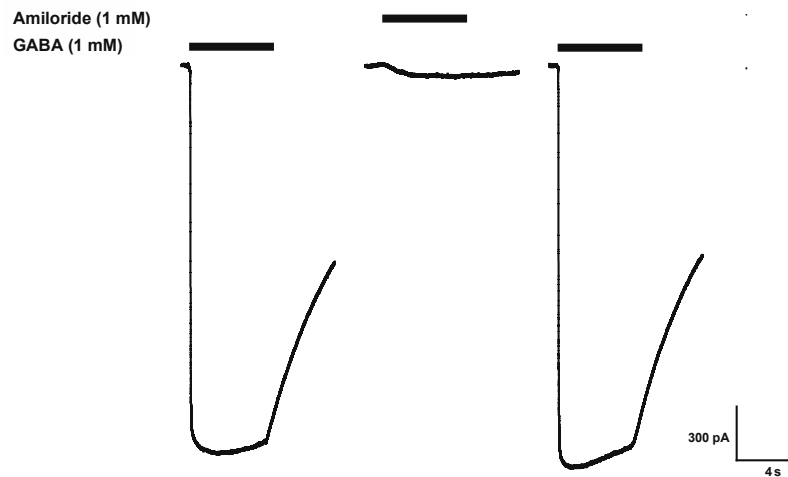


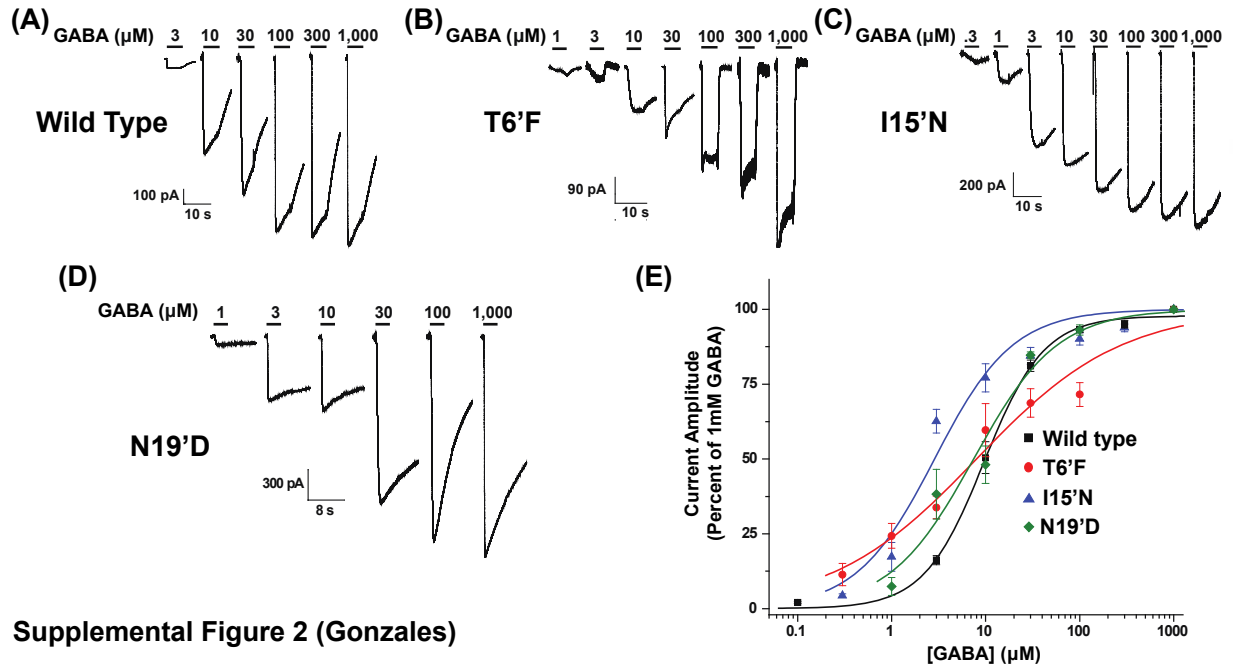
**Amiloride and GMQ allosteric modulation of the GABA-A  $\rho 1$  receptor: influences of the intersubunit site.**

Heather D. Snell and Eric B. Gonzales

SUPPLEMENTAL FIGURE 1

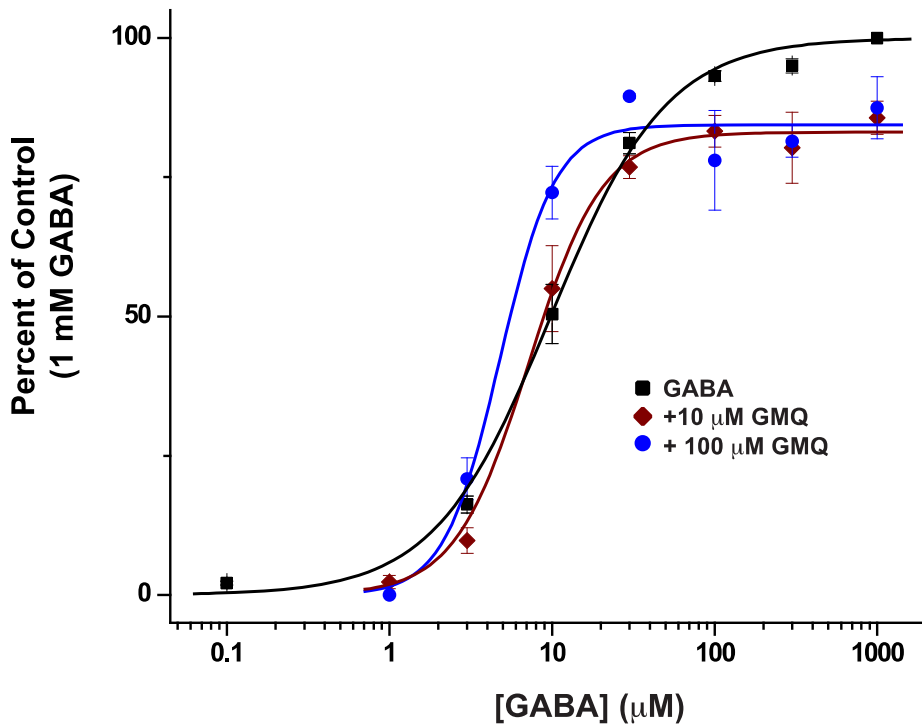


Supplemental Figure 1. Amiloride exhibits slight agonist activity in human GABA-A  $\rho 1$  receptors. Whole cell patch clamp electrophysiology recordings are shown. Patch-clamp recording traces of application of GABA (1 mM) used for control followed by application of amiloride (1mM). Current of the GABA-A  $\rho 1$  receptor was observed above an established cutoff of 20 pA ( $n \geq 4$ ).



Supplemental Figure 2. Concentration-response profile comparison of wild type hGABA-A  $\rho 1$ , T6'F, I15'N, and N19'D mutant receptors, transiently expressed in HEK293T cells.

(A) Representative traces of wild type hGABA-A  $\rho 1$ , (B) hGABA-A  $\rho 1$  T6'F, (C) hGABA-A  $\rho 1$  I15'N, and (D) hGABA-A  $\rho 1$  N19'D mutant receptors are shown. All activation currents generated by 5 second (s) exposures to increasing concentrations of GABA. (E) Summary of concentration-response profiles of GABA-mediated current in wild type hGABA-A  $\rho 1$ , T6'F, I15'N, and N19'D mutant receptors compared to the maximal response (1,000  $\mu\text{M}$  GABA). The determined GABA  $\text{EC}_{50}$ s for WT, T6'F, I15'N, and N19'D are  $9.4 \pm 0.1 \mu\text{M}$ ,  $8.0 \pm 1.7 \mu\text{M}$ ,  $2.9 \pm 0.6 \mu\text{M}$ , and  $7.4 \pm 1.5 \mu\text{M}$ , respectively. The respective Hill coefficients are  $1.1 \pm 0.2$ ,  $0.6 \pm 0.1$ ,  $1.0 \pm 0.2$ , and  $1.0 \pm 0.2$  respectively. Data is presented as the mean  $\pm$  SEM, with a sample size of  $n \geq 4$  cells.



**Supplemental Figure 3 (Gonzales)**

Supplemental Figure 3. Concentration-response profiles of increasing GABA concentrations in the presence of 10 µM and 100 µM GMQ. Each co-application was normalized to a maximal response of GABA (1,000 µM) in the absence of GMQ. The GABA + 10 µM GMQ and GABA + 100 µM GMQ  $EC_{50}$  values were  $7.2 \pm 0.6$  µM and  $4.7 \pm 0.9$  µM, respectively with Hill coefficients of  $1.9 \pm 0.3$  and  $2.6 \pm 0.7$ , respectively. Both GABA and GMQ concentration-response profiles have a reduced maximal efficacy as compared to the control GABA profile. Data are represented as the mean  $\pm$  SEM of a sample size of  $n \geq 4$  cells for each concentration evaluated.