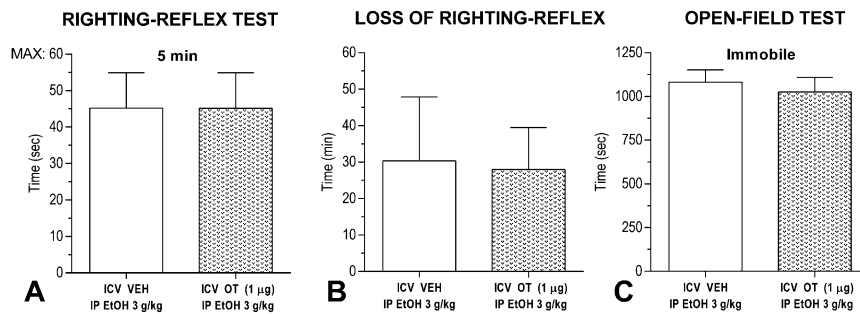
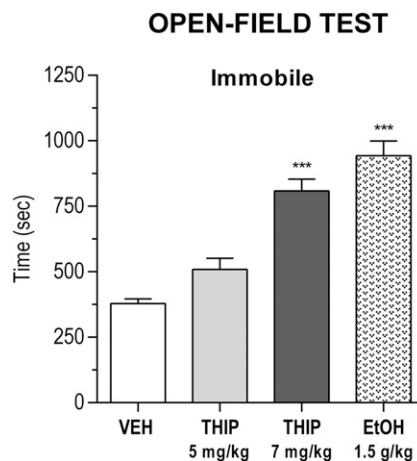


# Supporting Information

Bowen et al. 10.1073/pnas.1416900112



**Fig. S1.** Oxytocin (OT) does not prevent the severe motor impairment induced by a higher dose of ethanol (EtOH). Briefly, 16 male rats of the same strain, age, and weight used in the primary behavior experiments were randomly allocated to receive either i.c.v. VEH ( $n = 8$ ) or i.c.v. OT ( $1 \mu\text{g}$ ;  $n = 8$ ) + i.p. EtOH ( $3 \text{ mg/kg}$ ). (A) There was no significant difference between the VEH and OT conditions in time taken to right 5 min post-EtOH injection. (B) OT had no effect on the duration of the loss-of-righting reflex (LORR; the righting reflex was defined as being restored when the subject could right itself within 30 s). Note: all rats except four (two VEH, two OT) had a complete loss of the righting reflex. (C) OT had no impact on the time spent immobile in the 20-min open-field test conducted from 10–30 min post-EtOH administration.



**Fig. S2.** The  $7 \text{ mg/kg}$  4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP) produces a similar level of immobility to  $1.5 \text{ g/kg}$  ethanol (EtOH) in the open-field test. Briefly, 20 male rats of the same strain, age, and weight as used in the primary behavioral experiments were randomly allocated to receive i.p. VEH, THIP ( $5$  or  $7 \text{ mg/kg}$ ), or EtOH ( $1.5 \text{ g/kg}$ ) and were tested in the 20-min open-field test (as described in *Methods*) 10 min after drug administration. Time spent immobile was assessed, and the  $7 \text{ mg/kg}$  dose of THIP was chosen for use in the THIP–oxytocin experiment (Fig. 4) because it produced a similar sedative effect to  $1.5 \text{ g/kg}$  EtOH. \*\*\* $P < 0.001$  vs. VEH; THIP  $7 \text{ mg/kg}$  vs. EtOH  $1.5 \text{ g/kg}$ ,  $P > 0.05$ .

**Table S1.** Statistics from the contrast analysis of the OT–EtOH behavioral experiment

Test	Contrast (i.c.v. + i.p. vs. i.c.v. + i.p.)							
	VEH + VEH vs. VEH + EtOH		VEH + VEH vs. OT + VEH		VEH + VEH vs. OT + EtOH		VEH + EtOH vs. OT + EtOH	
	$F_{1,24}$	$P$	$F_{1,24}$	$P$	$F_{1,24}$	$P$	$F_{1,24}$	$P$
Wire-hanging 1	10.98	<b>0.003</b>	0.95	0.339	1.51	0.232	4.35	<b>0.048</b>
Righting-reflex 1	16.57	<b>&lt; 0.001</b>	0.12	0.728	1.77	0.196	7.51	<b>0.011</b>
Open-field: immobile	16.34	<b>&lt; 0.001</b>	0.74	0.398	2.92	0.1	5.44	<b>0.028</b>
Wire-hanging 2	21.11	<b>&lt; 0.001</b>	3.87	0.061	5.88	<b>0.023</b>	4.71	<b>0.04</b>
Righting-reflex 2	24.71	<b>&lt; 0.001</b>	0.15	0.699	7.155	<b>0.013</b>	5.27	<b>0.031</b>

$P$  values for significant differences are highlighted in bold.

**Table S2. Statistics from one-sample *t* tests examining change from response either to 30 nM GABA (nos. 1 and 2), 3 nM GABA (nos. 3–6), or 100 nM THIP (no. 7) applied alone or to baseline current (nos. 8 and 9)**

No.	Concentration of compound(s) applied	Cell											
		$\alpha 4\beta 1\delta$			$\alpha 4\beta 3\delta$			$\alpha 4\beta 1$			$\alpha 4\beta 3$		
		df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>
1	30 nM GABA + 30 mM EtOH	2	13.45	<b>0.005</b>	2	8.72	<b>0.013</b>	—	—	—	—	—	—
2	30 nM GABA + 30 mM EtOH + 10 $\mu$ M OT	2	1.5	0.272	2	0.39	0.731	—	—	—	—	—	—
3	3 nM GABA + 30 mM EtOH	8	12.35	<b>&lt; 0.001</b>	—	—	—	4	0.61	0.572	2	0.21	0.856
4	3 nM GABA + 30 mM EtOH + 10 $\mu$ M OT	4	2.12	0.11	—	—	—	—	—	—	—	—	—
5	3 nM GABA + 30 mM EtOH + 10 $\mu$ M AVP	3	4.05	<b>0.027</b>	—	—	—	—	—	—	—	—	—
6	3 nM GABA + 10 $\mu$ M OT	2	0.46	0.688	—	—	—	—	—	—	—	—	—
7	100 nM THIP + 10 $\mu$ M OT	2	3.08	0.091	—	—	—	—	—	—	—	—	—
8	30 mM EtOH	2	0.02	0.984	—	—	—	—	—	—	—	—	—
9	10 $\mu$ M OT	2	1.81	0.211	—	—	—	—	—	—	—	—	—

*P* values for significant changes are highlighted in bold. — indicates that the cell was not tested with the concentration and combination of compounds listed in that row.

**Table S3. Statistics for comparison of response to GABA + EtOH vs. GABA + EtOH + OT/AVP**

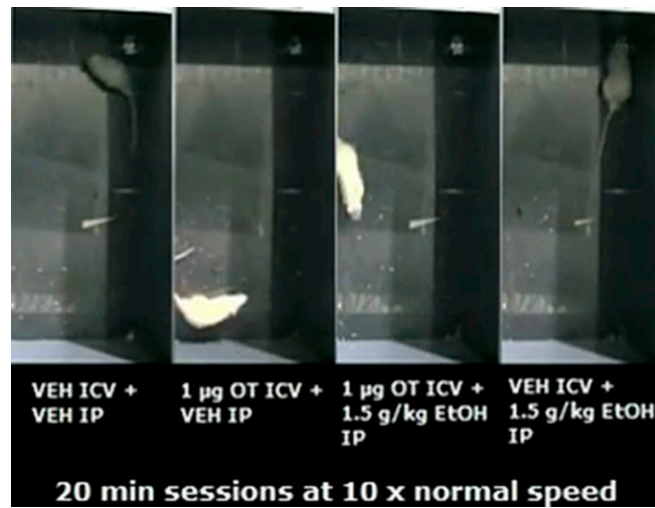
Comparison	Cell					
	$\alpha 4\beta 1\delta$			$\alpha 4\beta 3\delta$		
	df	<i>t</i>	<i>P</i>	df	<i>t</i>	<i>P</i>
3 nM GABA + 30 mM EtOH vs. 3 nM GABA + 30 mM EtOH + 10 $\mu$ M OT	4	10.91	<b>&lt; 0.001</b>	—	—	—
3 nM GABA + 30 mM EtOH vs. 3 nM GABA + 30 mM EtOH + 10 $\mu$ M AVP	3	0.82	0.475	—	—	—
30 nM GABA + 30 mM EtOH vs. 30 nM GABA + 30 mM EtOH + 10 $\mu$ M OT	2	23.47	<b>0.002</b>	2	8.18	<b>0.015</b>

*P* values for significant differences are highlighted in bold. — indicates that the cell was not tested with the concentration and combination of compounds listed in that row.

**Table S4. Statistics from the contrast analysis of the OT–THIP behavioral experiment**

Test	Contrast (i.c.v. + i.p. vs. i.c.v. + i.p.)							
	VEH + VEH vs. VEH + THIP		VEH + VEH vs. OT + VEH		VEH + VEH vs. OT + THIP		VEH + THIP vs. OT + THIP	
	$F_{1,27}$	<i>P</i>	$F_{1,27}$	<i>P</i>	$F_{1,27}$	<i>P</i>	$F_{1,27}$	<i>P</i>
Open-field: immobile	4.48	<b>0.044</b>	0.56	0.46	7.46	<b>0.011</b>	0.43	0.518

*P* values for significant differences are highlighted in bold.



**Movie S1.** The “sobering-up” effect of oxytocin (OT) in ethanol (EtOH)-treated rats. This movie shows rats tested in the open-field locomotor activity test conducted 10–30 min after i.p. injection of EtOH (1.5 g/kg) or vehicle (VEH), preceded by i.c.v. OT or VEH pretreatment. Movies are presented at 10× normal speed. The rat on the far left received i.c.v. VEH + i.p. VEH; in the middle left received i.c.v. OT (1 μg) + i.p. VEH; in the middle right received i.c.v. OT (1 μg) + i.p. EtOH (1.5 g/kg); and on the far right received i.c.v. VEH + i.p. EtOH (1.5 g/kg).

[Movie S1](#)