions (*P*=0.597) from rats receiving saline (Con group; 53.67 ± 11.99, *n*=6).