Addiction Biology

PRECLINICAL STUDY

doi:10.1111/j.1369-1600.2011.00360.x

Receptor subtype-dependent galanin actions on gamma-aminobutyric acidergic neurotransmission and ethanol responses in the central amygdala

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ABSTRACT

The neuropeptide galanin and its three receptor subtypes (GalR1-3) are expressed in the central amygdala (CeA), a brain region involved in stress- and anxiety-related behaviors, as well as alcohol dependence. Galanin also has been suggested to play a role in alcohol intake and alcohol dependence. We examined the effects of galanin in CeA slices from wild-type and knockout (KO) mice deficient of GalR2 and both GalR1 and GalR2 receptors. Galanin had dual effects on gamma-aminobutyric acid (GABA)-ergic transmission, decreasing the amplitudes of pharmacologically isolated GABAergic inhibitory postsynaptic potentials (IPSPs) in over half of CeA neurons but augmenting IPSPs in the others. The increase in IPSP size was absent after superfusion of the GalR3 antagonist SNAP 37889, whereas the IPSP depression was absent in CeA neurons of GalR1 × GalR2 double KO and GalR2 KO mice. Paired-pulse facilitation studies showed weak or infrequent effects of galanin on GABA release. Thus, galanin may act postsynaptically through GalR3 to augment GABAergic transmission in some CeA neurons, whereas GalR2 receptors likely are involved in the depression of IPSPs. Co-superfusion of ethanol, which augments IPSPs presynaptically, together with galanin caused summated effects of ethanol and galanin in those CeA neurons showing galanin-augmented IPSPs, suggesting the two agents act via different mechanisms in this population. However, in neurons showing IPSP-diminishing galanin effects, galanin blunted the ethanol effects, suggesting a preemptive effect of galanin. These findings may increase understanding of the complex cellular mechanisms that underlie the anxiety-related behavioral effects of galanin and ethanol in CeA.

Keywords Alcohol, antidepressant, anxiety, electrophysiology, inhibitory postsynaptic potential, synapse.

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INTRODUCTION

Galanin is a C-terminally amidated peptide of 29 amino acids in rodents that binds with high affinity to all three known galanin receptors (GalR1–3) belonging to the category of G-protein coupled receptors (GPCRs). Galanin-like immunoreactivity is widely distributed in the central nervous system, including the dorsal raphe nucleus (DRN), the locus coeruleus (LC), the central amygdala (CeA), several hypothalamic nuclei and the cholinergic cell bodies of nucleus basalis of Meynert (Melander *et al.* 1986). Galanin coexists with norepinephrine in the CeA and several other brain regions (Melander *et al.* 1986), and galanin binding sites or expression corresponding to GalR1, GalR2 and GalR3 are observed in the CeA (Melander *et al.* 1988). The CeA is implicated in several behaviors such as those related to fear, stress, anxiety and alcohol dependence (see e.g. Davis, Rainnie & Cassell 1994; Pich *et al.* 1995; Roberto *et al.* 2010b). This nucleus contains a high proportion of gamma-aminobutyric acid (GABA)-containing neurons (Cassell, Gray & Kiss 1986; Cassell, Freedman & Shi 1999) and is known to be involved in substance abuse and dependence (see e.g. Roberto *et al.* 2010b).

However, to our knowledge, there are no reports of electrophysiological effects of galanin on CeA neurons, despite behavioral studies suggesting that the galanin system in this nucleus could be important for the

treatment of anxiety (Moller *et al.* 1999; Khoshbouei *et al.* 2002). Effects of galanin have been studied electrophysiologically in several other brain regions. For example, galanin inhibits muscarinic excitatory postsynaptic potentials in the hippocampus via presynaptic galanin receptors inhibiting acetylcholine release (Fisone *et al.* 1987; Dutar, Lamour & Nicoll 1989). Galanin also excites dorsal root ganglia neurons (Kerekes *et al.* 2003), but hyperpolarizes noradrenergic neurons in LC (Pieribone *et al.* 1995; Ma *et al.* 2001). Galanin 2-11 (Gal 2-11), a GalR2-selective agonist, had no effect on LC neurons (Pieribone *et al.* 1995; Xu, Tong & Hokfelt 2001). In a recent study from our laboratory, in most DRN neurons, both galanin 2-11 and galanin 1-29 markedly decreased the amplitude of evoked GABAergic inhibitory postsynaptic potentials (IPSPs) (Sharkey *et al.* 2008). Paired-pulse facilitation (PPF) studies suggested that the galanin 1-29 effect was elicited presynaptically by reducing GABA release.

In addition to its suggested role in anxiety and depression (Swanson *et al.* 2005; Ogren *et al.* 2006; Lu, Sharkey & Bartfai 2007), galanin and its receptors also have been reported to play some role in food and ethanol consumption (Leibowitz *et al.* 2003; Lewis *et al.* 2004; Chang *et al.* 2007; Karatayev, Baylan & Leibowitz 2009; Karatayev *et al.* 2010) as well as opiate reinforcement (Picciotto *et al.* 2005). Thus, in rodents, intraventricular injection of galanin or galanin overexpression increases alcohol intake, whereas a galanin knockout (KO) decreases ethanol intake (Lewis *et al.* 2004; Chang *et al.* 2007; Karatayev *et al.* 2009, 2010); furthermore, ethanol intake increases galanin mRNA in the hypothalamus whereas ethanol withdrawal decreases it (Leibowitz *et al.* 2003).

Therefore, the present studies were designed to clarify the role of galanin and its receptors in the CeA and increase understanding of the role of CeA in stress, anxiety and ethanol effects. Our results suggest that galanin alters the electrophysiological properties of CeA neurons in a dual manner that may explain the reported mixed effects of intra-CeA galanin microinjection in anxiety tests in rodents (Moller *et al.* 1999; Khoshbouei *et al.* 2002). We find no postsynaptic effect of galanin on averaged voltage–current (V/I) curves, but it significantly alters the amplitude of evoked IPSPs in a dual manner probably involving multiple GalR subtypes. Our PPF studies suggest that these galanin effects may be explained by a postsynaptic action on GABA receptor function in some CeA neurons, but a small presynaptic effect in others. In addition, because we previously showed that superfusion of ethanol and the anxiety/stress-related peptide corticotrophin-releasing factor (CRF) both elicit the somewhat paradoxical effect of augmenting GABAergic IPSPs in CeA neurons (Roberto *et al.* 2003, 2010b; Nie *et al.* 2004), we also examined the effects of co-applied ethanol on the responses to galanin in CeA neurons and found complex interactions depending upon CeA neuron responses to galanin.

METHODS

Slice preparation

We prepared brain slices containing CeA from male C57Bl/6J mice (25–30 g) that were anesthetized with isoflurane (3%) and decapitated, as previously described (Roberto *et al.* 2003, 2010b; Nie *et al.* 2004). GalR1 and GalR2 double KO mice were obtained by crossing the GalR1 KO (Mitsukawa, Lu & Bartfai 2009) with GalR2 KO (Lu *et al.* 2008) mice. With the investigator blind to the mouse strain/genetics, the brains were rapidly removed and placed into ice-cold artificial cerebrospinal fluid (ACSF) gassed with 95% O_2 and 5% CO_2. We cut transverse slices 400 μm thick on a LeicaVT 1000S vibrating slicer (Leica Microsystems Inc., Buffalo Grove, IL, USA), incubated them in an interface configuration for about 30 minutes and then completely submerged and continuously superfused (flow rate of 2–4 ml/minute) them with warm (31°C), O_2/CO_2-gassed ACSF of the following composition in mM: NaCl, 130; KCl, 3.5; NaH_2PO_4, 1.25; $MgSO_4 \cdot 7H_2O$, 1.5; $CaCl_2$, 2.0; $NaHCO_3$, 24; glucose, 10. The inner chamber had a total volume of 0.8 ml; at the 2–4 ml/minute superfusion rates used, 90% replacement of the chamber solution could be obtained within 1 minute. We added drugs to the ACSF from stock solutions to obtain known concentrations in the superfusate.

Electrophysiology

We recorded from CeA neurons with sharp micropipettes containing 3 M KCl (65–80 mΩ resistance) using current-clamp mode. Data were acquired with an Axoclamp-2A preamplifier [Axon Instruments (now Molecular Devices, Sunnyvale, CA, USA)] and stored for offline analysis via pClamp software (Molecular Devices). We evoked pharmacologically isolated $GABA_A$ergic IPSPs by stimulating locally within the medial subdivision of the CeA with a bipolar stimulating electrode, while continuously superfusing the glutamate receptor blockers 6,7-dinitroquinoxaline-2,3-dione (DNQX; 20 μM) and DL-2-amino-5-phosphonopentanoic acid (DL-AP5, 30 μM), and the $GABA_B$ antagonist CGP 55845A (1 μM).

We held the CeA neurons near their resting membrane potentials (RMPs), which ranged between −64 and −84 mV (mean: −78.6 ± 0.7 mV, $n = 73$), and applied hyperpolarizing and depolarizing current steps (200 pA increments, 750 ms duration) to generate V/I curves. To

determine half-maximal IPSP amplitudes, we examined input/output (I/O) curves by measuring evoked IPSP amplitudes at five stimulus strengths, threshold to maximum stimulation. Subsequent analyses were done with averages of two IPSPs evoked with half-maximal stimuli. We measured the IPSP amplitudes before (control), during and after (washout) drug application, and we determined the percent change in IPSP amplitude at each stimulus intensity by the equation $(V_{drug}/V_{control}) * 100$. The criteria for accepting a galanin or ethanol effect on IPSP amplitudes were a change of $\geq 10\%$ of control at the half-maximal stimulus intensity over the 8- to 15-minute superfusion time points and following galanin/ethanol washout (10–25 minutes).

We examined PPF in multiple neurons using 100 ms interstimulus intervals and stimulus strength adjusted so that the amplitude of the first IPSP was 50% of maximal amplitude determined from the I/O relationship. We calculated PPF as the ratio of the second IPSP amplitude over that of the first IPSP, multiplied by 100. For PPF experiments, we took measurements before galanin or ethanol superfusion (control), during (8–15 minutes) and after galanin/ethanol washout (10–25 minutes).

We express all values as mean ± standard error of the mean. Statistical analysis was performed with GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). We analyzed the data using a one-way analysis of variance followed by a Dunnett's and Bonferroni *post hoc* test; $P < 0.05$ was considered statistically significant. In some cases, we also used Student's paired *t*-test; again, $P < 0.05$ was considered statistically significant.

Drugs

CGP 55845A was a gift from Novartis Pharma, Basel Switzerland. We purchased DL-AP5 and DNQX from Tocris Tocris-Bioscience (Ellisville, MO, USA) and bicuculline from Sigma-Aldrich (St Louis, MO, USA). Galanin (Gal 1-29; GWTLNSAGYLLGPHAIDNHRSFSDKHGLT amide) was synthesized by Biopeptide (San Diego, CA, USA) and the GalR3 antagonist SNAP 37889 was synthesized by Edward Roberts at The Scripps Research Institute. We obtained ethanol from Remet (La Mirada, CA, USA).

RESULTS

CeA neuron sample

We recorded from a total of 85 mouse CeA neurons and, for some of the ethanol-galanin studies, four rat CeA neurons. In our CeA slices, all cells were quiescent. The mean RMP was −78.6 ± 0.7 mV. Most neurons in the CeA appear to contain GABA, and may be either interneurons or projection neurons (Cassell *et al.* 1986, 1999). Therefore, the effects of galanin were initially assessed on the membrane properties and GABAergic IPSPs in these cells by superfusing a maximal 1 μM concentration of galanin (see Methods).

Little galanin effect on postsynaptic membrane properties in CeA neurons

We fully analyzed 23 mouse CeA neurons for the effects of superfused galanin (1 μM) on voltage/current measurements. Interestingly, in all cells so tested from the medial subdivision of the mouse CeA, and as shown by the virtual superposition of averaged V/I curves, we found no significant effect of 1 μM galanin on these V/I curves (Fig. 1), whether they were recorded from wild-type (WT) littermate controls or GalR2 KO mice (Fig. 1a), or from GalR1 × GalR2 KO mice or their WT littermate controls after block of GalR3 with SNAP 37889 (SNAP; Fig. 1b; see following discussion). SNAP was used to rule out the possibility that actions on GalR3 receptors might counteract actions of GalR1 or 2 on membrane conductance. As a first approximation, these findings suggest that galanin has little or no effect on membrane conductance in CeA neurons over a wide range of membrane potentials.

Galanin decreases $GABA_A$-IPSP amplitudes in a subpopulation of catecholamine neurons

We assessed the effects of galanin on pharmacologically isolated $GABA_A$-IPSPs evoked from CeA neurons by a stimulating electrode placed in the medial subdivision of the CeA. In our initial studies on CeA neurons from normal WT C57Bl/6 and WT littermate mice (of GalR2 and GalR1 × GalR2) using a relatively long superfusion time (8–15 minutes), a maximal concentration of galanin (1 μM) significantly ($P < 0.001$) decreased the mean-evoked IPSP amplitudes, in 11 of the 23 mouse CeA neurons studied (Fig. 2a, c), to a mean of 72% of control. In another subpopulation of five WT CeA neurons pretreated with SNAP to block GalR3 receptors, galanin still significantly ($P = 0.008$) decreased IPSP size (Fig. 3).

Galanin increases evoked IPSP amplitudes in another CeA subpopulation

In many WT CeA neurons galanin (1 μM) superfusion elicited an increase in IPSP amplitudes that was somewhat smaller but developed earlier (5–9 minutes) than the IPSP decrease seen in the neuronal population described above. The pooled data from 12 of 23 CeA cells from WT and littermate mice revealed that the mean evoked IPSP amplitude at half-maximal stimulus intensity was significantly increased (to 114% of control, $n = 12$; $P < 0.05$) by galanin superfused for 5–9 minutes (Fig. 2b & d). In both populations of neurons, subsequent

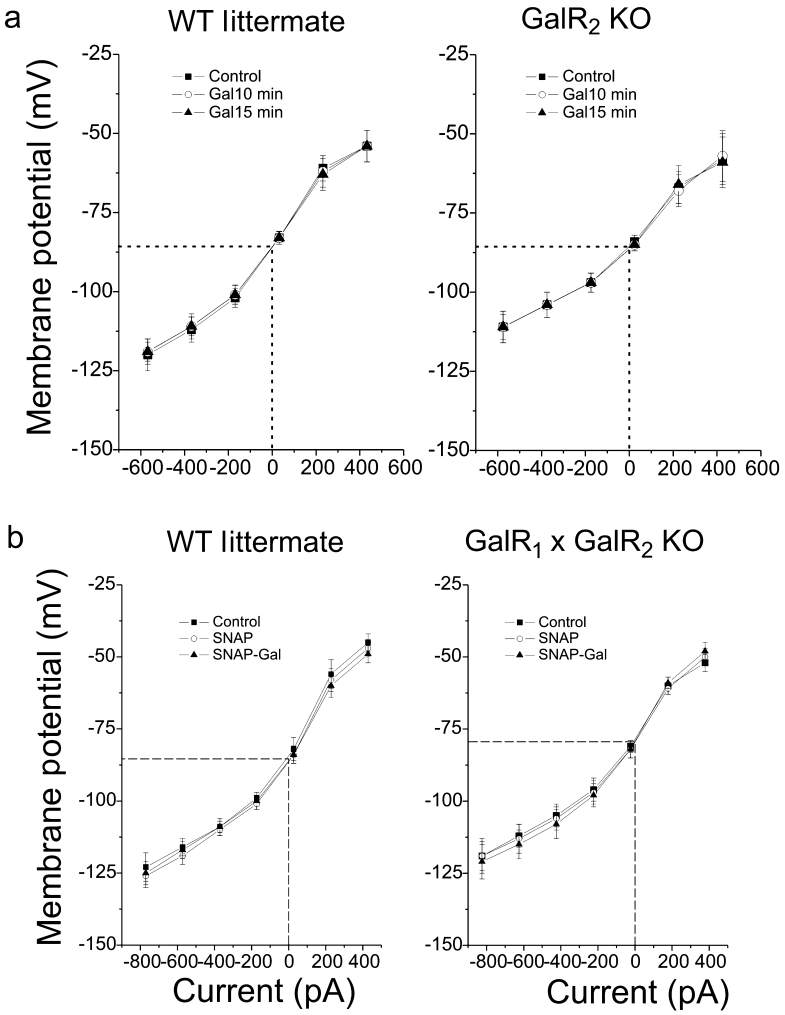

Figure 1 Galanin has little effect on voltage/current (V/I) relationships in central amygdala (CeA) neurons. (a) CeA neurons from the GalR2 knockout (KO) and their wild-type (WT) littermate controls. Galanin 1 μM had no effect on the V/I relationships in CeA neurons from either control ($n = 5$) or GalR2 KO ($n = 6$) mice. (b) CeA neurons from GalR1 × GalR2 double KO mice and their littermate controls. Galanin 1 μM in the presence of the GalR3 antagonist SNAP 37889 (SNAP, 200 nM) had no effect on the V/I relationships in CeA neurons from either control ($n = 6$) or GalR1 × GalR2 double KO ($n = 6$) mice. These data show that galanin has no effect on resting potential (dashed lines) in CeA, and as well they suggest little effect on voltage-dependent conductance. Each cell was recorded with sharp electrodes (filled with 3 M KCl) in current-clamp mode to obtain sweeps of electrotonic responses (V) to incrementally increasing current-step injections (I). Dashed lines indicate resting membrane potential (no holding current)

superfusion of 30 μM bicuculline for 5 minutes usually completely abolished or greatly reduced the evoked IPSPs (Fig. 2b), demonstrating that they were dependent on $GABA_A$ receptor activation.

Galanin has only weak, variable effects on PPF of evoked IPSPs

To initially assess the site of action for galanin in decreasing $GABA_A$-IPSP amplitudes, we examined its effect on PPF of IPSPs in CeA neurons. PPF, as determined from the paired-pulse ratio, has been shown to vary inversely with the presynaptic release of transmitter (Mennerick & Zorumski 1995; Salin, Malenka & Nicoll 1996; Bonci & Williams 1997). In brief, our paired-pulse studies showed highly variable or no effects of galanin on PPF of IPSPs in most CeA neurons of WT, GalR1 × GalR2 KO or their littermate control mice; when averaged according to the galanin effect on IPSP increases or decreases, there was no significant effect (data not shown). PPF was significantly changed (increased) by galanin only in CeA neurons of GalR2 KO mice, and only at the early superfusion time point (data not shown), despite the apparent lack of effect of galanin on IPSP amplitudes in this set of CeA neurons. Because our V/I studies showed no effect of galanin on membrane properties, these findings suggest that the changes in IPSP amplitudes might arise in most CeA neurons from modulation of postsynaptic $GABA_A$ receptors.

A GalR3 antagonist and GalR KOs differentially block the dual galanin effects

To assess the possible role of GalR subtype-dependent effects in the dual actions of galanin in CeA, we used mice with KO constructs of either GalR2 receptors alone or mice with KOs of both GalR1 and Gal2 receptors (GalR1 × GalR2 KOs). As mice with KOs of GalR3 receptors are not yet available, we also used one of the recently developed antagonists of these receptors (SNAP 37889; Ogren *et al.* 1992; Swanson *et al.* 2005) to block the GalR3 receptors pharmacologically.

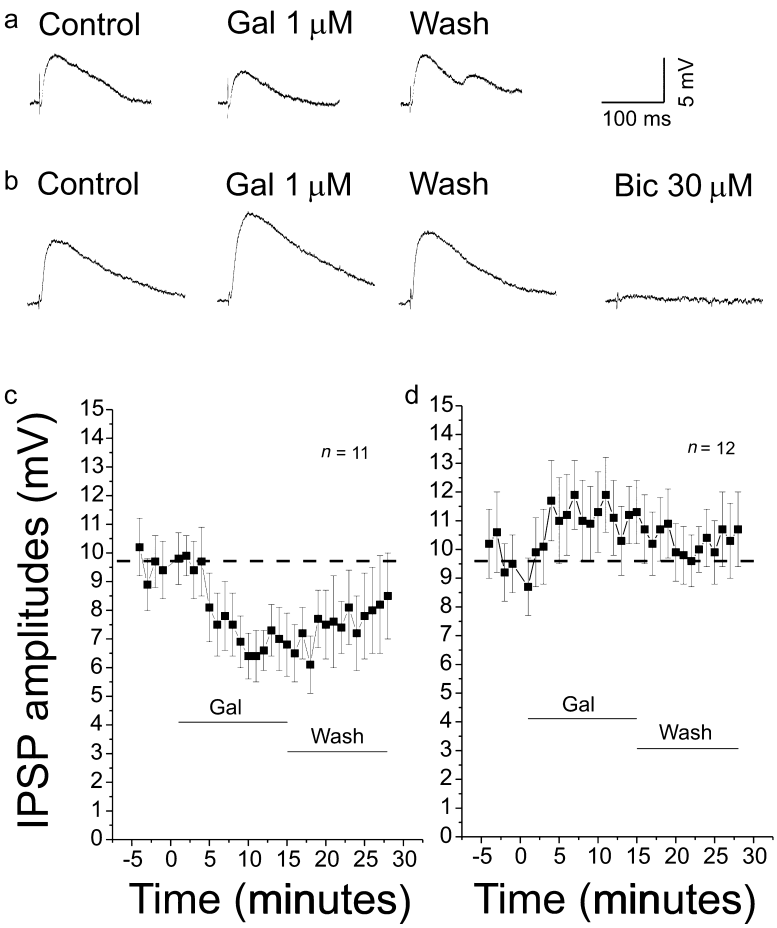

Figure 2 Brief galanin applications both decrease (a) and increase (b) the amplitudes of evoked gamma-aminobutyric acid $(GABA)_A$ inhibitory postsynaptic potentials (IPSPs) in different central amygdala (CeA) neuron populations. (a, b) Representative voltage traces (using half-maximal stimulus intensities) of evoked $GABA_A$-IPSPs from CeA neurons before (Control), during (Gal) and after (Wash) superfusion of 1 μM galanin for 5–8 minutes. (a) Galanin superfusion depresses IPSP amplitudes in this CeA neuron with recovery on washout of galanin. (b) In another CeA neuron, galanin superfusion augments IPSP amplitudes with recovery on washout. Note that the IPSPs in (b) are abolished (rightmost panel) by subsequent superfusion of bicuculline (Bic), verifying the mediation of IPSPs by $GABA_A$ receptor activation. (c, d) Averaged evoked IPSP data from the two populations of CeA neurons showing opposite responses to galanin. (c) Galanin superfusion decreases the mean IPSP amplitudes in one population of CeA neurons ($n = 11$) with partial recovery to baseline levels after galanin washout. (d) Galanin increases IPSP amplitudes in another population of CeA neurons ($n = 12$) with recovery on washout. Dashed lines represent mean baseline IPSP amplitudes prior to galanin superfusion

Thus, in an initial set of five CeA neurons from normal WT mice, we assessed the effect of SNAP 37889 on the two types of responses of evoked IPSP amplitudes to galanin. As shown in Fig. 3, after superfusion of 200 nM SNAP 37889, subsequent co-perfusion of 1 μM galanin had only depressant effects on IPSP amplitudes, suggesting that the IPSP augmentations and not the IPSP depressions involve GalR3 activation.

In initial studies using GalR KO mice, of five CeA neurons from the double GalR1 × GalR2 KO mice, four cells showed a clear galanin-evoked increase of IPSPs, suggesting involvement of GalR3, whereas one cell showed little change. No cells showed galanin-induced decreases of IPSP amplitudes. The mean IPSP increase for all five cells during galanin superfusion was $114 \pm 5\%$ of control, a significant effect ($P < 0.05$), again suggesting that the IPSP-augmenting galanin effect in WT mice may involve GalR3 receptors in most cells.

In another experiment, superfusing SNAP 37889 together with galanin onto CeA neurons of GalR1 × GalR2 KO and WT littermate controls ($n = 7$ and 8, respectively), SNAP prevented the IPSP increase but not the IPSP decrease in the WT littermate CeA neurons (Fig. 4). However, there was no significant galanin-induced effect on the IPSPs in the GalR1 × GalR2 KO neurons superfused with SNAP 37889. We conclude that the IPSP depression is absent in the CeA of double KO mice, but not in that of the WT littermates, even in the presence of the SNAP

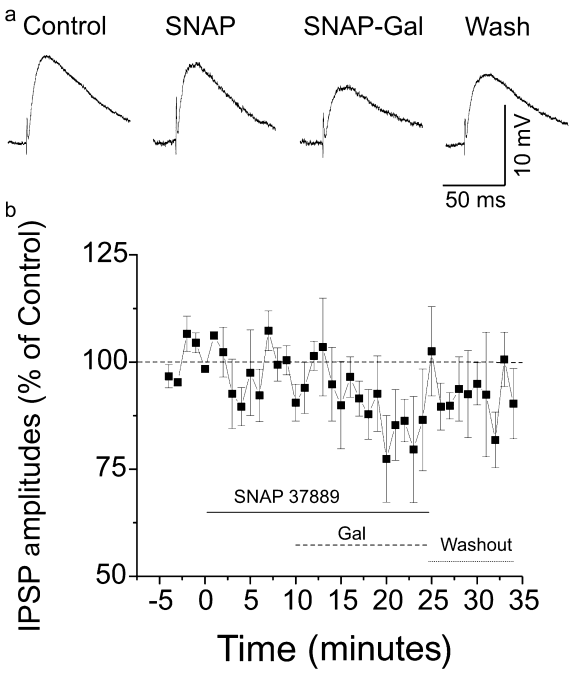

Figure 3 A GalR3 antagonist prevents the galanin increase of inhibitory postsynaptic potential (IPSP) amplitudes in WT central amygdala (CeA) neurons, but not the galanin-induced decrease. (a) Representative voltage traces from a CeA neuron taken from a normal WT mouse. In the presence of 200 nM SNAP 37889 (SNAP), superfusion of 1 μM galanin (14 minutes) still depresses the IPSP amplitude with recovery on washout of galanin (Wash; 14 minutes). (b) Time course of mean IPSP amplitudes from a pooled sample of five CeA neurons taken from normal WT mice. Application of SNAP alone had little direct effect on mean IPSP amplitudes but prevented 1 μM galanin-induced mean IPSP amplitude increase. However, in the presence of SNAP, galanin still significantly ($P = 0.008$; $n = 5$) decreased mean IPSP amplitudes. Upper dashed line indicates baseline pre-treatment IPSP amplitudes. Values are mean + standard error of the mean

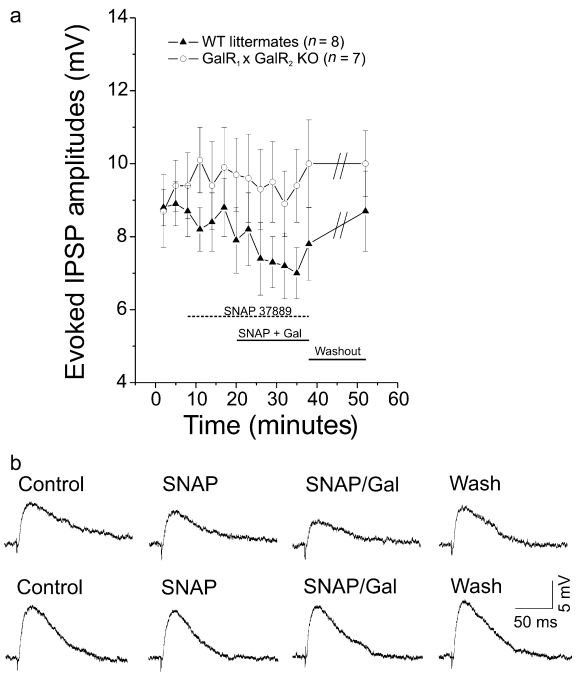

Figure 4 In the presence of a GalR3 antagonist, galanin diminishes central amygdala (CeA) inhibitory postsynaptic potentials (IPSPs) in wild-type (WT) but not in GalR1 × GalR2 knockout (KO) mice. (a) Time course of pooled intracellular IPSP data from CeA neurons of GalR1 × GalR2 KO and control WT littermate mice. In CeA of WT littermates, superfusion of the GalR3 antagonist SNAP 37889 (SNAP, 200 nM) alone slightly decreases mean IPSP amplitudes. Addition of 1 μM galanin (Gal) together with SNAP quickly and significantly ($P < 0.05$) reduced IPSP amplitudes with recovery on washout. By contrast, in CeA of GalR1 × R2 KO mice superfusion of 1 μM galanin with SNAP had no significant effect ($P = 0.66$) on evoked IPSP amplitudes. Error bars = ± standard error of the mean. (b) Top row: representative voltage traces from a CeA neuron of a WT mouse; superfusion of 200 nM SNAP for 15 minutes slightly decreases the evoked IPSPs, and addition of 1 μM galanin for 10–11 minutes further reduces IPSP amplitude with recovery on washout (Wash; 16–17 minutes). KCl electrode; resting membrane potential (RMP) = –79 mV. (b) Bottom row: representative-evoked IPSPs from a CeA neuron of a GalR1 × R2 KO mouse; superfusion of 200 nM SNAP for 13–14 minutes had little effect on IPSP amplitudes but the double GalR KO apparently prevented the IPSP decrease usually elicited by 12–13 minutes of 1 μM galanin. KCl electrode; RMP = –84 mV

antagonist, suggesting that this effect is the result of GalR1 and/or GalR2 receptor activation. The lack of an increase in IPSP amplitudes in the presence of the SNAP antagonist again suggests it is the result of activation of GalR3.

To tease out whether GalR1 or GalR2 is involved in the IPSP depression, we tested CeA from mice with deletion only of GalR2 receptors. Thus, in CeA neurons from GalR2 KO mice, although we found some variability from cell to cell, there was no significant decrease of evoked IPSP amplitudes ($n = 8$; $P = 0.15$), even after 15 minutes of 1 μM galanin superfusion. However, 1 μM galanin robustly and significantly ($n = 6$; $P < 0.05$) decreased mean IPSP amplitudes in CeA neurons from the WT littermate mice (Fig. 5).

Thus, by exclusion, our data showing that the GalR3 antagonist greatly reduces the early IPSP increase often seen in response to galanin, and that the more robust IPSP decrease is greatly diminished in CeA neurons from both GalR2 KO and the GalR1 × GalR2 KO mice, suggest that the IPSP decrease arises from activation of GalR2 receptors.

Ethanol interacts with galanin effects

Because of the suggested role of brain galanin in ethanol intake (see Introduction), we also examined possible interactions of ethanol and galanin in mouse and rat CeA neurons. Our previous studies of rat (Roberto

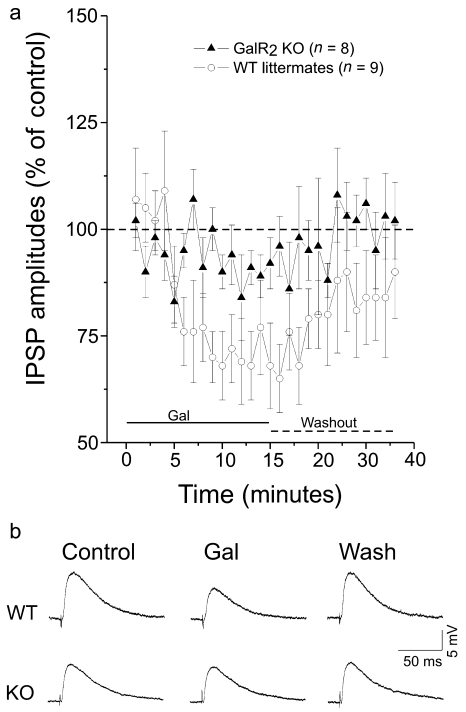

Figure 5 Galanin significantly decreases inhibitory postsynaptic potential (IPSP) amplitudes in wild-type (WT) littermates but not in GalR2 knockout (KO) mice. (a) Time course of pooled data from GalR2 KO and littermate WT control mice; mean-evoked IPSP amplitudes from central amygdala (CeA) neurons. Superfusion of 1 μM galanin (Gal) significantly decreases ($P = 0.001$; $n = 9$) the mean IPSP amplitudes in CeA neurons from WT controls with recovery on washout. Superfusion of 1 μM Gal only slightly but insignificantly ($P = 0.23$; $n = 8$) decreases mean IPSP amplitudes in CeA of GalR2 KO mice (error bars = ± standard error of the mean). (b) Top traces: representative voltage records of a CeA neuron from a WT littermate mouse; Gal superfusion for 12 minutes diminishes the IPSPs with recovery after 6 minutes of washout [KCl electrode; resting membrane potential (RMP) = −85 mV]. Bottom traces: In a CeA neuron of a GalR2 KO mouse, 14 minutes of galanin superfusion had little effect on IPSP amplitude (RMP = −86 mV with KCl electrodes)

et al. 2003, 2010b) and mouse (Nie *et al.* 2004, 2009; Bajo *et al.* 2008) CeA neurons indicate that ethanol reproducibly increases IPSP amplitudes, by increasing GABA release, in most CeA neurons. In the present studies, co-application of maximal concentrations for these two agents (1 μM galanin, 44 mM ethanol) resulted in a summation of effects only in a subpopulation of neurons (cf. Figs 6 & 7), suggesting that galanin and ethanol may interact on CeA GABAergic transmission via different cellular mechanisms. Thus, in a set of 12 CeA neurons from WT mice in which galanin decreased IPSP amplitudes, ethanol applied together with galanin only minimally reversed the IPSP amplitude decrease (to 87% of control) elicited by galanin alone (to 80%). Thus, galanin was somewhat preemptive over the ethanol effects (Fig. 6a, b), because ethanol alone generally augments CeA IPSPs to 130% or more of control levels. Interestingly, in this group of CeA neurons, both galanin and galanin with ethanol increased PPF to the same extent in most cells (Fig. 6c), similar to results with the GalR2 KOs, perhaps suggesting decreased GABA release; however, this effect was not statistically significant.

In seven WT mouse CeA neurons showing increases of evoked IPSP amplitudes by galanin alone, 44 mM ethanol together with 1 μM galanin further augmented the galanin effect by increasing IPSP amplitudes to 137% of baseline (from 117%; Fig. 7a, b), suggesting that ethanol and galanin effects involve different mechanisms of action (e.g. presynaptic versus postsynaptic) in this CeA population, with summation of maximal effects on IPSP size. Although again galanin alone seemed to increase PPF, this effect was not statistically significant; however, the difference between PPF during galanin alone and galanin with ethanol (which decreases PPF) was significant (Fig. 7c).

Of some interest is the rapid rebound reversal of ethanol effects on IPSP size in both neuron groups (i.e. showing either galanin-induced IPSP increases or decreases) on washout of the two agents. We interpret this rebound as resulting from a more rapid washout of ethanol compared with galanin, with the galanin effect persisting longer.

In a separate study (not shown), the co-perfusion of galanin and ethanol onto CeA neurons of Sprague-Dawley rats gave results equivalent to those in mouse CeA: in the pooled data from four rat CeA neurons showing galanin depression of IPSPs, ethanol co-superfusion slightly reversed the galanin effect in decreasing mean IPSP amplitudes, but only partially back toward baseline, as in mouse CeA.

DISCUSSION

The widespread localization of galanin and its various receptors in the amygdala (Melander *et al.* 1988; Kohler *et al.* 1989a,b; Jhamandas *et al.* 1996; Mennicken *et al.* 2002; Jungnickel & Gundlach 2005; Lu *et al.* 2005b) suggests that there are multiple cellular sites where the neuropeptide may influence CeA neuronal function. For example, the coexistence of galanin with noradrenaline in the LC and CeA (and with serotonin in the DRN; Xu & Hokfelt 1997) may suggest that galanin is involved in the interactions between these two monoaminergic regions that play key roles in mood regulation. In the case of the CeA, these monoaminergic interactions with galanin may influence the local GABAergic neurons known to comprise most of the CeA population (Cassell *et al.* 1986, 1999). Despite the evidence of norepinephrine and

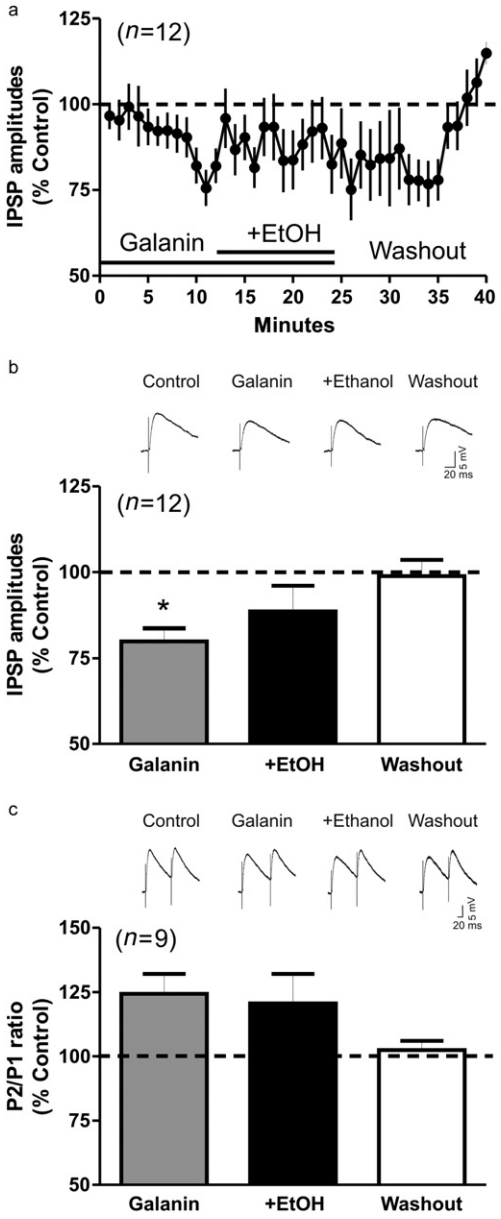

Figure 6 Co-application of galanin prevents ethanol-dependent augmentations of inhibitory postsynaptic potentials (IPSPs) in the central amygdala (CeA) cells responding to galanin with a decrease in IPSP amplitudes. (a) Time course of evoked IPSP responses of multiple individual mouse CeA neurons to superfusion of 1 μM galanin followed by co-superfusion of 44 mM ethanol (+EtOH). (b) Galanin decreased mean IPSP amplitudes by 20.0 ± 3.4% in CeA neurons (12 of 23 cells), and subsequent co-application of ethanol (+EtOH) returned the IPSP amplitudes toward but below control levels (to 87.3 ± 7.0% of control). The lower horizontal bars correspond to an average of the last 4 minutes of galanin, galanin + ethanol co-application and washout. Top panel: representative recordings from an individual CeA neuron. (c) Both galanin and galanin + ethanol co-application elicited increased paired-pulse facilitation (PPF) but the increases did not reach statistical significance. Top panel: representative recordings of PPF from an individual CeA neuron. The data represent mean ± standard error of the mean % of control, and asterisks denote significance at the $P < 0.05$ level (repeated analysis of variance and Bonferroni *post hoc* test)

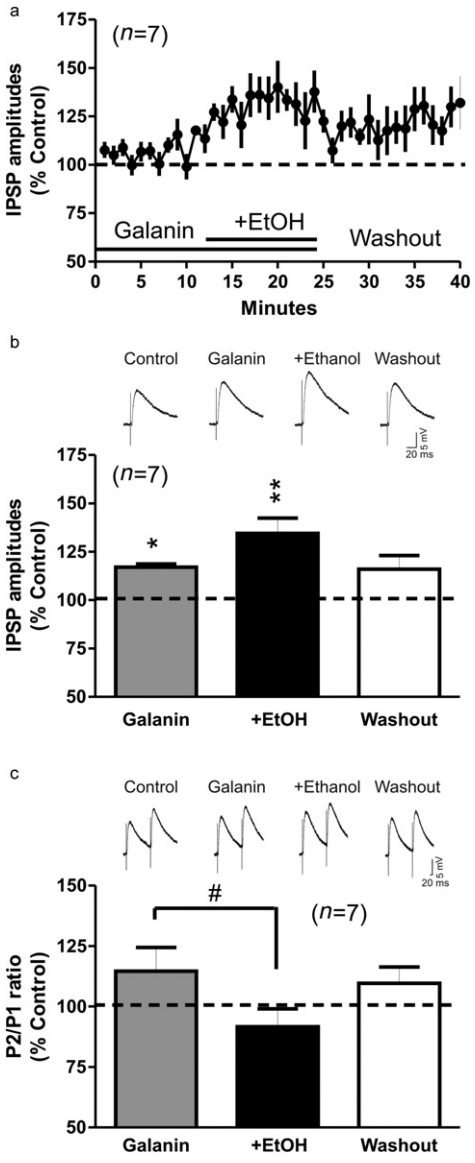

Figure 7 Co-superfusion of ethanol and galanin leads to additive effects on evoked inhibitory postsynaptic potential (IPSP) amplitudes in the cells responding to galanin with an increase in IPSP amplitudes. (a) Time course of evoked IPSP responses of multiple individual mouse central amygdala (CeA) neurons to superfusion of 1 μM galanin followed by co-superfusion of 44 mM ethanol (+EtOH). (b) Galanin increased mean IPSP amplitudes (to 117 ± 1.6% of baseline) and subsequent co-application of ethanol further increased amplitudes (to 136.7 ± 10.3%) with brief recovery on washout, followed by a rebound IPSP enhancement. Top panel: representative IPSP recordings from an individual CeA neuron. (c) Although neither galanin nor galanin + ethanol co-application significantly altered paired-pulse facilitation (PPF) compared with control, there was a significant decrease in PPF during galanin + ethanol co-application compared with galanin alone (#, $P < 0.05$). Top panel: representative recordings of PPF of IPSPs from an individual CeA neuron. The data represent mean ± standard error of the mean % of control; statistical significance, * and # corresponding to $P < 0.05$ and ** $P < 0.01$, respectively, were calculated by repeated analysis of variance and Bonferroni *post hoc* test. We interpret the washout rebound effects as a more rapid washout of ethanol effects compared with galanin

galanin coexistence in the CeA, the presence of multiple galanin receptor subtypes there and behavioral effects elicited by galanin microinjection into CeA (Moller *et al.* 1999; Khoshbouei *et al.* 2002), to our knowledge there are no data available on the electrophysiological effects of galanin in CeA neurons. Therefore, in the present study, we first examined the effect of the neuropeptide galanin and a GalR3-selective antagonist, in CeA neurons from WT and GalR1 and GalR2 KO mice, on the electrophysiological properties and GABAergic IPSPs of these neurons.

In contrast to previous reports of hyperpolarizing effects of galanin in DRN and several other neuron types (Hokfelt *et al.* 1998; Xu *et al.* 1998a,b, 2001; Swanson *et al.* 2005), galanin had no significant effect on current/voltage relationships in CeA neurons. However, we found that galanin clearly alters GABAergic neurotransmission in CeA neurons, but in a cell- and receptor-dependent manner. Interestingly, as with the effect of Gal 1-29 and Gal 2-11 in decreasing the amplitudes of isolated $GABA_A$-IPSPs in most DRN neurons (Sharkey *et al.* 2008), galanin (Gal 1-29) also decreased IPSP amplitudes in many CeA neurons. However, in a large percentage of CeA neurons, galanin measurably increased IPSP amplitudes, albeit to a lesser extent than the decrease in IPSPs seen in other cells. It should be noted that the long IPSP depressions seen in some CeA neurons, like those reported for Gal 1-29 decreases of IPSP amplitudes in DRN neurons (Sharkey *et al.* 2008), persisted and even increased over long application times, suggesting a stronger late effect and a lack of tachyphalaxis in both CeA and DRN neurons.

Our previous work on DRN neurons (Sharkey *et al.* 2008) suggested that the mechanisms whereby galanin receptor agonists altered GABAergic transmission may have depended on the receptor subtype activated: some GalR receptor subtypes may have been activated in a concentration-dependent manner such that longer exposure to the neuropeptide (and perhaps greater slice penetration) resulted in a greater effect. By contrast in CeA, selective activation of some receptor types (e.g. GalR3) may have a more transitory effect (e.g. increased IPSP amplitudes), peaking at shorter exposure times before desensitizing.

Further differences in the effects of galanin in CeA were seen in the inconsistent or lack of effects on PPF, known to be inversely related to neurotransmitter release (Mennerick & Zorumski 1995; Salin *et al.* 1996; Bonci & Williams 1997), except in CeA of GalR2 KO mice. In most CeA neurons from WT, GalR1 × GalR2 KO and littermate mice, galanin had little effect on PPF in CeA neurons, although CeA neurons of GalR2 KO mice showed galanin-evoked PPF increases, suggesting a possible presynaptic action on GalR1 and/or GalR3 receptors to regulate release of GABA, whereas galanin at GalR2 receptors may act postsynaptically. The localization of the GalR receptor subtypes is not known, because of lack of antibodies selective for receptor subtypes, but our data may suggest that some of the galanin receptors in the CeA may be on GABAergic terminals, whereas many could be on postsynaptic elements (e.g. GPCRs, other signalling pathways) that influence GABA receptor function.

The current study is the first to address the electrophysiological effects of galanin on the GABAergic system in the CeA. We have presented initial evidence for the differential activation of the CeA galanin receptors GalR2 and GalR3 that may have important implications for the development of novel galanin-based antidepressant or anti-anxiety treatments. Previous work showed that chronic antidepressant treatment (fluoxetine, 10 mg/kg i.p. 14 days and electroconvulsive shock treatment 2 days) produced a shift in the GalR1 and GalR2 ratio toward GalR2, as well as an elevation of galanin levels (Lu *et al.* 2005a), suggesting that the galaninergic system is affected by, and may be involved in, antidepressant effects. Support for this idea is the recent finding that mice with a GalR2 KO show a more persistent depressive-like phenotype than control WT mice (Lu *et al.* 2008). Differential activation of GalR2 and GalR3 by endogenous galanin may shed light on the mechanisms by which this antidepressant-induced change in GalR subtype ratio is important in the clinical efficacy of antidepressant treatments. Furthermore, the findings suggest that the development of subtype-specific GalR agonists or antagonists may be useful for novel antidepressant or anti-anxiety treatments, as recently shown for GalR3 antagonists as anti-anxiety agents (Swanson *et al.* 2005; Ogren *et al.* 2006; Lu *et al.* 2007).

In addition, our past (Nie *et al.* 2004; Roberto *et al.* 2010b) and present data may suggest that GalR2 activation or block of GalR3 favors reduction of GABAergic inhibitory function in CeA neurons, perhaps resulting in disinhibition of downstream GABAergic neurons (Bajo *et al.* 2008), that may provide a mechanism underlying this antidepressant action. Thus, the lack of an early increase in IPSP amplitudes in the presence of the SNAP antagonist suggests the increase is the result of activation of GalR3. Notably, these galanin effects are consistent with the anxiogenic/anxiolytic profile we have recently hypothesized for the GABAergic neurons predominant in CeA, based on our findings over the last 5–6 years: anxiolytic-like agents such as opioid (Kang-Park *et al.* 2007, 2009) and orphanin FQ (Roberto & Siggins 2006) agonists (e.g. enkephalin and nociceptin) and neuropeptide Y (NPY) and CB1 agonists (Roberto *et al.* 2010a, Gilpin*et al.* 2011) function in CeA to reduce presynaptic release of GABA, and thus should excite these GABAergic inhibitory neurons by disinhibition. By

contrast, the stress- and anxiety-related peptide CRF increases evoked IPSP amplitudes, via presynaptic increases in GABA release (Nie *et al*. 2004; Roberto *et al*. 2010b), and thus should counter the effects of the 'anxiolytic-like' agonists. Because most rodent CeA neurons contain GABA (with various co-localized peptides) and many of these GABA neurons project outside the nucleus (e.g. to bed nucleus of stria terminalis), local release of the opioids, nociceptin, NPY and cannabinoids (and now galanin acting on GalR2 receptors) in CeA will lead to the release of more GABA in downstream targets, thus inhibiting these areas. As the extended amygdala is known to be involved in stress-related disorders such as anxiety, our combined findings may help to solve the seeming paradox that anxiolytic-like substances reduce rather than enhance, and 'anxiogenic' peptides increase, GABAergic transmission within CeA: this CeA profile would have opposite (i.e. more conventional) effects in downstream areas like the bed nucleus of the stria terminalis. The dual effects of galanin on GABAergic IPSPs in CeA might then explain reports of mixed anxiolytic/anxiogenic behavioral effects seen after microinjection of galanin into CeA (Moller *et al*. 1999; Khoshbouei *et al*. 2002) and the anxiolytic-like effects of the GalR3 antagonists (Swanson *et al*. 2005; Ogren *et al*. 2006; Lu *et al*. 2007). The latter may reduce anxiety in part by antagonizing the GalR3 receptor-induced IPSP augmentation in CeA, leaving behind the decrease of IPSPs elicited by GalR2 receptors.

Notably, to date, ethanol was the only allegedly anxiolytic agent we tested that increased IPSP amplitudes in CeA (Roberto *et al*. 2003, 2010b; Roberto & Siggins 2006). As noted earlier, the galanin augmentation of IPSPs found in some CeA neurons suggests the activation of GalR3 receptors, now thought to be involved in anxiety. These considerations and emerging literature suggesting a role for galanin and GalR2 receptors in ethanol intake (Leibowitz *et al*. 2003; Lewis *et al*. 2004; Chang *et al*. 2007; Karatayev *et al*. 2009, 2010) led us to compare and assess possible interactions of ethanol with galanin effects in CeA neurons. We indeed found such interactions. These interactions were suggestive of a summation of like effects only in neurons showing IPSP augmentation by galanin. As no occlusive interactions occurred in this population of neurons, we interpret the summated interactions as indicating involvement of different cellular or molecular mechanisms for the two agents in these cells. This interpretation is also supported by the inconsistent or lack of significant alteration of PPF by galanin in CeA of WT mice, in contrast to the PPF decreases always associated with the increased presynaptic GABA release elicited by ethanol (Roberto *et al*. 2003, 2010b; Roberto & Siggins 2006). However, in those CeA cells where galanin diminished IPSPs, there was only slight summation of opposing effects by galanin plus ethanol; this interaction (see Fig. 6) suggests that galanin may have preemptive effects similar to those we previously found with nociceptin (Roberto & Siggins 2006). The suggestion of PPF increases by galanin in these cells may also indicate that ethanol and galanin act on the same mechanism (e.g. GABA release) in this neuronal population.

Further studies will be required to elucidate the exact cellular sites of the galanin effects, the possible CeA neuron types involved in the two galanin opposing effects on GABAergic transmission reported here and the possible cellular role of CeA galanin in ethanol effects. With respect to the latter, an important question is the effect of endogenous galanin on discrete Gal receptors in CeA and the effect of ethanol on the endogenous galanin-receptor interactions. The type of study reported by Lu *et al*. 2010, of the development of a putative GalR2-positive allosteric modulator (CYM2503) that would act on GalR2 receptors primarily via local endogenous galanin, may provide a path to answer such a question for the CeA.

Acknowledgements

We thank Drs. Scott Moore and Marisa Roberto for helpful comments on the article, Dr. Edward Roberts for providing SNAP 37889 and Novartis Pharma AG for the gift of CGP 55845A. This work has been supported by NIH grants from NIMH (MH074055) and NINDS (NS063560) to T.B., and from NIDA (DA03665) and NIAAA (U01 AA013498 under the INIA Consortium) to G.R.S.

Authors Contribution

M.B., S.G.M., X.L., T.B. and G.R.S. were responsible for the study concepts and design. MB, SGM and LS contributed to the data acquisition and analysis. X.L. and T.B. provided galanin and GalR KO mice. G.R.S. and M.B. drafted the article. MB, TB, SGM, LS and XL provided the critical revision of the article for important intellectual content. All authors critically reviewed the content and approved the final version for publication.

References

Bajo M, Cruz MT, Siggins GR, Messing R, Roberto M (2008) Protein kinase C epsilon mediation of CRF- and ethanol-induced GABA release in central amygdala. Proc Natl Acad Sci U S A 105:8410–8415.

Bonci A, Williams JT (1997) Increased probability of GABA release during withdrawal from morphine. J Neurosci 17:796–803.

Cassell MD, Gray TS, Kiss JZ (1986) Neuronal architecture in the rat central nucleus of the amygdala: a cytological, hodological, and immunocytochemical study. J Comp Neurol 246:478–499.

Cassell MD, Freedman LJ, Shi C (1999) The intrinsic organization of the central extended amygdala. Ann N Y Acad Sci 877:217–241.

Chang GQ, Karatayev O, Ahsan R, Avena NM, Lee C, Lewis MJ, Hoebel BG, Leibowitz SF (2007) Effect of ethanol on hypothalamic opioid peptides, enkephalin, and dynorphin: relationship with circulating triglycerides. Alcohol Clin Exp Res 31:249–259.

Davis M, Rainnie D, Cassell M (1994) Neurotransmission in the rat amygdala related to fear and anxiety. Trends Neurosci 17:208–214.

Dutar P, Lamour Y, Nicoll RA (1989) Galanin blocks the slow cholinergic EPSP in CA1 pyramidal neurons from ventral hippocampus. Eur J Pharmacol 164:355–360.

Fisone G, Wu CF, Consolo S, Nordstrom O, Brynne N, Bartfai T, Melander T, Hokfelt T (1987) Galanin inhibits acetylcholine release in the ventral hippocampus of the rat: histochemical, autoradiographic, in vivo, and in vitro studies. Proc Natl Acad Sci U S A 84:7339–7343.

Gilpin NW, Mirsa K, Herman MA, Cruz MT, Roberto M (2011) Neuropeptide y opposes alcohol effects on gamma-aminobutyric acid release in amygdala and blocks the transition to alcohol dependence. Biol Psychiatry 69:1091–1099.

Hokfelt T, Xu ZQ, Shi TJ, Holmberg K, Zhang X (1998) Galanin in ascending systems. Focus on coexistence with 5-hydroxytryptamine and noradrenaline. Ann N Y Acad Sci 863:252–263.

Jhamandas JH, Petrov T, Harris KH, Vu T, Krukoff TL (1996) Parabrachial nucleus projection to the amygdala in the rat: electrophysiological and anatomical observations. Brain Res Bull 39:115–126.

Jungnickel SR, Gundlach AL (2005) [125I]-Galanin binding in brain of wildtype, and galanin- and GalR1-knockout mice: strain and species differences in GalR1 density and distribution. Neuroscience 131:407–421.

Kang-Park MH, Kieffer BL, Roberts AJ, Siggins GR, Moore SD (2007) Presynaptic delta opioid receptors regulate ethanol actions in central amygdala. J Pharmacol Exp Ther 320:917–925.

Kang-Park MH, Kieffer BL, Roberts AJ, Roberto M, Madamba SG, Siggins GR, Moore SD (2009) Mu-opioid receptors selectively regulate basal inhibitory transmission in the central amygdala: lack of ethanol interactions. J Pharmacol Exp Ther 328:284–293.

Karatayev O, Baylan J, Leibowitz SF (2009) Increased intake of ethanol and dietary fat in galanin overexpressing mice. Alcohol 43:571–580.

Karatayev O, Baylan J, Weed V, Chang S, Wynick D, Leibowitz SF (2010) Galanin knockout mice show disturbances in ethanol consumption and expression of hypothalamic peptides that stimulate ethanol intake. Alcohol Clin Exp Res 34:72–80.

Kerekes N, Mennicken F, O'Donnell D, Hokfelt T, Hill RH (2003) Galanin increases membrane excitability and enhances Ca(2+) currents in adult, acutely dissociated dorsal root ganglion neurons. Eur J Neurosci 18:2957–2966.

Khoshbouei H, Cecchi M, Dove S, Javors M, Morilak DA (2002) Behavioral reactivity to stress: amplification of stress-induced noradrenergic activation elicits a galanin-mediated anxiolytic effect in central amygdala. Pharmacol Biochem Behav 71:407–417.

Kohler C, Hallman H, Melander T, Hokfelt T, Norheim E (1989a) Autoradiographic mapping of galanin receptors in the monkey brain. J Chem Neuroanat 2:269–284.

Kohler C, Persson A, Melander T, Theodorsson E, Sedvall G, Hokfelt T (1989b) Distribution of galanin-binding sites in the monkey and human telencephalon: preliminary observations. Exp Brain Res 75:375–380.

Leibowitz SF, Avena NM, Chang GQ, Karatayev O, Chau DT, Hoebel BG (2003) Ethanol intake increases galanin mRNA in the hypothalamus and withdrawal decreases it. Physiol Behav 79:103–111.

Lewis MJ, Johnson DF, Waldman D, Leibowitz SF, Hoebel BG (2004) Galanin microinjection in the third ventricle increases voluntary ethanol intake. Alcohol Clin Exp Res 28:1822–1828.

Lu X, Barr AM, Kinney JW, Sanna P, Conti B, Behrens MM, Bartfai T (2005a) A role for galanin in antidepressant actions with a focus on the dorsal raphe nucleus. Proc Natl Acad Sci U S A 102:874–879.

Lu X, Mazarati A, Sanna P, Shinmei S, Bartfai T (2005b) Distribution and differential regulation of galanin receptor subtypes in rat brain: effects of seizure activity. Neuropeptides 39:147–152.

Lu X, Sharkey L, Bartfai T (2007) The brain galanin receptors: targets for novel antidepressant drugs. CNS Neurol Disord Drug Targets 6:183–192.

Lu X, Ross B, Sanchez-Alavez M, Zorrilla EP, Bartfai T (2008) Phenotypic analysis of GalR2 knockout mice in anxiety- and depression-related behavioral tests. Neuropeptides 42:387–397.

Lu X, Roberts E, Xia F, Sanchez-Alavez M, Liu T, Baldwin R, Wu S, Chang J, Wasterlain CG, Bartfai T (2010) GalR2-positive allosteric modulator exhibits anticonvulsant effects in animal models. Proc Natl Acad Sci U S A 107:15229–15234.

Ma X, Tong YG, Schmidt R, Brown W, Payza K, Hodzic L, Pou C, Godbout C, Hokfelt T, Xu ZQ (2001) Effects of galanin receptor agonists on locus coeruleus neurons. Brain Res 919:169–174.

Melander T, Hokfelt T, Rokaeus A, Cuello AC, Oertel WH, Verhofstad A, Goldstein M (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS. J Neurosci 6:3640–3654.

Melander T, Kohler C, Nilsson S, Hokfelt T, Brodin E, Theodorsson E, Bartfai T (1988) Autoradiographic quantitation and anatomical mapping of 125I-galanin binding sites in the rat central nervous system. J Chem Neuroanat 1:213–233.

Mennerick S, Zorumski CF (1995) Paired-pulse modulation of fast excitatory synaptic currents in microcultures of rat hippocampal neurons. J Physiol 488:85–101.

Mennicken F, Hoffert C, Pelletier M, Ahmad S, O'Donnell D (2002) Restricted distribution of galanin receptor 3 (GalR3) mRNA in the adult rat central nervous system. J Chem Neuroanat 24:257–268.

Mitsukawa K, Lu X, Bartfai T (2009) Bidirectional regulation of stress responses by galanin in mice: involvement of galanin receptor subtype 1. Neuroscience 160:837–846.

Moller C, Sommer W, Thorsell A, Heilig M (1999) Anxiogenic-like action of galanin after intra-amygdala administration in the rat. Neuropsychopharmacology 21:507–512.

Nie Z, Schweitzer P, Roberts AJ, Madamba SG, Moore SD, Siggins GR (2004) Ethanol augments GABAergic transmission in the central amygdala via CRF1 receptors. Science 303:1512–1514.

Nie Z, Zorrilla EP, Madamba SG, Rice KC, Roberto M, Siggins GR (2009) Presynaptic CRF1 receptors mediate the ethanol

enhancement of GABAergic transmission in the mouse central amygdala. ScientificWorldJournal 9:68–85.

Ogren SO, Hokfelt T, Kask K, Langel U, Bartfai T (1992) Evidence for a role of the neuropeptide galanin in spatial learning. Neuroscience 51:1–5.

Ogren SO, Kuteeva E, Hokfelt T, Kehr J (2006) Galanin receptor antagonists: a potential novel pharmacological treatment for mood disorders. CNS Drugs 20:633–654.

Picciotto MR, Hawes JJ, Brunzell DH, Zachariou V (2005) Galanin can attenuate opiate reinforcement and withdrawal. Neuropeptides 39:313–315.

Pich EM, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF, Weiss F (1995) Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. J Neurosci 15:5439–5447.

Pieribone VA, Xu ZQ, Zhang X, Grillner S, Bartfai T, Hokfelt T (1995) Galanin induces a hyperpolarization of norepinephrine-containing locus coeruleus neurons in the brainstem slice. Neuroscience 64:861–874.

Roberto M, Siggins GR (2006) Nociceptin/orphanin FQ presynaptically decreases GABAergic transmission and blocks the ethanol-induced increase of GABA release in central amygdala. Proc Natl Acad Sci U S A 103:9715–9720.

Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR (2003) Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. Proc Natl Acad Sci U S A 100:2053–2058.

Roberto M, Cruz M, Bajo M, Siggins GR, Parsons LH, Schweitzer P (2010a) The endocannabinoid system tonically regulates inhibitory transmission and depresses the effect of ethanol in central amygdala. Neuropsychopharmacology 35:1962–1972.

Roberto M, Cruz MT, Gilpin NW, Sabino V, Schweitzer P, Bajo M, Cottone P, Madamba SG, Stouffer DG, Zorrilla EP, Koob GF, Siggins GR, Parsons LH (2010b) Corticotropin releasing factor-induced amygdala gamma-aminobutyric acid release plays a key role in alcohol dependence. Biol Psychiatry 67:831–839.

Salin PA, Malenka RC, Nicoll RA (1996) Cyclic AMP mediates a presynaptic form of LTP at cerebellar parallel fiber synapses. Neuron 16:797–803.

Sharkey LM, Madamba SG, Siggins GR, Bartfai T (2008) Galanin alters GABAergic neurotransmission in the dorsal raphe nucleus. Neurochem Res 33:285–291.

Swanson CJ, Blackburn TP, Zhang X, Zheng K, Xu ZQ, Hokfelt T, Wolinsky TD, Konkel MJ, Chen H, Zhong H, Walker MW, Craig DA, Gerald CP, Branchek TA (2005) Anxiolytic- and antidepressant-like profiles of the galanin-3 receptor (Gal3) antagonists SNAP 37889 and SNAP 398299. Proc Natl Acad Sci U S A 102:17489–17494.

Xu ZQ, Bartfai T, Langel U, Hokfelt T (1998a) Effects of three galanin analogs on the outward current evoked by galanin in locus coeruleus. Ann N Y Acad Sci 863:459–465.

Xu ZQ, Hokfelt T (1997) Expression of galanin and nitric oxide synthase in subpopulations of serotonin neurons of the rat dorsal raphe nucleus. J Chem Neuroanat 13:169–187.

Xu ZQ, Tong YG, Hokfelt T (2001) Galanin enhances noradrenaline-induced outward current on locus coeruleus noradrenergic neurons. Neuroreport 12:1779–1782.

Xu ZQ, Zhang X, Pieribone VA, Grillner S, Hokfelt T (1998b) Galanin-5-hydroxytryptamine interactions: electrophysiological, immunohistochemical and in situ hybridization studies on rat dorsal raphe neurons with a note on galanin R1 and R2 receptors. Neuroscience 87:79–94.