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Supplemental information

Inhibitory maturation and ocular dominance plasticity in mouse visual cortex require astrocyte CB1 receptors

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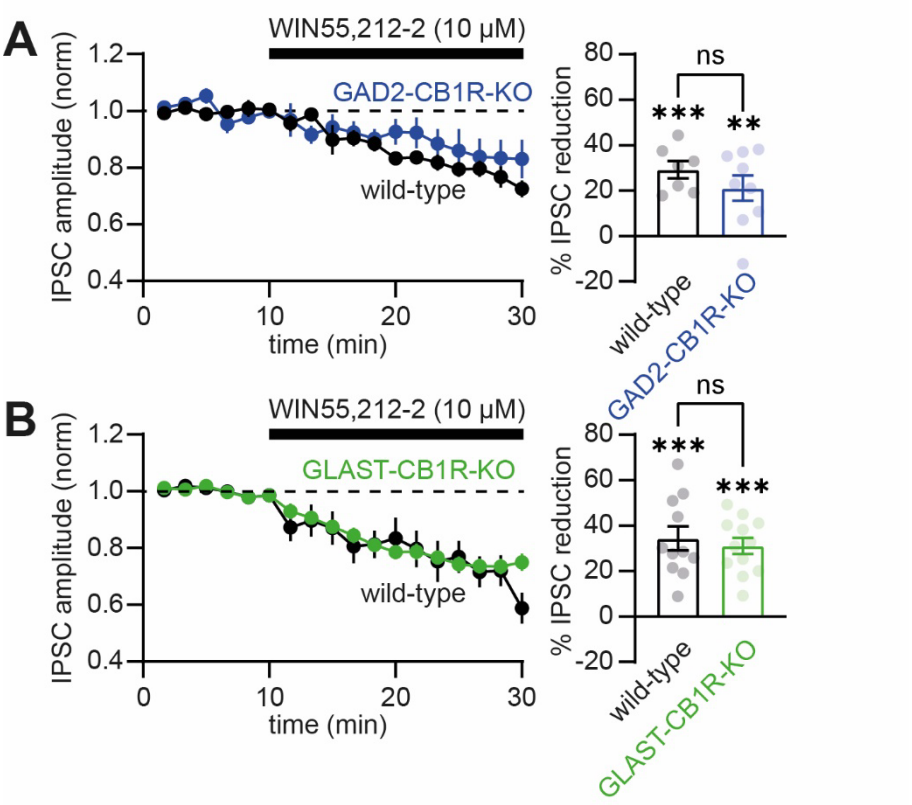

Figure S1. Synaptic depression by application of the synthetic CB1R agonist WIN55,212-2 is unaffected by removal of astrocyte or interneuron CB1 receptors, Related to Figure 3. (A) Left: Averaged time course of the IPSC amplitude in response to washin of WIN55,212-2 (10 µM), normalized to baseline. All recordings in GAD2-CB1R-KO mice (blue) and their wild-type littermates (black) are averaged. Right: Averaged amount of IPSC reduction (% reduction of the IPSC amplitude after application of WIN55,212-2) for all individual recorded neurons (GAD2-CB1R-KO IPSC reduction: 21.0±0.1%, n=9/N=5, IPSC reduction baseline vs after WIN55,212-2: $P<0.05$, paired t-test; wild-type littermates iLTD: 29.2±3.8% iLTD, n=7/N=3, IPSC amplitude baseline vs after WIN55,212-2: $P<0.01$; % reduction wild-type vs GAD2-CB1R-KO: $P=0.27$, unpaired t-test). (B) Same as in A, but now for GLAST-CB1R-KO mice (green) and their wild-type littermates (black; GLAST-CB1R-KO IPSC reduction: 29.4±3.5%, n=9/N=5, IPSC reduction baseline vs after WIN55,212-2: $P<0.001$, paired t-test; wild-type littermates iLTD: 34.4±5.3% iLTD, n=11/N=4, IPSC amplitude baseline vs after WIN55,212-2: $P<0.001$; % reduction wild-type vs GLAST-CB1R-KO: $P=0.39$, unpaired t-test). Error bars indicate SEM. *: P < 0.05; **: P < 0.01; ***: P < 0.001.

Table S1. Statistical details, Related to Figures 1-4.

FIGURE 2

	comparison	*test*	*P-value*	*one- or two-tailed?*	*Sum of Ranks (wt, KO)*	*Mann-Whitney U*
Figure 2A	steady state IPSC, littermate control vs GAD2-CB1R-KO	Mann-Whitney test	0,7069	two-tailed	276 , 354	140
Figure 2B	steady state IPSC, littermate control vs GLAST-CB1R-KO	Mann-Whitney test	0,0075	two-tailed	191 , 134	29

FIGURE 3

	comparison	*test*	*P-value*	*one- or two-tailed?*	*t, df*
Figure 3A	IPSC amplitude, baseline vs after iLTD induction, control	paired t-test	0,0002	two-tailed	t=4,366, df=28
	IPSC amplitude, baseline vs after iLTD induction, AM251	paired t-test	0,9221	two-tailed	t=0,09999, df=11
	% iLTD, control vs AM251	unpaired t-test	0,0023	two-tailed	t=3,264, df=39
Figure 3B	IPSC amplitude, baseline vs after iLTD induction, littermate control	paired t-test	<0.0001	two-tailed	t=4,719, df=39
	IPSC amplitude, baseline vs after iLTD induction, GAD2-CB1R-KO	paired t-test	0,0002	two-tailed	t=4,197, df=36
	% iLTD, littermate control vs GAD2-CB1R-KO	unpaired t-test	0,6464	two-tailed	t=0,4607, df=75
Figure 3C	IPSC amplitude, baseline vs after iLTD induction, littermate control	paired t-test	<0,0001	two-tailed	t=5,098, df=36
	IPSC amplitude, baseline vs after iLTD induction, GLAST-CB1R-KO	paired t-test	<0,0001	two-tailed	t=4,316, df=43
	% iLTD, littermate control vs GLAST-CB1R-KO	unpaired t-test	0,9056	two-tailed	t=0,1190, df=79

FIGURE S1

	comparison	*test*	*P-value*	*one- or two-tailed?*	*t, df*
Figure S1A	IPSC amplitude, baseline vs after WIN55,212-2 washin, littermate control	paired t-test	0,0006	two-tailed	t=6,468, df=6
	IPSC amplitude, baseline vs after WIN55,212-2 washin, GAD2-CB1R-KO	paired t-test	0,0053	two-tailed	t=3,791, df=8
	% reduction by WIN55,212-2, littermate control vs GAD2-CB1R-KO	unpaired t-test	0,2734	two-tailed	t=1,140, df=14
Figure S1B	IPSC amplitude, baseline vs after WIN55,212-2 washin, littermate control	paired t-test	0,0005	two-tailed	t=5,023, df=10
	IPSC amplitude, baseline vs after WIN55,212-2 washin, GLAST-CB1R-KO	paired t-test	<0,0001	two-tailed	t=7,544, df=11
	% reduction by WIN55,212-2, littermate control vs GLAST-CB1R-KO	unpaired t-test	0,6062	two-tailed	t=0,5234, df=21

FIGURE 4

	comparison	*test*	*P-value*	*SS (Type III)*	*DF*	*MS*	*F (DFn, DFd)*
Figure 4A	genotype (littermate control vs GAD2-CB1R-KO)	two-way ANOVA:					
	& monocular deprivation (no MD vs MD)	*Interaction*	0,9283	0,00008892	1	0,00008892	F (1, 17) = 0,008341
		Monocular Deprivation	<0,0001	0,3957	1	0,3957	F (1, 17) = 37,12
		Genotype	0,5307	0,004366	1	0,004366	F (1, 17) = 0,4096
		Residual		0,1812	17	0,01066	
	multiple comparisons:	*test*	*P-value*	*Q*	*DF*		
	non MD:GAD2Cre-WT vs. non MD:GAD2Cre-KO	Tukey's multiple comparison	0,9807	0,5371	17		
	non MD:GAD2Cre-WT vs. MD:GAD2Cre-WT	Tukey's multiple comparison	0,0023	6,136	17		
	non MD:GAD2Cre-WT vs. MD:GAD2Cre-KO	Tukey's multiple comparison	0,0012	6,591	17		
	non MD:GAD2Cre-KO vs. MD:GAD2Cre-WT	Tukey's multiple comparison	0,0052	5,575	17		
	non MD:GAD2Cre-KO vs. MD:GAD2Cre-KO	Tukey's multiple comparison	0,0026	6,054	17		
	MD:GAD2Cre-WT vs. MD:GAD2Cre-KO	Tukey's multiple comparison	0,9509	0,7477	17		

	comparison	*test*	*P-value*	*SS (Type III)*	*DF*	*MS*	*F (DFn, DFd)*
Figure 4B	genotype (littermate control vs GAD2-CB1R-KO)	two-way ANOVA:					
	& monocular deprivation (no MD vs MD)	*Interaction*	0,022	0,0879	1	0,0879	F (1, 24) = 5,998
		Monocular Deprivation	0,0003	0,2697	1	0,2697	F (1, 24) = 18,40
		Genotype	0,0756	0,05056	1	0,05056	F (1, 24) = 3,450
		Residual		0,3517	24	0,01465	
	multiple comparisons:	*test*	*P-value*	*Q*	*DF*		
	non MD:GLASTCre-WT vs. non MD:GLASTCre-KO	Tukey's multiple comparison	0,9772	0,5715	24		
	non MD:GLASTCre-WT vs. MD:GLASTCre-WT	Tukey's multiple comparison	0,0006	6,508	24		
	non MD:GLASTCre-WT vs. MD:GLASTCre-KO	Tukey's multiple comparison	0,34	2,42	24		
	non MD:GLASTCre-KO vs. MD:GLASTCre-WT	Tukey's multiple comparison	0,0011	6,179	24		
	non MD:GLASTCre-KO vs. MD:GLASTCre-KO	Tukey's multiple comparison	0,5407	1,911	24		
	MD:GLASTCre-WT vs. MD:GLASTCre-KO	Tukey's multiple comparison	0,0205	4,471	24		

	comparison	*test*	*P-value*	*SS (Type III)*	*DF*	*MS*	*F (DFn, DFd)*
Figure 4C	genotype (littermate control vs GAD2-CB1R-KO)	two-way ANOVA:					
	& monocular deprivation (no MD vs MD)	*Interaction*	0,0014	1,025	1	1,025	F (1, 434) = 10,31
		Monocular Deprivation	<0,0001	7,207	1	7,207	F (1, 434) = 72,44
		Genotype	0,0055	0,7742	1	0,7742	F (1, 434) = 7,781
		Residual		43,18	434	0,0995	
	multiple comparisons:	*test*	*P-value*	*Q*	*DF*		
	non MD:GLASTCre-WT vs. non MD:GLASTCre-KO	Tukey's multiple comparison	0,9886	0,453	434		
	non MD:GLASTCre-WT vs. MD:GLASTCre-WT	Tukey's multiple comparison	<0,0001	11,64	434		
	non MD:GLASTCre-WT vs. MD:GLASTCre-KO	Tukey's multiple comparison	0,0002	5,99	434		
	non MD:GLASTCre-KO vs. MD:GLASTCre-WT	Tukey's multiple comparison	<0,0001	10,84	434		
	non MD:GLASTCre-KO vs. MD:GLASTCre-KO	Tukey's multiple comparison	0,001	5,34	434		
	MD:GLASTCre-WT vs. MD:GLASTCre-KO	Tukey's multiple comparison	0,0005	5,625	434		