

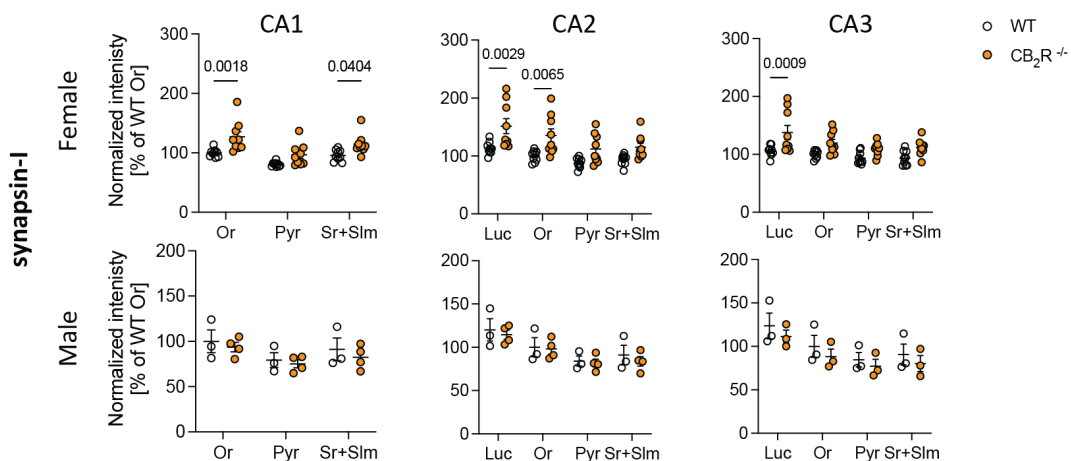
Supplementary Table S1. Detailed results from 2-way ANOVA analysis.

Figure	Panel	Statistical test		Posthoc test	
		Test name	result	Test name	result
1. Synapsin-I levels are increased in the hippocampus after CB2R deletion in female mice	1c: females: CA1	2-way ANOVA	layer "F (2, 171) = 25.16", $p < 0.0001$; genotype "F (1, 171) = 64.42", $p < 0.0001$	Šídák's multiple comparisons test	Or $p < 0.00001$; Pyr $p = 0.0013$; Sr+SIm $p < 0.0001$
	1c: males: CA1	2-way ANOVA	layer "F (2, 75) = 9.820", $p = 0.0002$		
	1c: females: CA2	2-way ANOVA	layer "F (3, 236) = 22.06", $p < 0.0001$; genotype "F (1, 236) = 102.4", $p < 0.0001$	Šídák's multiple comparisons test	Luc $p < 0.0001$; Or $p < 0.00001$; Pyr $p = 0.0002$; Sr+SIm $p = 0.0019$
	1c: males: CA2	2-way ANOVA	layer "F (3, 104) = 27.58", $p < 0.0001$		
	1c: females: CA3	2-way ANOVA	layer "F (3, 236) = 51.37", $p < 0.0001$; genotype "F (1, 236) = 110.2", $p < 0.0001$; layer x genotype "F (3, 236) = 3.557", $p = P=0.0150$	Šídák's multiple comparisons test	Luc $p < 0.0001$; Or $p < 0.00001$; Pyr $p = 0.0008$; Sr+SIm $p < 0.0001$
	1c: males: CA3	2-way ANOVA	layer "F (3, 104) = 27.58", $p < 0.0001$		
2. Average size of Synapsin-I particles is increased after CB2R deletion in females.	2b: females: CA2	2-way ANOVA	layer "F (3, 228) = 31.96", $p < 0.0001$; genotype "F (1, 228) = 4.343", $p = 0.0383$	Šídák's multiple comparisons test	-
	2b: males: CA2	2-way ANOVA	layer "F (3, 104) = 214.0", $p < 0.0001$		
	2b: females: CA3	2-way ANOVA	layer "F (3, 276) = 43.86", $p < 0.0001$; genotype "F (1, 276) = 16.07", $p < 0.0001$	Šídák's multiple comparisons test	Luc $p = 0.0112$
	2b: males: CA3	2-way ANOVA	layer "F (3, 104) = 220.4", $p < 0.0001$		
3. vGLUT1 levels are slightly increased in female CB2R-/- mice.	3c: females: CA1	2-way ANOVA	layer "F (2, 162) = 130.6", $p < 0.0001$		
	3c: males: CA1	2-way ANOVA	layer "F (2, 78) = 172.2", $p < 0.0001$		
	3c: females: CA2	2-way ANOVA	layer "F (3, 224) = 94.36", $p < 0.0001$; genotype "F (1, 224) = 6.166", $p = 0.0138$	Šídák's multiple comparisons test	-
	3c: males: CA2	2-way ANOVA	layer "F (3, 104) = 61.08", $p < 0.0001$		
	3c: females: CA3	2-way ANOVA	layer "F (3, 224) = 91.73", $p < 0.0001$; genotype "F (1, 224) = 5.725", $p = 0.0175$	Šídák's multiple comparisons test	-
	3c: males: CA3	2-way ANOVA	layer "F (3, 104) = 104.0", $p < 0.0001$		
4. vGAT levels are increased in female CB2R-/- mice, but not in male.	4c: females: CA1	2-way ANOVA	layer "F (2, 155) = 18.93", $p < 0.0001$; genotype "F (1, 155) = 36.59", $p < 0.0001$	Šídák's multiple comparisons test	Or $p = 0.0111$; Pyr $p = 0.0001$; Sr+SIm $p = 0.0031$
	4c: males: CA1	2-way ANOVA	layer "F (2, 66) = 8.762", $p = 0.0004$; genotype "F (1, 66) = 13.55", $p = 0.0005$	Šídák's multiple comparisons test	Pyr $p = 0.0470$
	4c: females: CA2	2-way ANOVA	layer "F (3, 216) = 17.29", $p < 0.0001$; genotype "F (1, 216) = 55.13", $p < 0.0001$	Šídák's multiple comparisons test	Luc $p = 0.0141$; Or $p = 0.0057$; Pyr $p < 0.0001$; Sr+SIm $p = 0.0017$
	4c: males: CA2	2-way ANOVA	layer "F (3, 100) = 11.19", $p < 0.0001$; genotype "F (1, 100) = 3.711", $p = 0.0569$		
	4c: females: CA3	2-way ANOVA	layer "F (3, 216) = 6.922", $p = 0.0002$; genotype "F (1, 216) = 53.83", $p < 0.0001$	Šídák's multiple comparisons test	Luc $p = 0.0055$; Or $p = 0.0084$; Pyr $p < 0.0001$; Sr+SIm $p = 0.0004$
	4c: males: CA3	2-way ANOVA	layer "F (3, 100) = 2.986", $p = 0.0348$; genotype "F (1, 100) = 4.463", $p = 0.0371$		

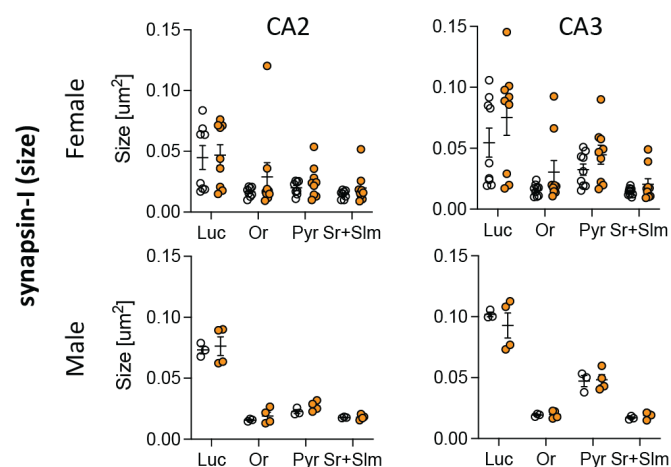
Supplementary Table S2. Detailed results from 3-way ANOVA analysis.

Dateset	Area	Statistical test	
		Test name	result
Synapsin-I levels in the hippocampus	CA1	3-way ANOVA	layer "F (2, 159) = 20.80", $p < 0.0001$; genotype "F (1, 159) = 15.83", $p = 0.0001$; sex "F (1, 159) = 26.71", $p < 0.0001$; genotype x sex "F (1, 159) = 42.42", $p < 0.0001$
	CA2	3-way ANOVA	layer "F (3, 224) = 53.35", $p < 0.0001$; genotype "F (1, 224) = 70.77", $p < 0.0001$; sex "F (1, 224) = 41.64", $p < 0.0001$; genotype x sex "F (1, 224) = 113.3", $p < 0.0001$
	CA3	3-way ANOVA	layer "F (3, 224) = 63.22", $p < 0.0001$; genotype "F (1, 224) = 19.94", $p = 0.0001$; sex "F (1, 224) = 45.02", $p < 0.0001$; genotype x sex "F (1, 224) = 76.18", $p < 0.0001$

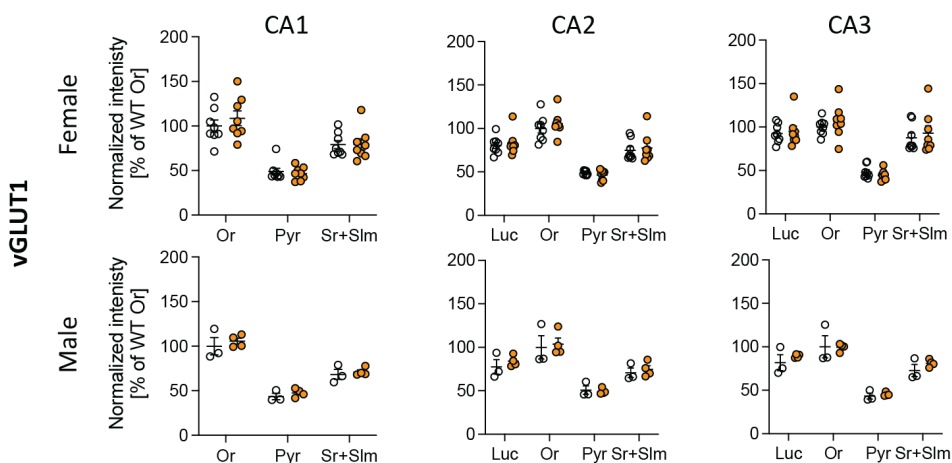
A



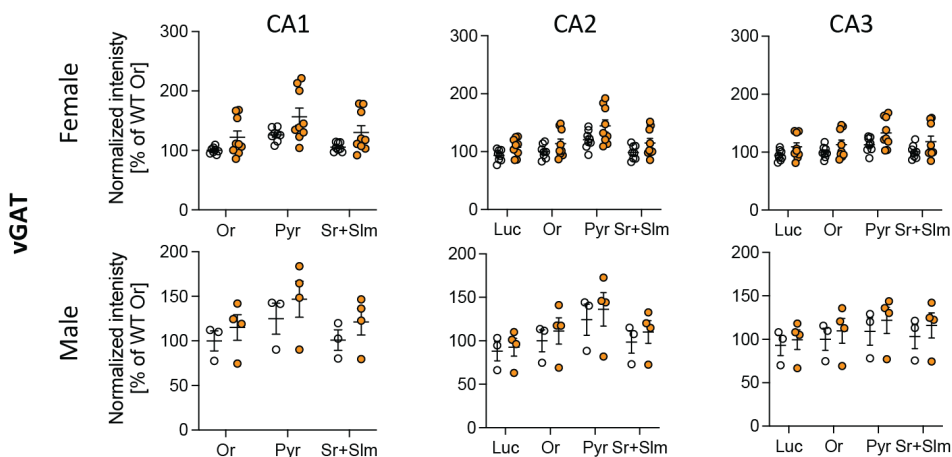
B



C



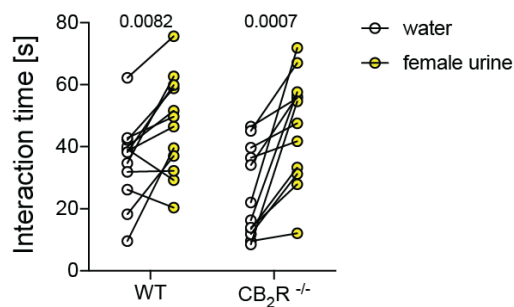
D



Supplement Figure S1. Mean per animal values for all presented data in Figures 1-4. (A) Figure 1: synapsin-I (green) **(B)** Figure 2: average size of synapsin-I particles; **(C)** Figure 3: vGLUT1; **(D)** Figure 4: vGAT. Or - stratum oriens; Luc – stratum lucidum; Pyr – stratum pyramidale; Sr+SIm – stratum radiatum and stratum lacunosum-moleculare. White circle – WT; orange circle – CB₂R^{-/-} mice. Each datapoint represents one mouse. Upper panel – female mice (WT: N = 8-9 mice; CB₂R^{-/-}: N = 9 mice); right panel - male mice (WT: N = 3 mice; CB₂R^{-/-}: N = 4 mice). Left panel – CA1 region; middle panel – CA2 region; right panel – CA3 region. Data is displayed as mean value $\pm$ SEM. Data was analyzed by two-way ANOVA followed by Sidak's multiple comparison test. The exact p-values are indicated on the graph and reported in Supplementary Table S3.

Supplementary Table S3. Detailed results from 2-way ANOVA analysis for Supplement Figure S2.

Figure	Panel	Statistical test		Posthoc test	
		Test name	result	Test name	result
S2. Mean per animal	a: females: CA1	2-way ANOVA	layer "F (2, 48) = 11.77", p < 0.0001; genotype "F (1, 48) = 23.39", p < 0.0001	Šídák's multiple comparisons test	Or p = 0.0018; Sr+SIm p = 0.0404
	a: females: CA2	2-way ANOVA	layer "F (3, 64) = 7.389", p = 0.0003; genotype "F (1, 64) = 30.75", p < 0.0001	Šídák's multiple comparisons test	Luc p = 0.0029; Or p = 0.0065
	a: females: CA3	2-way ANOVA	layer "F (3, 64) = 5.153", p = 0.0030; genotype "F (1, 64) = 27.28", p < 0.0001	Šídák's multiple comparisons test	Luc p = 0.0009
	a: males: CA2	2-way ANOVA	layer "F (3, 20) = 8.041", p = 0.0010		
	a: males: CA3	2-way ANOVA	layer "F (3, 16) = 4.876", p = 0.0135		
	b: females: CA2	2-way ANOVA	layer "F (3, 60) = 7.020", p = 0.0004		
	b: females: CA3	2-way ANOVA	layer "F (3, 64) = 13.05", p < 0.0001; genotype "F (1, 64) = 5.207", p = 0.0258		
	b: males: CA2	2-way ANOVA	layer "F (3, 20) = 106.7", p < 0.0001		
	b: males: CA3	2-way ANOVA	layer "F (3, 20) = 111.0", p < 0.0001		
	c: females: CA1	2-way ANOVA	layer "F (2, 42) = 49.37", p < 0.0001		
	c: females: CA2	2-way ANOVA	layer "F (3, 56) = 58.52", p < 0.0001		
	c: females: CA3	2-way ANOVA	layer "F (3, 56) = 45.78", p < 0.0001		
	c: males: CA1	2-way ANOVA	layer "F (2, 15) = 77.35", p < 0.0001		
	c: males: CA2	2-way ANOVA	layer "F (3, 20) = 22.92", p < 0.0001		
	c: males: CA3	2-way ANOVA	layer "F (3, 20) = 38.20", p < 0.0001		
	d: females: CA1	2-way ANOVA	layer "F (2, 45) = 5.779", p = 0.0058; genotype "F (1, 45) = 11.39", p = 0.0015		
	d: females: CA2	2-way ANOVA	layer "F (3, 60) = 8.730", p < 0.0001; genotype "F (1, 60) = 12.57", p = 0.0008	Šídák's multiple comparisons test	Pyr p = 0.0632
	d: females: CA3	2-way ANOVA	layer "F (3, 60) = 3.615", p = 0.0181; genotype "F (1, 60) = 11.51", p = 0.0012		



Supplement Figure S2. CB₂R^{-/-} have an intact olfaction. Interaction time of mice with water-stained (white circle) or female urine-stained (yellow circle) cotton swab. Increase in the interaction time suggests lack of olfactory deficits. Circles represent data from individual mice (N = 12 mice/genotype). Data was analyzed separately for each genotype by paired t-test. The exact p-values are indicated on the graph.

Supplementary Materials and Methods

Olfaction test protocol was modified from “Simple Behavioral Assessment of Mouse Olfaction”; The Olfactory Habituation/Dishabituation Test (Yang & Crawley, 2009). The test was performed only on the male group after the partner recognition test. A cotton swab was inserted from the top of the cage around 2 - 3 cm from the grid top. In the first trial, 10 µl of water was placed on the cotton swab and for 2 minutes interaction time of the mouse with a cotton swab was recorded. Then the cotton swab was removed from the cage for 1 minute and another cotton swab was inserted with a 10 µl drop of female urine. Once again, the interaction time was recorded for 2 minutes. Proper sense of smell would cause no change or an increase in interaction time in the second trial. Decrease of interaction time would suggest a habituation to the cotton swab and olfactory disfunction.

Bibliography

Yang, M., & Crawley, J. N. (2009). Simple Behavioral Assessment of Mouse Olfaction
 Mouse Olfaction. *Current Protocols in Neuroscience*, (CHAPTER: Unit-8.24), 1–14.
<https://doi.org/10.1002/0471142301>