

Oxytocin prevents ethanol actions at δ subunit-containing GABA_A receptors and attenuates ethanol-induced motor impairment in rats

Michael T. Bowen^{a,b,1}, Sebastian T. Peters^{c,1}, Nathan Absalom^b, Mary Chebib^b, Inga D. Neumann^c, and Iain S. McGregor^{a,2}

^aSchool of Psychology, University of Sydney, Sydney, NSW 2006, Australia; ^bFaculty of Pharmacy, University of Sydney, Sydney, NSW 2006, Australia; and ^cDepartment of Behavioral and Molecular Neurobiology, University of Regensburg, Regensburg 93053, Germany

Edited* by Bruce S. McEwen, The Rockefeller University, New York, NY, and approved January 22, 2015 (received for review September 2, 2014)

Even moderate doses of alcohol cause considerable impairment of motor coordination, an effect that substantially involves potentiation of GABAergic activity at δ subunit-containing GABA_A receptors (δ -GABA_ARs). Here, we demonstrate that oxytocin selectively attenuates ethanol-induced motor impairment and ethanol-induced increases in GABAergic activity at δ -GABA_ARs and that this effect does not involve the oxytocin receptor. Specifically, oxytocin (1 μ g i.c.v.) given before ethanol (1.5 g/kg i.p.) attenuated the sedation and ataxia induced by ethanol in the open-field locomotor test, wire-hanging test, and righting-reflex test in male rats. Using two-electrode voltage-clamp electrophysiology in *Xenopus* oocytes, oxytocin was found to completely block ethanol-enhanced activity at $\alpha 4\beta 1\delta$ and $\alpha 4\beta 3\delta$ recombinant GABA_ARs. Conversely, ethanol had no effect when applied to $\alpha 4\beta 1$ or $\alpha 4\beta 3$ cells, demonstrating the critical presence of the δ subunit in this effect. Oxytocin had no effect on the motor impairment or in vitro effects induced by the δ -selective GABA_AR agonist 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol, which binds at a different site on δ -GABA_ARs than ethanol. Vasopressin, which is a nonapeptide with substantial structural similarity to oxytocin, did not alter ethanol effects at δ -GABA_ARs. This pattern of results confirms the specificity of the interaction between oxytocin and ethanol at δ -GABA_ARs. Finally, our in vitro constructs did not express any oxytocin receptors, meaning that the observed interactions occur directly at δ -GABA_ARs. The profound and direct interaction observed between oxytocin and ethanol at the behavioral and cellular level may have relevance for the development of novel therapeutics for alcohol intoxication and dependence.

oxytocin | alcohol | GABA | motor impairment | electrophysiology

Extrasynaptic δ subunit-containing γ -aminobutyric acid type A receptors (δ -GABA_ARs) play a major role in regulating tonic inhibition in the central nervous system (CNS) (1). The δ -GABA_ARs also represent one of the major targets of ethanol in the CNS, particularly at relatively low ethanol concentrations, and mediate some of the rewarding and ataxic effects of ethanol (2–8). For instance, a genetic polymorphism that exaggerates the actions of ethanol at δ -GABA_ARs also potentiates ethanol-induced motor impairment (2). Further, knockdown of δ subunit expression in the medial shell of the nucleus accumbens reduces alcohol intake in rats (6). Finally, overstimulation and subsequent internalization of $\alpha 4\beta \delta$ extrasynaptic GABA_ARs after persistent stimulation by ethanol seem to underlie the development of rapid tolerance to the ataxic effects of ethanol (4).

The neuropeptide oxytocin (OT) is well-known for its regulatory role in mammalian sociability and various other central and peripheral physiological processes (9). Early preclinical studies suggested that OT can prevent the development of tolerance to the sedative and ataxic effects of ethanol in rodents (10) and also modulate the severity of ethanol withdrawal (11). More recent studies show that OT reduces alcohol intake in rats (12) and reduces the severity of alcohol withdrawal and craving in dependent humans during detoxification (13). However, the

mechanism underlying these actions is largely uncharacterized, including whether the modulation of various alcohol-related effects by OT involves the OT receptor (OTR).

Given the role of $\alpha 4\beta \delta$ extrasynaptic GABA_ARs in mediating tolerance to the ataxic effects of ethanol (4), and the modulation of ethanol tolerance by OT (14), we hypothesized that OT might act to prevent ethanol actions directly at δ -GABA_ARs. Such an action would be expected to reduce alcohol-induced motor impairment. We therefore assessed whether centrally administered OT inhibits ethanol-induced motor impairment in rodents. After this experiment, we tested OT effects on ethanol-potentiated GABA-gated current in $\alpha 4\beta \delta$ subunit-containing recombinant GABA_ARs. To ascertain whether any OT effects were due to general characteristics shared by similar neuropeptides or were specific to OT, we also tested the effect of the structurally related neuropeptide arginine vasopressin (AVP) at these recombinant receptors. To determine whether the δ subunit was crucial for any ethanol and OT effects, we also assessed ethanol actions at $\alpha 4\beta$ recombinant GABA_ARs. Finally, to determine whether the inhibitory action of OT was specific to effects induced by ethanol, we tested the impact of OT on the sedation and currents induced by the GABA_AR agonist 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP), which has high selectivity for the δ subunit but at a distinct binding site to ethanol (15).

Significance

Even moderate doses of alcohol can cause considerable motor impairment. This effect has been linked to ethanol-induced potentiation of GABA actions at δ subunit-containing GABA_A receptors (δ -GABA_ARs). Here, we demonstrate that the neuropeptide oxytocin selectively attenuates ethanol-induced motor impairment in rats as well as ethanol-induced potentiation of GABAergic activity at δ -GABA_ARs. This effect of oxytocin is shown to be independent of the oxytocin receptor (OTR) and involves a direct action at δ -GABA_ARs. To our knowledge, this study provides the first evidence of oxytocin having a direct, non-OTR-mediated effect on GABA-ethanol interactions. Recent preclinical and clinical studies indicate that oxytocin may also attenuate alcohol consumption, craving, and withdrawal, and the present study shows a previously unidentified mechanism through which some of these effects may occur.

Author contributions: M.T.B., S.T.P., N.A., M.C., I.D.N., and I.S.M. designed research; M.T.B. and S.T.P. performed research; M.C., I.D.N., and I.S.M. contributed new reagents/analytic tools; M.T.B. and S.T.P. analyzed data; and M.T.B., S.T.P., N.A., M.C., I.D.N., and I.S.M. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

¹M.T.B. and S.T.P. contributed equally to this work.

²To whom correspondence should be addressed. Email: iain.mcgregor@sydney.edu.au.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1416900112/-DCSupplemental.

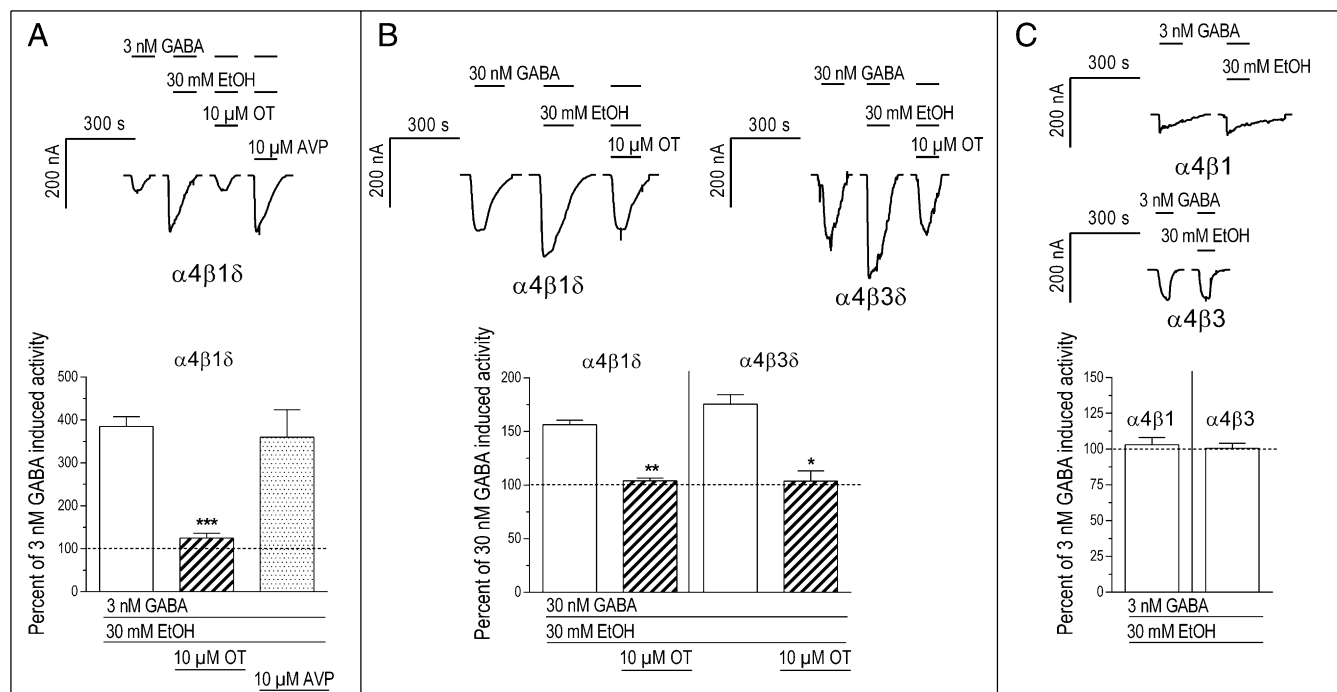


Fig. 2. Oxytocin (OT) prevents the action of ethanol (EtOH) at δ -GABA_ARs. (A) The 30 mM EtOH coapplied with 3 nM GABA onto GABA_A receptor α 4 β 1 δ subunit-expressing *X. laevis* oocytes increased the magnitude of the GABA-gated current to almost 400% of the response elicited by GABA alone ($P < 0.001$). This effect was prevented by coapplication of 10 μ M OT with EtOH and GABA ($***P < 0.001$), such that no significant EtOH-induced effect was observed ($P > 0.1$). AVP did not affect EtOH-induced enhancement of GABA-gated currents ($P > 0.1$). (B) EtOH potentiation of GABA-gated currents in α 4 β 1 δ -expressing cells and its antagonism by OT ($**P < 0.01$) were also seen when a higher concentration of GABA (30 nM) was used, but the effects of EtOH were less pronounced. The interaction of EtOH, GABA, and OT also occurred with α 4 β 3 δ -expressing cells ($*P < 0.05$). (C) EtOH (30 mM) had no effect on 3 nM GABA-gated current in α 4 β 1 ($P > 0.1$) or α 4 β 3 ($P > 0.1$) cells.

behavioral and receptor-level effects of the GABA_A agonist 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP). THIP has high selectivity for the δ subunit but occupies a different binding site to ethanol (15). When injected into rats, THIP (7 mg/kg, i.p.) caused significant sedation (immobility) in the open-field test, but this sedation was unaffected by pretreatment with OT (1 μ g, i.c.v.) (Fig. 4A and Table S4). Application of 100 nM THIP induced currents in α 4 β 1 δ -expressing cells, but coapplication of 10 μ M OT did not affect these THIP-induced currents (Fig. 4B and C and Table S2).

Further emphasizing that OT has a highly specific impact on ethanol actions, rather than a more general effect on the δ subunit, we found that OT did not alter the GABA-gated current induced by 30 nM GABA alone (Fig. 5A and Table S2) and induced no current when applied on its own (Fig. 5B and Table S2). Ethanol also had no effect on the cells when infused alone at a concentration of 30 mM (Fig. 5C and Table S2).

Discussion

The present study reports a hitherto uncharacterized interaction between the neuropeptide OT and one of the most widely used recreational drugs in the world, ethanol. This finding comes at a time when there is substantial interest in the potential of OT as a therapeutic for various psychiatric problems (18), including substance-use disorders (19–23). A robust antagonistic action of OT on moderate doses of ethanol was evident in the present report at both the behavioral and cellular level of analysis. Thus, OT provided a striking attenuation of the characteristic intoxicating effects of acute injection of a moderate dose of ethanol in rats. At the same time, OT prevented the potentiating action of ethanol on GABA-gated currents at δ -GABA_ARs, a cellular action that is mechanistically linked to ethanol-induced motor impairment. In contrast, OT had no impact on either the behavioral

impairment or in vitro effects of THIP, which has similar in vivo and in vitro effects to ethanol but binds at a distinct site on the δ subunit (15). Furthermore, OT did not alter the more profound motor impairment induced by a much higher dose of ethanol. This result would be expected based on our electrophysiological findings because high doses of ethanol exert their extreme ataxic effects primarily through a non- δ -GABA_AR-mediated mechanism (7).

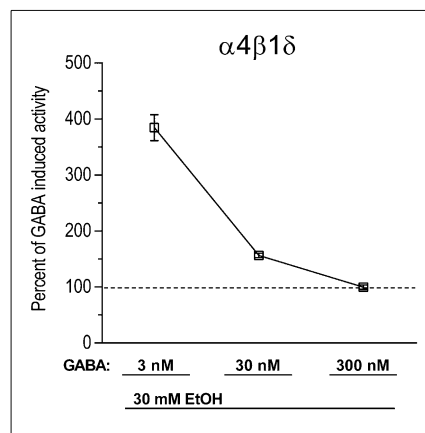


Fig. 3. Ethanol (EtOH) potentiation of GABA-gated currents at α 4 β 1 δ recombinant receptors depends on the concentration of GABA. The effectiveness of 30 mM EtOH in potentiating GABA-gated current was strongly related to GABA concentrations, being highly effective at 3 nM ($P < 0.001$), less effective at 30 nM ($P < 0.01$), and ineffective at 300 nM ($P > 0.1$).

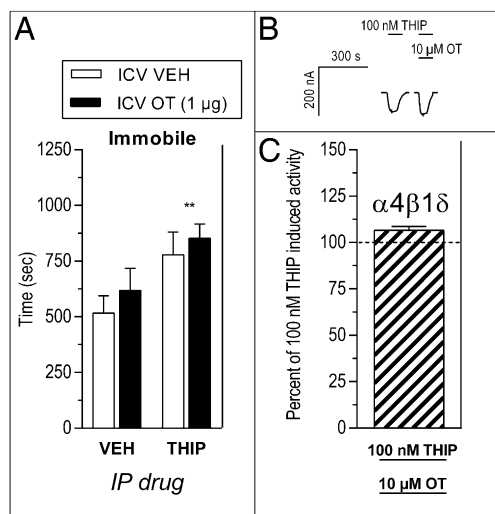


Fig. 4. Oxytocin (OT) does not alter the in vivo or in vitro effects of the GABA_A agonist 4,5,6,7-tetrahydroisoxazolo(5,4-c)pyridin-3-ol (THIP). (A) THIP (7 mg/kg, i.p.) increased immobility in the open-field test in rats (** $P < 0.01$, THIP vs. VEH), and this increased immobility was unaffected by i.c.v. OT (1 µg; $P > 0.1$). (B and C) Current induced in $\alpha 4\beta 1\delta$ cells by application of 100 nM THIP was unaffected by coapplication of 10 µM OT ($P > 0.05$).

The motor impairment induced by ethanol was clearly evident across three different behavioral models and at two different time points. Strikingly, OT (1 µg/5 µL, i.c.v.) administered before an intoxicating, moderate dose of ethanol (1.5 g/kg, i.p.) markedly antagonized the ataxia induced by ethanol in the wire-hanging test and in the righting-reflex test and reversed ethanol-induced hypoactivity in an open-field test. Observation of behavior on the latter test (Movie S1) suggests a remarkable “sobering-up” effect of OT in ethanol-treated rats.

At a molecular level, OT completely prevented the characteristic enhancement of GABA-gated currents by ethanol at recombinant δ -GABA_ARs. The δ -GABA_ARs, sometimes called the “one-glass-of-wine” receptors (24), are located in several brain regions, including the thalamus, cerebellum, hippocampus, and striatum (25, 26), and show high sensitivity to relatively low doses of ethanol (2–8, 27). A number of previous studies demonstrate the involvement of δ -GABA_ARs in a range of ethanol-induced functional effects, including ataxia, sedation, and reward (2–8). We established that OT blocked ethanol enhancement at both $\alpha 4\beta 1\delta$ and $\alpha 4\beta 3\delta$ subunit-containing receptors. However, ethanol had no effect on currents when applied to $\alpha 4\beta 1$ or $\alpha 4\beta 3$ subunit-containing receptors, indicating that the δ subunit is likely to be the key site of OT–ethanol interactions.

Specificity was also shown, in that AVP, a closely related neuropeptide to OT, did not influence ethanol-induced potentiation of GABA-induced currents in $\alpha 4\beta 1\delta$ -expressing cells. OT and AVP are nonapeptides with a similar molecular mass (OT, 1,007 Da; AVP, 1,084 Da) and differ by only two amino acids (third and eighth position). The absence of an AVP effect on ethanol’s actions at δ -GABA_ARs suggests that the key structural features allowing OT to inhibit ethanol actions at these receptors may involve the isoleucine (third position) or leucine (eighth position) segments of the OT molecule (i.e., those amino acids that differ from AVP).

Of additional interest in the present study was the observation that 30 mM ethanol enhanced GABA-gated current at δ -GABA_ARs only in the presence of low (3 nM) or moderate (30 nM) concentrations of GABA. When 300 nM GABA was applied, no potentiating effects of ethanol were observed, most likely due to a ceiling effect whereby maximal stimulation was

achieved by the high dose of GABA alone. Two populations of δ -GABA_ARs with unknown stoichiometry have been identified—one with a high-potency GABA-binding site and the other with a lower potency GABA-binding site (28, 29). Therefore, both ethanol effects and OT–ethanol interactions likely occur at the high-potency subpopulation of δ -GABA_ARs that respond to low nM concentrations of GABA. Therefore, the inability of some studies to observe potentiation of GABA-gated current at δ -GABA_ARs by low and moderate concentrations of ethanol (e.g., ref. 30) may be explained by the clear GABA concentration dependency of ethanol effects demonstrated in the present study, highlighting the importance of GABA concentration dependency for future cellular studies of ethanol effects and OT–ethanol interactions at δ -GABA_ARs.

OT exerted its striking antagonism of ethanol effects at δ -GABA_ARs via a mechanism that seems independent of the OTR. *X. laevis* oocytes do not endogenously express OTRs (31), and OTR RNA was not coexpressed into the oocytes because we injected cells with RNA only for the $\alpha 4$, β , and δ GABA_AR subunits. This finding is consistent with recent studies demonstrating that a range of OT functional effects rely on receptors other than the OTR (32–36). OT has, however, been found to alter the expression and function of GABA_ARs, albeit via an OTR-mediated mechanism (37–39). Specifically, GABA is the primary excitatory neurotransmitter in immature neurons, but, shortly before parturition, OT, via an OTR-mediated action, triggers a transient reduction in intracellular chloride concentration that switches GABA actions from excitatory to inhibitory (38, 39).

The effects of OT at both the behavioral and cellular level highlighted in the present study bear a striking similarity to those reported with the benzodiazepine inverse agonist Ro15-4513, which is a competitive antagonist and an antidote to ethanol. This compound reverses the ataxic effects of ethanol (40) and blocks the effects of ethanol at δ -GABA_ARs (3, 8). Initially there were hopes that Ro15-4513 would have a significant impact on the clinical treatment of alcohol-use disorders. However, due to its actions as a benzodiazepine inverse agonist, Ro15-4513 was found to be anxiogenic (41) and to potentiate seizures during and after alcohol withdrawal (42). In contrast, OT has a well-characterized anxiolytic effect (9) and, when dosed at a specific level, powerfully reduces seizures and lethality during ethanol withdrawal in mice (11). In a recent preliminary clinical study, OT also attenuated symptoms of alcohol withdrawal in dependent

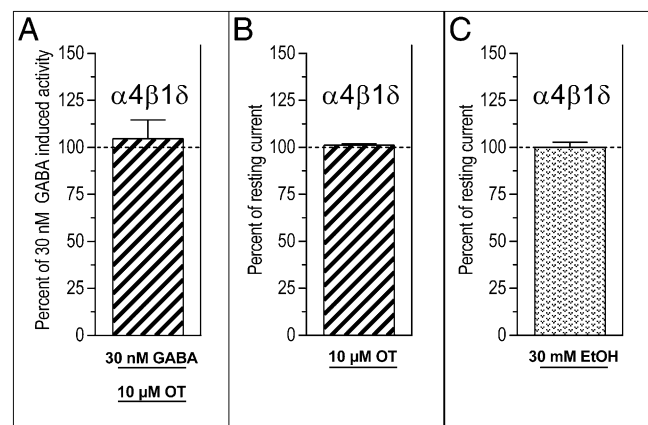


Fig. 5. Oxytocin (OT) does not affect current induced by GABA alone and does not induce current when applied alone. (A) OT (10 µM) had no effect on the current induced by 30 nM GABA alone at $\alpha 4\beta 1\delta$ recombinant receptors ($P > 0.1$). Neither 10 µM OT (B) nor 30 mM ethanol (EtOH) (C) applied alone induced any change in current in these receptors ($P > 0.1$).

humans to the point where a standard clinical intervention for anxiety and seizures (lorazepam) was not required (13).

Because OT lacks the benzodiazepine inverse agonist effects of Ro15-4513 and the molecules have considerable structural differences, OT may have a distinct binding site to both Ro15-4513 and ethanol at $\alpha 4\beta\delta$ GABA_ARs. However, given that Ro15-4513 and OT share considerable similarity in their antagonism of ethanol effects at the level of both behavior and receptor pharmacology, it is also possible that they share the same binding site or an overlapping binding site. Future work could explore these possibilities.

The present study extends early and more recent observations of OT-ethanol interactions. The capacity of centrally administered OT to delay tolerance to the sedative and ataxic effects of ethanol (10, 14) has been known since the late 1980s. The present study suggests that this effect of OT on the development of tolerance may reflect the antagonistic action of OT on acute ethanol-induced sedation and ataxia and its blockade of ethanol potentiation of GABA-gated current at δ -GABA_ARs. It is through overstimulation and subsequent internalization of these receptors that rapid tolerance to ethanol's effects seems to develop (4). It is also possible that the effects described here are relevant to the inhibitory action of OT on ethanol consumption (12, 19). A regionally specific knockdown of δ subunits in the medial shell of the nucleus accumbens reduces ethanol consumption in rats (6), whereas δ knockout mice are known to consume less ethanol than wild types (5). Future studies will hopefully determine whether the anticraving actions of OT across a range of addiction-relevant behaviors (12, 13) reflect binding to δ -GABA_ARs in reward-relevant areas such as the nucleus accumbens. The present study demonstrates that OT has a profound inhibitory effect on the acute behavioral and cellular effects of ethanol.

Methods

All work involving rats was approved by the University of Sydney Animal Ethics Committee and the Committee on Animal Health and Care of the Government of Oberpfalz, Germany. All work involving *X. laevis* was approved by the University of Sydney Animal Ethics Committee. All work was conducted in accordance with the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (43) and the *Guide for the Care and Use of Laboratory Animals* (44).

Behavioral Experiments.

Subjects. Adult male Albino Wistar rats weighing 328–414 g and aged 10–12 wk at the time of testing were used for the OT-ethanol ($N = 28$, $n = 7$) and the OT-THIP ($N = 32$, $n = 8$) behavioral experiments. Subjects were group housed on arrival under standard laboratory conditions (12:12 h light:dark cycle, 22 °C, 60% humidity, food and water available ad libitum). After surgery, rats were transferred to observation cages and remained single-housed for the remainder of the experiment. All behavioral testing took place from 2 to 5 h into the light cycle. Rats were randomly assigned to experimental conditions.

Surgery. For intracerebroventricular (i.c.v.) drug infusion, guide cannulae were stereotaxically implanted 1 mm posterior and 1.6 mm lateral to Bregma and at a depth of 1.8 mm. Rats were anesthetized using isoflurane and injected with an antibiotic (oxytetracycline, 10 mg/kg, i.p.) at the conclusion of surgery. The i.c.v. guide cannula (21 G, 12-mm length) was fixed to the skull using jewelers' screws and dental cement and was closed by a stainless steel stylet (25 g). After surgery, rats were handled daily (stroking, holding, cleaning of stylets) for five days to allow adequate recovery and to minimize nonspecific stress responses during the experiments.

I.c.v. infusions and i.p. injections. For the OT-ethanol and the OT-THIP experiments, rats received an i.c.v. infusion of either synthetic OT (1 μ g/5 μ l; AusPep Ltd.) or vehicle (5 μ l of sterile Ringer's solution) via an infusion cannula that extended 2 mm beyond the guide cannula and that was connected via polyethylene tubing to a Hamilton syringe. The infusion cannula was left in place for 30 s after the infusion. Immediately after the i.c.v. infusion, rats received an i.p. injection of either ethanol (1.5 g/kg; 15% wt/vol) or vehicle (equivalent volume of isotonic saline). We replicated the OT-ethanol experiment (with slight modifications) using a higher dose of

ethanol (3 g/kg; 20% wt/vol) (Fig. S1). For the OT-THIP experiment, rats received either THIP (7 mg/kg, i.p.), or vehicle (an equivalent volume of isotonic saline). The dose of THIP was based on pilot experiments comparing the inhibition of locomotor activity induced by various doses of THIP relative to that induced by 1.5 g/kg ethanol (Fig. S2). The 7 mg/kg THIP dose caused similar sedation to 1.5 g/kg ethanol and is a dose that has selectivity for the δ subunit (15). One rat was excluded from the OT-THIP experiment due to a blocked guide cannula. Rats were euthanized using CO₂ and India ink (5 μ l) was infused i.c.v. before removal of the brain to allow visualization of infusion sites. Brains were sliced coronally on a cryostat to confirm staining of the ventricle.

Wire-hanging test. The wire-hanging test is a standard behavioral assay of motor impairment and is sensitive to ethanol-induced deficits (45–47). It is particularly useful for assessing the effect of chemical substances on muscle strength. At the start of each trial, rats were placed standing on a wire mesh platform. The platform was briefly shaken by the experimenter to cause the rat to grip onto the bars through the platform's being inverted and placed 750 mm above a landing box that was filled with wood shavings to prevent any injury to the rat from the fall. The length of time that the rat was able to hang from the inverted platform was recorded by the experimenter. If a duration of 60 s was reached, then the trial was ended and a time of 60 s recorded. Rats were tested on three consecutive trials at two time points (5 and 35 min after i.p. injection of ethanol or vehicle), with the average time across the trials at each time point used for analysis.

Righting-reflex test. The righting-reflex test is a widely used assay of motor coordination and sedation (48). Rats were placed inside a tub with corncob bedding and were placed on their back by the experimenter. The time taken to right (defined as time taken to place all four paws on the ground after release by the experimenter) was recorded. If subjects were unable to right by 60 s after being placed on their back, a time of 60 s was recorded and the animal was returned to all fours. Righting reflex was assessed immediately after the final trial of the wire-hanging test. The average of three trials at each time point was used for analysis.

With the moderate dose of ethanol (1.5 g/kg, i.p.), a delay in the righting reflex was observed but not a total loss. However, when we administered the higher dose of ethanol (3 g/kg, i.p.) (Fig. S1), a total loss of righting reflex was observed in the vast majority of rats. Rats were defined as having a loss of the righting reflex when they were unable to right within 30 seconds after being placed on their back. The duration of the loss of the righting reflex was also assessed in the higher ethanol dose experiment (Fig. S1) by measuring how long after ethanol administration it took rats to regain the ability to right within 30 s after being turned on their back.

Open-field test. The open-field test was used to examine general locomotor activity and is sensitive to the effects of both stimulants and depressants (49). For 3 d before the test session, rats were placed individually in a rectangular test arena [200 mm (height) \times 400 mm (width) \times 800 mm (length)] made of marine plywood and painted matt black for 20 min to habituate them to the arena and to minimize stress effects. On test day, rats were placed in the arena 10–30 min after i.p. injection of ethanol or vehicle. Test sessions were recorded, and videos were scored by an observer who was blind to experimental conditions for time spent immobile (a measure of sedation) and time spent grooming. Immobility was defined as a rat being completely stationary: i.e., engaging in no body, head, or limb movement. For the OT-THIP experiment, the open-field test was run as described above.

Statistics. Behavioral data were analyzed using a 2 \times 2 ANOVA with planned contrast analysis. The assumption of homogeneity of variance was violated for the wire-hanging tests and 35-min righting-reflex test data (Levene's test, $P < 0.05$). As such, these data were log transformed for analysis (50), which restored homogeneity of variance.

Electrophysiology. Electrophysiological procedures with GABA_ARs were as recently reported (51) and are described briefly below.

Expression of recombinant GABA_A receptors in *X. laevis* oocytes. cDNAs containing the $\alpha 4$, $\beta 1$, $\beta 3$, and δ subunits were subcloned into suitable vectors as previously described (28). Plasmids containing the $\alpha 4$, $\beta 1$, and δ subunits were linearized using the restriction enzyme NotI, and the $\beta 3$ subunits were linearized using NheI. mRNA for injection into the oocytes was transcribed using the T7 mMessage machine kit and poly-A tailed using the poly-A tailing kit (Life Technologies). Gel electrophoresis was used to visualize RNA before and after poly-A tailing, and RNA concentrations were quantified using UV/Vis spectrophotometry with a Nanodrop 2000 (Nanodrop Instruments). Staged V–VI oocytes were microinjected with 0.5–5 ng of mRNA using the following ratios of mRNA: $\alpha 4\beta 1\delta$ (1:1:5); $\alpha 4\beta 3\delta$ (1:1:5); $\alpha 4\beta 1$ (1:1); and $\alpha 4\beta 3$ (1:1). After injection, oocytes were maintained at 18 °C in ND96 wash solution (96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, and 5 mM

hemosodium salt augmented with 2.5 mM sodium pyruvate, 0.5 mM theophylline, 50 μ g/mL gentamycin and tetracycline).

Two-electrode voltage-clamp recordings. Whole-cell currents were measured 3–5 d after injection of cRNA by two-electrode voltage clamp with a Digidata 1200, Geneclamp 500B amplifier together with a Powerlab/200 and Chart version 3.5 (AD Instruments). Oocytes were voltage-clamped at -60 mV. The recording microelectrodes were filled with 3 M KCl and had resistance between 0.2 and 1 M Ω . A minimum of four cells were recorded for each recombinant receptor type. Substances were bath applied until a plateau was reached and the peak currents were measured. A 7-min wash with ND96

between successive applications was performed to prevent recordings being compromised by the effects of desensitization.

Statistics. One-sample *t* tests were used to assess the significance of percent change in peak current from baseline (test value = 100). Paired-sample *t* tests were used to compare percent change in current between different combinations of substances (e.g., GABA + ethanol vs. GABA + ethanol + OT).

ACKNOWLEDGMENTS. This research was supported by National Health and Medical Research Council grants (to M.C. and I.S.M.) and fellowships (M.T.B and I.S.M.), a Deutscher Akademischer Austausch Dienst/Group of Eight grant (to I.S.M. and I.D.N.), and Deutsche Forschungsgemeinschaft grants (to I.D.N.).

- Brickley SG, Mody I (2012) Extrasynaptic GABA_A receptors: Their function in the CNS and implications for disease. *Neuron* 73(1):23–34.
- Hanchar HJ, Dodson PD, Olsen RW, Otis TS, Wallner M (2005) Alcohol-induced motor impairment caused by increased extrasynaptic GABA_A receptor activity. *Nat Neurosci* 8(3):339–345.
- Wallner M, Hanchar HJ, Olsen RW (2006) Low-dose alcohol actions on $\alpha 4\beta 3\delta$ GABA_A receptors are reversed by the behavioral alcohol antagonist Ro15-4513. *Proc Natl Acad Sci USA* 103(22):8540–8545.
- Liang J, et al. (2007) Mechanisms of reversible GABA_A receptor plasticity after ethanol intoxication. *J Neurosci* 27(45):12367–12377.
- Mihalek RM, et al. (2001) GABA(A)-receptor δ subunit knockout mice have multiple defects in behavioral responses to ethanol. *Alcohol Clin Exp Res* 25(12):1708–1718.
- Nie H, Reval M, Gill TM, Ron D, Janak PH (2011) Extrasynaptic δ -containing GABA_A receptors in the nucleus accumbens dorsomedial shell contribute to alcohol intake. *Proc Natl Acad Sci USA* 108(11):4459–4464.
- Wallner M, Hanchar HJ, Olsen RW (2003) Ethanol enhances $\alpha 4\beta 3\delta$ and $\alpha 6\beta 3\delta$ γ -aminobutyric acid type A receptors at low concentrations known to affect humans. *Proc Natl Acad Sci USA* 100(25):15218–15223.
- Hanchar HJ, et al. (2006) Ethanol potently and competitively inhibits binding of the alcohol antagonist Ro15-4513 to $\alpha 4\beta 3\delta$ GABA_A receptors. *Proc Natl Acad Sci USA* 103(22):8546–8551.
- Neumann ID, Landgraf R (2012) Balance of brain oxytocin and vasopressin: Implications for anxiety, depression, and social behaviors. *Trends Neurosci* 35(11):649–659.
- Pucilowski O, Kostowski W, Trzaskowska E (1985) The effect of oxytocin and fragment (MIF-) on the development of tolerance to hypothermic and hypnotic action of ethanol in the rat. *Peptides* 6(1):7–10.
- Szabó G, Kovács GL, Telegdy G (1987) Effects of neurohypophysial peptide hormones on alcohol dependence and withdrawal. *Alcohol Alcohol* 22(1):71–74.
- Bowen MT, Carson DS, Spiro A, Arnold JC, McGregor IS (2011) Adolescent oxytocin exposure causes persistent reductions in anxiety and alcohol consumption and enhances sociability in rats. *PLoS ONE* 6(11):e27237.
- Pedersen CA, et al. (2013) Intranasal oxytocin blocks alcohol withdrawal in human subjects. *Alcohol Clin Exp Res* 37(3):484–489.
- Kovács GL, Sarnyai Z, Szabó G (1998) Oxytocin and addiction: A review. *Psychoneuroendocrinology* 23(8):945–962.
- Meera P, Wallner M, Otis TS (2011) Molecular basis for the high THIP/gaboxadol sensitivity of extrasynaptic GABA(A) receptors. *J Neurophysiol* 106(4):2057–2064.
- Walker BM, Ehlers CL (2009) Age-related differences in the blood alcohol levels of Wistar rats. *Pharmacol Biochem Behav* 91(4):560–565.
- Davson H (1969) The cerebrospinal fluid. *Handbook of Neurochemistry*, ed Lajtha A (Springer, New York), pp 23–48.
- Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M (2011) Oxytocin and vasopressin in the human brain: Social neuropeptides for translational medicine. *Nat Rev Neurosci* 12(9):524–538.
- McGregor IS, Bowen MT (2012) Breaking the loop: Oxytocin as a potential treatment for drug addiction. *Horm Behav* 61(3):331–339.
- Zanos P, et al. (2014) The oxytocin analogue carbetocin prevents emotional impairment and stress-induced reinstatement of opioid-seeking in morphine-abstinent mice. *Neuropsychopharmacology* 39(4):855–865.
- Buisman-Pijlman FT, et al. (2014) Individual differences underlying susceptibility to addiction: Role for the endogenous oxytocin system. *Pharmacol Biochem Behav* 119:22–38.
- Sarnyai Z (2011) Oxytocin as a potential mediator and modulator of drug addiction. *Addict Biol* 16(2):199–201.
- McGregor IS, Bowen MT (2013) Oxytocin and addiction: Recent preclinical advances and future clinical potential. *Oxytocin, Vasopressin and Related Peptides in the Regulation of Behavior*, eds Choleris E, Pfaff DW, Kavaliers M (Cambridge Univ Press, Cambridge, UK), pp 270–287.
- Olsen RW, Hanchar HJ, Meera P, Wallner M (2007) GABA_A receptor subtypes: The “one glass of wine” receptors. *Alcohol* 41(3):201–209.
- Wisden W, Laurie DJ, Monyer H, Seeburg PH (1992) The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* 12(3):1040–1062.
- Laurie DJ, Seeburg PH, Wisden W (1992) The distribution of 13 GABA_A receptor subunit mRNAs in the rat brain. II. Olfactory bulb and cerebellum. *J Neurosci* 12(3):1063–1076.
- Sundstrom-Poromaa I, et al. (2002) Hormonally regulated $\alpha(4)\beta(2)\delta$ GABA(A) receptors are a target for alcohol. *Nat Neurosci* 5(8):721–722.
- Karim N, et al. (2012) Low nanomolar GABA effects at extrasynaptic $\alpha 4\beta 1\beta 3\delta$ GABA(A) receptor subtypes indicate a different binding mode for GABA at these receptors. *Biochem Pharmacol* 84(4):549–557.
- Eaton MM, et al. (2014) γ -aminobutyric acid type A $\alpha 4$, $\beta 2$, and δ subunits assemble to produce more than one functionally distinct receptor type. *Mol Pharmacol* 86(6):647–656.
- Borghese CM, et al. (2006) The δ subunit of γ -aminobutyric acid type A receptors does not confer sensitivity to low concentrations of ethanol. *J Pharmacol Exp Ther* 316(3):1360–1368.
- Kimura T, Tanizawa O, Mori K, Brownstein MJ, Okayama H (1992) Structure and expression of a human oxytocin receptor. *Nature* 356(6369):526–529.
- Ramos L, et al. (2013) Acute prosocial effects of oxytocin and vasopressin when given alone or in combination with 3,4-methylenedioxyamphetamine in rats: Involvement of the V1A receptor. *Neuropsychopharmacology* 38(11):2249–2259.
- Sala M, et al. (2011) Pharmacologic rescue of impaired cognitive flexibility, social deficits, increased aggression, and seizure susceptibility in oxytocin receptor null mice: A neurobehavioral model of autism. *Biol Psychiatry* 69(9):875–882.
- Bowen MT, McGregor IS (2014) Oxytocin and vasopressin modulate the social response to threat: A preclinical study. *Int J Neuropsychopharmacol* 17(10):1621–1633.
- Song Z, et al. (2014) Oxytocin induces social communication by activating arginine-vasopressin V1a receptors and not oxytocin receptors. *Psychoneuroendocrinology* 50:14–19.
- Suraev AS, et al. (2014) Adolescent exposure to oxytocin, but not the selective oxytocin receptor agonist TGOT, increases social behavior and plasma oxytocin in adulthood. *Horm Behav* 65(5):488–496.
- Koksma JJ, et al. (2003) Oxytocin regulates neurosteroid modulation of GABA(A) receptors in supraoptic nucleus around parturition. *J Neurosci* 23(3):788–797.
- Tyzio R, et al. (2006) Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *Science* 314(5806):1788–1792.
- Tyzio R, et al. (2014) Oxytocin-mediated GABA inhibition during delivery attenuates autism pathogenesis in rodent offspring. *Science* 343(6171):675–679.
- Suzdak PD, et al. (1986) A selective imidazobenzodiazepine antagonist of ethanol in the rat. *Science* 234(4781):1243–1247.
- Harris CM, Benjamin D, Lal H (1987) Anxiety-like subjective effect of ethanol antagonist RO 15-4513 demonstrated in pentylenetetrazol discrimination. *Neuropharmacology* 26(10):1545–1547.
- Mhatre MC, Gonzalez LP (1999) Increased Ro15-4513-induced seizures following multiple ethanol withdrawals. *Pharmacol Biochem Behav* 63(1):93–99.
- NHMRC (2004) *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* (National Health and Medical Research Council, Canberra, ACT, Australia), 7th Ed.
- National Research Council (2011) *Guide for the Care and Use of Laboratory Animals* (National Academies Press, Washington, DC), 8th Ed.
- Shukitt-Hale B, Mouzakis G, Joseph JA (1998) Psychomotor and spatial memory performance in aging male Fischer 344 rats. *Exp Gerontol* 33(6):615–624.
- Boyce-Rustay JM, Holmes A (2006) Ethanol-related behaviors in mice lacking the NMDA receptor NR2A subunit. *Psychopharmacology (Berl)* 187(4):455–466.
- Crabbe JC, et al. (2003) Strain differences in three measures of ethanol intoxication in mice: the screen, dowel and grip strength tests. *Genes Brain Behav* 2(4):201–213.
- Little PJ, Kuhn CM, Wilson WA, Swartzwelder HS (1996) Differential effects of ethanol in adolescent and adult rats. *Alcohol Clin Exp Res* 20(8):1346–1351.
- Correa M, Arizzi MN, Betz A, Mingote J, Salamone JD (2003) Open field locomotor effects in rats after intraventricular injections of ethanol and the ethanol metabolites acetaldehyde and acetate. *Brain Res Bull* 62(3):197–202.
- Keene ON (1995) The log transformation is special. *Stat Med* 14(8):811–819.
- Abshalom N, et al. (2012) $\alpha 4\beta 3\delta$ GABA(A) receptors are high-affinity targets for γ -hydroxybutyric acid (GHB). *Proc Natl Acad Sci USA* 109(33):13404–13409.