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Supplementary Materials for

Social isolation–related depression accelerates ethanol intake via microglia-derived neuroinflammation

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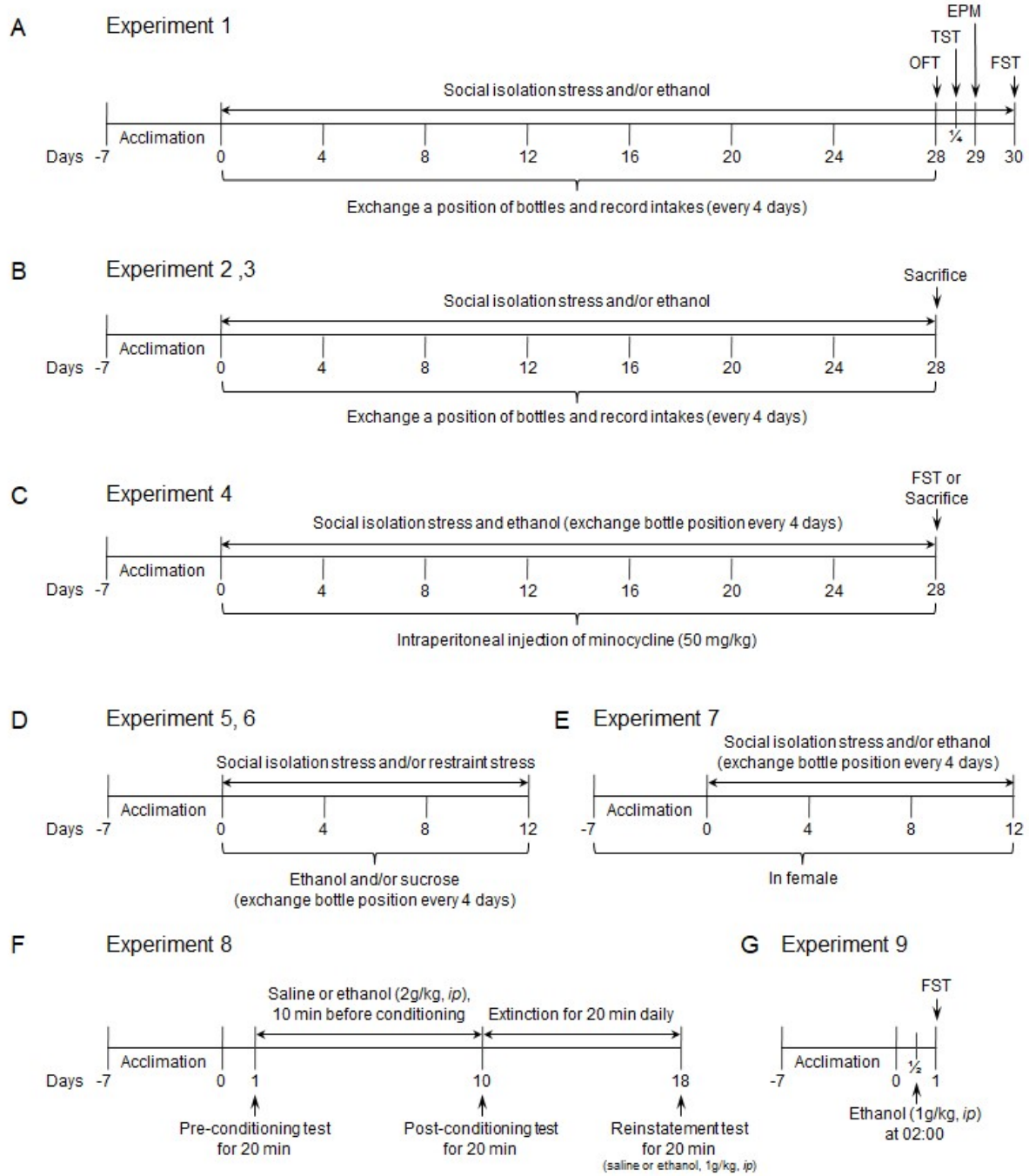
The PDF file includes:

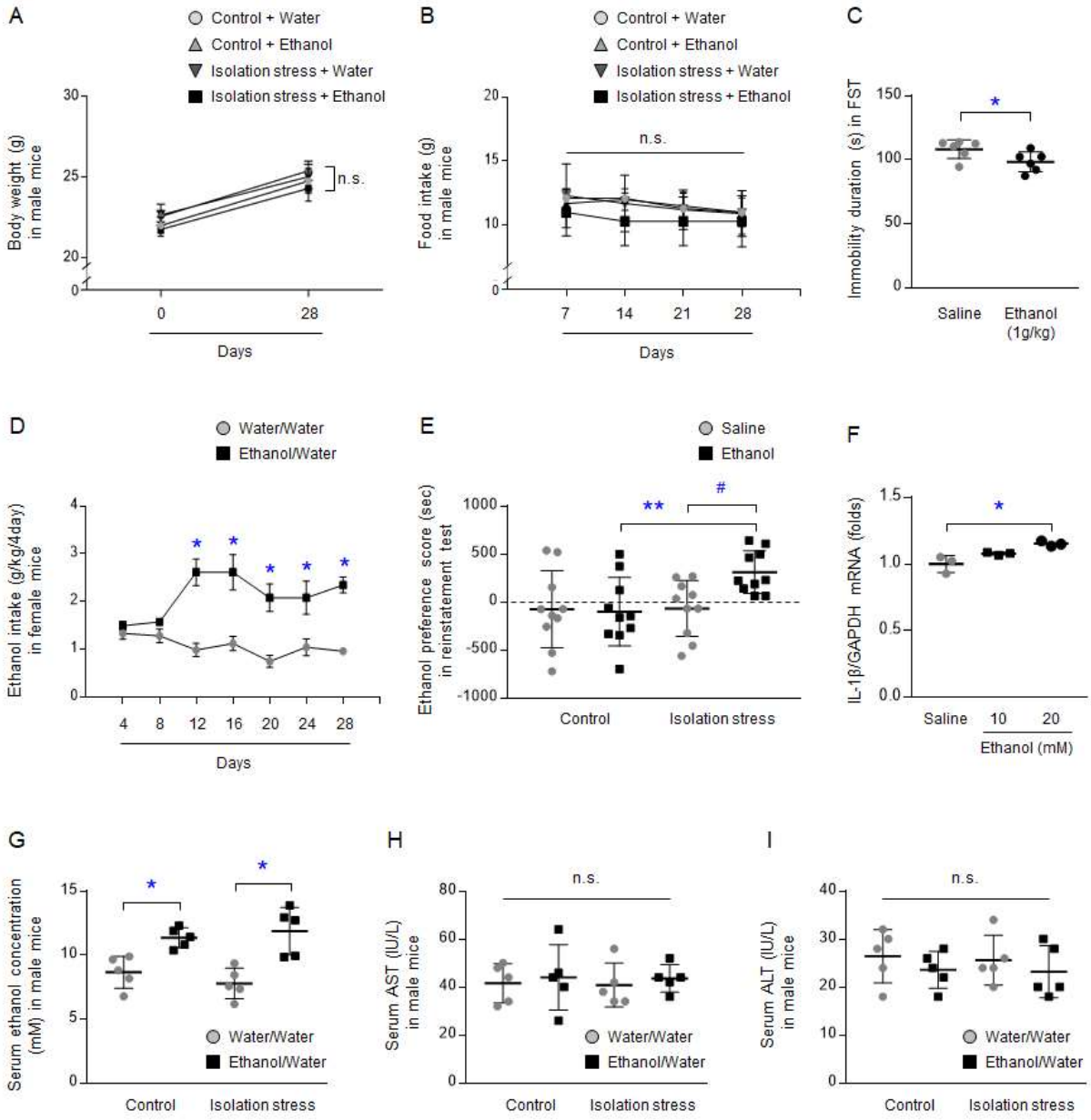
Other Supplementary Material for this manuscript includes the following:

Fig. S1. Experimental designs.



Experiments for behavioral tests (A), cytokine analysis, protein expression, serum biochemistry and immunohistology (B) were performed. Experiments for evaluating influences of minocycline (C), stress-type specificity or material specificity (D), gender specificity (E), ethanol-seeking behavioral test (F) and acute effects of ethanol on the behaviors (G) were additionally performed.

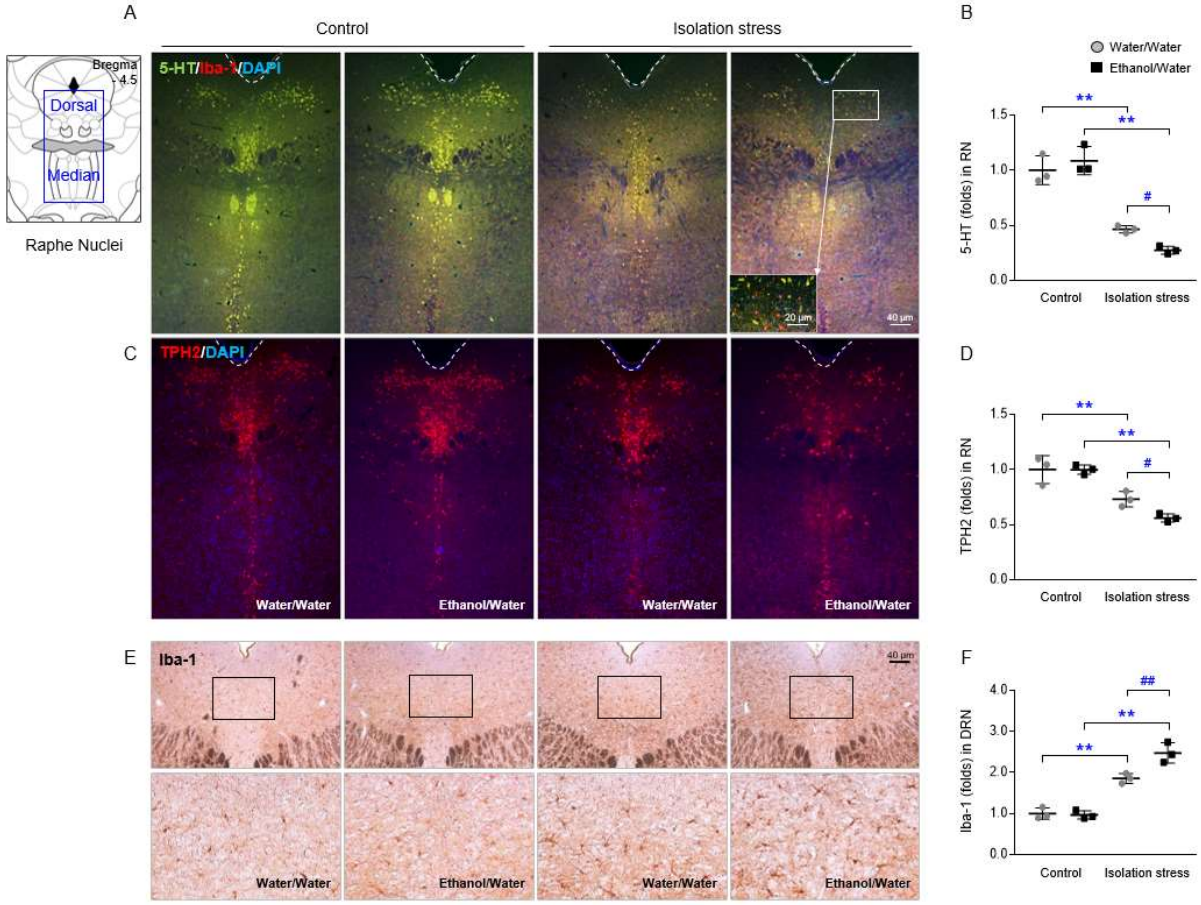
Fig. S2. Body weight, food intake, acute ethanol effects on depressive-like behavior, ethanol intake in female, ethanol seeking behavior and serum biochemistries.



During experimental days, body weight (A) and food intake (B) of male mice were recorded. Acute effects of ethanol on the depressive-like behavior were confirmed (C). The data are expressed as the mean ± SD (n = 5 or 8). $^{*}p < 0.05$ compared to the mice not exposed to ethanol. For confirmation in female mice, the ethanol intake of female mice that underwent isolation stress was recorded for 28 days (D). The data are expressed as the mean ± SD (n = 3). $^{*}p < 0.05$ compared to the unstressed female mice. To verify ethanol seeking behaviors during IS, ethanol-conditioned place preference was scored in reinstatement test (E). The data are expressed as the mean ± SD (n = 10). $^{**}p < 0.01$ compared to the unstressed mice, $^{\#}p < 0.05$ compared to the mice not injected to

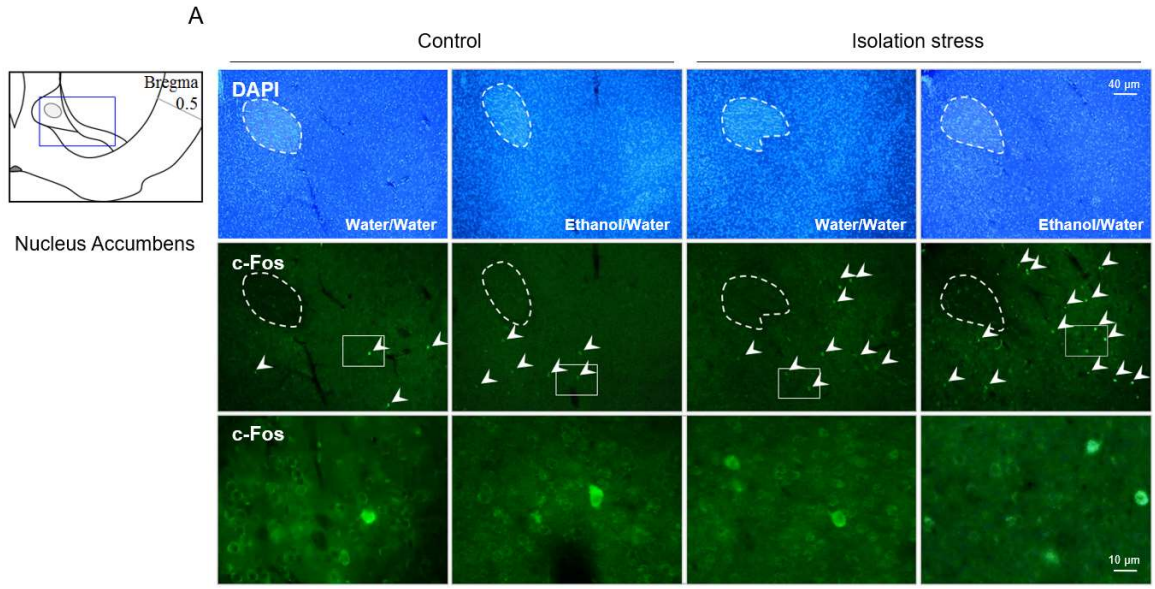
ethanol. Microglial activation upon the low dose of ethanol (10 and 20 mM) was monitored based on the mRNA level of IL-1β in lysate of BV2 cells (F). The data are expressed as the mean ± SD (n = 3). $^{*}p < 0.05$ and compared to the vehicle-treated cells. Serum levels of ethanol concentration, AST and ALT were determined using a UV spectrometer or autoanalyzer (G to I). The data are expressed as the mean ± SD (n = 5). $^{*}p < 0.05$ compared to the unstressed mice. $^{\#}p < 0.05$ compared to the mice not exposed to ethanol.

Fig. S3. Serotonergic signals and microglia in the raphe nuclei.



After isolation stress with or without ethanol exposure for 28 days, the mice were sacrificed by transcardial perfusion. Serotonergic signals and microglial activation were confirmed by immunofluorescence or immunohistochemistry analysis of 5-HT, TPH and Iba-1 (A, C and E) in the raphe nuclei, and their intensities were semi-quantified (B, D and F). The data are expressed as the mean ± SD (n = 3). $^{**}p < 0.01$ compared to the unstressed mice, $^{\#}p < 0.05$ or $^{\#\#}p < 0.01$ compared to the mice not exposed to ethanol.

Fig. S4. Dopaminergic system.



After isolation stress with or without ethanol exposure for 28 days, the mice were sacrificed by transcardial perfusion. The VTA-Dopaminergic projecting to NAc were confirmed by immunofluorescence analysis of c-Fos translocation (A). The data are expressed as the mean ± SD (n = 3).

Fig. S5. Summary of the study.

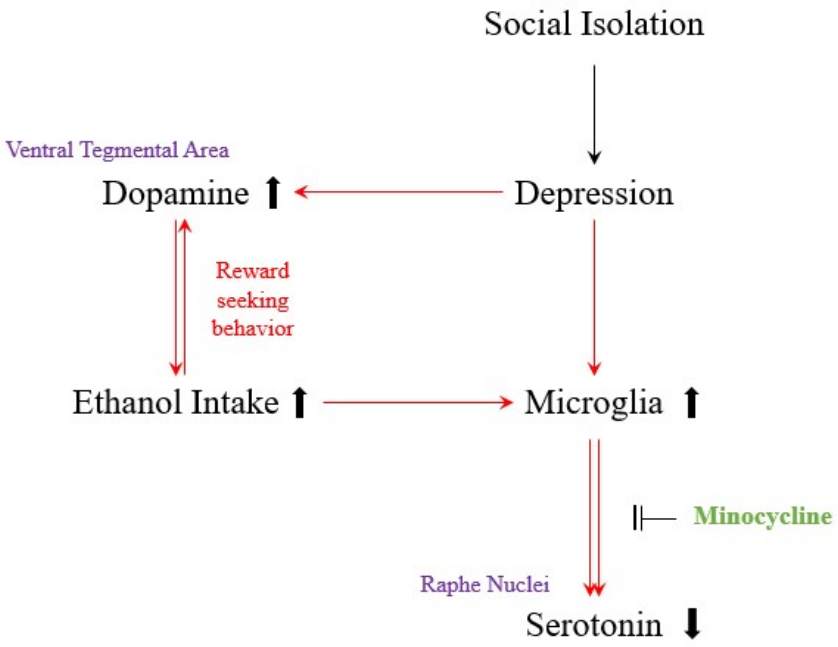
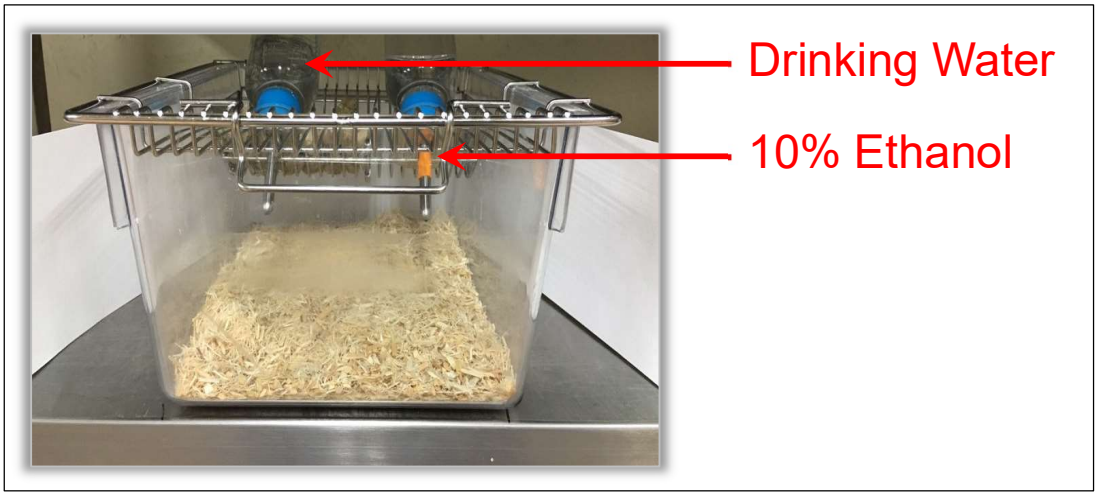


Table S1. Sequence of the primers used in real-time PCR analysis.

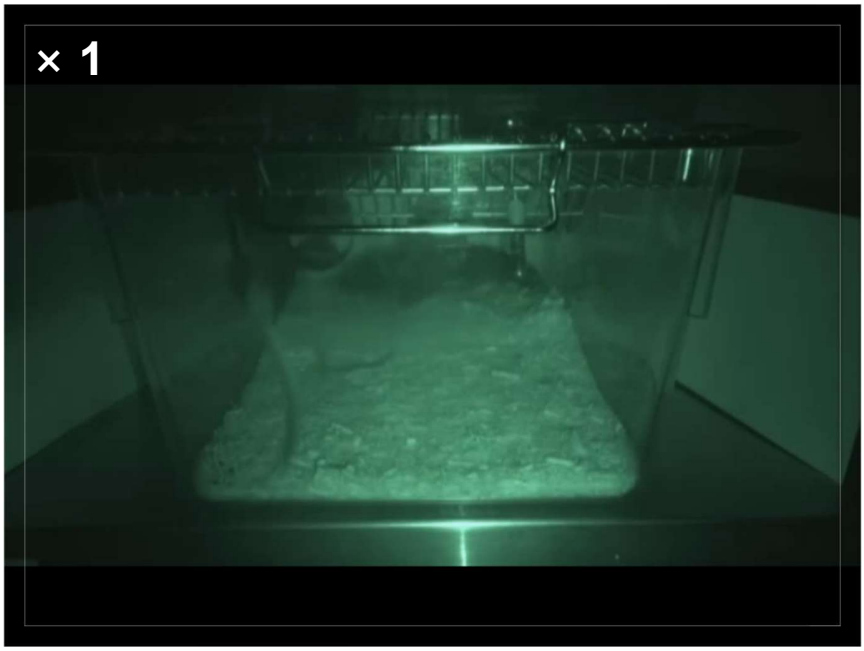
Gene (number)	Primer sequencing (Forward and Reverse)
TNF-α (NM_001278601)	5′-CCA AAT GGC CTC CCT CTC-3′ 5′-TTG CTA CGA CGT GGG CTA CA-3′
IL-1β (NM_008361)	5′-AAG TTG ACG GAC CCC AAA AGA-3′ 5′-TTG ATG TGC TGC TGC GAG AT-3′
C1qa (NM_007572)	5'-CTC AGG GAT GGC TGG TGG CC-3' 5'-CCT TTG AGA CCC GGC CTC CCC-3
GAPDH (NM_001289726)	5'-ACA TCA TCC CTG CAT CCA CT-3' 5'-AGA TCC ACG ACG GAC ACA TT-3'

TNF; tumor necrosis factor, IL; interleukin, C1qa; complement component 1q subunit a, GAPDH as a housekeeping gene.

Movie S1. Ethanol intake of control group



Control



Movie S2. Ethanol intake of social isolation stress group

Data S1. Figure 4A full-length gel and blots