

Supplementary Materials for:

**NEONATAL ETHANOL EXPOSURE TRIGGERS APOPTOSIS IN THE MURINE
RETROSPLENIAL CORTEX: ROLE OF INHIBITION OF NMDA RECEPTOR-DRIVEN
ACTION POTENTIAL FIRING**

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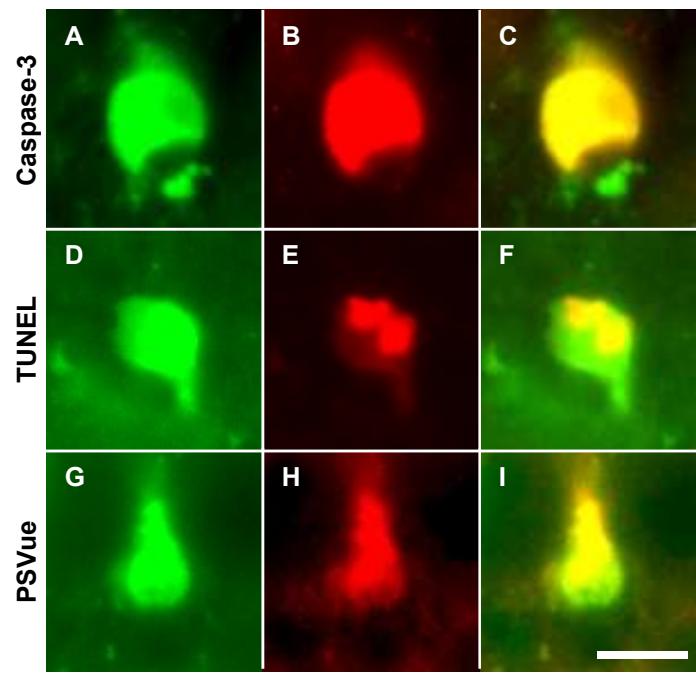
Supplementary Table 1
Supplementary Figures 1 to 5

Supplemental Table 1. Comprehensive collection of all statistical analyses performed.

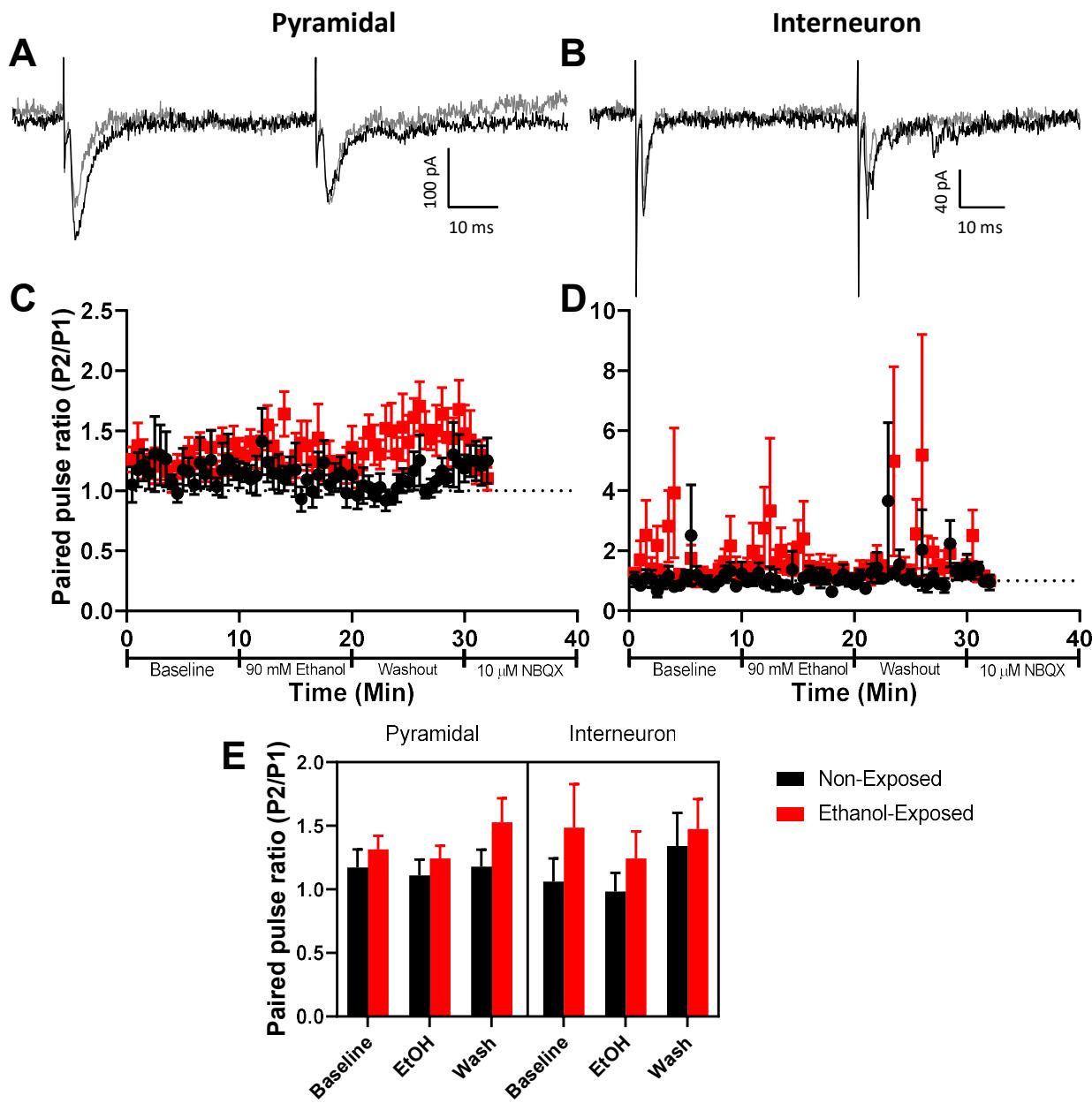
Figure or results section	Experiment	Test	Measure	df	Test statistic	p value	Effect size measure	Effect size	Notes	Passes SW normality test?	
Figure 2	Blood ethanol concentrations during and after vapor chamber exposure	One-way ANOVA	Effect of vapor chamber exposure on BECs	F(5,18)	40.029	<0.0001	Partial eta squared	0.917			
			0 h vs 2 h	t(18)	3.148	0.0278	Hedges' g	6.286	Bonferroni adjusted p-value		
			0 h vs 4 h	t(18)	9.202	<0.0001	Hedges' g	5.152	Bonferroni adjusted p-value	Yes: residuals pass	
			0 h vs 8 h	t(18)	10.140	<0.0001	Hedges' g	6.066	Bonferroni adjusted p-value		
			0 h vs 12 h	t(18)	7.019	<0.0001	Hedges' g	5.494	Bonferroni adjusted p-value		
			0 h vs 24 h	t(18)	0.154	>0.9999	Hedges' g	0.615	Bonferroni adjusted p-value		
		Adjusted rank transform	Interaction (Layer X Time Point)	F(8,63)	5.185	<0.0001	Partial eta squared	0.397			
		Scheirer-Ray-Hare Test	Layer	H(2)	21.760	<0.0001	Eta squared	0.123			
			Time Point	H(4)	33.393	<0.0001	Eta squared	0.308			
			Layer 1 Control vs. 0 h	n/a	0.165	>0.9999	r	0.052	Bonferroni adjusted p-value		
Figure 3I	Caspase cell counts	Dunn's multiple comparison: comparing time points within layer	Layer 1 Control vs. 2 h	n/a	1.034	>0.9999	r	0.327	Bonferroni adjusted p-value	No: residuals fail normality test	
			Layer 1 Control vs. 4 h	n/a	1.530	0.5043	r	0.484	Bonferroni adjusted p-value		
			Layer 1 Control vs. 8 h	n/a	1.835	0.2658	r	0.553	Bonferroni adjusted p-value		
			Layers 2-4 Control vs. 0 h	n/a	0.620	>0.9999	r	0.196	Bonferroni adjusted p-value		
			Layers 2-4 Control vs. 2 h	n/a	2.233	0.1023	r	0.706	Bonferroni adjusted p-value		
			Layers 2-4 Control vs. 4 h	n/a	3.266	0.0044	r	1.033	Bonferroni adjusted p-value		
			Layers 2-4 Control vs. 8 h	n/a	3.563	0.0015	r	1.074	Bonferroni adjusted p-value		
			Layer 5 Control vs. 0 h	n/a	1.240	0.8594	r	0.392	Bonferroni adjusted p-value		
			Layer 5 Control vs. 2 h	n/a	2.439	0.0589	r	0.771	Bonferroni adjusted p-value		
			Layer 5 Control vs. 4 h	n/a	2.729	0.0254	r	0.863	Bonferroni adjusted p-value		
Figure 3J	Caspase interneuron counts	Dunn's multiple comparison: comparing time points within layer	Layer 5 Control vs. 8 h	n/a	4.246	<0.0001	r	1.280	Bonferroni adjusted p-value		
			Adjusted rank transform	Interaction (Layer X Time Point)	F(8,63)	3.880	0.0009	Partial eta squared	0.330		
			Scheirer-Ray-Hare Test	Layer	29.093	<0.0001	Eta squared	0.240			
				H(4)	21.704	0.0002	Eta squared	0.122			
			Layer 1 Control vs. 0 h	n/a	1.298	0.7769	r	0.410	Bonferroni adjusted p-value	No: residuals fail normality test	
			Layer 1 Control vs. 2 h	n/a	0.085	>0.9999	r	0.027	Bonferroni adjusted p-value		
			Layer 1 Control vs. 4 h	n/a	0.830	>0.9999	r	0.262	Bonferroni adjusted p-value		
			Layer 1 Control vs. 8 h	n/a	2.312	0.0832	r	0.697	Bonferroni adjusted p-value		
			Layers 2-4 Control vs. 0 h	n/a	1.447	0.5915	r	0.458	Bonferroni adjusted p-value		
			Layers 2-4 Control vs. 2 h	n/a	2.357	0.0738	r	0.745	Bonferroni adjusted p-value		
Figure 4I	TUNEL cell counts	Dunn's multiple comparison: comparing time points within layer	Layers 2-4 Control vs. 4 h	n/a	3.101	0.0077	r	0.981	Bonferroni adjusted p-value	No: residuals fail normality test	
			Layers 2-4 Control vs. 8 h	n/a	2.879	0.0160	r	0.868	Bonferroni adjusted p-value		
			Layer 5 Control vs. 0 h	n/a	1.488	0.5466	r	0.471	Bonferroni adjusted p-value		
			Layer 5 Control vs. 2 h	n/a	3.018	0.0102	r	0.954	Bonferroni adjusted p-value		
			Layer 5 Control vs. 4 h	n/a	2.729	0.0254	r	0.863	Bonferroni adjusted p-value		
			Layer 5 Control vs. 8 h	n/a	3.527	0.0017	r	1.063	Bonferroni adjusted p-value		
Figure 4J	TUNEL interneuron counts	Dunn's multiple comparison: comparing time points within layer	Adjusted rank transform	Interaction (Layer X Time Point)	F(2,30)	16.215	<0.0001	Partial eta squared	0.519		
			Scheirer-Ray-Hare Test	Layer	3.008	0.222	Eta squared	<0.001			
				H(1)	22.249	<0.0001	Eta squared	0.575			
			Layer 1 Control vs. 8 h	U(n1=n2=6)	4	0.0780	r	0.647	Bonferroni adjusted p-value	No: residuals fail normality test	
			Layers 2-4 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value		
			Layer 5 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value		
Figure 5I	PSVue cell counts	Dunn's multiple comparison: comparing time points within layer	Adjusted rank transform	Interaction (Layer X Time Point)	F(2,30)	5.149	0.0120	Partial eta squared	0.256		
			Scheirer-Ray-Hare Test	Layer	6.980	0.0305	Eta squared	0.066			
				H(1)	19.610	<0.0001	Eta squared	0.487			
			Layer 1 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value	No: residuals fail normality test	
			Layers 2-4 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value		
			Layer 5 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value		
Figure 5J	PSVue interneuron counts	Dunn's multiple comparison: comparing time points within layer	Adjusted rank transform	Interaction (Layer X Time Point)	F(2,30)	44.306	<0.0001	Partial eta squared	0.747		
			Scheirer-Ray-Hare Test	Layer	17.935	0.0001	Eta squared	0.431			
				H(1)	13.616	0.0002	Eta squared	0.287			
			Layer 1 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value	No: residuals fail normality test	
			Layers 2-4 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value		
			Layer 5 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value		
Figure 5J	PSVue interneuron counts	Dunn's multiple comparison: comparing time points within layer	Adjusted rank transform	Interaction (Layer X Time Point)	F(2,30)	19.027	<0.0001	Partial eta squared	0.559		
			Scheirer-Ray-Hare Test	Layer	20.406	<0.0001	Eta squared	0.514			
				H(1)	10.296	0.0013	Eta squared	0.177			
			Layer 1 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value	No: residuals fail normality test	
			Layers 2-4 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value		
			Layer 5 Control vs. 8 h	U(n1=n2=6)	0	0.0066	r	0.832	Bonferroni adjusted p-value		

Figure or results section	Experiment	Test	Measure	df	Test statistic	p value	Effect size measure	Effect size	Notes	Passes SW normality test?
Table 1	AMPA current Characteristics	Mann-Whitney U	AMPA pyramidal rise time	U(n1 = n2 = 8)	29.5	0.7984	r	0.066		Both fail normality test
		Mann-Whitney U	AMPA interneuron rise time	U(n1 = 6, n2 = 7)	14.500	0.3805	r	0.259		Non-exposed fails normality test
		Mann-Whitney U	AMPA pyramidal decay tau	U(n1 = n2 = 8)	31	0.9591	r	0.026		Non-exposed fails normality test
		Mann-Whitney U	AMPA interneuron decay tau	U(n1 = 6, n2 = 7)	18	0.7308	r	0.119		Non-exposed fails normality test
		Unpaired t-test	AMPA pyramidal peak current	t(14)	0.650	0.5262	Hedges' g	0.307		Yes
		Mann-Whitney U	AMPA interneuron peak current	U(n1 = 6, n2 = 7)	12	0.2343	r	0.357		Non-exposed fails normality test
		Unpaired t-test	AMPA pyramidal current density	t(14)	0.439	0.6671	Hedges' g	0.208		Yes
		Unpaired t-test	AMPA interneuron current density	t(11)	1.127	0.2838	Hedges' g	0.591		Yes
		Mann-Whitney U	AMPA pyramidal PPR	U(n1 = n2 = 8)	22	0.3282	r	0.263		Non-exposed fails normality test
		Unpaired t-test	AMPA Interneuron PPR	t(11)	1.343	0.2062	Hedges' g	0.711		Yes
		Mann-Whitney U	AMPA pyramidal membrane resistance	U(n1 = n2 = 8)	26	0.5737	r	0.158		Non-exposed fails normality test
		Unpaired t-test	AMPA interneuron membrane resistance	t(11)	0.315	0.7588	Hedges' g	0.165		Yes
		Unpaired t-test	AMPA pyramidal membrane capacitance	t(14)	0.568	0.5791	Hedges' g	0.268		Yes
		Unpaired t-test	AMPA interneuron membrane capacitance	t(11)	0.948	0.3634	Hedges' g	0.502		Yes
Table 1	NMDA current characteristics	Mann-Whitney U	NMDA pyramidal rise time	U(n1 = 9; n2 = 8)	27	0.4234	r	0.210		Non-exposed fails normality test
		Mann-Whitney U	NMDA interneuron rise time	U(n1 = n2 = 7)	19	0.5350	r	0.188		Non-exposed fails normality test
		Unpaired t-test	NMDA pyramidal decay tau	t(15)	1.104	0.2869	Hedges' g	0.515		Yes
		Mann-Whitney U	NMDA interneuron decay tau	U(n1 = n2 = 7)	21	0.7104	r	0.120		Non-exposed fails normality test
		Unpaired t-test	NMDA pyramidal peak current	t(15)	0.740	0.4705	Hedges' g	0.335		Yes
		Unpaired t-test	NMDA interneuron peak current	t(12)	1.397	0.1878	Hedges' g	0.629		Yes
		Unpaired t-test	NMDA pyramidal current density	t(8,116)	0.839	0.4257	Hedges' g	0.396	Welch's correction for unequal variances	Yes
		Mann-Whitney U	NMDA interneuron current density	U(n1 = n2 = 7)	16	0.3176	r	0.290		Ethanol-exposed fails normality test
		Unpaired t-test	NMDA pyramidal membrane resistance	t(15)	1.024	0.3221	Hedges' g	0.434		Yes
		Mann-Whitney U	NMDA interneuron membrane resistance	U(n1 = n2 = 7)	20	0.6200	r	0.154		Non-exposed fails normality test
		Unpaired t-test	NMDA pyramidal membrane capacitance	t(15)	0.123	0.9035	Hedges' g	0.057		Yes
		Unpaired t-test	NMDA interneuron membrane capacitance	t(12)	0.578	0.5738	Hedges' g	0.289		Yes
Figure 6C	Pyramidal neurons Evoked NMDA current amplitude (effect of 90 mM ethanol)	Repeated measures two-way ANOVA: Effect of 90 mM ethanol application X vapor chamber exposure	Interaction (exposure X acute effect)	F(1,15)	0.056	0.8166	Partial eta squared	0.004		
			Main effect: exposure	F(1,15)	1.426	0.2509	Partial eta squared	0.087	Baseline + Wash Average	Yes: residuals pass
Figure 6D	Interneurons Evoked NMDA current amplitude (effect of 90 mM ethanol)	Repeated measures two-way ANOVA: Effect of 90 mM ethanol application X vapor chamber exposure	Main effect: acute effect	F(1,15)	18.173	0.0007	Partial eta squared	0.548		
			Interaction (exposure X acute effect)	F(1,12)	0.685	0.4241	Partial eta squared	0.054		
Figure 6 C-D	Effect of ethanol on evoked NMDA EPSCs	Repeated measures three-way ANOVA	Main effect: exposure	F(1,12)	0.959	0.3467	Partial eta squared	0.074	Baseline + Wash Average	Yes: residuals pass
			Main effect: acute effect	F(1,12)	10.859	0.0064	Partial eta squared	0.475		
Figure 6 G-H	Pyramidal neurons Evoked AMPA current amplitude (effect of 90 mM ethanol)	Repeated measures two-way ANOVA: Effect of 90 mM ethanol application X vapor chamber exposure	Interaction (acute ethanol X Vapor chamber exposure X cell type)	F(1,27)	0.409	0.5279	Partial eta squared	0.015		
			Interaction (acute ethanol X Vapor chamber exposure)	F(1,27)	0.072	0.7898	Partial eta squared	0.003	Three way ANOVA: Acute ethanol X Vapor chamber exposure X cell type	Yes: residuals pass
			Interaction (acute ethanol X cell type)	F(1,27)	2.354	0.1366	Partial eta squared	0.080		
			Interaction (vapor chamber exposure X cell type)	F(1,27)	0.177	0.6775	Partial eta squared	0.007		
			Main effect: acute ethanol exposure	F(1,27)	26.552	<0.0001	Partial eta squared	0.496		
			Main effect: vapor chamber exposure	F(1,27)	2.156	0.1536	Partial eta squared	0.074		
			Main effect: cell type	F(1,27)	11.018	0.0026	Partial eta squared	0.290		
			Interaction (exposure X acute effect)	F(1,14)	4.033	0.0643	Partial eta squared	0.224		
			Main effect: exposure	F(1,14)	0.400	0.5373	Partial eta squared	0.028	Baseline + Wash Average	Yes: residuals pass
			Main effect: acute effect	F(1,14)	19.664	0.0006	Partial eta squared	0.584		
Figure 6 H	Interneurons Evoked AMPA current amplitude (effect of 90 mM ethanol)	Repeated measures two-way ANOVA: Effect of 90 mM ethanol application X vapor chamber exposure	Interaction (exposure X acute effect)	F(1,11)	0.193	0.6689	Partial eta squared	0.017		
			Main effect: exposure	F(1,11)	1.046	0.3284	Partial eta squared	0.087		
Figure 6 G-H	Effect of ethanol on evoked AMPA EPSCs	Repeated measures three-way ANOVA	Main effect: acute effect	F(1,11)	6.111	0.0310	Partial eta squared	0.357		
			Interaction (acute ethanol X Vapor chamber exposure X cell type)	F(1,25)	2.038	0.1658	Partial eta squared	0.075	Three way ANOVA: Acute ethanol X Vapor chamber exposure X cell type	Yes: residuals pass
			Interaction (acute ethanol X Vapor chamber exposure)	F(1,25)	0.382	0.5421	Partial eta squared	0.015		
			Interaction (acute ethanol X cell type)	F(1,25)	<0.001	0.9852	Partial eta squared	<0.001		
			Interaction (vapor chamber exposure X cell type)	F(1,25)	1.601	0.2175	Partial eta squared	0.060		
			Main effect: acute ethanol exposure	F(1,25)	20.571	0.0001	Partial eta squared	0.451		
			Main effect: vapor chamber exposure	F(1,25)	0.392	0.5372	Partial eta squared	0.015		
			Main effect: cell type	F(1,25)	5.260	0.0305	Partial eta squared	0.174		
			% change after 90 mM ethanol	n/a	3.019	0.0025	r	0.910	Wilcoxon signed-rank test compared to 0	No
Figure 7C	Pyramidal synaptic excitability	One sample t-test or Wilcoxon signed-rank test (% change vs. 0 for both tests) One-way ANOVA: % Change in excitability following drug application	% change after 90 mM ethanol	t(9)	3.093	0.0129	Hedges' g	0.790	One sample t-test compared to 0	Yes
			% change after 5 μM AP5	n/a	2.293	0.0220	r	0.691	Wilcoxon signed-rank test compared to 0	No
			% change after 50 nM NBQX	t(12)	6.031	<0.0001	Hedges' g	1.431	One sample t-test compared to 0	Yes
			Drug	F(3,41)	20.356	<0.0001	Partial eta squared	0.598	Is there a difference in how drugs affected AP#?	
			90 μM ethanol vs 5 μM AP5	t(41)	3.014	0.0264	Hedges' g	1.336	Bonferroni adjusted p-value	
			90 μM ethanol vs 50 nM NBQX	t(41)	7.342	<0.0001	Hedges' g	3.304	Bonferroni adjusted p-value	Yes: residuals pass
			90 μM ethanol vs 5 μM AP5 + 50 nM NBQX	t(41)	1.547	0.7775	Hedges' g	0.668	Bonferroni adjusted p-value	
			5 μM AP5 vs. 50 nM NBQX	t(41)	4.151	0.0010	Hedges' g	1.617	Bonferroni adjusted p-value	
			5 μM AP5 vs. 5 μM AP5 + 50 nM NBQX	t(41)	1.624	0.6718	Hedges' g	0.609	Bonferroni adjusted p-value	
			50 nM NBQX vs. 5 μM AP5 + 50 nM NBQX	t(41)	6.095	<0.0001	Hedges' g	2.293	Bonferroni adjusted p-value	

Figure or results section	Experiment	Test	Measure	df	Test statistic	p value	Effect size measure	Effect size	Notes	Passes SW normality test?
Figure 7D	Interneuron synaptic excitability	One sample t-test or Wilcoxon signed-rank test (% change vs. 0 for both tests)	% change after 90 mM ethanol	t(10)	5.189	0.0004	Hedges' g	1.294	One sample t-test compared to 0	Yes
			% change after 5μM AP5	t(10)	4.868	0.0007	Hedges' g	1.214	One sample t-test compared to 0	Yes
			% change after 50 nM NBQX	n/a	0.652	0.5147	r	0.206	Wilcoxon signed-rank test compared to 0	No
			% change after 5 μM AP5 + 50 nM NBQX	t(10)	7.456	<0.0001	Hedges' g	1.859	One sample t-test compared to 0	Yes
			Drug	$\chi^2(3)$	18.377	<0.0001	Partial eta squared	0.320	Is there a difference in how drugs affected AP#?	
		Dunn's multiple comparison: Comparing how drugs changed AP#	90 μM ethanol vs 5 μM AP5	n/a	0.331	>0.9999	r	0.071	Bonferroni adjusted p-value	
			90 μM ethanol vs 50 nM NBQX	n/a	2.686	0.0433	r	0.586	Bonferroni adjusted p-value	No: residuals fail normality test
			90 μM ethanol vs 5 μM AP5 + 50 nM NBQX	n/a	1.605	0.6507	r	0.342	Bonferroni adjusted p-value	
			5 μM AP5 vs. 5 μM AP5 + 50 nM NBQX	n/a	2.363	0.1088	r	0.516	Bonferroni adjusted p-value	
			50 nM NBQX vs. 5 μM AP5 + 50 nM NBQX	n/a	1.936	0.3169	r	0.413	Bonferroni adjusted p-value	
Figure 7 C-D	Effect of ethanol on action potential number	Repeated measures two-way ANOVA	Interaction: Acute ethanol X cell type	F(1,20)	14.860	0.0010	Partial eta squared	0.426	Did 90 mM inhibit action potentials differently between cell types?	
			Main effect: Acute ethanol	F(1,20)	124.647	<0.0001	Partial eta squared	0.452		Yes: residuals pass
			Main effect: Cell type	F(1,20)	14.860	0.0010	Partial eta squared	0.426		
Supplemental figure 2C	AMPA PPR	Repeated measures two-way ANOVA: Effect of 90 mM ethanol application X vapor chamber exposure	Interaction (exposure X acute effect)	F(2,28)	0.781	0.4678	Partial eta squared	0.053	Yes: residuals pass	
			Main effect: exposure	F(1,14)	1.663	0.2077	Partial eta squared	0.114		
			Main effect: acute effect	F(2,28)	0.202	0.1136	Partial eta squared	0.106		
Supplemental figure 2D	AMPA PPR	Repeated measures two-way ANOVA: Effect of 90 mM ethanol application X vapor chamber exposure	Interaction (exposure X acute effect)	F(2,22)	0.904	0.4194	Partial eta squared	0.076	Yes: residuals pass	
			Main effect: exposure	F(1,11)	0.714	0.4162	Partial eta squared	0.061		
			Main effect: acute effect	F(2,22)	3.757	0.0395	Partial eta squared	0.255		
Supplemental Figure 2E	Effect of ethanol on AMPA PPR	Repeated measures three-way ANOVA	Interaction (phase X vapor chamber exposure X cell type)	F(2,50)	1.524	0.2278	Partial eta squared	0.057	Three way ANOVA: Phase(Baseline, Acute ethanol, Wash) X Vapor chamber exposure X cell type	
			Interaction (phase X vapor chamber exposure)	F(2,50)	0.173	0.8414	Partial eta squared	0.007		Yes: residuals pass
			Interaction (phase X cell type)	F(2,50)	0.379	0.6864	Partial eta squared	0.015		
			Main effect: phase	F(2,50)	5.256	0.0085	Partial eta squared	0.174		
			Main effect: vapor chamber exposure	F(1,25)	2.041	0.1655	Partial eta squared	0.075		
		Multiple comparison	Main effect: cell type	F(1,25)	0.002	0.968	Partial eta squared	<0.001	Phase(Baseline, Acute ethanol, Wash) X Vapor chamber exposure X cell type	
			Baseline vs. ethanol	t(50)	1.560	0.3754	Hedges' g	0.230		Bonferroni adjusted p-value
			Baseline vs. Wash	t(50)	1.682	0.2965	Hedges' g	0.214		
			ethanol vs. Wash	t(50)	3.242	0.0064	Hedges' g	0.482		
			Interaction (phase X current injected)	F(1,629,13,030)	2.316	0.1437	Partial eta squared	0.224		Yes: residuals pass
Supplemental Figure 3	Effect of ethanol exposure on mini EPSC characteristics: pyramidal neurons	Unpaired t-test	mini EPSC amplitude	t(8,545)	1.935	0.0867	Hedges' g	0.865	Welch's correction for unequal variances	Yes
			mini EPSC frequency	t(7,454)	1.207	0.264	Hedges' g	0.601	Welch's correction for unequal variances	Yes
		Mann-Whitney U	mini EPSC decay tau	U(n=7,n2=9)	9	0.0164	r	0.596	Ethanol-exposed fails normality test	
Supplemental Figure 4C	Effect of ethanol on Intrinsic excitability: Pyramidal neurons	Unpaired t-test	mini EPSC amplitude	t(13)	1.293	0.2185	Hedges' g	0.627		Yes
		Mann-Whitney U	mini EPSC frequency	U(n=7,n2=8)	27	0.9551	r	0.030	Ethanol-exposed fails normality test	
		Mann-Whitney U	mini EPSC decay tau	U(n=7,n2=8)	27	0.9551	r	0.030		Non-exposed fails normality test
Supplemental Figure 4D	Effect of ethanol on Intrinsic excitability: Interneurons	Repeated measures two-way ANOVA	Interaction (phase X current injected)	F(1,423,8,539)	0.761	0.4530	Partial eta squared	0.113	Greenhouse-Geisser corrected p-value and F-ratio	
		Interaction (phase X current injected)	Main effect of phase (baseline, ethanol, wash)	F(1,889,11,340)	3.395	0.0534	Partial eta squared	0.394		Yes: residuals pass
		Interaction (phase X current injected)	Main effect of current injected (200,400,600 pA)	F(1,064,6,386)	340.6	<0.0001	Partial eta squared	0.983		
Supplemental Figure 5A	Mimicking 90 mM ethanol inhibition of NMDA currents with 5 μM AP5	Unpaired t-test	% inhibition: 90 mM ethanol vs. 5 μM AP5	t(14)	1.209	0.2468	Hedges' g	0.593	Yes	
Supplemental Figure 5B	Mimicking 90 mM ethanol inhibition of non-NMDA currents with 50 nM NBQX	Unpaired t-test	% inhibition: 90 mM ethanol vs. 50 nM NBQX	t(11)	0.667	0.5183	Hedges' g	0.389		Yes

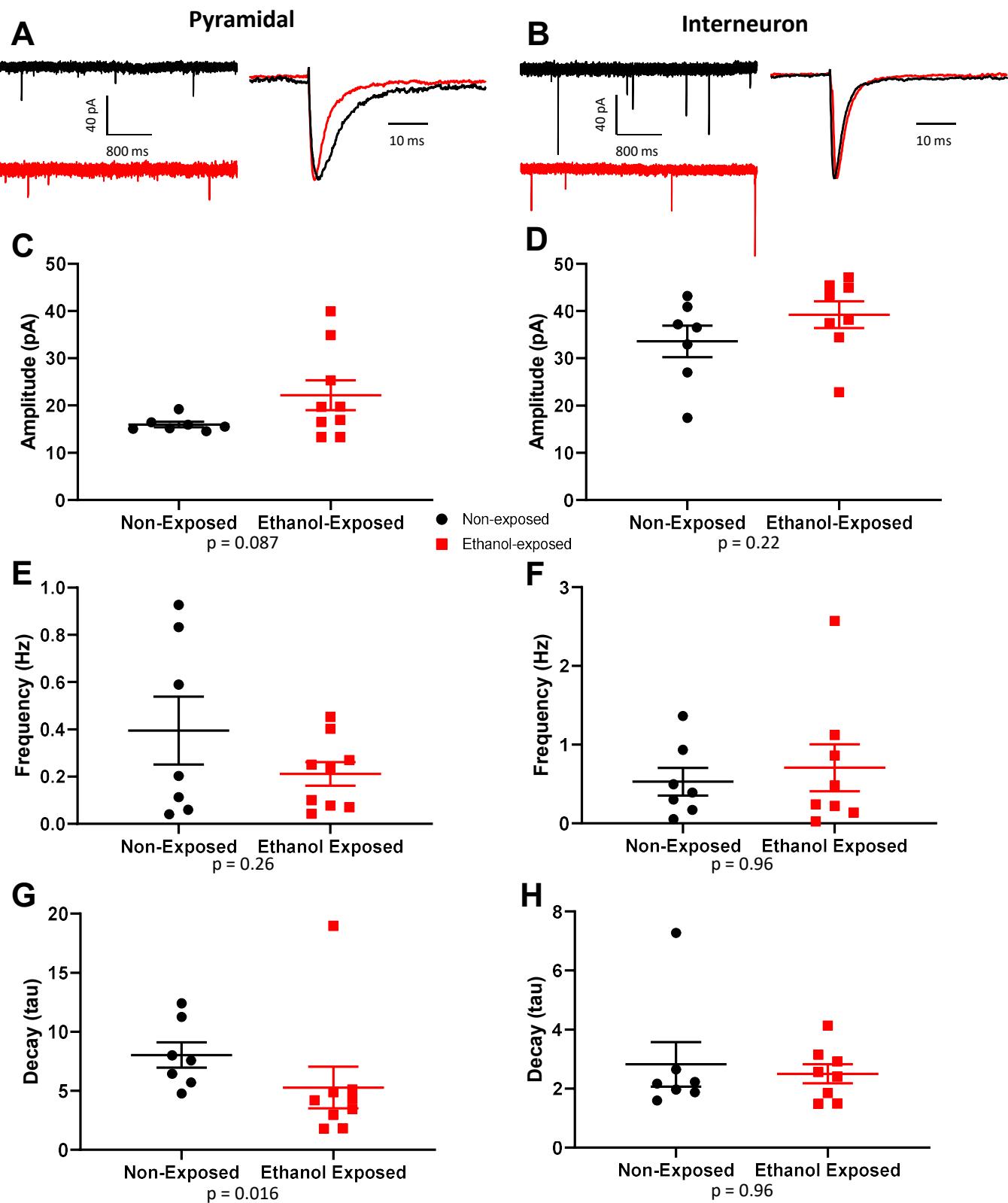


Supplemental Figure 1. High magnification images showing colocalization of apoptotic markers with Venus-positive interneurons.
(40X objective, scale bar = 10 μ m) Co-localization of Venus fluorescence (panels A, D, and G) with cleaved caspase-3 (B), TUNEL (E) and PSVue (H) immunofluorescence. The corresponding merged images are shown in panels C, F, and I.

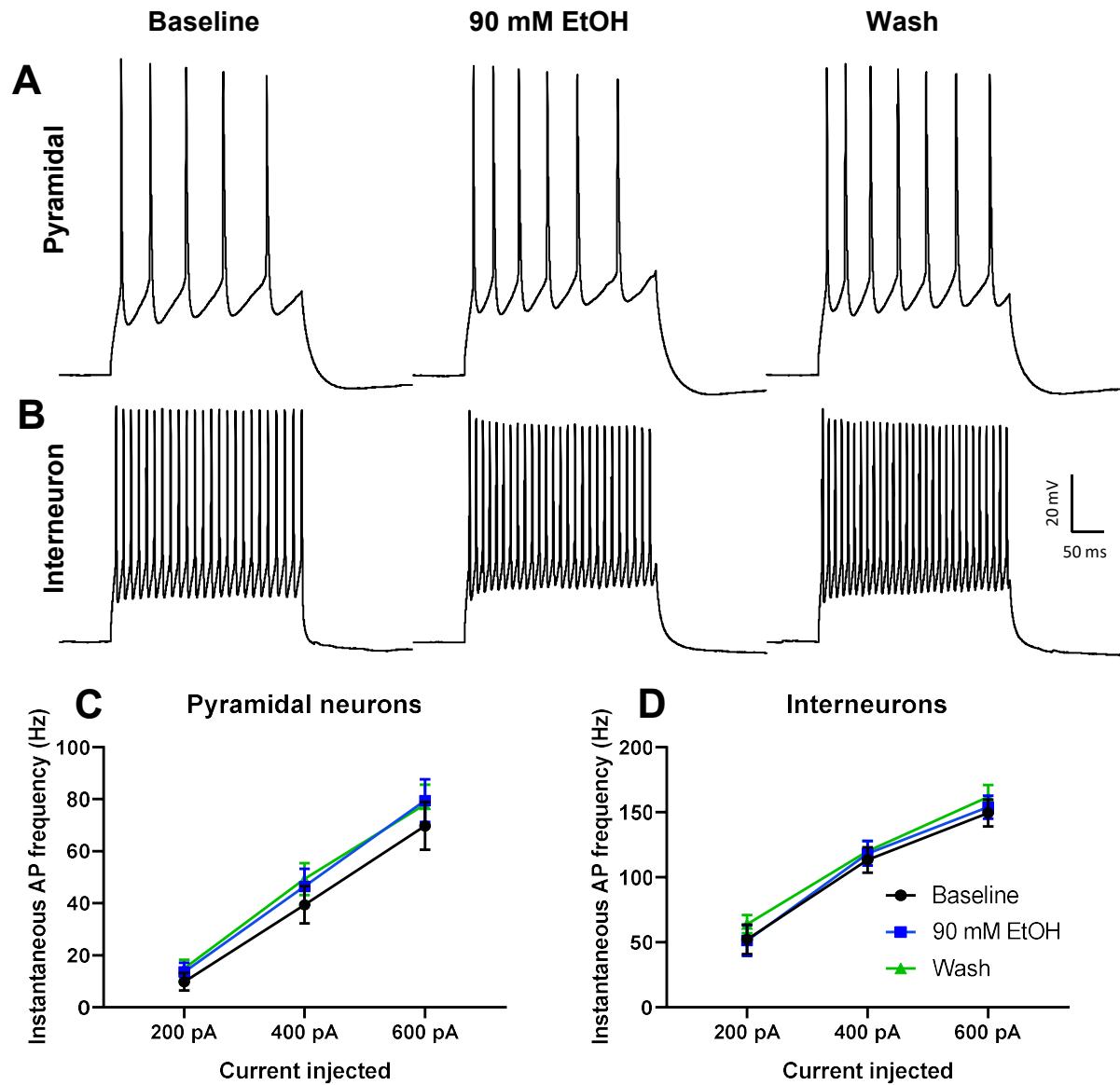


Supplemental Figure 2. Non-NMDA EPSC paired pulse ratios in pyramidal neurons and interneurons from control and ethanol exposed animals.

Representative evoked AMPA paired pulse traces are shown for (A) pyramidal neurons and (B) interneurons during baseline (black traces) and acute 90 mM ethanol bath application (grey traces) in animals not exposed to vaporized ethanol *in vivo*. Effect of 90 mM ethanol on paired pulse ratios in (C) pyramidal neurons and (D) interneurons from control animals (black data points) and 4-h ethanol vapor-exposed animals (red data points). E) Average paired pulse ratios from the last 3 min of each phase (baseline, 90 mM ethanol application, and washout) are shown for both pyramidal neurons and interneurons from non-exposed and 4-h ethanol vapor-exposed animals. Pyramidal neuron non-exposed and ethanol-exposed n = 8 animals (1 cell per animal, each from a different litter); interneuron non-exposed n = 6 animals (7 cells from 6 litters), ethanol-exposed n = 7 animals (1 cell per animal, each from a different litter). Data shown are mean \pm SEM.

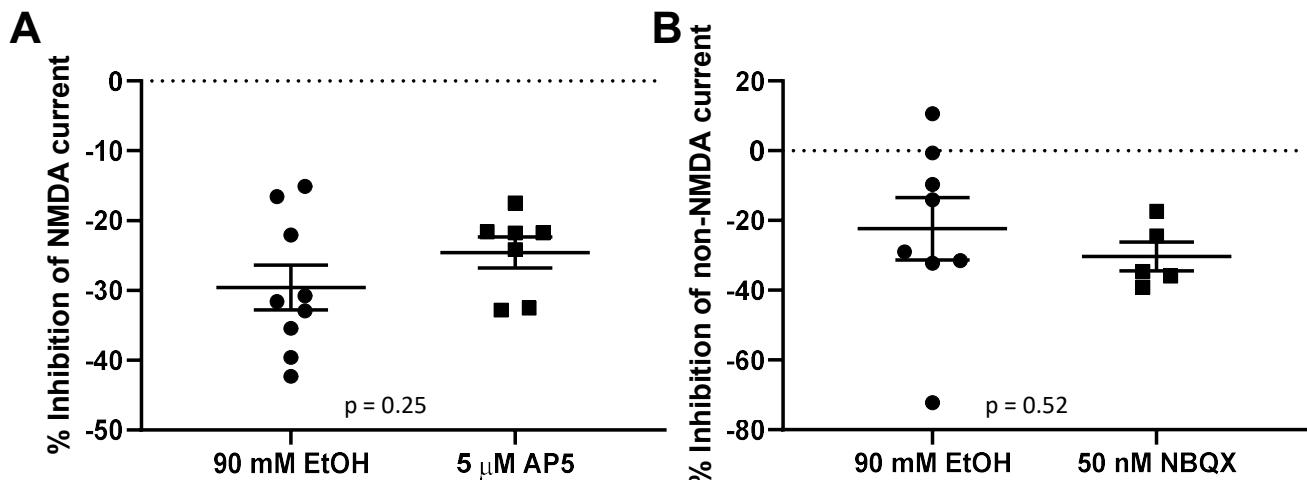


Supplemental Figure 3. mEPSC amplitude, frequency, and decay time constants in pyramidal neurons and interneurons from control and ethanol exposed animals. Representative mEPSC traces and average mEPSC waveforms (amplitudes normalized) from (A) pyramidal neurons and (B) interneurons from control (black traces) and ethanol exposed (red traces) animals. The average amplitude of mEPSCs in C) pyramidal neurons and D) interneurons is shown. E-F) The average frequency of mEPSCs in E) pyramidal neurons and F) interneurons is shown. The average decay constant (τ from a single-exponential curve fit) is shown for (G) pyramidal neurons and (H) interneurons. p-values shown are from appropriate parametric or non-parametric tests. For a detailed presentation of statistical tests performed please see Supplemental Table 1. Pyramidal non-exposed n = 7 animals (1 cell per animal, each from a different litter), ethanol-exposed n = 9 animals (1 cell per animal, each from a different litter); interneuron non-exposed n = 7 animals (1 cell per animal, each from a different litter), ethanol-exposed n = 8 animals (1 cell per animal, each from a different litter). Data shown are individual values from each cell along with the mean \pm SEM.



Supplemental Figure 4. Acute effect of 90 mM ethanol on action potential firing induced by current injection in pyramidal neurons and interneurons.

Shown are representative voltage traces recorded from (A) pyramidal neurons and (B) interneurons during a 400 pA, 300 ms current injection during baseline, acute application of 90 mM ethanol, and washout (all from animals not exposed to ethanol vapor). Also shown are plots illustrating that acute bath application of ethanol had little effect on the instantaneous action potential firing frequency in pyramidal neurons (C) and interneurons (D). For a detailed presentation of statistical tests performed please see Supplemental Table 1. Pyramidal neuron n = 9 cells (from 5 animals from 2 litters); interneuron n = 7 cells (from 5 animals from 2 litters). Data presented are mean \pm SEM.



Supplemental Figure 5. Mimicking the effects of acute bath application of 90 mM ethanol on NMDA and non-NMDA evoked EPSC amplitude with 5 μ M DL-AP5 and 50 nM NBQX in pyramidal neurons, respectively. A) The percent inhibition on NMDA EPSC amplitude caused by 90 mM ethanol application (calculated from the data shown in Fig 6) and 5 μ M DL-AP5 are compared. B) The percent inhibition on non-NMDA current amplitude caused by 90 mM ethanol application (calculated from the data shown in Fig 6) and 50 nM NBQX are compared. p-values presented are from unpaired t-tests. NMDA current 90 mM EtOH n = 9 cells (1 cell per animal, each from a different litter), 5 μ M DL-AP5 n = 7 cells (1 cell per animal, each from a different litter). Non-NMDA current 90 mM EtOH n = 8 cells (1 cell per animal, each from a different litter), 50 nM NBQX n = 5 (1 cell per animal, each from a different litter). Data presented are individual values along with the mean \pm SEM.