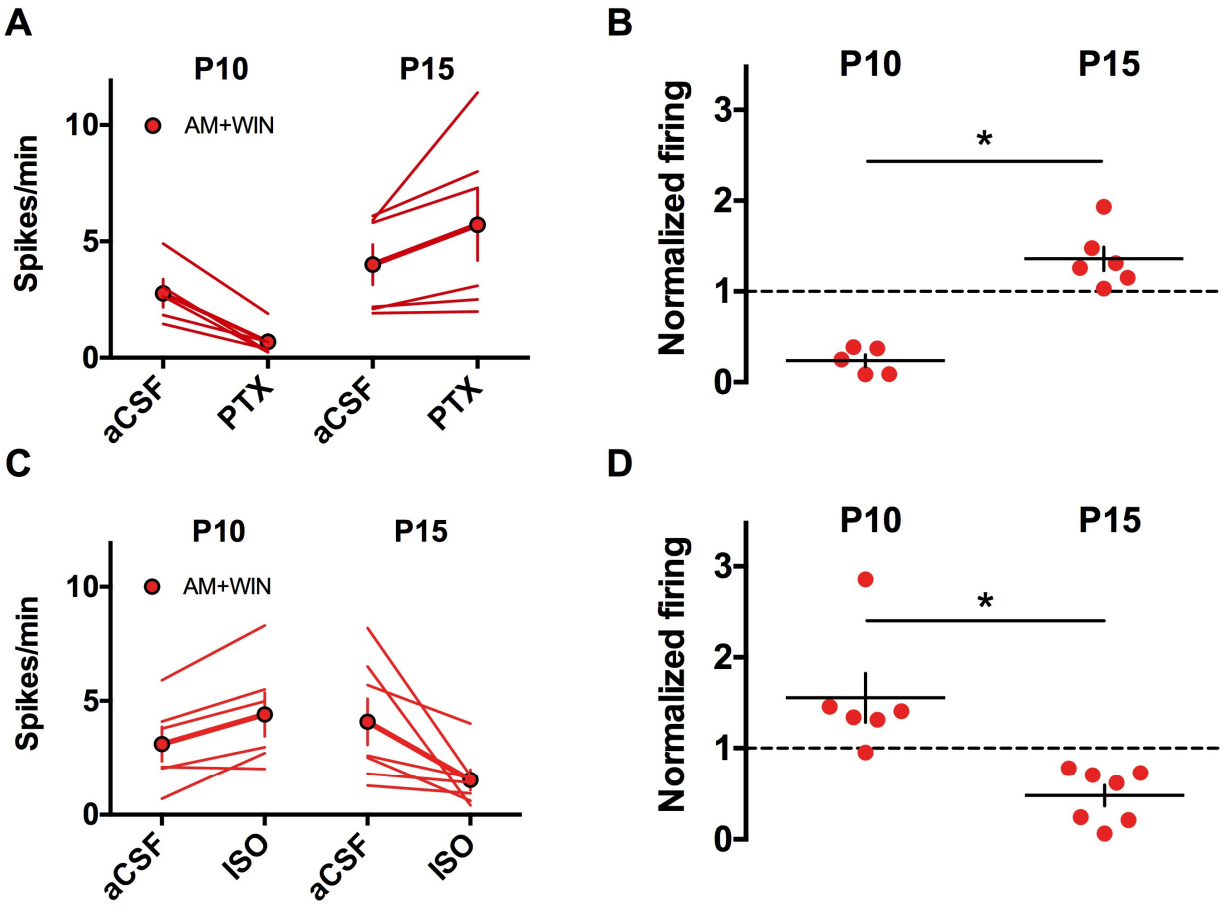
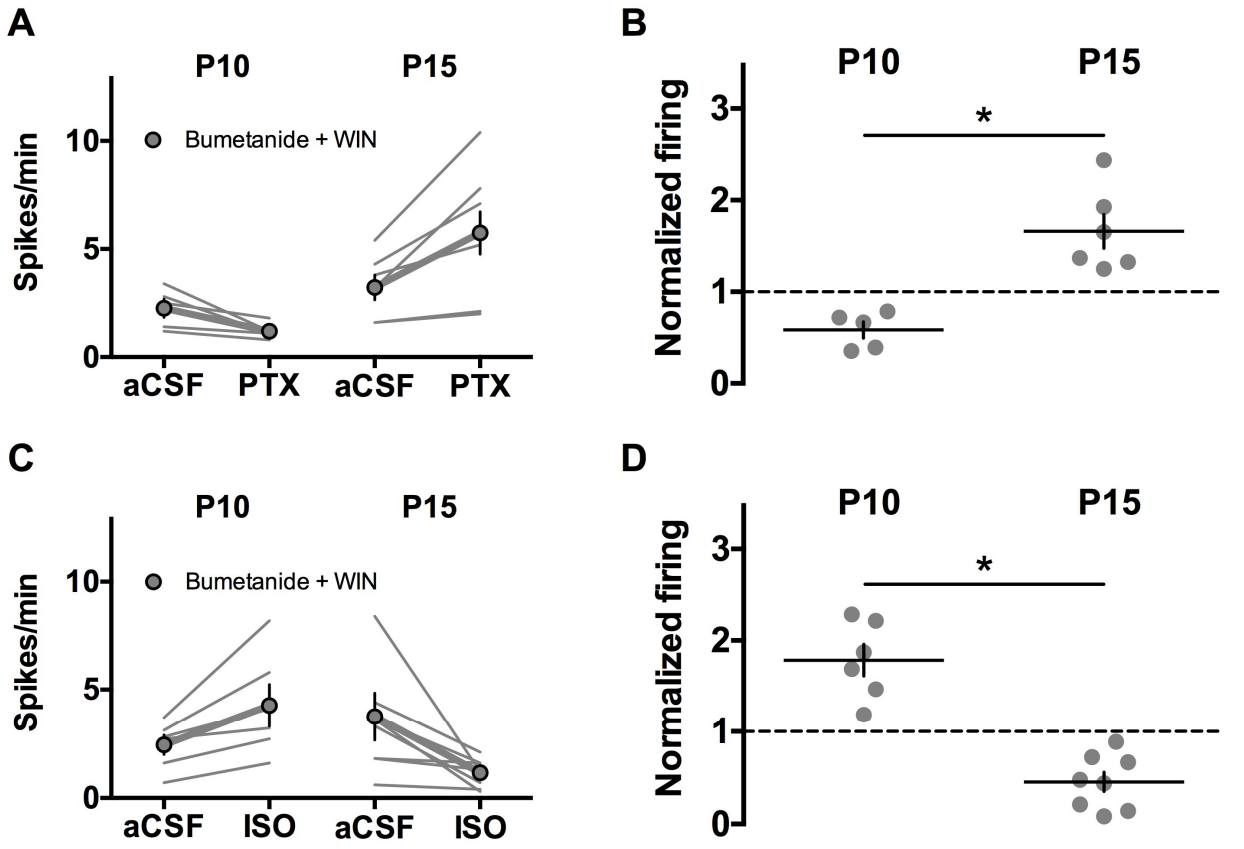
Cannabinoid Exposure via Lactation in Rats Disrupts Perinatal Programming of the Gamma-Aminobutyric Acid Trajectory and Select Early-life Behaviors

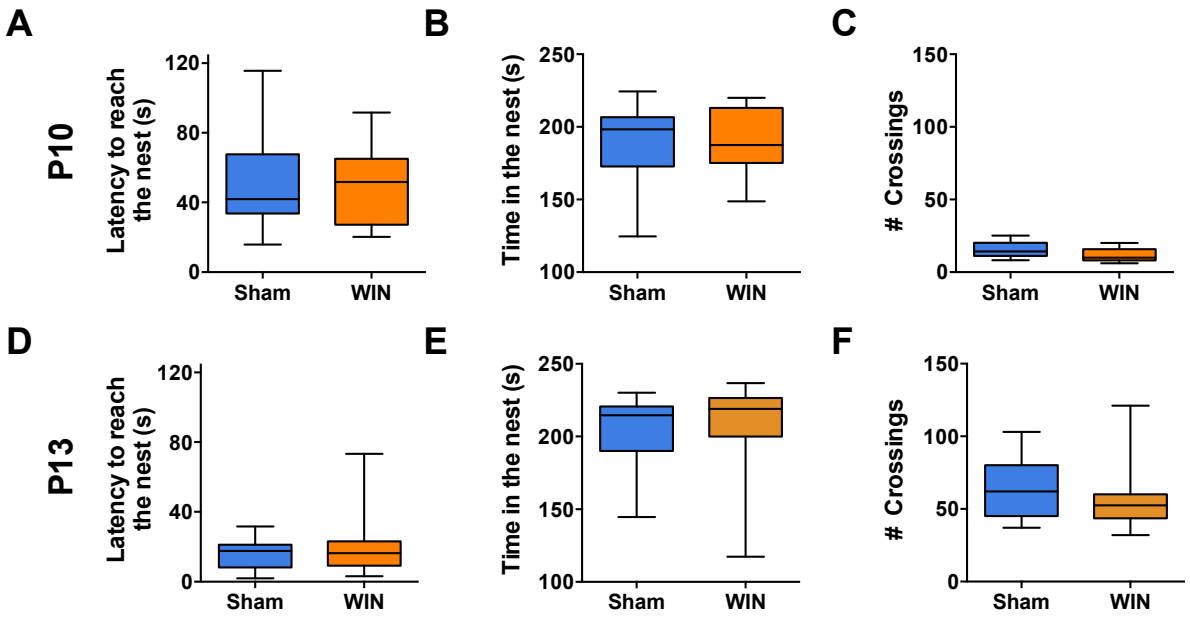
Supplemental Information



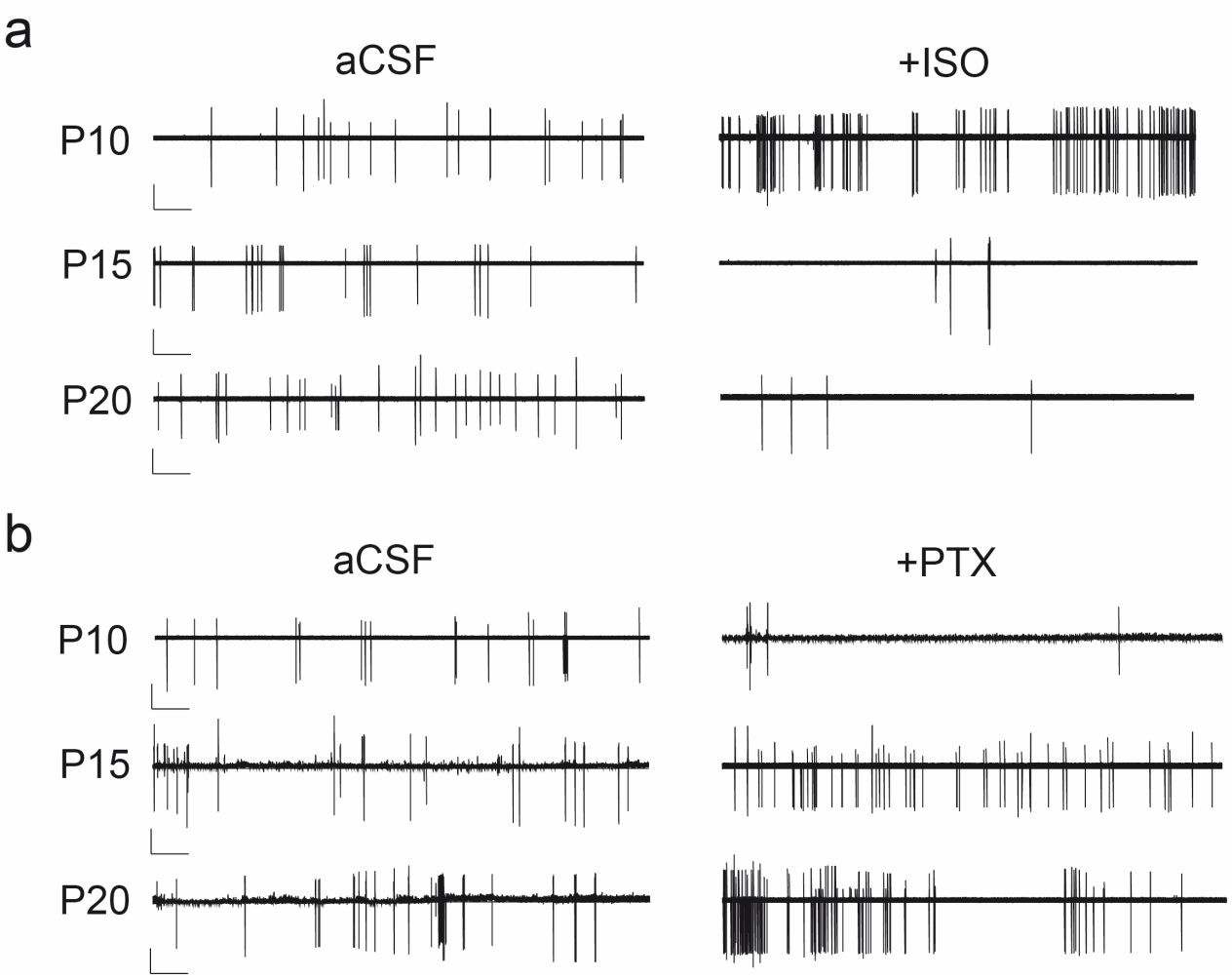
Supplementary Figure S1: The delayed excitatory/inhibitory switch in progeny of dams exposed to WIN is mediated by CB1R. Action potentials were recorded in cell-attached (I=0) layer 5 pyramidal neurons in standard aCSF. After 10 min of baseline recording, picrotoxin (20 µM; GABA-A receptor antagonist, PTX) or isoguvacine (7 µM; GABA-A receptor agonist, ISO) was bath-applied. Spiking activity was calculated as an average of spikes per minute (10 min baseline) compared to the last 10 min of drug application. AM251 co-administration prevents the delayed GABA shift induced by perinatal WIN treatment. **A, B**: GABA-A receptor antagonism is inhibitory at P09-10 in AM+WIN-exposed rats. At P09-10, PTX decreased spike frequency in slices obtained from AM+WIN rats (N=5 cells/3 rats). At P15-16 however, PTX increased spike frequency in AM+WIN-exposed progeny (N=6 cells/4 rats). *P<0.0001, Student's t-test with Welch's correction. **C, D**: At P09-10, ISO application increased spike frequency in AM+WIN (N=6 cells/4 rats) rats. Conversely, at P15-16 ISO application decreased spiking in slices obtained from AM+WIN-exposed rats (N=7 cells/4 rats). *P=0.0084, Student's t-test with Welch's correction. Example traces shown in supplementary Figure S7.



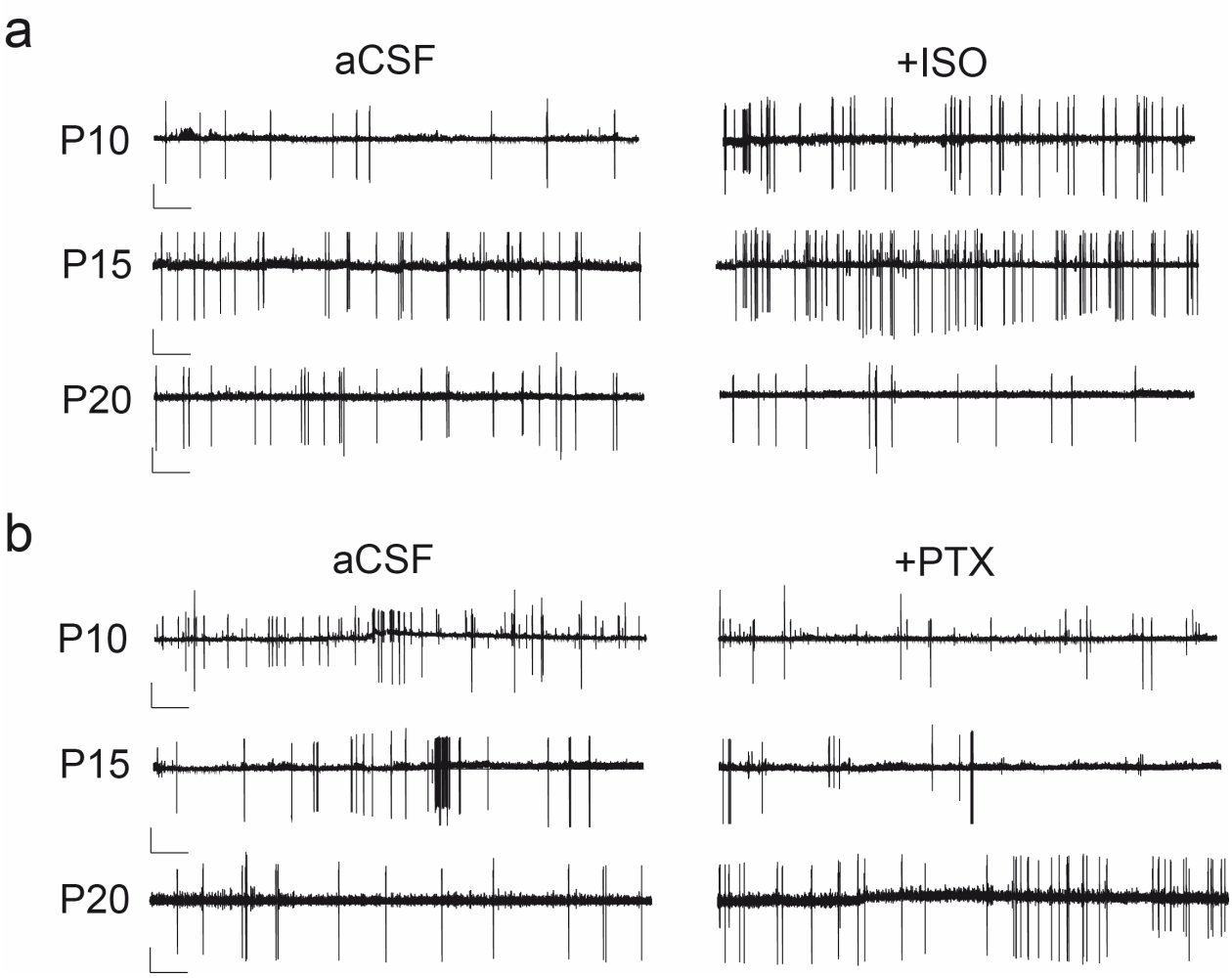
Supplementary Figure S2: The delayed excitatory/inhibitory switch in progeny of dams exposed to WIN is prevented by perinatal (P1-P15) bumetanide treatment. Action potentials were recorded in cell-attached (I=0) layer 5 pyramidal neurons in standard aCSF. After 10 min of baseline recording, picrotoxin (20 µM; GABA-A receptor antagonist, PTX) or isoguvacine (7 µM; GABA-A receptor agonist, ISO) was bath-applied. Spiking activity was calculated as an average of spikes per minute (10 min baseline) compared to the last 10 min of drug application. **A, B**: GABA-A receptor antagonism is inhibitory at P09-10 in WIN+bumetanide, but excitatory at P15-16. At P09-10, PTX decreased spike frequency in slices obtained from WIN+bumetanide rats (N=5 cells/3 rats). Conversely, at P15-16 PTX decreased spike frequency in WIN+bumetanide-exposed progeny (N=6 cells/4 rats). *P=0.0012, Student's t-test with Welch's correction, P=0.0156. **C, D**: At P09-10, ISO application increased spike frequency in WIN+bumetanide rats (N=6 cells/4 rats). However, at P15-16 ISO application increased spike frequency in slices obtained from WIN+bumetanide exposed progeny (N=8 cells/6 rats). *P=0.0001, Student's t-test with Welch's correction.



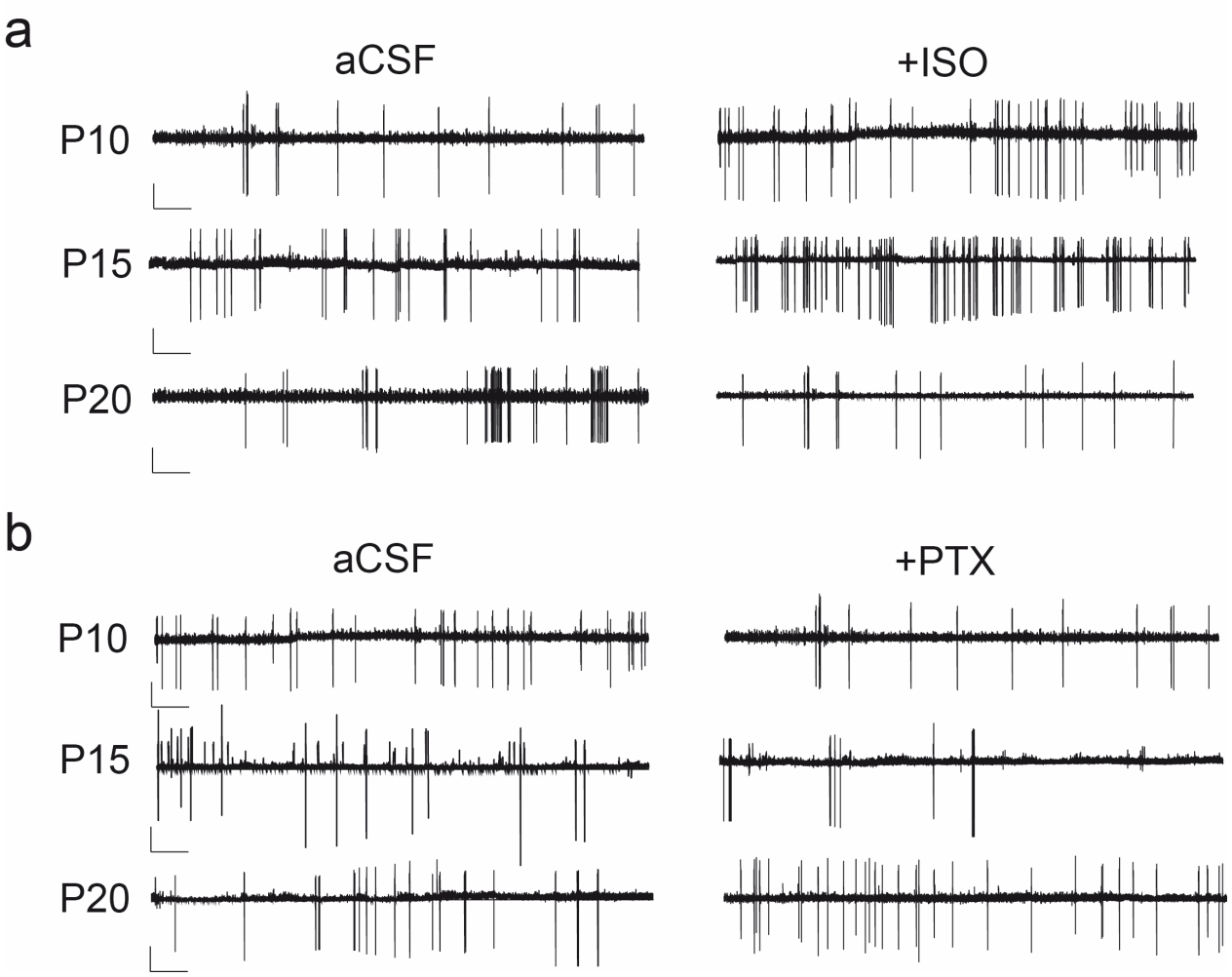
Supplementary Figure S3: Homing behavior is unaffected in pups exposed to WIN during the lactation period. a: The latency to reach the nest in the homing apparatus does not differ between pups from Sham- or WIN-treated dams at P10 (P=0.8754, Mann-Whitney) or P13 (P=0.8707, Mann-Whitney). **b:** Similarly, no significant difference was found for the total time spent in the nest between pups from Sham- or WIN-treated dams at P10 (P=0.8411, Mann-Whitney) or P13 (P=0.2318, Mann-Whitney). **c:** Finally, no difference was found in the total number of crossings made during the test by pups from Sham- vs WIN-treated dams at P10 (P=0.0586, Mann-Whitney) or P13 (P=0.1364, Mann-Whitney). P10: Sham, N=11 pups/4 litters; WIN, N=14 pups/3 litters. P13: Sham, N= 21 pups/2 litters; WIN, N= 22 pups/2 litters). Data are shown as median with interquartile ranges and minimal to maximal values.



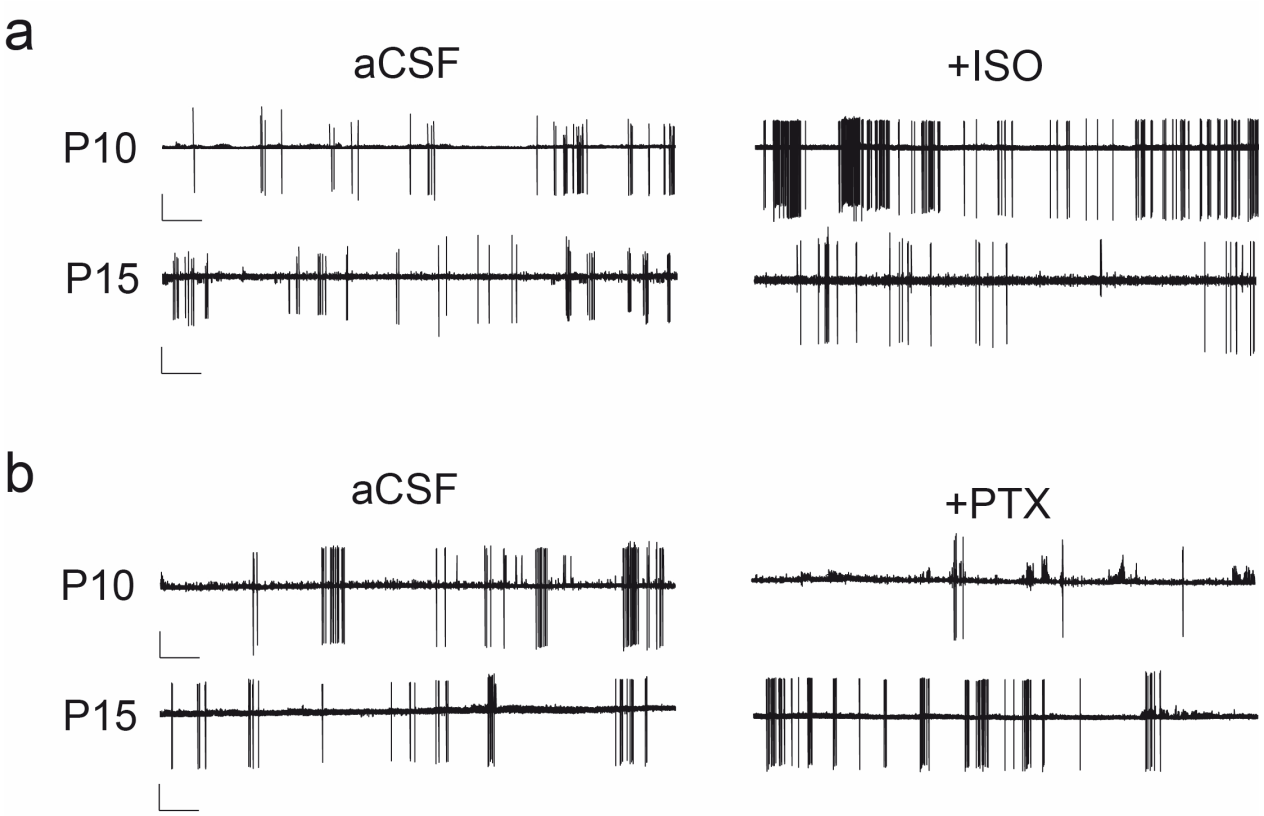
Supplementary Figure S4: Example traces for cell-attached recordings in Figure 1 (Sham recordings). Scale bars represent 50pA x 1min.



Supplementary Figure S5: Example traces for cell-attached recordings in Figure 2 (WIN recordings). Scale bars represent 50pA x 1min.



Supplementary Figure S6: Example traces for cell-attached recordings in Figure 2 (THC). Scale bars represent 50pA x 1min.



Supplementary Figure S7: Example traces for cell-attached recordings in Figure 3 (AM+WIN). Scale bars represent 50pA x 1min.

KCC2/GAPDH	P10	P15	P21
Male Sham	N=4 1.227	N=4 3.033	N=4 4.098
Female Sham	N=4 1.164	N=4 3.805	N=4 4.581
Student's T-test (P)	M vs F P=0.8114	M vs F P=0.4325	M vs F P=0.2321
Male WIN	N=3 1.363	N=4 2.011	N=5 2.977
Female WIN	N=3 1.409	N=4 2.679	N=5 3.315
Student's T-Test (P)	M vs F P=0.9382	M vs F P=0.3912	M vs F P=0.3296

NKCC1/GAPDH	P10	P15	P21
Male Sham	N=4 1.000	N=4 0.983	N=4 1.000
Female Sham	N=4 1.000	N=4 1.022	N=4 0.999
Student's T-test (P)	M vs F P>0.9999	M vs F P=0.6065	M vs F P>0.9999
Male WIN	N=3 0.941	N=4 0.917	N=5 0.989
Female WIN	N=3 0.904	N=4 0.872	N=5 0.877
Student's T-Test (P)	M vs F P=0.6885	M vs F P=0.6912	M vs F P=0.1990

Supplementary Table S1: Sex-distribution of KCC2 and NKCC1 Western Blot analyses. Samples used in Western blot analyses were collected from both male and female rats at all ages. Values for individual groups are expressed as rats (N) and protein/GAPDH outcomes. No significant differences were found between sexes within treatment groups at any of the tested ages for either KCC2 or NKCC1.

KCC2 fold-change	**P10**	**P15**	**P21**
Male Sham	N=6 1.097	N=5 1.665	N=5 1.662
Female Sham	N=5 1.233	N=4 1.516	N=5 1.667
Student's T-test (P)	M vs F P=0.2981	M vs F P=0.2873	M vs F P=0.9685
Male WIN	N=6 1.158	N=7 1.671	N=5 1.541
Female WIN	N=4 1.153	N=4 1.602	N=5 1.652
Student's T-Test (P)	M vs F P=0.9837	M vs F P=0.8342	M vs F P=0.5731

Supplementary Table S2: Sex-distribution of qPCR data. Samples used in qPCR data were collected from both male and female rats at all ages. Values for individual groups are expressed as rats (N) and KCC2 fold-change. No significant differences were found between sexes within treatment groups responding to either ISO or PTX in slice conditions.

Behavior	Parameter	Treatment	Sex	n	Mean	SEM	P value (unpaired t test)
USV	Dominant frequency	Sham P09 Sham P15	Male	21 6	39.59 42.09	0.44 0.89	0.7497 0.8166
		Sham P09 Sham P15	Female	14 4	39.34 41.73	0.53 1.12	
		WIN P09 WIN P15	Male	13 8	41.84 38.59	0.47 0.84	0.7093 0.3368
		WIN P09 WIN P15	Female	18 6	42.08 38.52	0.41 0.65	
		THC P09 THC P15	Male	19 5	42.22 39.05	0.50 1.16	0.8882 0.9648
		THC P09 THC P15	Female	17 5	42.12 39.11	0.58 0.70	
		AM+WIN P09	Male	9	39.01	0.30	0.8396
		AM+WIN P09	Female	7	39.16	0.75	
		Bumetanide P09	Male	3	41.26	2.38	0.9898
		Bumetanide P09	Female	3	41.22	0.57	
		Bumetanide + WIN P09	Male	9	40.46	0.84	0.7482
		Bumatanide + WIN P09	Female	6	40.97	1.41	

Supplementary Table S3: Sex-distribution of USV data. Samples used in USV data were collected from both male and female rats at all ages. Values for individual groups are expressed as rats (N) and dominant frequency. No significant differences were found between sexes within treatment groups.

Behavior	Parameter	Treatment	Sex	n	Mean	SEM	P value (unpaired t test)
Homing	Latency (s)	Sham P10 Sham P13	Male	10 4	15.67 64.24	2.867 18.76	0.9421 0.3337
		Sham P10 Sham P13	Female	11 7	15.39 42.17	2.510 6.785	
		WIN P10 WIN P13	Male	10 8	11.33 37.65	2.179 5.808	0.0622 0.0090
		WIN P10 WIN P13	Female	10 6	18.01 65.23	2.551 6.515	
Homing	Time in nest (s)	Sham P10 Sham P13	Male	10 4	205.3 176.1	7.881 18.76	0.8996 0.3337
		Sham P10 Sham P13	Female	10 7	206.6 198.1	5.937 6.785	
		WIN P10 WIN P13	Male	10 8	220.8 202.7	5.022 5.808	0.1950 0.0072
		WIN P10 WIN P13	Female	10 6	202.3 174.6	12.46 6.327	
Homing	Crossings	Sham P10 Sham P13	Male	10 4	62.70 11.50	7.025 1.936	0.6989 0.0638
		Sham P10 Sham P13	Female	10 7	66.09 17.29	4.984 1.848	
		WIN P10 WIN P13	Male	10 8	51.30 11.63	3.232 1.762	0.2261 0.8571
		WIN P10 WIN P13	Female	10 6	62.80 11.17	8.396 1.759	

Supplementary Table S4: Sex-distribution of homing data. Samples used in homing data were collected from both male and female rats at all ages. Values for individual groups are expressed as rats (N) and units (seconds for latency and time in nest or centimeters for distance travelled).

Supplementary Methods

Electrophysiology slice-preparation and acquisition: rats were anesthetized with isoflurane and 300 µm-thick coronal slices were prepared in a sucrose-based solution (in mM: 87 NaCl, 75 sucrose, 25 glucose, 2.5 KCl, 4 $MgCl_2$, 0.5 $CaCl_2$, 23 $NaHCO_3$ and 1.25 NaH_2PO4) at 4˚C using an Integraslice vibratome (Campden Instruments). Slices were stored for one hour at 32˚C in artificial cerebrospinal fluid (ACSF; in mM: 130 NaCl, 2.5 KCl, 2.4 $MgCl_2$, 1.2 $CaCl_2$, 23 $NaHCO_3$, 1.2 NaH_2PO_4 and 11 glucose), equilibrated with 95% O_2/5% CO_2. Slices were then stored at room temperature until recording. All experiments were conducted at 30–32˚C in ACSF. Cell-attached patch-clamp recordings were made in PFC layer 5, collected using an Axopatch-1D amplifier (Molecular Devices) and acquired with Clampex 10.2 acquisition Software via a Digidata 1440A (Molecular Devices). Pyramidal neurons in PFC layer 5 were visualized using an infrared illuminated upright microscope (Olympus BX51WI). Slices were superfused at 2ml/min with ACSF.

Single-channel pipette solution, in mM: 120 NaCl, 20 TEA-Cl, 5 KCl, 5 4-Aminopyridine, 0.1 $CaCl_2$, 10 $MgCl_2$, 10 Glucose, 10 HEPES, 0.01 GABA.