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## Negative modulation of the GABA<sub>A</sub>1 receptor function by L-Cysteine

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## Keywords

GABA receptor, GABA<sub>A</sub>ρ1 receptors, L-Cysteine, retina.

## Abbreviations

GABA, γ-aminobutyric acid	L-Cys, L-Cysteine	NEM, N-ethyl maleimide	CNS,
central nervous system	C-R, concentration-response	Cl <sup>-</sup> , Chloride	

## Abstract

L-Cysteine is an endogenous Sulphur containing amino acid with multiple and varied roles in the central nervous system, including neuroprotection and the maintenance of the redox balance. However, it was also suggested as an excitotoxic agent implicated in the pathogenesis of neurological disorders such as Parkinson's and Alzheimer's disease. L-Cysteine can modulate the activity of ionic channels, including voltage-gated calcium channels and glutamatergic NMDA receptors, whereas its effects on GABAergic neurotransmission had not been studied before. In the present work, we analyzed the effects of L-Cysteine on responses mediated by homomeric GABA<sub>A</sub>ρ1 receptors, which are known for mediating tonic GABA responses in retinal neurons. GABA<sub>A</sub>ρ1 receptors were expressed in *Xenopus laevis* oocytes and GABA-evoked chloride currents recorded by two-electrode voltage-clamp in the presence or absence of L-Cysteine. L-Cysteine antagonized GABA<sub>A</sub>ρ1 receptor-mediated responses; inhibition was dose-dependent, reversible, voltage independent and susceptible to GABA concentration. Concentration-response curves for GABA were shifted to the right in the presence of L-Cysteine without a substantial change in the maximal response. L-Cysteine inhibition was insensitive to chemical protection of the sulfhydryl

groups of the  $\rho 1$  subunits by the irreversible alkylating agent N-ethyl maleimide. Our results suggest that redox modulation is not involved during L-Cysteine actions and that L-Cysteine might be acting as a competitive antagonist of the GABA $\rho 1$  receptors.

## Introduction

L-Cysteine (L-Cys) is an endogenous Sulphur containing amino acid that plays key roles in the central nervous system (CNS). Under physiological conditions, it is a rate-limiting precursor for glutathione synthesis and takes part in the maintenance of the redox balance (Armstrong et al., 2004; Banerjee, 2012; Lu, 2013).

L-Cys can exert contrasting effects on brain and retina, including neuroprotective and antioxidant activities (Miyamoto et al., 1989; de Melo Reis et al., 2007), as well as neurotoxic actions which were suggested to be involved in the pathogenesis of neurological disorders such as amyotrophic lateral sclerosis, Parkinson's and Alzheimer's disease, hypoxic/ischemic and hypoglycemic brain damage and retinal degeneration (Pedersen and Karlsen, 1980; Karlsen and Pedersen, 1982; Heafield et al., 1990; Slivka and Cohen, 1993; Janáky et al., 2000; Gazit et al., 2004; Sawamoto et al., 2004). These and other evidence (Peana et al., 2010; McBean et al., 2015), together with the fact that L-Cys is present in many dietary supplements (McPherson and Hardy, 2011), indicate that effects of L-Cys on neurotransmission might have clinical importance.

L-Cys was also proposed as neuromodulator, based on studies showing that it is released on depolarization in a Ca $^{2+}$ -dependent manner from rat brain slices (Keller et al., 1989) and produces synaptic actions, like inhibition of glutamate reuptake, activation of glutamate NMDA receptors or modulation of the activity of voltage-gated calcium channels

(Ferkany and Coyle, 1986; Pace et al., 1992; Nelson et al., 2005). However, the possible targets and action mechanisms of L-Cys throughout the CNS are not entirely understood and its actions on pre or post-synaptic elements of the GABAergic synapses had not been examined before.

GABA<sub>A</sub> receptors are GABA-gated pentameric chloride (Cl<sup>-</sup>) channels, members of the *Cys-loop* receptor superfamily, mediating most of the inhibitory neurotransmission in the CNS (Farrant and Nusser, 2005). Functionally distinct neuronal GABA<sub>A</sub> receptors arise from the combination of a variety of subunit subtypes ( $\alpha$ 1-6,  $\beta$ 1-3,  $\gamma$ 1-3,  $\delta$ ,  $\epsilon$ ,  $\pi$ ,  $\theta$ ,  $\rho$ 1-3). For example, heteromeric GABA<sub>A</sub> $\alpha$ 1 $\beta$ 2 $\gamma$ 2 receptors are widely localized in brain and retina, where they mediate desensitizing ionic currents typically antagonized by bicuculline and modulated by benzodiazepines and barbiturates (Olsen and Sieghart, 2009). Meanwhile homomeric GABA<sub>A</sub> $\rho$  receptors, which are typically involved in several modes of inhibitory signaling in the retina (Matthews, 1994; Lukasiewicz et al., 2004; Jones and Palmer, 2009), display high affinity for GABA and mediate non-desensitizing responses which are highly resistant to bicuculline (Cutting et al., 1991; Polenzani et al., 1991; Naffa et al., 2017). GABA<sub>A</sub> $\rho$  receptors are highly expressed in retinal bipolar cells (Wässle et al., 1998; McCall et al., 2002). They play an important role in the control of axon terminal excitability mediating reciprocal synapses with amacrine cells (Dong and Werblin, 1998). They also mediate tonic inhibitory currents, which arise in response to ambient GABA locally controlled by GABA transporter 1 (GAT-1) transporters located on amacrine cells (Zhang and Slaughter, 1995; Lukasiewicz et al., 2004; Hull et al., 2006; Jones and Palmer, 2009).

Based on the relevance of tonic GABAergic inhibition for retinal function we examined whether or not GABA<sub>A</sub> $\rho$  receptors can be target of L-Cys modulatory actions. In

the present work we analyzed the effects of L-Cys on responses mediated by human homomeric GABA<sub>A</sub>ρ1 receptors in an *in vitro* cell model (Beltrán González et al., 2014). GABA<sub>A</sub>ρ1 receptors were heterologously expressed in *Xenopus laevis* oocytes and GABA-induced Cl<sup>-</sup> currents electrophysiologically recorded. Our results showed that L-Cys inhibits the activity of GABA<sub>A</sub>ρ1 receptors. Experiments involving the chemical modification of sulfhydryl groups suggest that redox mechanisms were not involved in L-Cys actions and that inhibitory actions are more likely explained by a competitive antagonism operating on the GABA<sub>A</sub>ρ1 receptors.

### Materials and methods

Experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health (<https://grants.nih.gov/grants/olaw/Guide-for-the-Care-and-Use-of-Laboratory-Animals.pdf>), and were approved by the Institution's Animal Care and use Committee.

#### *RNA preparation, oocyte isolation and cell injection*

Human cDNA encoding the ρ1 GABA<sub>A</sub> receptor subunit, cloned in the *in vitro* transcription-suitable vector pGEM, was used as a template to synthesize cRNAs *in vitro*. cRNA solutions (0.3-1 ng/nl) were prepared in RNase-free H<sub>2</sub>O and stored at -70°C. *Xenopus laevis* (Nasco, Modesto, CA, USA) oocytes at stages V and VI were used for expression of exogenous cRNAs. Isolation and maintenance of cells were carried out as previously described (Goutman et al., 2005). Briefly, frogs were anesthetized with 3-aminobenzoic-acid ethylester (~1 mg/ml) and ovaries surgically removed. Ovaries were incubated with 400 U/ml collagenase for 4 h at 23–24°C and isolated oocytes maintained in an incubator at 18°C in Barth's medium (in mM: 88 NaCl; 0.33 Ca(NO<sub>3</sub>)<sub>2</sub>; 0.41 CaCl<sub>2</sub>; 1 KCl; 0.82 MgSO<sub>4</sub>; 2.4 NaHCO<sub>3</sub>; 10 HEPES , 0.05 mg/ml gentamicin and 0.05 mg/ml

tetracycline ; pH adjusted to 7.4 with NaOH). After 1 day, each oocyte was manually microinjected (microinjector Drummond Sci. Co., Broomall, PA, USA) with 50 nl of a solution containing 5–50 ng of cRNA.

### *Electrophysiological recordings*

TEVC recordings were performed 3–7 days after oocyte injection, with an Axoclamp 2B amplifier (Axon Instruments, Union City, CA, USA). Standard glass recording electrodes were made in a Narishige PB-7 puller (Narishige Scientific Instrument Lab., Tokyo, Japan) and filled with 3 M KCl. Pipette resistance values were approximately 1 M $\Omega$ . The holding potential was set to -70 mV and current traces acquired by a PC through a Labmaster TL-1 DMA interface (Scientific solutions Inc., Solon, OH, USA) using AXOTAPE software (Axon Instruments). I-V curves consisted of voltage stepped ramps from -120 to +40 mV, in 2 sec steps, starting from a holding potential of -70 mV. Ramps were delivered at the plateau of the GABA responses, in the presence or absence of L-Cys. Leak currents were estimated through voltage ramps performed on the baseline (before and after GABA application) and then were digitally subtracted. Cells were placed in a chamber (volume 100  $\mu$ l) continuously superfused (12 ml/min) with frog Ringer's solution (in mM: 115 NaCl; 2 KCl; 1.8 CaCl<sub>2</sub>; 5 HEPES; pH 7.0). GABA and other drugs were applied through the perfusion system. Drugs were freshly prepared each day as concentrated stocks and dissolved in frog Ringer's solution. The pH of each test solution was always checked and adjusted if necessary to pH=7.0. All the experiments were carried out at room temperature (23–24°C) and were replicated in at least 5 different oocytes isolated from at least two different frogs.

### *Materials*

The transcription kit mMessage mMachine was purchased from Ambion (Austin, TX, USA) and type II collagenase from Worthington (Freehold, NJ, USA). All chemicals and salts were purchased from Sigma-Aldrich (St Louis, MO, USA).

### *Data analysis*

Data were analyzed with Prism v. 6.0 (Graphpad Software, Inc. San Diego, CA, USA). Concentration–response curves for GABA were fit with a logistic equation of the following form:  $I_{GABA}/B = [A^n/(A^n+EC_{50}^n)] \times 100$  where  $A$  is the agonist concentration,  $B$  the maximal response,  $EC_{50}$  the concentration of agonist that elicits half-maximal responses and  $n$  the Hill coefficient. Percentage of control response was calculated as  $[(I_{GABA\beta 1L-Cys} \times 100 / I_{GABA\beta 1control})]$ , where  $I_{GABA\beta 1L-Cys}$  indicates the current amplitude evoked at each particular GABA concentration in the presence of L-Cys and  $I_{GABA\beta 1control}$  the corresponding responses in the absence of modulator. Percentage of inhibition was calculated as  $[100 - (I_{GABA\beta 1L-Cys} \times 100 / I_{GABA\beta 1control})]$ . Student's t-tests (two tailed) were employed to evaluate significant differences between parameters. In all cases errors were expressed as SEM.

## **Results**

### *Inhibition of GABA-mediated currents by L-Cysteine*

GABA applications to oocytes expressing homomeric GABA<sub>A</sub>β1 receptors induced non-desensitizing inward Cl<sup>-</sup> currents displaying all features of the bicuculline resistant GABA receptor mediated responses typically found in the retina (Polenzani et al., 1991; Zhang et al., 2001; Hull et al., 2006). They were antagonized by TPMPA and picrotoxin and displayed the characteristic pharmacological profile for agonists (data not shown) (Kusama et al., 1993; Woodward et al., 1993; Ragozzino et al., 1996; Naffa et al., 2017). Fig. 1 illustrates the effects of L-Cys on GABA<sub>A</sub>β1 receptor-mediated responses evoked by 1 μM

GABA, a concentration close to the EC<sub>50</sub> of GABA for these receptors, at -70 mV. Short bath applications of L-Cys significantly inhibited GABA responses (Fig. 1A and 1B). Antagonistic actions were dose-dependent, showed fast onset and completely reversed after washout. In the absence of GABA, L-Cys applications (200 μM) performed on either mRNA-injected or sham oocytes did not produce noticeable effects on oocyte properties such as membrane potential, membrane resistance or current baseline under voltage-clamp ( $n=7$ ; data not shown). No changes in the degree of inhibition were observed when consecutive GABA-evoked responses (1 μM GABA) were recorded in the continued presence of a single concentration of L-Cys (200 μM) (Fig. 1C), indicating that L-Cys effects on GABA<sub>A</sub>ρ1 receptors are not use-dependent (Goutman and Calvo, 2004).

Concentration-response (C-R) curves for GABA were performed either in the absence or presence of L-Cys (Fig. 2A; parameters detailed in Table 1). L-Cys shifted the C-R curve for GABA to the right, that is increased the EC<sub>50</sub> without a substantial change in the maximal response. These results indicated that in the presence of L-Cys the apparent affinity of GABA<sub>A</sub>ρ1 receptors decreased. L-Cys inhibition strongly depended on GABA concentration (Fig. 2B), with the degree of antagonism declining as GABA concentration increased. Currents evoked by 0.3 μM GABA were inhibited in a  $76.8 \pm 4.4$  % ( $n = 8$ ,  $p < 0.0001$ ), whereas currents evoked by 10 μM GABA were inhibited in a  $2.6 \pm 0.9$  % ( $n = 13$ ,  $ns$ ). For GABA concentrations lower than 3 μM, inhibition induced by 200 μM L-Cys was always significant ( $p < 0.001$ ). The effects of increasing doses of L-Cys were tested on responses evoked by different GABA concentrations: 0.3 μM (A), 1 μM (B) and 10 μM (C) ( $n = 5-10$ ) (Fig. 2C). The effects of L-Cys on GABA<sub>A</sub>ρ1 receptor-mediated responses were particularly pronounced at GABA concentrations within the dynamic range of activation, but almost negligible at saturating values of GABA. At 0.3 μM GABA, inhibition was significant for all L-Cys concentrations tested (10 μM to 3 mM) and at 1 μM GABA, effects



were significant for concentrations above 20  $\mu\text{M}$  ( $n = 6$ ,  $p < 0.02$ ). Meanwhile, at 10  $\mu\text{M}$  GABA, inhibition was only significant for levels of L-Cys above 500  $\mu\text{M}$  ( $n = 10$ ,  $p < 0.02$ ).

Current-voltage relationships (I-V curves), performed in the presence or absence of 200  $\mu\text{M}$  L-Cys, showed a change in the slope without alterations in the linearity of the I-V relationship or the reversal potential (between -120 and 40 mV for 1  $\mu\text{M}$  GABA-elicited responses), demonstrating that effects on GABA<sub>A</sub> $\rho$ 1 receptors did not depend on the membrane potential and neither were due to variations in intracellular Cl<sup>-</sup> levels (Fig. 2D).

#### *Modulation of GABA<sub>A</sub> $\rho$ 1 receptors by L-Cysteine is not affected by N-ethyl-maleimide treatment*

L-Cys had been previously shown to be a redox modulator of T-type calcium channels in rat peripheral nociceptors (Todorovic et al., 2001; Nelson et al., 2005). In addition, we had also observed that reducing and oxidizing thiol agents are effective modulators of the GABA<sub>A</sub> $\rho$ 1 receptor function (Calero and Calvo, 2008; Gasulla et al. 2012; Beltrán González et al., 2014). In fact, many different ionic channels sensitive to redox modulation usually are chemically modified through oxidation of cysteine residues. Based on all these data, we decided to test if redox modulation of cysteine residues might also be involved in the actions of L-Cys on GABA<sub>A</sub> $\rho$ 1 receptors.

At first view, the rapid onset and reversible effect of L-Cys on the GABA<sub>A</sub> $\rho$ 1 receptor mediated responses would be consistent with a direct redox modulatory action and thiol groups located at the  $\rho$ 1 subunits would be the most reactive redox candidates. Each  $\rho$ 1 subunit contains only two molecular sites contributing potential reactive cysteines, namely the extracellular Cys-loop and the single intracellular (C364) (Sedelnikova et al., 2005; Lo et al., 2008). However, it is important to state that redox modulation of the GABA<sub>A</sub> $\rho$ 1 receptors

mediated by these cysteine residues was sensitive to N-ethyl-maleimide (NEM) treatment in all cases examined before (Calero et al., 2011; Gasulla et al., 2012; Beltrán González et al., 2014). NEM is a membrane permeable irreversible alkylating reagent which selectively forms covalent bonds with free sulfhydryl groups, preventing any further chemical reaction at these sites (at pH = 7.0) (Means and Feeney, 1971). During the present experiments NEM concentration was kept as low as possible and incubation periods maintained very short in order to avoid non-specific effects (Calero et al., 2011). Pre-incubation for 2 min with 30  $\mu$ M and 300  $\mu$ M NEM failed to prevent L-Cys induced inhibition (% Control Response L-Cys =  $32.7 \pm 4.7$  % ( $n = 5$ ), + NEM<sub>30 $\mu$ M</sub> =  $40.1 \pm 6.8$  % ( $n = 6$ ), + NEM<sub>300 $\mu$ M</sub> =  $40.8.1 \pm 6.4$  % ( $n = 6$ ), *ns*) (Fig. 3). Taken together, these results suggest that redox modulation is not involved during GABA<sub>A</sub> $\rho$ 1 responses inhibition by L-Cys.

#### *GABA<sub>A</sub> $\rho$ 1 receptors function is insensitive to L-Cystine and L-Homocysteine*

The specificity of L-Cys actions on GABA<sub>A</sub> $\rho$ 1 responses was analyzed by testing the closely related compounds L-Cystine and L-Homocysteine, which also have physiological relevance (Cherqui et al., 2002; Mattson and Shea, 2003; Isobe et al., 2005; Ajith and Ranimenon, 2015) and whose actions on GABA<sub>A</sub> $\rho$ 1 receptor mediated responses had not been previously examined. Application of L-Cystine (200  $\mu$ M) did not produce significant effects on responses elicited by 0.3  $\mu$ M ( $103.8 \pm 5.3$  % ( $n = 8$ ) *ns*) or 1  $\mu$ M GABA ( $101.7 \pm 1.2$  % ( $n = 5$ ) *ns*). L-Homocysteine (200  $\mu$ M) showed no significant effect either on responses elicited by 0.3  $\mu$ M ( $107.7 \pm 6.3$  % ( $n = 5$ ) *ns*) or 1  $\mu$ M GABA ( $99.3 \pm 0.8$  % ( $n = 6$ ) *ns*). Representative experiments are illustrated in Fig 4.

## Discussion

The present findings are the first to demonstrate that the ubiquitous and endogenous amino acid L-Cys can modulate the function of GABA<sub>A</sub> receptors. Our results show that L-Cys is capable to induce functional changes on homomeric GABA<sub>A</sub>ρ1 receptors, a GABA<sub>A</sub> receptor subtype highly expressed in the retina that mediates tonic GABA responses in bipolar cells.

### *Mechanisms underlying the inhibition of GABA<sub>A</sub>ρ1 receptors by L-Cysteine*

Inhibition of GABA<sub>A</sub>ρ1 receptors by L-Cys was reversible, dose-dependent, voltage-independent and strongly susceptible to GABA concentration. The rapid onset of inhibition was consistent with an extracellular mechanism of action taking place. Experiments with NEM suggested that antagonistic actions of L-Cys are not due to redox modulation of cysteine residues at the GABA<sub>A</sub>ρ1 receptors. Interactions of thiol compounds, like L-Cys, with traces of transition metal ions can potentially generate oxygen free-radical species by Haber-Weiss type reactions (Janáky et al., 2000). However, an indirect action of L-Cys on GABA<sub>A</sub>ρ1 receptors through this mechanism seems not to be the case, because previous studies in our laboratory showed that oxygen free radicals potentiated GABA<sub>A</sub>ρ1 responses with a slow onset through an interaction with the intracellular cysteine residues of the ρ1 subunits (Beltrán González et al., 2014), in sharp contrast to the fast inhibition observed here following L-Cys applications.

L-Cys induced a rightward parallel shift in the C-R curve for GABA without changing the maximum response and the Hill coefficient. In addition, GABA<sub>A</sub>ρ1 receptor antagonism by L-Cys was surmounted by increasing the GABA concentration and did not involve use-dependent processes. Taken together, these results suggest that L-Cys shares the

characteristics of other competitive antagonists acting at on the GABA<sub>A</sub>ρ1 receptors (Woodward et al., 1993). However, binding studies will be necessary to confirm this assumption. Finally, neither L-Cystine nor L-Homocysteine, structural analogs of L-Cys, had effect on GABA<sub>A</sub>ρ1 responses evoked at concentrations in which L-Cys exhibited significant effects. Thus, structural determinants are essential for L-Cys modulation.

***Possible physiological relevance of the modulation of ionotropic GABA<sub>A</sub>ρ1 receptors by L-Cysteine.***

GABA<sub>A</sub>ρ1 receptors provide significant inhibitory drive to the synaptic terminals of retinal bipolar cells, including tonic, reciprocal and lateral inhibition (Zhang and Slaughter, 1995; Lukasiewicz et al., 2004; Hull et al., 2006; Chávez et al., 2010). In this context, the inhibition of GABA<sub>A</sub>ρ1 receptors responses by L-Cys could eventually increase the excitability of the bipolar cells, shaping ganglion cell responses and raising the excitability of the visual pathway. However, whether or not L-Cys modulation represents a relevant mechanism for controlling the activity of these and other ionotropic GABA receptors in retinal neuronal circuits, under physiological or pathological conditions, will need to be assessed by using both retinal slices and *in vivo* models.

The study of endogenous agents, such as L-Cys, as modulators of ionotropic GABA receptors broadens the understanding of the control of the phasic and tonic GABAergic neurotransmission and could eventually shed light on the etiology of several neurological disorders and the development of therapeutic applications. The extracellular concentrations of L-Cys used here were far below the range of those commonly used in previous work (Pace et al., 1992; Todorovic et al., 2001; Nelson et al., 2005). Even though effects on GABA<sub>A</sub>ρ1 receptors were significant L-Cys is normally found in relatively low levels in brain (ca. 0.05

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μmol/g), compared for example to the cysteine-containing tripeptide, glutathione (GSH, ca. 1.5 μmol/g). However, L-Cys was estimated to reach up to a level equivalent to a concentration of 700 μM in the course of ischemic brain damage (Slivka and Cohen, 1993). In our study, antagonistic actions of L-Cys on GABA<sub>A</sub>ρ1 receptors were detected even at 35-fold lower concentration than that. Given the potential clinical relevance of L-Cys effects on GABA neurotransmission, the extensive use of L-Cys as food additive or nutraceutical (McPherson and Hardy, 2012), together with the fact that ionotropic GABA receptors are the most important inhibitory receptors in the brain, it will be important in future studies to establish if L-Cys can serve as signaling mediator regulating the activity of different GABA<sub>A</sub> receptors subtypes in the CNS.

Involves human subjects:

If yes: Informed consent & ethics approval achieved:

=> if yes, please ensure that the info "Informed consent was achieved for all subjects, and the experiments were approved by the local ethics committee." is included in the Methods.

ARRIVE guidelines have been followed:

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Conflicts of interest: None

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### Conflict of interest disclosure

None

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**Table 1**

	EC <sub>50</sub> (μM)	nH	Max Response (%)	n
Control	0.92 ± 0.07	2.26 ± 0.05	99.34 ± 0.28	11
+ 50 μM L-Cys	1.21 ± 0.14	2.06 ± 0.09	100.60 ± 0.83	6
+ 200 μM L-Cys	2.24 ± 0.12	2.09 ± 0.09	102.30 ± 0.97	5
+ 1 mM L-Cys	3.95 ± 0.17	2.62 ± 0.12	102.20 ± 0.82	5
+ 2 mM L-Cys	6.71 ± 0.14	3.13 ± 0.09	101.00 ± 0.45	6

**Legends****Fig 1**

Antagonistic effects of L-Cys on responses mediated by GABA<sub>A</sub>ρ1 receptors expressed in *Xenopus laevis* oocytes. Representative traces of GABA<sub>A</sub>ρ1 receptor-mediated Cl<sup>-</sup> currents elicited by 1 μM GABA. Applications of 20 μM (**A**) and 200 μM (**B**) L-Cys, indicated as bars, were performed at the plateau of the GABA-evoked responses. (**C**) Representative traces of currents recorded during repeated co-applications of 1 μM GABA and 200 μM L-Cys. L-Cys was applied 1 min before the first co-application with GABA and kept continuously present until the end of the third GABA application. Recording of the GABA responses were separated by 2 min interval. In this and the following figures oocytes were voltage-clamped at -70 mV. Scale bars indicate current amplitude (y-axis) and time (x-axis).

**Fig 2**

Analysis of L-Cys effects on GABA<sub>A</sub>ρ1 receptors. (**A**) C-R curves for GABA, in the presence and absence (●) of 50 μM (□), 200 μM (▲), 1 mM (▽) and 2 mM (■) L-Cys. Response amplitudes were expressed as a fraction of maximal current values, evoked at 100 μM GABA (*n* = 5-11). (**B**) Inhibition produced by 200 μM L-Cys on GABA<sub>A</sub>ρ1 receptor currents induced by increasing concentrations of GABA. Data were expressed as percentage of inhibition (*n* = 5-13). (**C**) Inhibition curves performed for GABA<sub>A</sub>ρ1 receptor currents elicited by 0.3 μM (▲), 1 μM (■) and 10 μM (●) GABA in the presence of increasing

concentrations of L-Cys. Data were expressed as percentage of change relative to control values ( $n = 5-10$ ). **(D)** Representative trace of an I-V relationship for GABA<sub>A</sub>ρ1 receptor responses evoked by 1 μM GABA in the presence (▲) or absence (●) of 200 μM L-Cys. The reversal potential of GABA currents was not modified.

### Fig 3

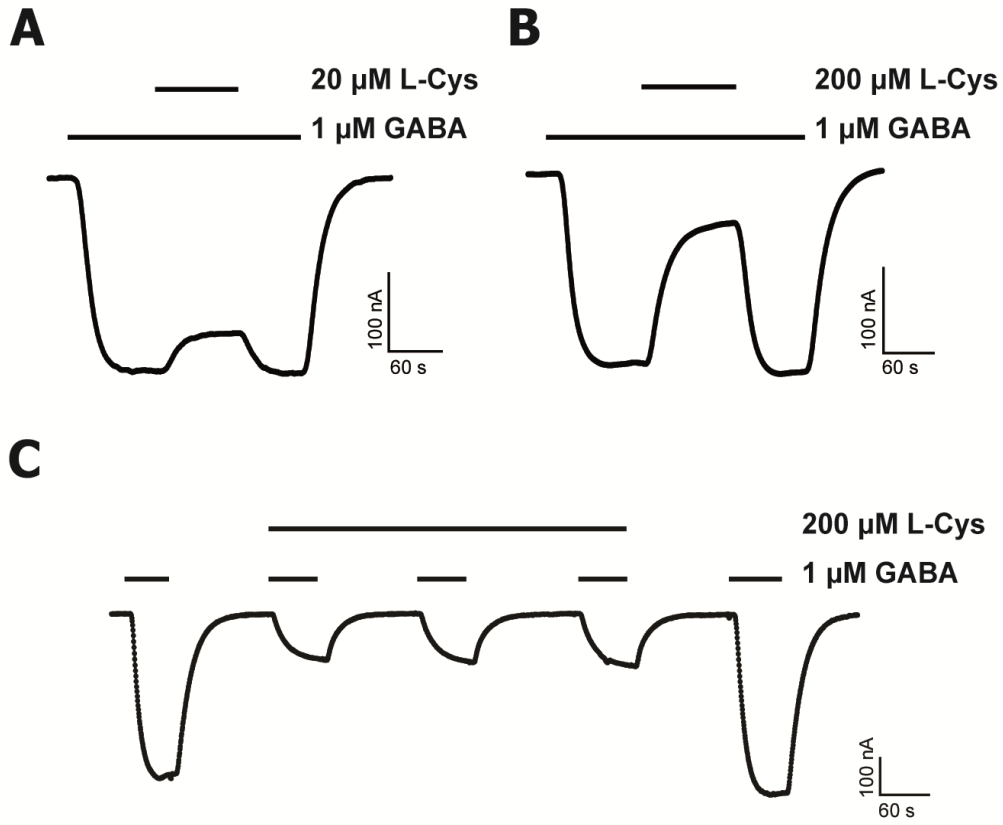
Cysteine thiols are not involved in L-Cys inhibition of GABA<sub>A</sub>ρ1 receptor-mediated responses. **(A)** Representative trace of two successive GABA<sub>A</sub>ρ1 responses elicited by 1 μM GABA. The control response was followed by a pre-treatment of the oocyte with 30 μM NEM (for 150 sec). Irreversible cysteine blockade by NEM did not prevent the inhibition induced on GABA responses by applications of 200 μM L-Cys. **(B)** Data expressed as percentage of change relative to control values. Equivalent results were obtained using 30 μM or 300 μM NEM ( $n = 5-6$ ).

### Fig 4

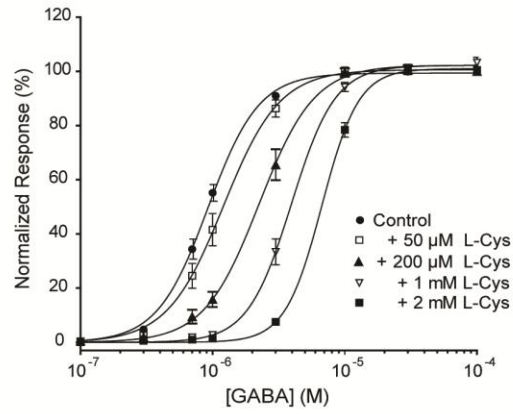
L-Cystine and L-Homocysteine did not produce effects on GABA<sub>A</sub>ρ1 receptor-mediated responses. Representative traces of GABA<sub>A</sub>ρ1 responses elicited by 0.3 μM GABA during application of 200 μM L-Cystine **(A)** and 200 μM L-Homocystine **(B)**.

### Table I

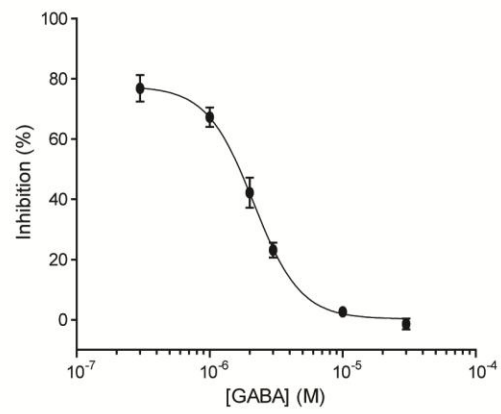
Parameters estimated from the fit of C-R curves for GABA, performed in the absence or presence of L-Cys. EC<sub>50</sub> is the concentration that evokes a half maximal response,  $nH$  the Hill coefficient and  $n$  the number of data sets. Data are the mean ± S.E.M.



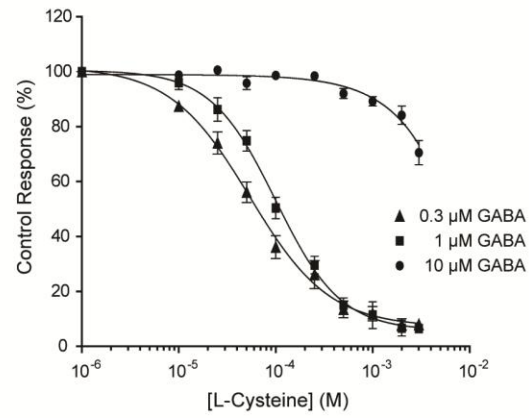
**A**



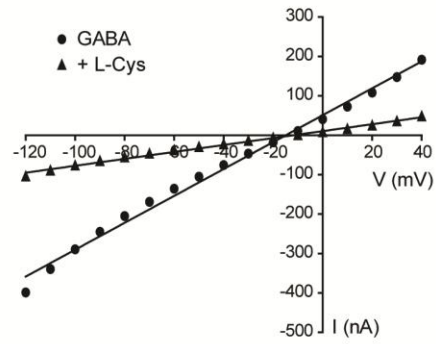
**B**

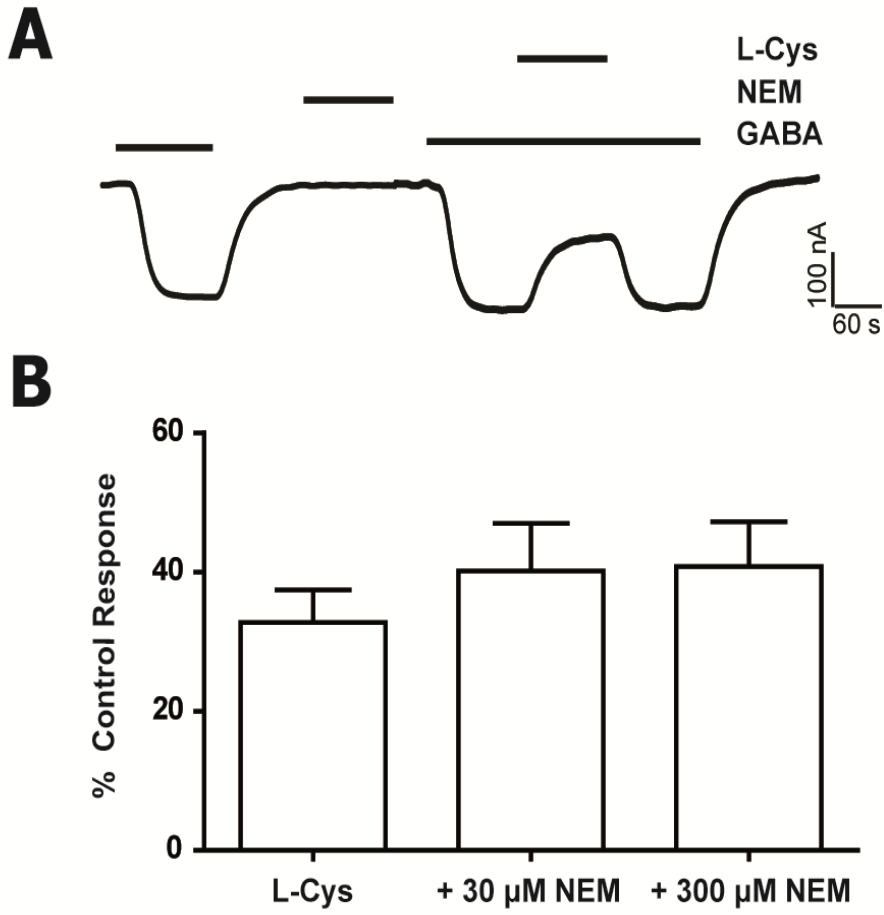


**C**

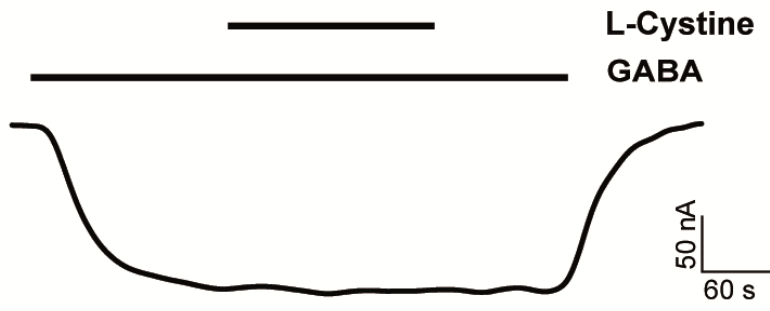


**D**





**A**



**B**

