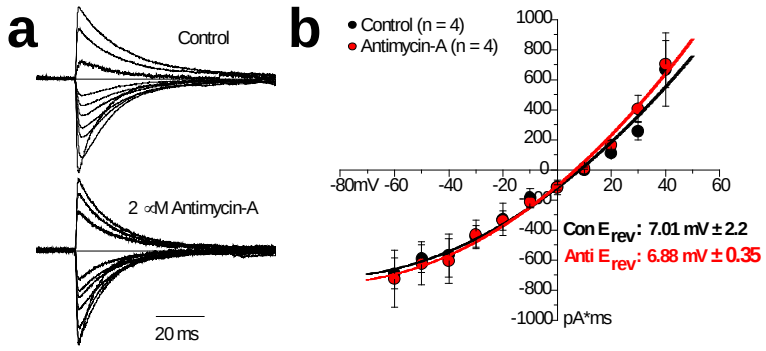
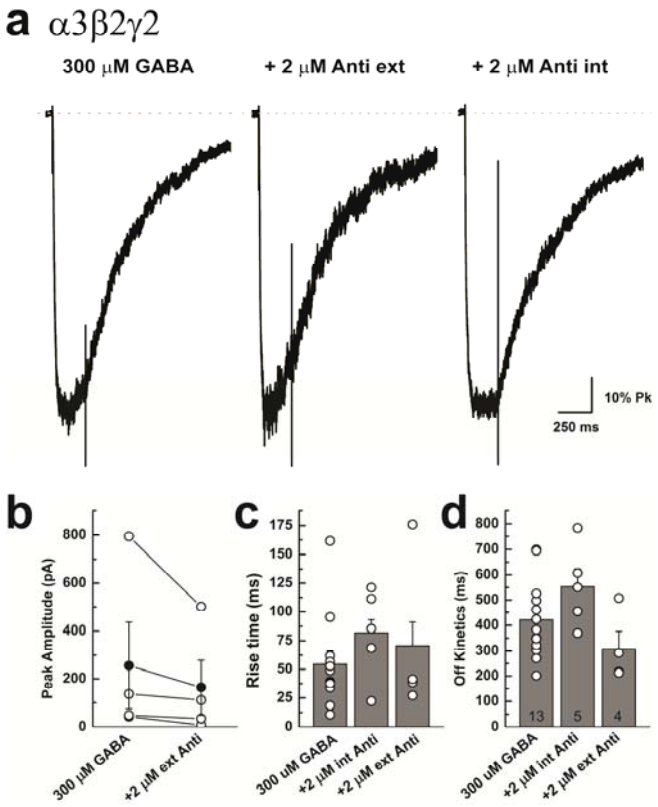
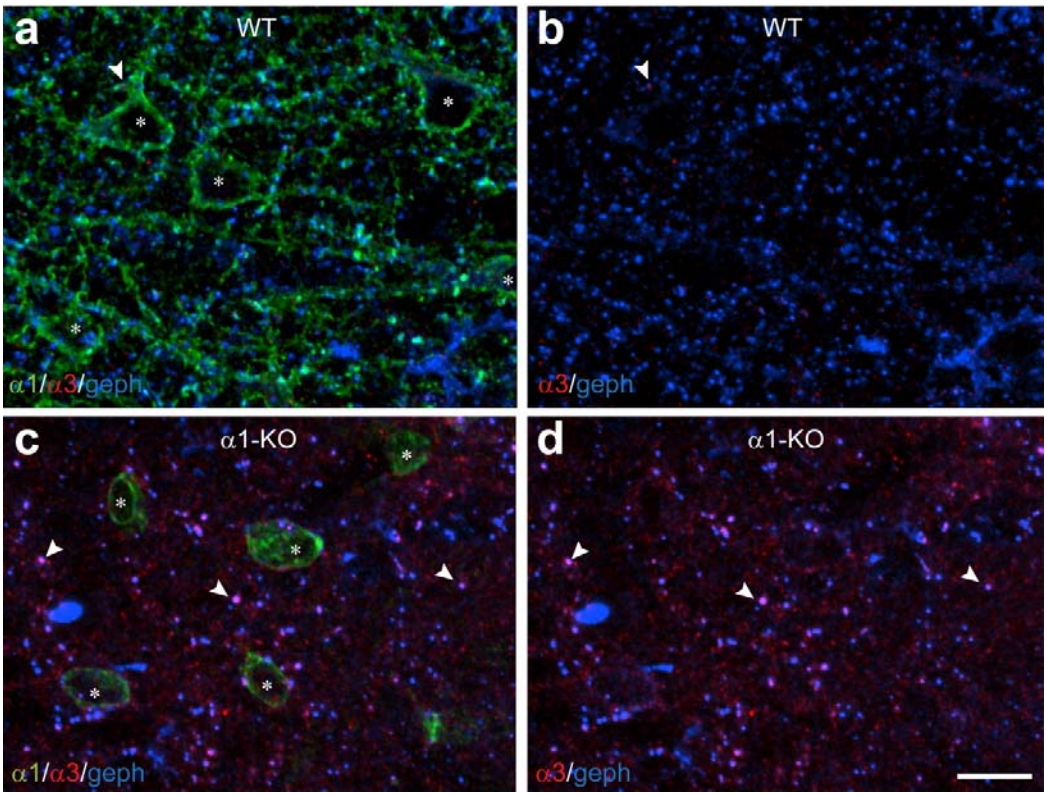
Supplementary figures



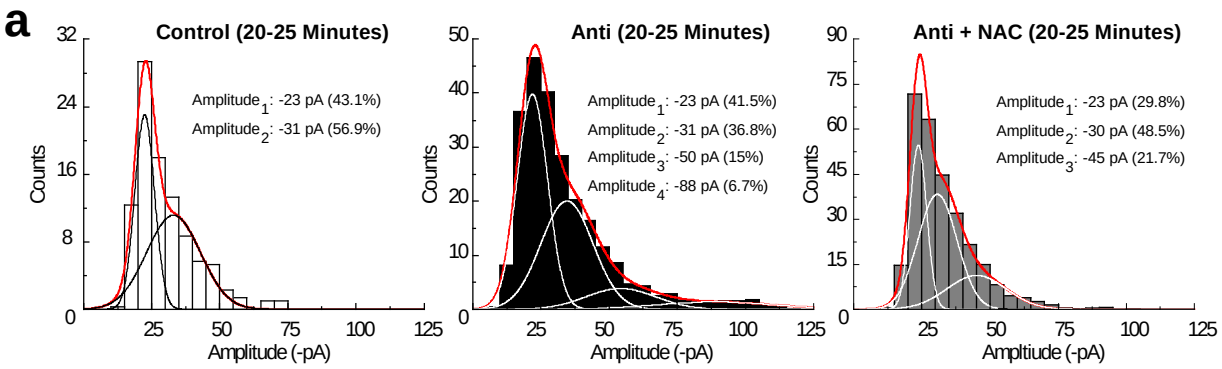
Supplementary Figure 1. Antimycin-A does not shift the chloride equilibrium potential. (**a**) Typical experiment showing averaged mIPSCs at a range of membrane potentials (range: -80 mV to +40 mV, 20 mV increments) in the presence (red circles, n = 4) or absence (black circles, n = 4) of 2 μM antimycin-A. (**b**) Equilibrium potential for chloride ions (i.e. E_{Cl}) estimated by measurement of the $GABA_A$ receptor reversal potential from current-voltage relationships was similar in the presence (red circles, E_{rev}: + 6.88 ± 0.35 mV, P = 0.96) or absence (black circles, E_{rev}: + 7.01 ± 2.2 mV) of antimycin-A. Error bars, ± s.e.m.



Supplementary Figure 2. Antimycin-A does not directly affect α3β2γ2 $GABA_A$ receptors. (**a**) Typical electrophysiological responses for α3β2γ2 receptors evoked by 300 μM GABA (250 ms). The left and middle panels show GABA responses before (left) and during (middle) external application of 2 μM antimycin-A (patch # 130719p2). In the right panel, 2 μM antimycin-A was included in the internal solution (patch # 130715p2). (**b**) Peak amplitudes for responses evoked by 300 μM GABA (250 ms) before and during external application of 2 μM antimycin-A. The slight decrease in amplitude is due to normal response rundown. Empty circles represent individual patches (n = 4), black circles represent the mean. Error bars, s.e.m. (**c**, **d**) Bar graphs showing rise times (**c**) and off-kinetics (**d**) of individual patches (circles, 300 μM GABA, 250 ms). Error bars, s.e.m. Numbers indicate the number of patches.



Supplementary Figure 3. α3-containing $GABA_A$ receptors are more abundant at inhibitory synapses in α1-null mice. (**a** and **b**) Triple immunofluorescence staining of the α1- (green) and α3- (red) $GABA_A$ receptor subunits with gephyrin (blue) in the molecular layer of the cerebellum of wild-type mice. Panels on the right show staining of the α3 subunit and gephyrin only. Note that the intense α1 subunit staining outlines the dendrites of stellate cells (*) and Purkinje cells. Gephyrin clusters are co-localized with the α1 subunit (cyan) with the α3 subunit-immunofluorescence is nearly undetectable with the exception of a single cluster (arrowhead). (**c** and **d**) In α1-KO mice, loss of the α1 subunit is compensated for by an increase in α3 subunit-immunoreactivity which forms clusters with gephyrin (arrowheads). Some of these clusters are observed at the surface of stellate cell somata. Residual α1 subunit staining represents truncated protein that forms an N-terminal epitope that is recognized by the antibody. Note the decreased density of gephyrin clusters compared to wild-type mice. Scale bar, 10 μm.



Supplementary Figure 4. N-acetylcysteine inhibits the antimycin-A induced increase in mIPSC response amplitudes of α1-KO animals. (**a**) Amplitude distributions of mIPSCs observed during the last 5 minutes (i.e. 20-25 minutes) of stellate cell recordings from α1-KO mice in the absence (left, n = 5) and presence (middle, n = 5) of 2 μM antimycin-A (Anti) as well as in the concomitant presence of 2 μM antimycin-A and 1 mM N-acetylcysteine (NAC, right, n = 6). Averaged data has been fit with the sum of 2 to 4 Gaussian functions (red line) with individual Gaussians shown in either black (left) or white (middle and right panels).

Supplementary Table 1

Kinetics of α1β2γ2 and α3β2γ2 $GABA_A$ receptors.

1 ms pulse

α1β2γ2	Components	t1	±	s.e.m.	%A1	t2	±	s.e.m.	%A2	t3	±	s.e.m.	%A3	n
10 mM	**2**	2.3	±	n/a	45	55.4		n/a	55					1
	3	3.1	±	0.3	42	30.2	±	2.9	30	152.9	±	15.7	28	11
300 µM	**2**	3.5	±	0.5	45	86.5		11.2	55					2
	3	2.9	±	0.2	40	29.5	±	2.2	30	153.3	±	9.5	30	14
α3β2γ2	**Components**	**t1**	**±**	**s.e.m.**	**%A1**	**t2**	**±**	**s.e.m.**	**%A2**	**t3**	**±**	**s.e.m.**	**%A3**	**n**
10 mM	**2**	40.2	±	n/a	23	1586	±	n/a	77					1
	3	2.9	±	0.4	23	53.3	±	13.0	23	728.1	±	252.6	54	7
300 µM	**2**	6.5	±	1.7	36	258.5	±	38.6	64					7
	3	2.3	±	n/a	30	22.7	±	n/a	17	393.5	±	n/a	53	1

250 ms pulse

α1β2γ2	Components	t1	±	s.e.m.	%A1	t2	±	s.e.m.	%A2	t3	±	s.e.m.	%A3	n
10 mM	**2**	3.9	±	0.7	55	154.9	±	33.5	45					4
	3	2.3	±	0.3	37	17.6	±	3.4	32	258.4	±	61.1	31	6
300 µM	**2**	4.2	±	0.6	53	174.7	±	70.2	47					8
	3	2.0	±	0.3	23	15.3	±	2.3	33	317.2	±	90.1	45	9
α3β2γ2	**Components**	**t1**	**±**	**s.e.m.**	**%A1**	**t2**	**±**	**s.e.m.**	**%A2**	**t3**	**±**	**s.e.m.**	**%A3**	**n**
10 mM	**1**	133	±	26	100									3
	2	14.1	±	4.1	30	283.3	±	101.3	70					5