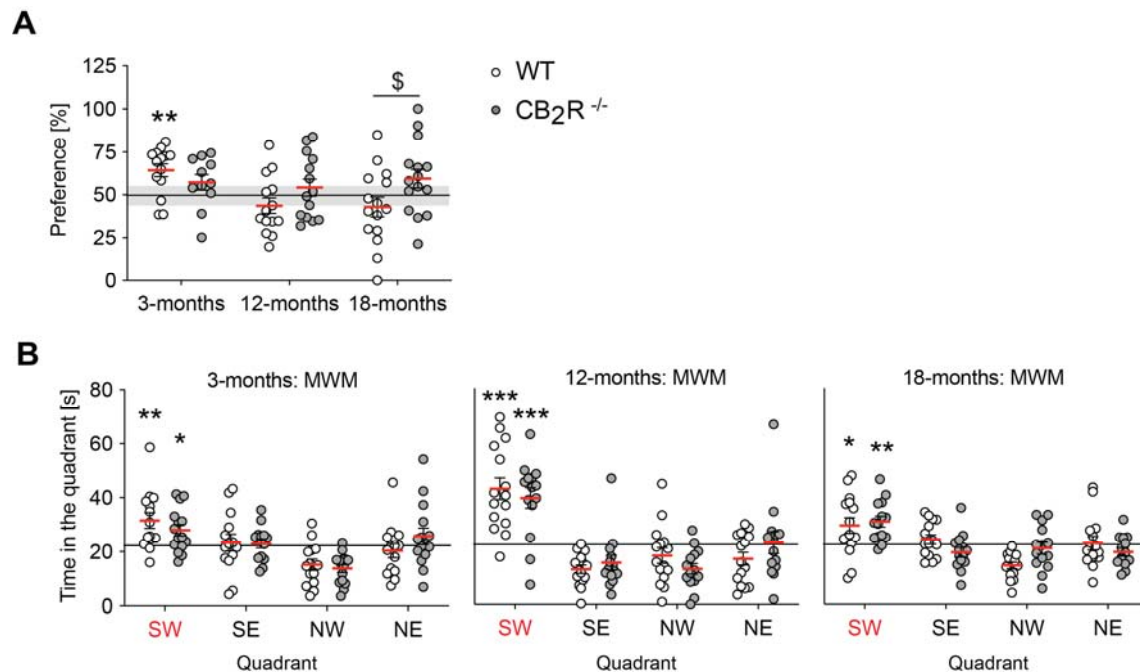
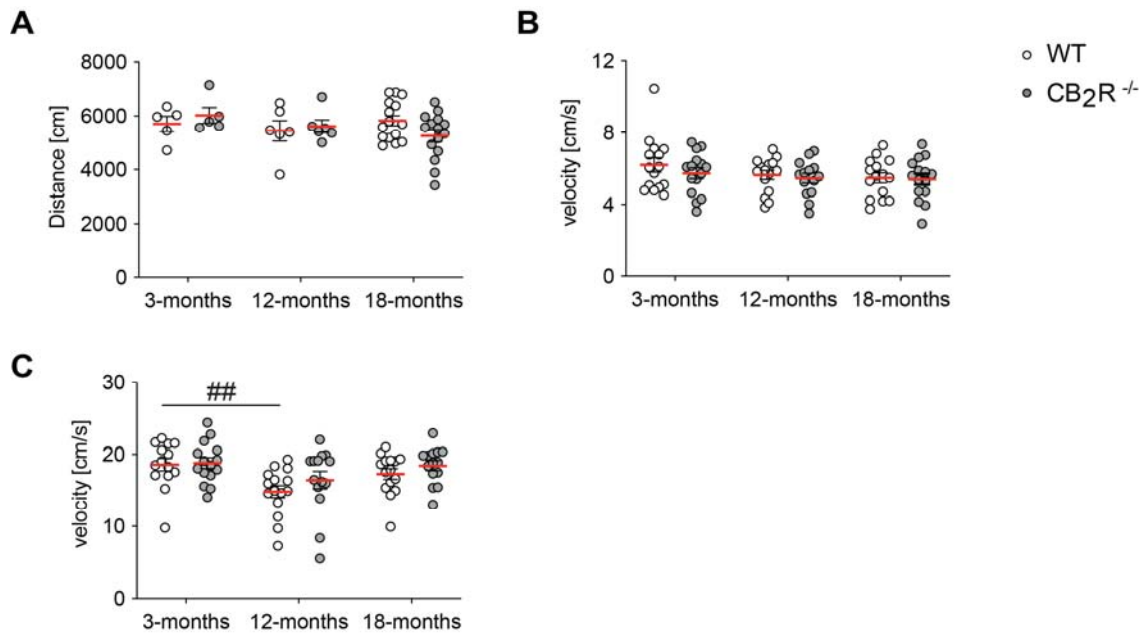


Supplementary Material



Supplementary Figure S1: Memory (A) Preference for the novel partner (after 30 minutes separation) was calculated as time with the novel partner mouse over total interaction time. Each group was analysed individually by one-sample t-test (hypothetical mean = 50). Significant difference from the 50 % chance level indicated learning with $**p < 0.01$. Each point represents a single mouse. Red line indicates the mean value \pm SEM. Line indicates a 50 % chance level. Grey box indicates 5 % variance around the chance level. Two-way ANOVA followed by Sidak's multiple comparison test with \$ $p < 0.05$ significance between genotypes within the same age group. (B) Probe trial of the MWM. Panels from left to right: 3-, 12- and 18-months old mice. Each group was analysed individually by one-sample t-test (hypothetical mean = 22.5). Line indicates a 22.5 s chance level with total swim time 90 s. Quadrant in which the platform was localized (SW) indicated in red. SW - southwest; SE - southeast; NW - northwest; NE - northeast. Significant difference from the 22.5 s chance level indicated intact memory recall with $p < 0.05$; $**p < 0.01$; $***p < 0.001$

WT mice – white circle; CB₂R^{-/-} mice – grey circles. N=14-15 mice / genotype / age group.



Supplementary Figure S2: Motility (A) Total distance travelled during open-field like test (habituation of the PR test, 10-minute free exploration of the open-field box). (B) Mice velocity during trial 1 of the PR test. (C) Mice velocity during probe trail of the MWM test. Two-way ANOVA followed by Sidak's multiple comparison test with ## $p < 0.01$ significance between age groups within the same genotype. WT mice – white circle; CB₂R^{-/-} mice – grey circles. N=5-15 mice / genotype / age group.

Supplementary Table S1. Statistical analysis.

		Statistical test		Posthoc test	
Figure	Panel	Test name	result	Test name	result
1. CB2R deletion has a moderate age-dependent effect on cognition.	1b: Sociability (30 min)	one-sample t-test (hypothetical mean = 50)	all groups $p < 0.0001$		
		two-way ANOVA	Interaction age x genotype $F(2, 71) = 0.8353$, $p = 0.4379$; age $F(2, 71) = 1.018$, $p = 0.3664$; genotype $F(1, 71) = 1.041$, $p = 0.3111$		
	1c: Novelty preference (30 min)	one-sample t-test (hypothetical mean = 50)	3-month WT $p = 0.0468$, CB2R-/- $p = 0.9620$; 12-month WT $p = 0.0035$, CB2R-/- $p = 0.0906$; 18-month WT $p = 0.7849$, CB2R-/- $p = 0.0472$		
		two-way ANOVA	age x genotype $F(2, 71) = 3.560$; $p = 0.0336$; age $F(2, 71) = 1.136$, $p = 0.3270$; genotype $F(1, 71) = 0.7894$, $p = 0.3773$	Šídák's multiple comparisons test	WT 3-month vs 18-month $p = 0.0391$
	1d: 3-month old (Acquisition)	two-way RM Anova	interaction time x genotype $F(5, 135) = 0.6925$; $p = 0.6300$; time $F(5, 135) = 12.29$; $p < 0.0001$; genotype $F(1, 27) = 0.0006$; $p = 0.9800$	Šídák's multiple comparisons test	WT Day 1 vs Day 4 $p = 0.0245$; Day 1 vs Day 6 $p = 0.0010$; Day 2 vs Day 4 $p = 0.0427$; Day 2 vs Day 6 $p = 0.0019$; CB2R-/- Day 1 vs Day 4 $p = 0.0047$; Day 1 vs Day 5 $p = 0.0001$; Day 1 vs Day 6 $p < 0.0001$; Day 2 vs Day 6 $p = 0.0213$
	1d: 3-month old (Reversal)	two-way RM Anova	interaction time x genotype $F(2, 54) = 0.711$; $p = 0.4957$; time $F(2, 54) = 8.366$; $p = 0.0007$; genotype $F(1, 27) = 0.1297$; $p = 0.7215$	Šídák's multiple comparisons test	CB2R-/- Day 8 vs Day 9 $p = 0.0124$; Day 8 vs Day 10 $p = 0.0024$
	1d: 12-month old (Acquisition)	two-way RM Anova	interaction time x genotype $F(5, 135) = 2.06$; $p = 0.0743$; time $F(5, 135) = 33.06$; $p < 0.0001$; genotype $F(1, 27) = 0.0017$; $p = 0.8950$	Šídák's multiple comparisons test	WT Day 1 vs Day 2/3/4/5/6 $p < 0.0001$; Day 2 vs Day 5 $p = 0.0255$; Day 2 vs Day 6 $p = 0.420$; CB2R-/- Day 1 vs Day 3 $p = 0.0027$; Day 1 vs Day 4/5/6 $p < 0.0001$; Day 2 vs Day 3 $p = 0.0269$; Day 2 vs Day 4 $p = 0.0006$; Day 2 vs Day 5/6 $p < 0.0001$; Day 3 vs Day 6 $p = 0.0310$
	1d: 12-month old (Reversal)	two-way RM Anova	interaction time x genotype $F(2, 54) = 3.92$; $p = 0.0257$; time $F(2, 54) = 20.04$; $p < 0.0001$; genotype $F(1, 27) = 1.299$; $p = 0.2644$	Šídák's multiple comparisons test	Day 9 WT vs CB2R-/- $p = 0.0610$; WT Day 8 vs Day 9/10 $p < 0.0001$; CB2R-/- Day 8 vs Day 10 $p = 0.0072$; Day 9 vs Day 10 $p = 0.0402$

		Statistical test		Posthoc test	
Figure	Panel	Test name	result	Test name	result
	1d: 18-month old (Acquisition)	two-way RM Anova	interaction time x genotype F(5, 135)= 1.081; p = 0.3739; time F(5,135) = 31.62; p< 0.0001; genotype F(1,27)= 0.907; p = 0.3494	Šídák's multiple comparisons test	WT Day 1 vs Day 2 p = 0.0080; Day 1 vs Day 3/4/5/6 p < 0.0001; Day 2 vs Day 5 p = 0.0412; Day 2 vs Day 6 p = 0.0003; CB2R-/- Day 1 vs Day 2/3/4/5/6 p< 0.0001
	1d: 18-month old (Reversal)	two-way RM Anova	interaction time x genotype F(2, 54)= 1.936; p = 0.1541; time F(2, 54)= 12.82; p < 0.0001; genotype F(1, 27) = 0.07016; p = 0.7931	Šídák's multiple comparisons test	CB2R-/- Day 8 vs Day 9 p= 0.0113, Day 8 vs Day 10 p< 0.0001
2. CB2R deletion results in a decreased anxiety phenotype in o-maze	2a: % of time spent in the open compartment	two-way ANOVA	Interaction age x genotype F(2, 82) = 0.3559, p = 0.7016; age F(2, 82) = 10.26; p = 0.0001; genotype F(1, 82) = 4.427; p = 0.0384	Šídák's multiple comparisons test	WT 3-months vs 18-months p= 0.0026; CB2R-/- 3-months vs 12-months p= 0.0372, 3-months vs 18-months p= 0.0306
	2b: Distance traveled in the open compartment	two-way ANOVA	Interaction age x genotype F(2, 82) = 0.5281, p= 0.5917; age F(2,82) = 17; p < 0.0001; genotypeF(1,82) = 7.009, p= 0.0097	Šídák's multiple comparisons test	WT 3-months vs 18-months p= 0.0004; CB2R-/- 3-months vs 12-months p= 0.0012, 3-months vs 18-months p= 0.0007
	2c: Number of stretched posture behaviours	two-way ANOVA	Interaction age x genotype F(2, 82) = 3.004, p = 0.0552, age F(2, 81) = 4.152; p= 0.0192; genotype F(1, 81) = 13.24; p = 0.0005	Šídák's multiple comparisons test	WT vs CB2R-/- 3-months p= 0.0071, WT vs CB2R-/- 18-months p= 0.0072; CB2R-/- 12-month vs 18-months p =0.0083
	2d: Number of looking down behaviors	two-way ANOVA	Interaction age x genotype F(2, 81) = 0.6890, p = 0.5050, age F(2, 81) = 2.702; p =0.0731, genotype F(1, 81) = 33.24; p < 0.0001	Šídák's multiple comparisons test	WT vs CB2R-/- 3-months p= 0.0039, WT vs CB2R-/- 12-months p= 0.0451; WT vs CB2R-/- 18-months p= 0.0002
3. Accumulation of Lipofuscin in pyramidal neurons during aging is not altered by CB2 deletion	3b: Area covered	two-way ANOVA	interaction, genotype x age F(2,66)= 0.4423; p = 0.6445; age F(2,66)= 81.41; p < 0.0001; genotypeF(1,66)= 0.5129; p = 0.4764	Šídák's multiple comparisons test	WT 3-months vs 12-months p = 0.0001, 3-months vs 18-months p < 0.0001, 12-months vs 18-months p < 0.0001; CB2R-/- 3-months vs 12-months p < 0.0001, 3-months vs 18-months p < 0.0001, 12-month vs 18-month p = 0.0034
	3c: Particle density	two-way ANOVA	interaction genotype x age F(2, 65) = 0.7953; p = 0.4558; age F(2, 65) = 82.98; p< 0.0001; genotype F(1, 65) = 1.269; p = 0.2641	Šídák's multiple comparisons test	WT 3-months vs 12-months p < 0.0001; 3-months vs 18-months p < 0.0001; 12-months vs 18-months p = 0.0010; CB2R-/- 3-months vs 12-months p < 0.0001; 3-months vs 18-months p<0.0001; 12-months vs 18-months p = 0.0527

		Statistical test		Posthoc test	
Figure	Panel	Test name	result	Test name	result
4. Accumulation of Lipofuscin in microglia is increased after CB2 deletion	4b: Area covered	two-way ANOVA	interaction genotype x age $F(2, 419) = 7.514$; $p = 0.0006$; age $F(2, 419) = 47.06$; $p < 0.0001$; genotype $F(1, 419) = 7.857$; $p = 0.0053$	Šídák's multiple comparisons test	WT vs CB2R-/- 12 months $p < 0.0001$; WT 3-months vs 18-months $p < 0.0001$, 12-months vs 18-months $p < 0.0001$; CB2R-/- 3-months vs 12-months $p < 0.0001$, 3-months vs 18-months $p < 0.0001$
	4c: Particle density	two-way ANOVA	interaction genotype x age $F(2, 429) = 1.085$; $p = 0.3388$; age $F(2, 429) = 48.34$; $p < 0.0001$; genotype $F(1, 419) = 3.209$; $p = 0.0740$	Šídák's multiple comparisons test	WT 3-months vs 12-months $p < 0.0001$, 3-months vs 18-months $p < 0.0001$; CB2R-/- 3-months vs 12-months $p < 0.0001$; 3-months vs 18-months $p < 0.0001$; 12-months vs 18-months $p = 0.0385$
5. Iba1 and CD68 intensity is enhanced in CB2-/- microglia	5b: Iba1 mean grey value	two-way ANOVA	interaction genotype x age $F(2, 345) = 3.525$; $p = 0.0305$; age $F(2, 345) = 11.43$; $p < 0.0001$; genotype $F(1, 345) = 36.48$; $p < 0.0001$	Šídák's multiple comparisons test	WT vs CB2R-/- 3 months $p < 0.0001$; WT vs CB2R-/- 18 months $p < 0.0001$; WT 3-months vs 18-months $p < 0.0001$, 3-months vs 18-months $p = 0.0465$; 3-months vs 18-months $p = 0.0511$; CB2R-/- 3-months vs 18-months $p = 0.0373$
	5c: % of soma covered by CD68	two-way ANOVA	interaction genotype x age $F(2, 334) = 2.424$; $p = 0.0901$; age $F(2, 334) = 18.11$; $p < 0.0001$; genotype $F(1, 334) = 2.794$; $p = 0.0956$	Šídák's multiple comparisons test	WT vs CB2R-/- 18 months $p = 0.0342$; WT 3-months vs 12-months $p < 0.0001$, 3-months vs 18-months $p = 0.0347$; CB2R-/- 3-months vs 12-months $p = 0.0046$; 3-months vs 18-months $p < 0.0001$
6. Age-dependent alteration in expression of inflammatory mediators.	6a: <i>tnfa</i> relative expression	two-way ANOVA	interaction genotype x age $F(2, 28) = 2.191$; $p = 0.1306$; age $F(2, 28) = 7.804$; $p = 0.002$; genotype $F(1, 28) = 3.673$; $p = 0.0656$	Šídák's multiple comparisons test	CB2R-/- 3-month vs 12-month $p = 0.0095$, 3-month vs 18-month $p = 0.0022$
	6b: <i>il-6</i> relative expression	two-way ANOVA	interaction genotype x age $F(2, 28) = 1.152$; $p = 0.3304$; age $F(2, 28) = 15.08$; $p < 0.0001$; genotype $F(1, 28) = 1.089$; $p = 0.3057$	Šídák's multiple comparisons test	WT 3-month vs 12-month $p = 0.0025$; 12-month vs 18-month $p = 0.0052$; CB2R-/- 3-month vs 12-month $p = 0.0018$
	6a: <i>ccl2</i> relative expression	two-way ANOVA	interaction genotype x age $F(2, 29) = 4.738$; $p = 0.0166$; age $F(2, 29) = 12.29$; $p = 0.0001$; genotype $F(1, 29) = 0.5880$; $p = 0.4494$	Šídák's multiple comparisons test	WT vs CB2R-/- $p = 0.0266$; WT 3-month vs 18-month $p < 0.0001$, 12-month vs 18-month $p = 0.0001$
	6b: <i>arg1</i> relative expression	two-way ANOVA	interaction genotype x age $F(2, 30) = 0.3524$; $p = 0.7059$; age $F(2, 28) = 1.803$; $p = 0.1823$; genotype $F(1, 30) = 0.0531$; $p = 0.8193$		

		Statistical test		Posthoc test	
Figure	Panel	Test name	result	Test name	result
	6b: <i>nos2</i> relative expression	two-way ANOVA	interaction genotype x age F(2,26) = 0.08149; p = 0.9220; age F(2,26) = 4.275; p = 0.0248; genotype F(1,26) = 0.5170; p = 0.4785	Šídák's multiple comparisons test	-
7. CB2R deletion changes microglial morphology	7b: Soma size	two-way ANOVA	interaction genotype x age F(2,1000) = 1.786; p = 0.1681; age F(2,1000) = 22.97; p < 0.0001; genotype F(1,1000) = 15.99; p < 0.0001	Šídák's multiple comparisons test	WT vs CB2R-/- 18 months p = 0.0006; WT 3-months vs 18-months p < 0.0001, 12-months vs 18-months p < 0.0001; CB2R-/- 3-months vs 18-months p = 0.0276; 12-months vs 18-months p = 0.0029
	7c: Volume	two-way ANOVA	interaction genotype x age F(2,316) = 0.1823; p = 0.8334; age F(2,316) = 1.076; p = 0.3422; genotype F(1,316) = 11.6; p = 0.0007	Šídák's multiple comparisons test	WT vs CB2R-/- 3 months p = 0.0462
	7d: Ramification index	two-way ANOVA	interaction genotype x age F(2,317) = 0.4927; p = 0.6114; age F(2,317) = 1.279; p = 0.2797; genotype F(1,317) = 15.18; p = 0.0001	Šídák's multiple comparisons test	WT vs CB2R-/- 3 months p = 0.0101
	7e: Polarity index	two-way ANOVA	interaction genotype x age F(2,340) = 0.09658; p = 0.9080; age F(2,340) = 2.283; p = 0.1035; genotype F(1,340) = 15.27; p = 0.0001	Šídák's multiple comparisons test	WT vs CB2R-/- 3 months p = 0.0298
	7f: Tree length	two-way ANOVA	interaction genotype x age F(2,329) = 1.426; p = 0.2418; age F(2,329) = 0.4238; p = 0.6549; genotype F(1,329) = 13.02; p = 0.0004	Šídák's multiple comparisons test	WT vs CB2R-/- 3 months p = 0.0025
	7g: Average branch length	two-way ANOVA	interaction genotype x age F(2,326) = 3.519; p = 0.0308; age F(2,326) = 1.295; p = 0.2754; genotype F(1,326) = 5.847; p = 0.0161	Šídák's multiple comparisons test	WT vs CB2R-/- 18 months p = 0.0054; CB2R-/- 12-months vs 18-months p = 0.0187
S1. Memory	S1a: Novelty preference (1h)	one-sample t-test (hypothetical mean = 50)	3-month WT p = 0.0021		
	S1a: Novelty preference (1h)	two-way ANOVA	age x genotype F(2,77) = 2.910; p = 0.0605; age F(2,77) = 3.041; p = 0.0535	Šídák's multiple comparisons test	WT vs CB2R-/- 18-months p = 0.0483
	1b: MWM probe trial (preference for platform-associated quadrant)	one-sample t-test (hypothetical mean = 22.5)	3-month WT p = 0.0088, CB2R-/- p = 0.0219; 12-month WT p = 0.0002, CB2R-/- p = 0.0008; 18-month WT p = 0.0421, CB2R-/- p = 0.0018		
S2. Motility	S2a: Distance: open-field like test	two-way ANOVA	interaction genotype x age F(2,46) = 1.743; p = 0.1864; age F(2,46) = 0.7420; p = 0.4818; genotype F(1,46) = 0.01203; p = 0.9131		

		Statistical test		Posthoc test	
Figure	Panel	Test name	result	Test name	result
	S2a: Velocity: PR test	two-way ANOVA	interaction genotype x age $F(2,83) = 0.2221$; $p = 0.8013$; age $F(2,83) = 1.701$; $p = 0.1888$; genotype $F(1,83) = 0.9010$; $p = 0.3453$		
	S2c: Velocity: MWM test	two-way ANOVA	interaction genotype x age $F(2,81) = 0.3312$; $p = 0.7190$; age $F(2,81) = 6.678$; $p = 0.0021$; genotype $F(1,81) = 1.954$; $p = 0.1660$		WT 3-months vs 12-months $p = 0.0083$