Supporting Information

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SI Methods

Locomotor Activity Testing. On activity test days, animals were transferred in their home cages to the procedure room, 45–60 min before behavioral testing to allow acclimation to the environment. Locomotion was tested in clear acrylic plastic boxes (40 × 40 × 30 cm) covered with plastic lids (44 × 44 cm with 0.64-cm holes for ventilation) and placed in AccuScan activity monitors. Consecutive interruptions of two sets of eight intersecting photocell beams, situated 2 cm above the floor, measured the distance traveled. Interruptions were recorded and later translated by AccuScan software to horizontal distance traveled (in centimeters). The monitors were set inside individual black acrylic chambers (Flair Plastics), each containing foam insulation for exclusion of external noise. A fluorescent light (15 W) illuminated the chambers during testing, and a fan provided ventilation and background noise to mask extraneous laboratory sounds.

Determination of Blood EtOH Concentration (BEC). Blood samples from the tail vein (20 μl), to determine whether these mutations or pharmacological treatments modified EtOH levels at the time of behavioral testing, were collected on day 11 immediately after activity testing (15 min after EtOH injection) by using calibrated capillary tubes. BEC was determined by gas chromatography with previously published methods (1). The blood sample was added to 50 μl of chilled 5% $ZnSO_4$ and stored on ice. Fifty microliters of 0.3 N $Ba(OH)_2$ and 300 μl of distilled water were later added to each sample and were centrifuged for 5 min at 29,000 × *g*. Supernatants were transferred to glass vials and analyzed for EtOH concentration by gas chromatography (Agilent 6890N) with flame ionization detection. Five pairs of external standards of known EtOH concentrations (0.47–2.96 mg/ml) were used to establish a standard curve.

Corticosterone (CORT) RIA. A second tail blood sample (20 μl) was collected from each mouse and placed into heparinized capillary tubes. These tubes were centrifuged to separate the plasma from other blood constituents. Plasma was stored at −20°C until assayed. All samples were run by using an ImmuChem [^{125}I] CORT RIA from MP Biomedicals. All samples were diluted 1:200 with a phosphosaline buffer (provided with the kit), per kit instructions, before being assayed. Counts per minute were normalized and fit to a least-squares regression equation produced by log-logit transformation of the standards (25–1,000 ng). Sample concentration was calculated by interpolation of the standards. The detectable range of the assay was from 0.7 to 130 μg of CORT per 100 ml of plasma. Intra- and interassay coefficients of variation were <10%. Specificity of the assay: 0.34% cross-reactivity to deoxycorticosterone, and <0.15% cross-reactivity to other endogenous steroids.

CP-154,526 and Receptor Occupancy. CP-154,526 binds with high affinity to CRF_1 receptors ($K < 10$ nM) and blocks CRF-stimulated adenylate cyclase activity in membrane preparations from rat cortex and pituitary (2). Systemically administered CP-154,526 crosses the blood–brain barrier and reaches peak brain concentrations 20–30 min after administration (3). Administration of 30 mg/kg of this compound produces a profound reduction (almost a complete blockade) of CRF-stimulated ACTH levels (2), whereas a dose of 10 mg/kg produces only a 50% reduction in CRF effects.

1. Boehm SL II, Schafer GL, Phillips TJ, Browman KE, Crabbe JC (2000) Sensitivity to ethanol-induced motor incoordination in 5-HT(1B) receptor null mutant mice is task-dependent: Implications for behavioral assessment of genetically altered mice. *Behav Neurosci* 114:401–409.
2. Schulz DW, *et al.* (1996) CP-154,526: A potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. *Proc Natl Acad Sci USA* 93:10477–10482.
3. Keller C, Bruelisauer A, Lemaire M, Enz A (2002) Brain pharmacokinetics of a nonpeptidic corticotropin-releasing factor receptor antagonist. *Drug Metab Dispos* 30:173–176.

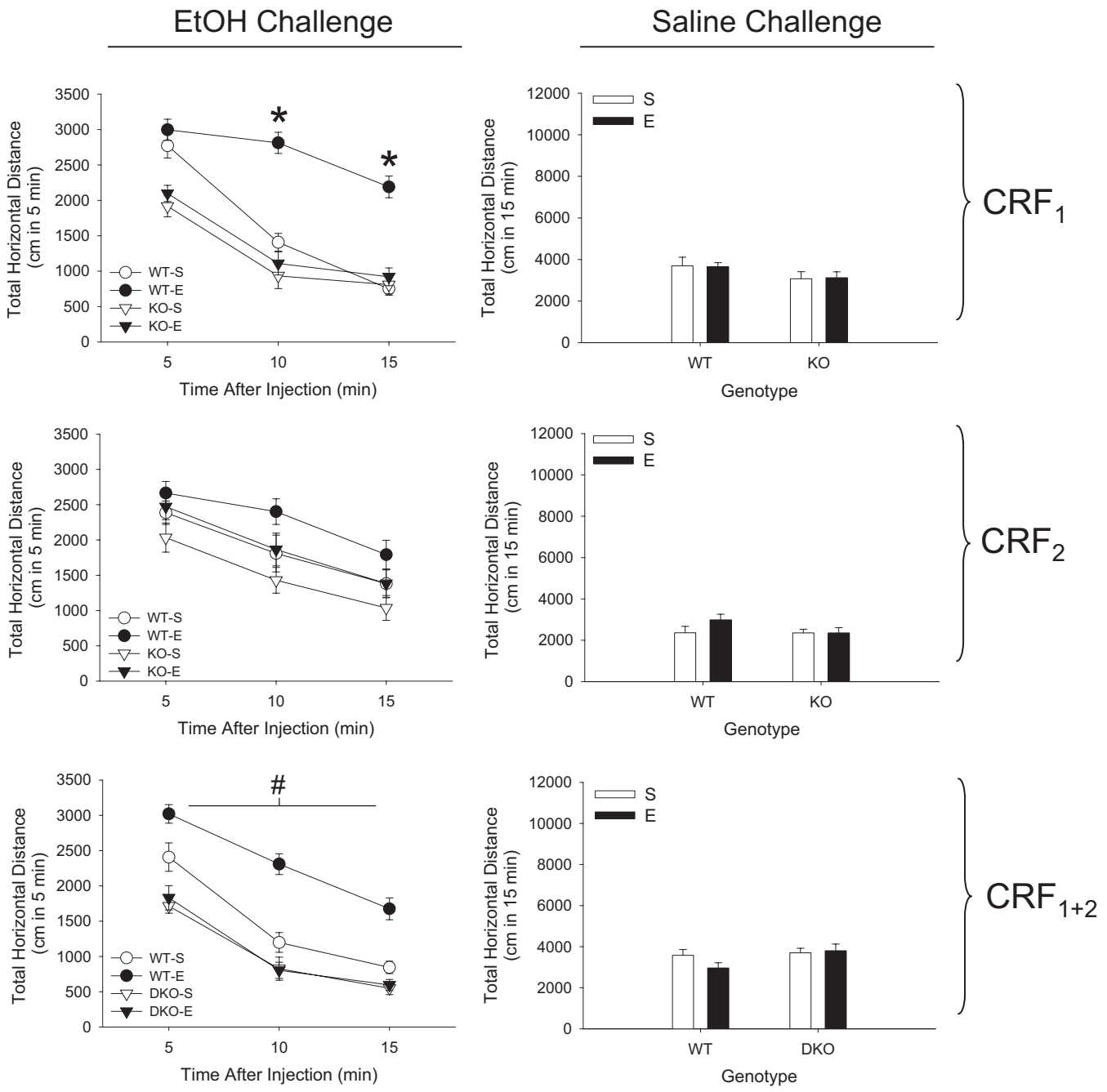

Fig. S1. Absence of sensitization to EtOH in animals lacking CRF_1 and CRF_{1+2} but not CRF_2 receptors. (*Left*) Time course (3 × 5-min epochs) of the locomotor response (mean ± SEM) to 1.5 g/kg EtOH in CRF_1 (*Top*), CRF_2 (*Middle*), and CRF_{1+2} (*Bottom*) WT and KO mice pretreated for 10 days with saline (S) or 2.5 g/kg EtOH (E). *, different from saline-pretreated WT and EtOH-pretreated KO mice (S.M.E. $P < 0.01$); #, main effect of EtOH pretreatment in WT mice only (no interaction with time). (*Right*) Summary of activity levels (mean ± SEM for total 15 min) after saline on day 12 for the three genotypes; no statistically significant differences among groups were found. $n = 13–15$ per group for CRF_1, 9–15 for CRF_2, and 12–15 for CRF_{1+2}. (See main article for statistical results.)

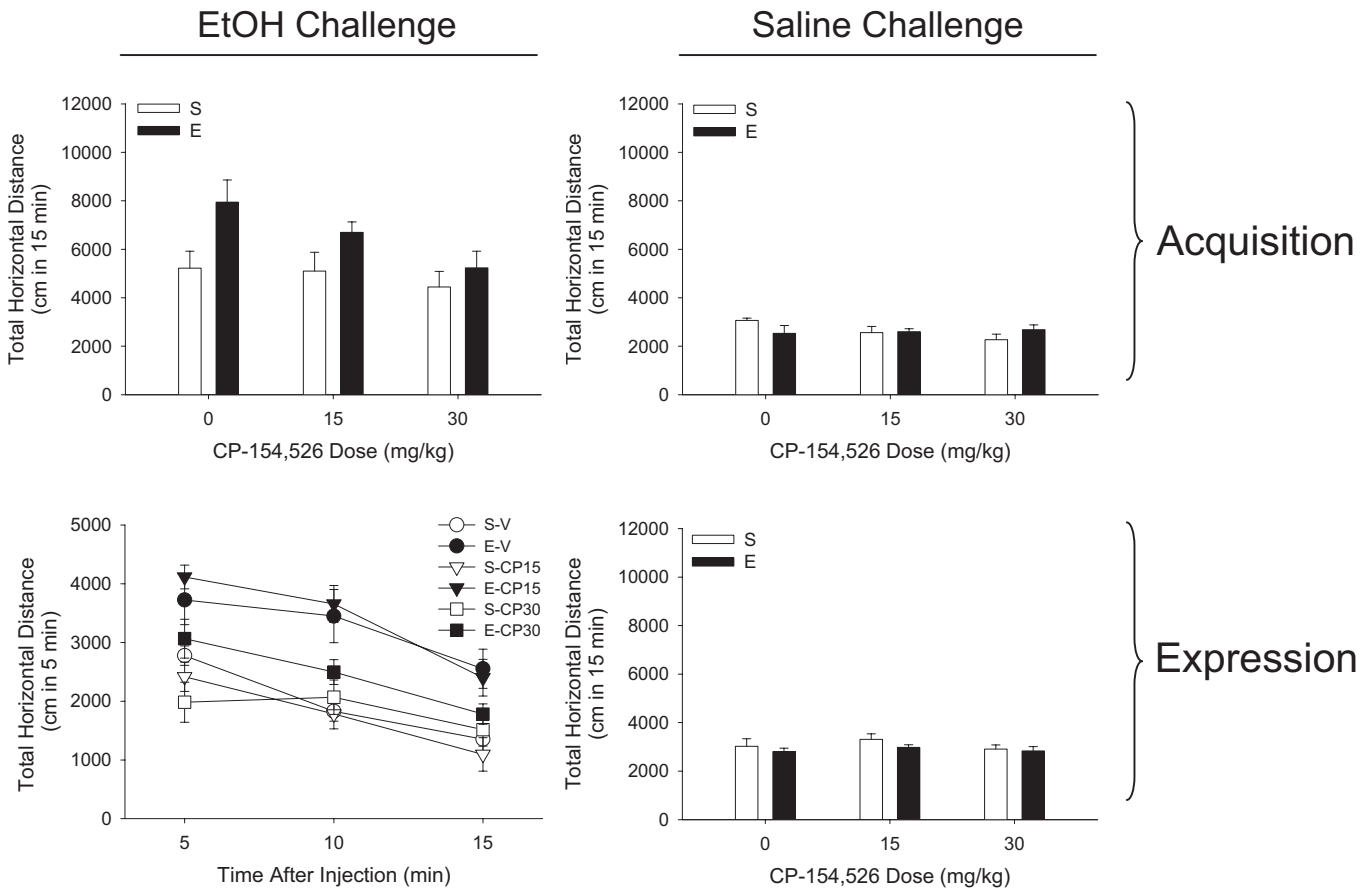

Fig. S2. The CRF_1 receptor antagonist CP-154,526 attenuates the acquisition and blocks the expression of psychomotor sensitization to EtOH. (*Left*) Mean ± SEM for total of 15 min or the time course after an injection of 1.5 g/kg EtOH in D2 mice treated as follows: for the acquisition study (*Upper*), on days 1–10 mice received saline (S) or 1.5 g/kg EtOH (E), 30 min after vehicle (V), 15 or 30 mg/kg CP-154,526 (CP15 and CP30, respectively). There was a significant effect of day 1–10 EtOH vs. saline treatment ($F_{1,70} = 7.4$; $P < 0.01$) and a statistical trend toward an interaction between CP-154,526 pretreatment dose and EtOH treatment dose ($P = 0.07$). $n = 10$–15 per group. For the expression study (*Lower*), on days 1–10, mice received saline (S) or 1.5 g/kg EtOH, 30 min after a vehicle injection and were then tested for their response to 1.5 g/kg EtOH, 30 min after vehicle (V), 15 or 30 mg/kg CP-154,526 (CP15 and CP30, respectively) on day 11. An interaction between CP-154,526 pretreatment dose on day 11 and EtOH treatment dose administered on days 1–10 was found; this result was not altered by the inclusion of time in the analysis. $n = 12$–14 per group. For both the acquisition and expression studies, the response to saline (*Right*; mean ± SEM for total of 15 min) was not altered by the previous treatments. (See main article for statistical results.)

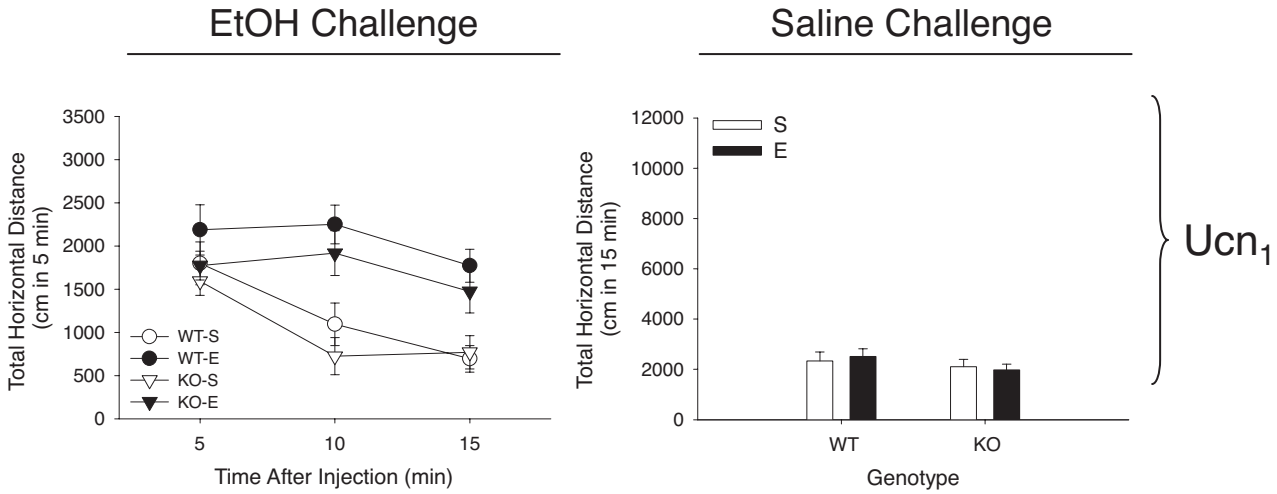

Fig. S3. Absence of an effect of Ucn_1 deletion on EtOH sensitization. Shown are mean ± SEM for time course (*Left*, 3 × 5-min epochs) of the locomotor stimulant response to 1.5 g/kg EtOH in Ucn_1 WT and KO mice pretreated with saline (S) or 2.5 g/kg EtOH (E) for 10 days. No differences between genotypes were found. (*Right*) Shown are activity levels (mean ± SEM for total of 15 min) after administration of saline on day 12. No statistically significant differences among groups were found. $n = 9–10$ per group. (See main article for statistical results.)

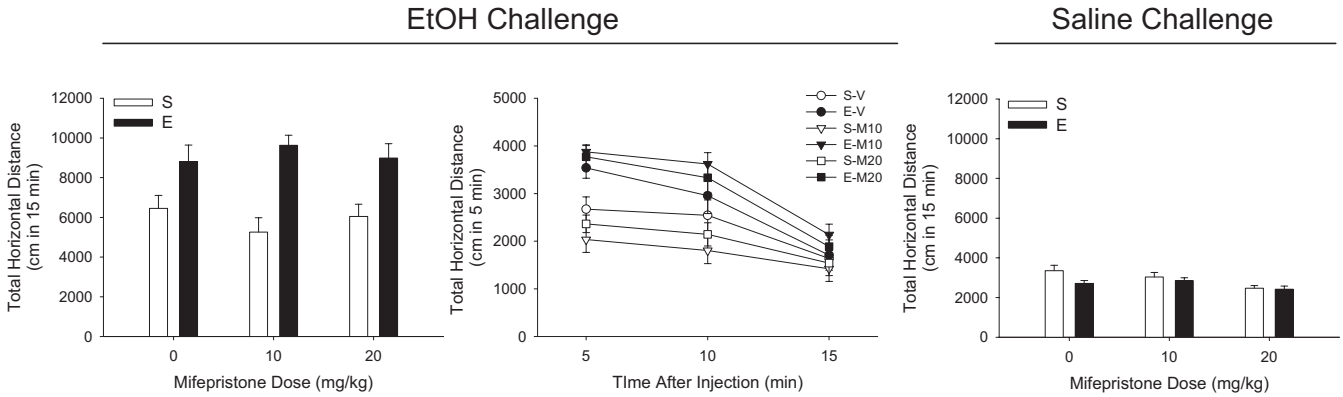

Fig. S4. Expression of sensitization to EtOH was not affected by glucocorticoid receptor blockade by mifepristone in D2 mice. Both vehicle- and mifepristone-pretreated D2 mice expressed sensitization to EtOH (*Left*; main effect of EtOH pretreatment dose: $F_{1,73} = 26.25$; $P < 0.01$). Shown is mean ± SEM for total of 15 min (*Left*) and time course (*Center*; 3 × 5-min epochs) of the locomotor stimulant response to 1.5 g/kg EtOH after vehicle (V), 10 or 20 mg/kg mifepristone (M10 and M20, respectively) in animals that received, on days 1–10, saline (S) or 1.5 g/kg EtOH (E), 30 min after vehicle. The response to EtOH (*Center*) changed across time ($F_{2,146} = 128.4$; $P < 0.01$) and time interacted with EtOH pretreatment dose ($F_{2,146} = 18.14$; $P < 0.01$). The sensitized response to EtOH was limited to the first 10 min after EtOH challenge. (Right) Shown is that locomotor behavior after saline on day 12 (mean ± SEM for total of 15 min) was also not affected by prior treatments. $n = 13–14$ per group. (See Table S3, showing that BEC did not differ among groups).

Table S1. BEC (mean ± SEM mg/ml) obtained 15 min after 1.5 g/kg EtOH on day 11 in knockout (KO) mice for CRF_1, CRF_2, CRF_{1+2}, and Ucn_1 and their respective wild-type (WT) controls

Genotype	WT (S)	WT (E)	KO (S)	KO (E)
CRF_1	1.6 ± 0.18	1.8 ± 0.17	1.3 ± 0.17	1.5 ± 0.14
CRF_2	1.4 ± 0.06	1.3 ± 0.07	1.2 ± 0.07	1.2 ± 0.11
CRF_{1+2}	1.3 ± 0.13	1.6 ± 0.16	1.1 ± 0.14	1.3 ± 0.16
Ucn_1	1.2 ± 0.08	1.1 ± 0.11	1.2 ± 0.10	1.2 ± 0.08

Data shown include groups treated repeatedly with saline (S) or 2.5 g/kg EtOH (E) in their home cages on days 1–10. For each particular genetic model, no statistically significant treatment effects were found.

Table S2. BEC (mean ± SEM mg/ml) obtained 15 min after 1.5 g/kg EtOH on day 11 in the studies of CP-154,526 effects on the acquisition and expression of EtOH sensitization

CP-154,526 dose	S	E
	Acquisition*	
0	1.1 ± 0.08	1.2 ± 0.05
15	1.1 ± 0.07	1.1 ± 0.07
30	1.0 ± 0.09	1.1 ± 0.08
	Expression†	
0	1.0 ± 0.08	1.1 ± 0.07
15	1.1 ± 0.07	1.1 ± 0.09
30	1.0 ± 0.06	1.1 ± 0.08

*Mice received 0, 15, or 30 mg/kg of CP-154,526, 30 min before saline (S) or 1.5 g/kg EtOH (E) on days 1–10, then were challenged with E, 30 min after vehicle on day 11 (CP-154,526 was not administered on this day).

†On days 1–10 mice received vehicle then saline (S), or vehicle then 1.5 g/kg EtOH (E), with injection pairs spaced 30 min apart. On day 11 they received vehicle-E, 15 mg/kg CP154,526-E or 30 mg/kg CP154,526-E, with injections spaced 30 min apart.

Table S3. BEC (mean ± SEM mg/ml) obtained 15 min after 1.5 g/kg EtOH on day 11 in the study of mifepristone effects on the expression of EtOH sensitization

Mifepristone dose	S	E
0	1.1 ± 0.07	1.0 ± 0.04
10	1.0 ± 0.06	1.1 ± 0.07
20	1.0 ± 0.1	1.1 ± 0.07

On days 1–10 mice received vehicle (0) then saline (S), or vehicle then 1.5 g/kg EtOH (E), with injection pairs spaced 30 min apart. On day 11 they received vehicle-E, 10 mg/kg mifepristone-E or 20 mg/kg mifepristone-E, with injections spaced 30 min apart.