Opposite actions of alcohol on tonic $GABA_A$ receptor currents mediated by nNOS and PKC activity.

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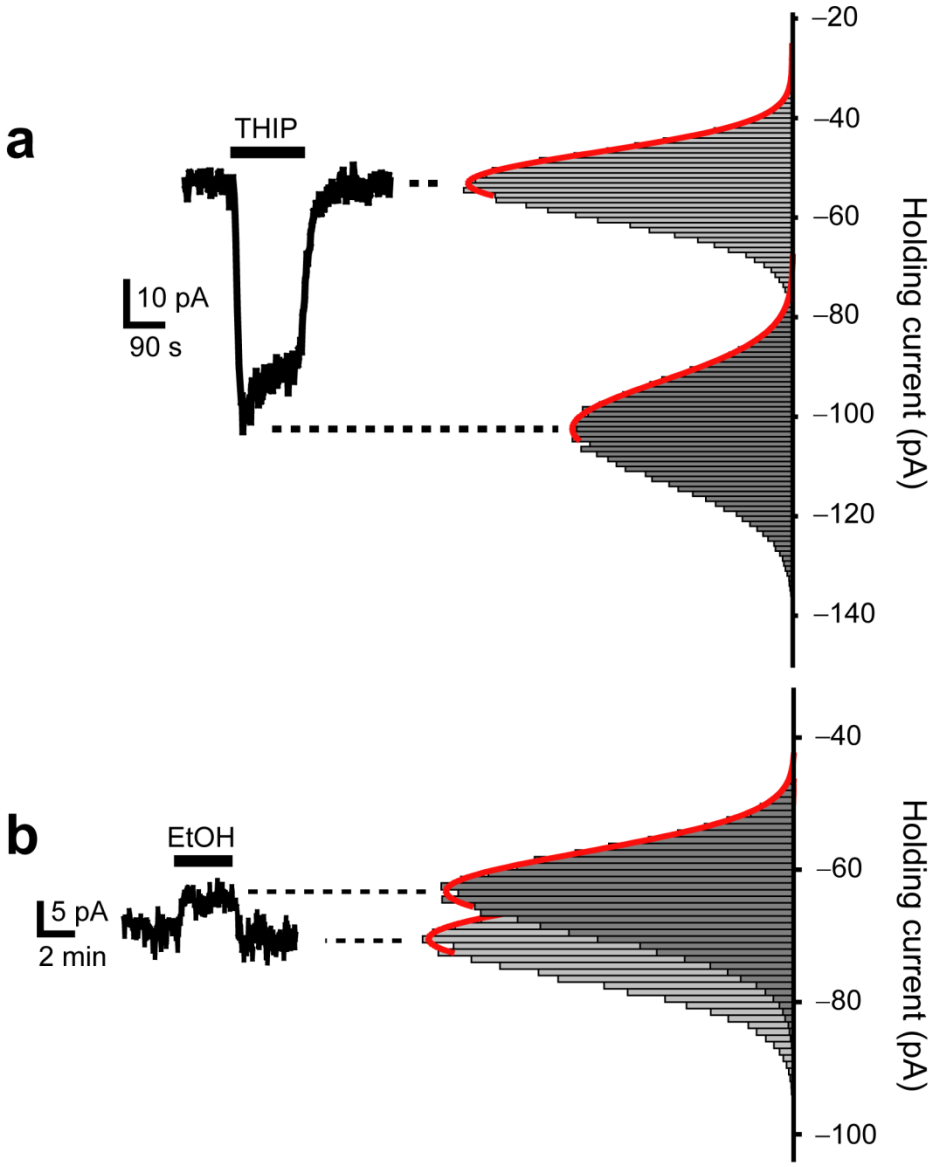
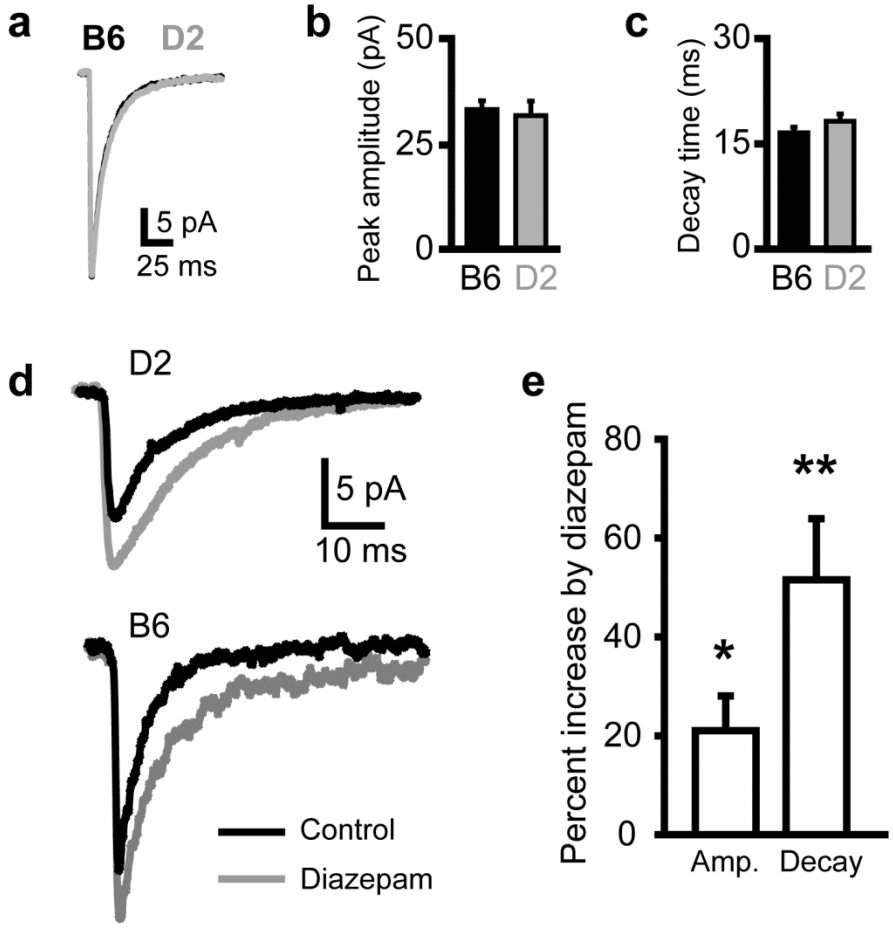
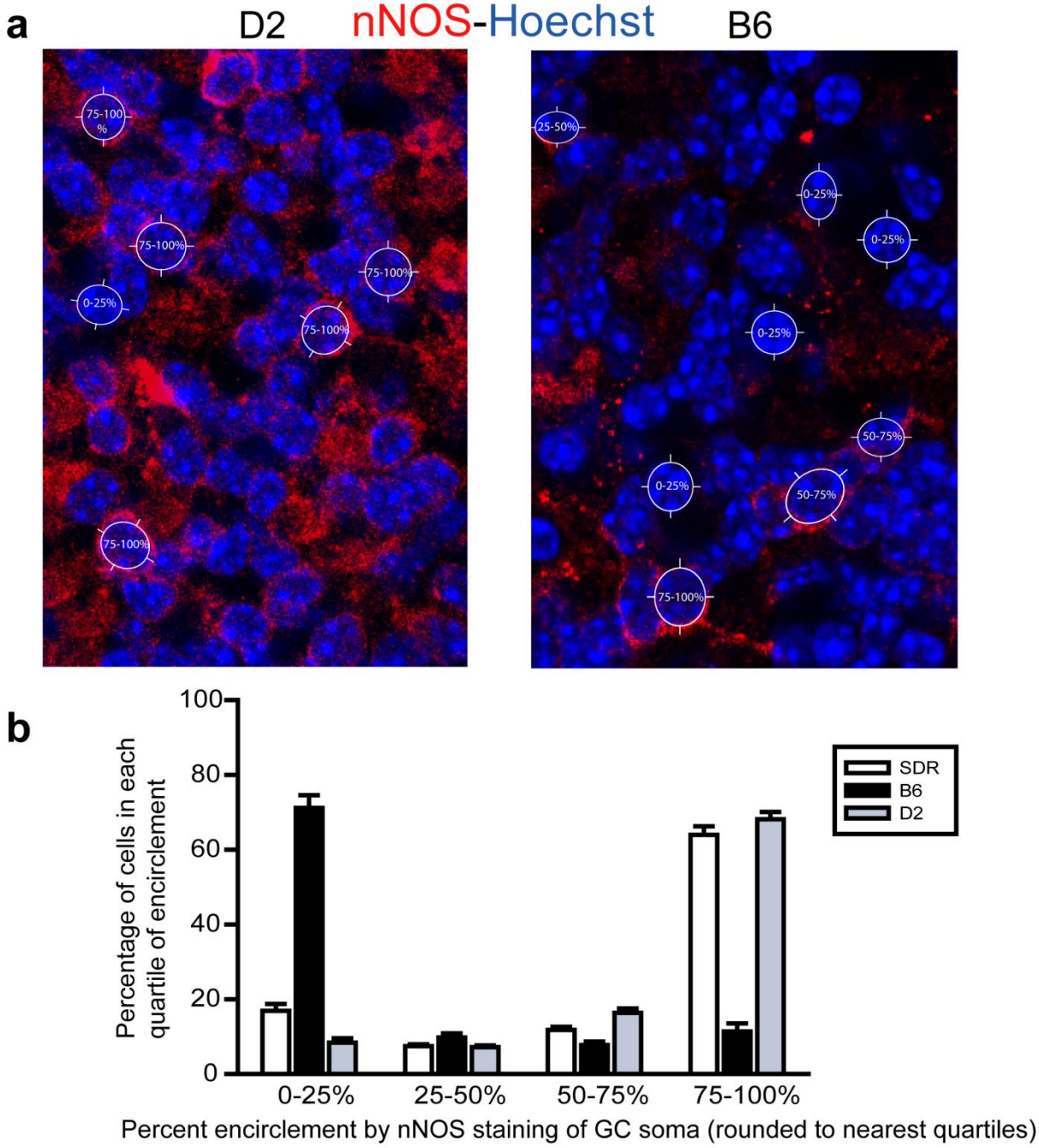


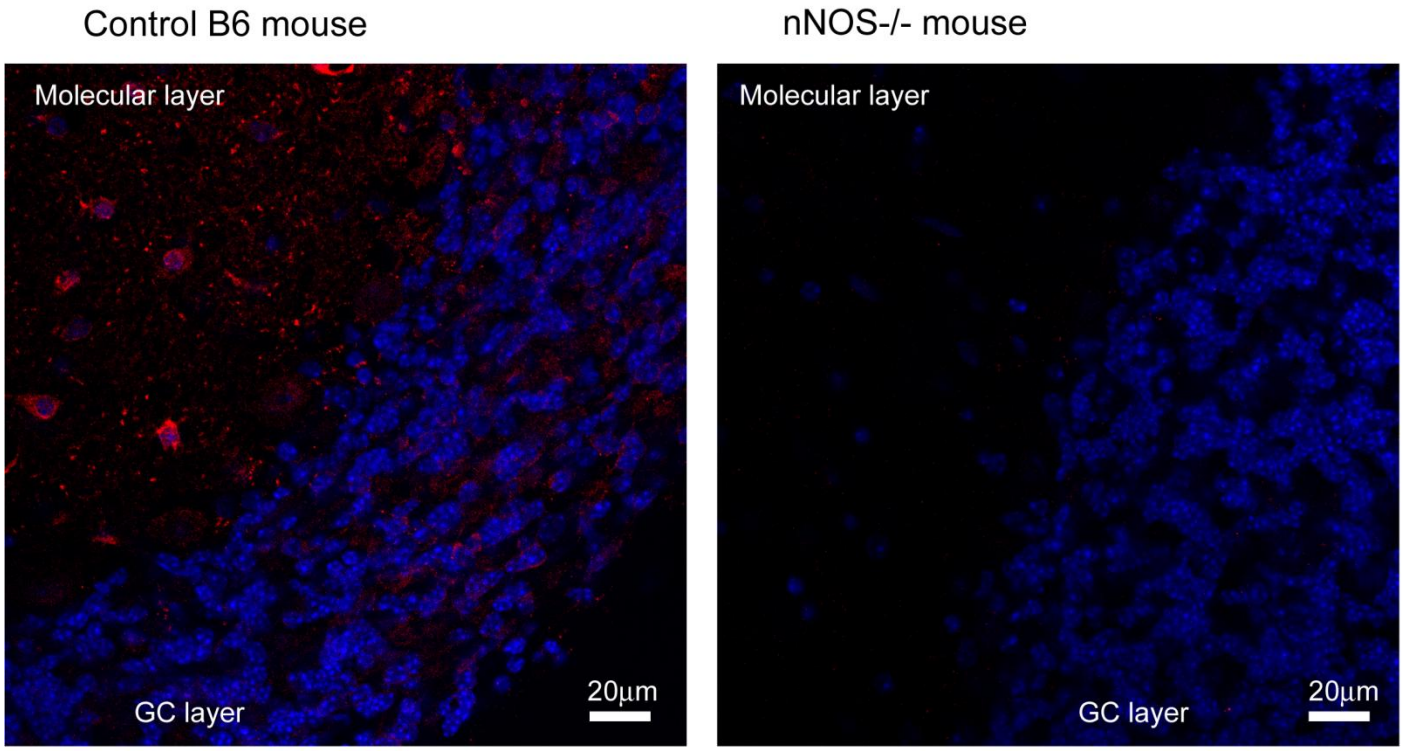
Supplementary Figure 1: Quantification of tonic current magnitudes. a,b. Representative examples of Gaussian fits to all points histograms of tonic current enhancement (**a,** induced by THIP, 300nM) and suppression (**b,** induced by EtOH, 52mM). For quantification of drug-induced changes in tonic current magnitude, all point histograms were generated for 20 second segments of the GC holding current, just prior to drug application, during the peak response to drug (**a,** only for THIP whose large currents showed desensitization) or steady state plateau just prior to wash (**b,** for all drugs other than THIP), and at a point with a post-wash time interval equivalent to the time interval between pre-drug control and drug measurement. Such all point histograms were fitted with a single Gaussian function, from a point 3 pA to the left of the peak value to the rightmost (smallest) value of the histogram distribution, thereby excluding potential skewing of the tonic current measurement by overlapping sIPSCs. The magnitude of drug-induced change was quantified as the difference between the peak of the Gaussian fit of the all point histogram derived for the drug segment and the mean of the peak of the Gaussian fits of the all point histograms derived for the pre- and post-drug segments. Note, as for all figures in this manuscript, to enable clear presentation of the whole time course of such small currents, the traces shown are heavily filtered (1 Hz), but all quantification was done on minimally filtered (10 KHz) raw data traces.



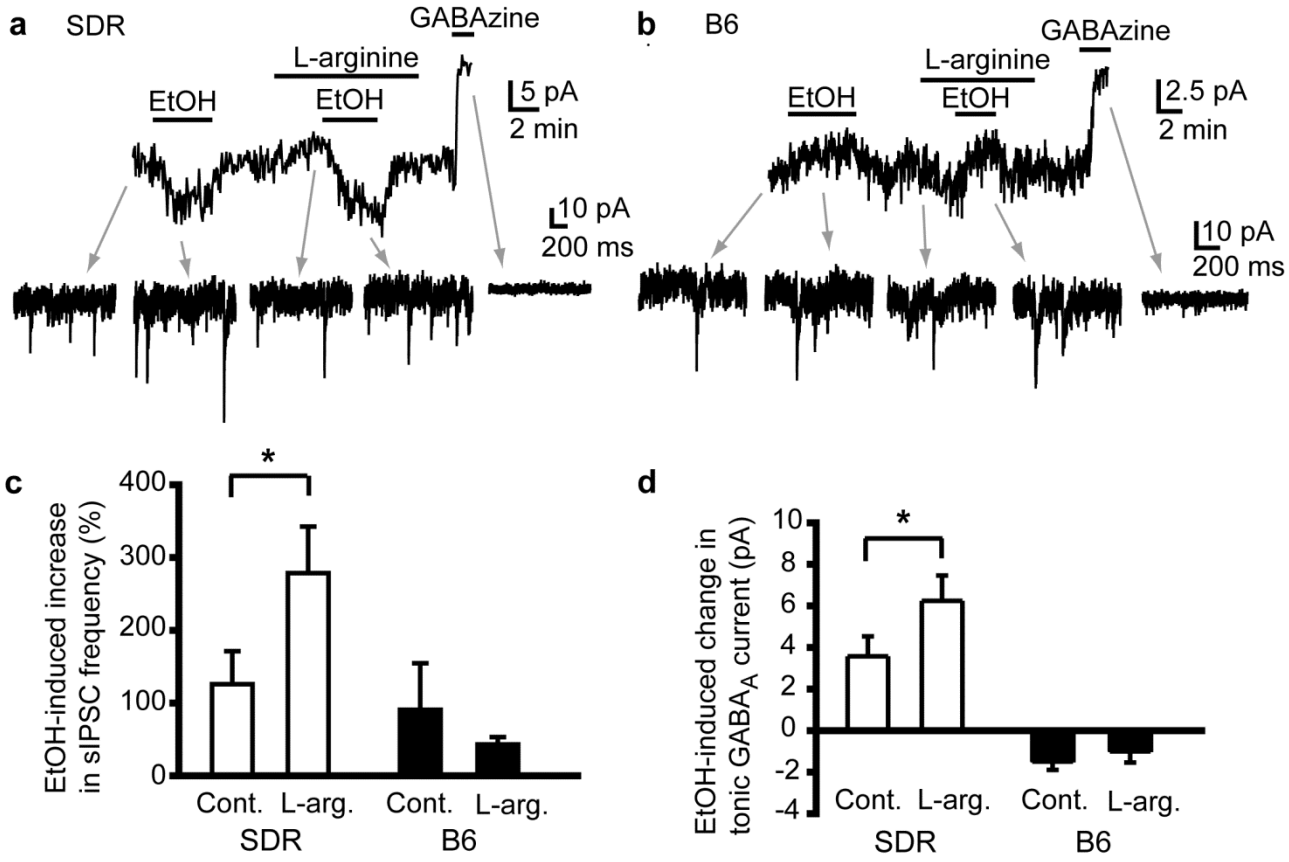
Supplementary figure 2: $GABA_AR$ mediated sIPSCs in B6 and D2 mouse GCs have similar physiological properties and are enhanced by diazepam. a. Overlaid traces show the average of all sIPSCs from B6 (black) and D2 (gray) mouse GCs. **b,c.** Plots display mean amplitude (B6: 33.14 ± 2.07; D2: 31.68 ± 3.47) and 10-90% decay time of sIPSCs (B6: 16.56 ± 0.82; D2: 18.22 ± 1.03) in B6 (n = 14) and D2 (n = 16) mouse GCs. **d.** Representative traces show that diazepam (300nM) increases the amplitude and decay time of sIPSCs in D2 (top) and B6 (bottom) mouse GCs. **e.** Plot of mean percent enhancement by diazepam of sIPSC amplitude and decay time. Percent enhancement was not significantly different between D2 (n = 6) and B6 (n = 7) GCs (amplitude: P = .73; decay: P = .23, unpaired t-tests) so data are combined (% increase in amplitude = 21.04 ± 7.02%, P = .01, signified by *; % increase in decay: 51.53 ± 12.36%, P = .001, n = 13, single-sample t-tests, signified by **).



Supplementary Figure 3: Quantification of GC encirclement by nNOS. a. Representative confocal images of immunocytochemistry for nNOS in D2 (left) and B6 (right) cerebellar granule cell layer with examples of how the percent encirclement of GC somas by nNOS was determined by an experimenter blind to the animal's genotype. Circles divided into quartiles were stretched to closely circumscribe the GC nucleus (stained blue with nuclear stain Hoechst), and each nucleus in the image plain was categorized as being 0-25%, 25-50%, 50-75%, or 75-100% encircled by visibly detectable nNOS staining. Note, for each image the red channel gain was adjusted so that ~5% of the pixels were saturated, thereby normalizing fluorescence emission intensity across slices. **b.** Distribution of percent encirclement is skewed toward the top quartile for SDR and D2 GCs, but to the bottom quartile for B6 GCs. The mean percent encirclement (shown in main figure 6d) is significantly smaller for B6 GCs compared to SDR or D2 GCs.



Supplementary Figure 4: Immunostaining signal for nNOS is absent in nNOS knockout mice. Representative examples of confocally acquired images of cerebellar slices stained with nuclear stain, Hoecsht (blue) and antibody for nNOS (red) from a wildtype B6 mouse (left) and from a mouse in which nNOS was genetically deleted (right; -/-). Images are representative of 3 separate slices for each genotype. Note, because there is relatively little nNOS signal in the GC layer of B6 mice (see main text and fig. 6), the images include a portion of the overlying molecular layer which, in wildtype B6 mice showed more intense staining than the underlying GC layer, highlighting the complete lack of nNOS signal in the nNOS knockout mouse.



Supplementary Figure 5: Providing exogenous NOS substrate increases the EtOH-induced enhancement of GC GABA$_A$R currents in SDRs but not in B6 mice. a. Representative trace showing that adding the NOS substrate, L-arginine (100μM) to the bath increases the degree to which EtOH enhances SDR GC sIPSC frequency (insets) and the tonic GABA$_A$R current magnitude. **b.** Representative trace showing that L-arginine does not affect B6 GC responses to EtOH. **c,d.** Plot of mean percent change in sIPSC frequency (SDRs in white: 125.85 ± 45.41% in control, n = 14, versus 278.53 ± 63.97% in L-arginine, n = 14, **P* = .038; B6s in black: 90.56 ± 64.03% in control, n = 6, versus 42.75 ± 10.33% in L-arginine, n = 6, *P* = .48) (**c**) and mean increase in tonic GABA$_A$R current magnitude (SDRs in white: 3.57 ± 0.96 pA in control, n = 16, versus 6.24 ± 1.22 pA in L-arginine, n = 16, **P* = .04; B6s in black: -1.50 ± 0.40 pA in control, n = 7, versus -1.00 ± 0.53 pA in L-arginine, n = 7, *P* = .48, by paired *t*-tests) (**d**) induced by EtOH (52mM) under control conditions or in the presence of L-arginine (100μM) in the same cell, in SDRs and B6 GCs.