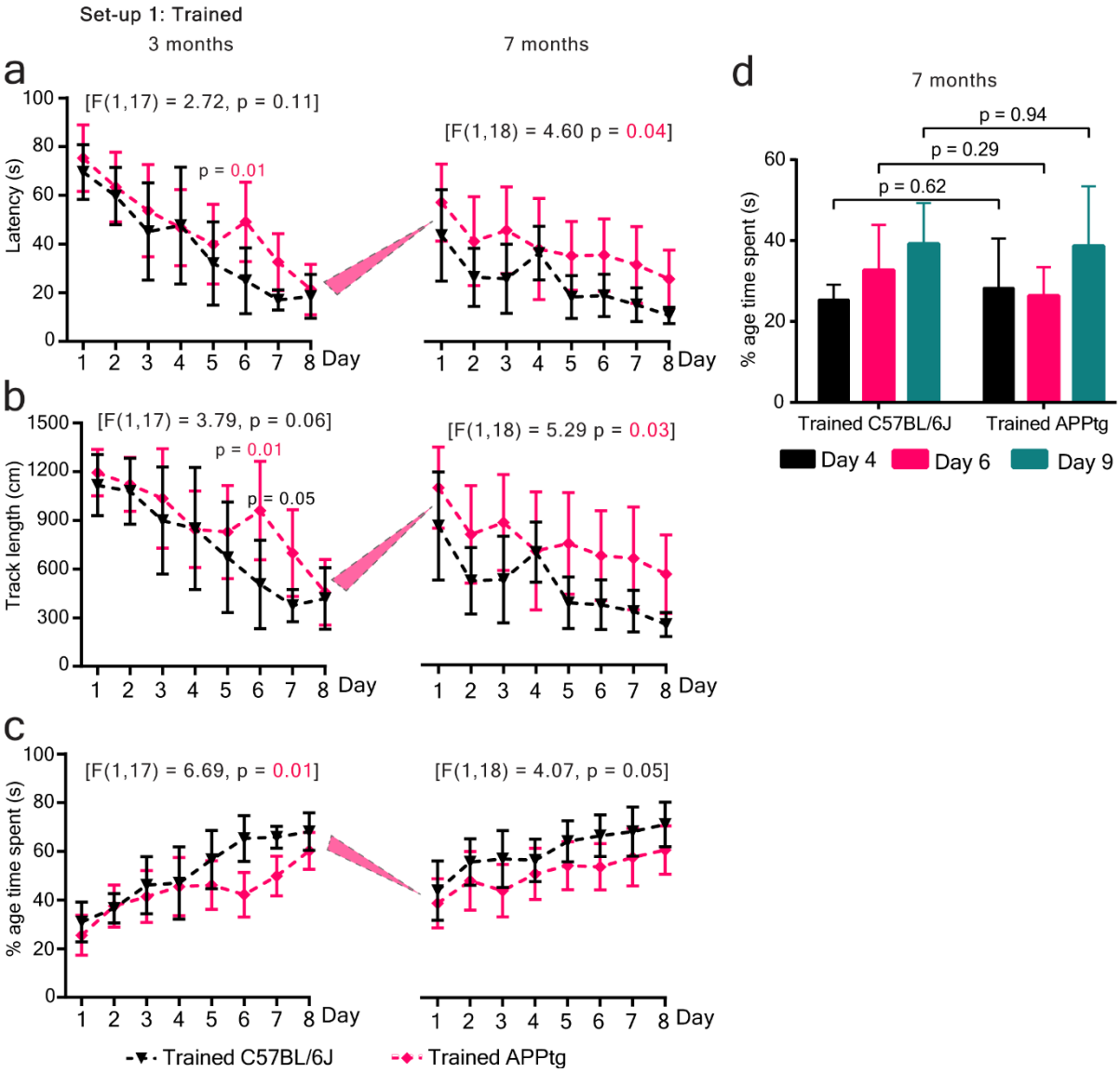
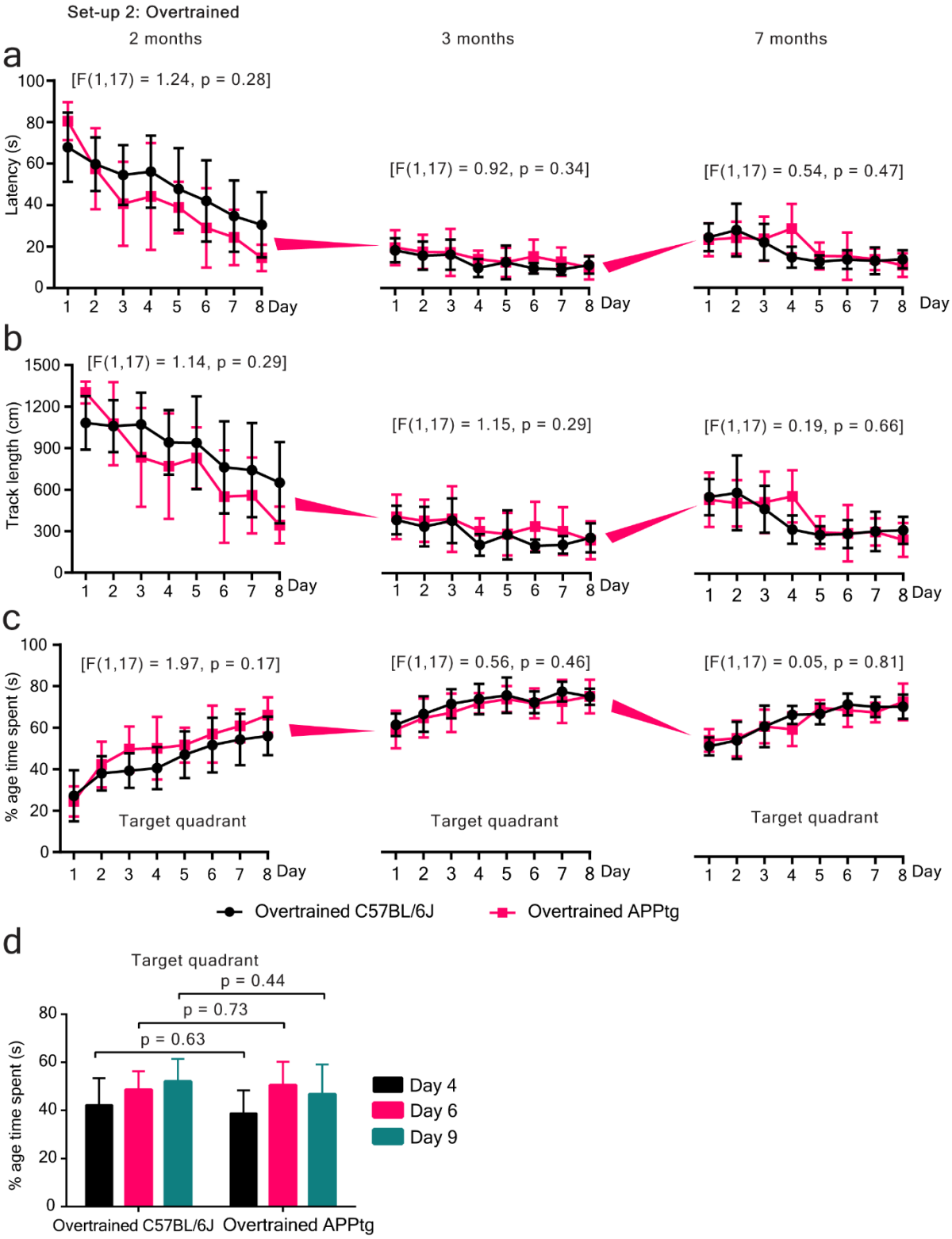
Supplementary Material

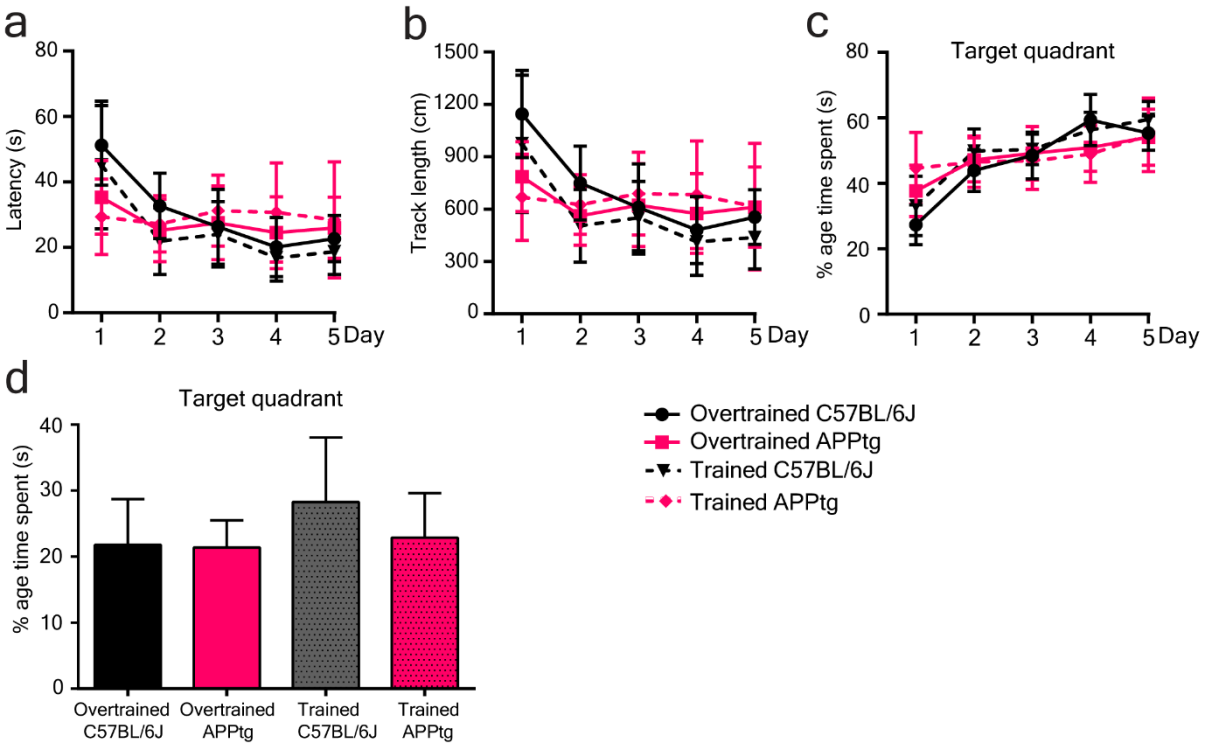
Early Cognitive Training Rescues Remote Spatial Memory but Reduces Cognitive Flexibility in Alzheimer's Disease Mice



Supplementary Figure 1. Assessment of WM performance using different measures in 7-month-old APPtg mice. The data were related to set-up 1 experiment. Mice that were trained at 3 months of age were retrained at 7 months of age. Different measures such as a) latency, b) track length, and c) % age time spent on the target quadrant were used to assess spatial learning during training trials. ANOVA on the mixed model analysis showed significant group difference between trained C57BL/6J and APPtg mice during initial training task as well as retraining task. b) Assessment of spatial memory using probe trials at 7 months of age. Two-tailed unpaired t-test was performed. Values are mean ± 95% confidence interval.



Supplementary Figure 2. Cognitive overtraining and WM performance in APPtg mice. The result is based on set-up II (over-trained group). Here, we trained mice at 2 months of age, retrained them at 3 months, and assessed their performance at 7 months of age. The WM performance was assessed via a) latency, b) track length, and c) % age time spent on target quadrant. ANOVA result of the mixed model analysis were provided alongside each figure. d) Performance of animals during probe trials. Three probe trials were given on day 4, 6, and 9, respectively, in which mice were allowed to swim for 30 s. Two-tailed unpaired t-tests were performed between groups on each probe trial. Values are mean ± 95% confidence interval.



Supplementary Figure 3. WM performance during WM-reversal task. Additional measures such as latency, track length, and % age time spent on the target quadrant were used to assess learning and memory. Later, ANOVA on a mixed model for learning measures were conducted at the p-value < 0.05 for four different training groups. a) Latency: main effect [$F(3,34) = 0.69$, $p = 0.56$]. b) Track length: main effect [$F(3,34) = 1.01$, $p = 0.39$]. c) % age time spent on target quadrant: main effect [$F(3,34) = 0.40$, $p = 0.75$]. d) Finally, a single probe trial was conducted on day 15 by allowing mice to swim for 30 sec in a water pool. A one-way ANOVA on % age time spent on target quadrant data was conducted at the p-value < 0.05 for four different training groups [$F(3,34) = 0.95$, $p = 0.42$]. Values are mean ± 95% confidence interval.

Supplementary Table 1. Summary table of one-way ANOVA followed by post hoc Tukey's comparison for different training groups on a first trial of day 1. Latency, track length, and % age time spent in target quadrant were used as measures of spatial learning and memory. Values were taken from 7-month-old mice. Red values are $p < 0.05$; TQ, target quadrant

	Group	estimate	t-ratio	p
Main effect on Trial 1- Latency: [F(3,35) = 4.10, p = **0.01**]				
1	Overtrained APPtg versus Overtrained C57BL/6J	-12.40	-1.02	0.73
2	Overtrained APPtg versus trained APPtg	-39.90	-3.22	**0.04**
3	Overtrained C57BL/6J versus trained C57BL/6J	-15.6	-1.365	0.52
4	Trained APPtg versus trained C57BL/6J	11.9	1.017	0.74
Main effect on Trial 1- Track length: [F(3,35) = 3.96, p = **0.01**]				
1	Overtrained APPtg versus Overtrained C57BL/6J	-326	-1.16	0.65
2	Overtrained APPtg versus trained APPtg	-926	-3.22	**0.01**
3	Overtrained C57BL/6J versus trained C57BL/6J	-316	-1.19	0.63
4	Trained APPtg versus trained C57BL/6J	283	1.04	0.72
Main effect on Trial 1- % age time spent on TQ: [F(3,35) = 3.44, p = **0.02**]				
1	Overtrained APPtg versus Overtrained C57BL/6J	13.84	1.70	0.33
2	Overtrained APPtg versus trained APPtg	19.97	2.40	0.09
3	Overtrained C57BL/6J versus trained C57BL/6J	11.71	1.53	0.42
4	Trained APPtg versus trained C57BL/6J	5.58	0.71	0.89

Supplementary Table 2. Summary table of one-way ANOVA followed by post hoc Tukey's comparison for different groups in day 1. Latency, track length, and % age time spent in target quadrant were used as measures of learning and memory and values were taken from 7-month-old mice. Red values are p <0.05; TQ, target quadrant

	Main effect on Day 1- Latency: [F(3,35) = 7.41, p = **0.0005**]			
	Group	**Estimate**	**t-ratio**	**p**
1	Overtrained APPtg versus Overtrained C57BL/6J	-1.22	-0.14	0.99
2	Overtrained APPtg versus trained APPtg	-33.97	-3.83	**0.002**
3	Overtrained C57BL/6J versus trained C57BL/6J	-19.25	-2.35	0.10
4	Trained APPtg versus trained C57BL/6J	13.50	1.61	0.38
	Main effect on Day 1- Track length: [F(3,35) = 6.34, p = **0.001**]			
1	Overtrained APPtg versus Overtrained C57BL/6J	-19.2	-0.12	0.99
2	Overtrained APPtg versus trained APPtg	-559.1	-3.56	**0.005**
3	Overtrained C57BL/6J versus trained C57BL/6J	-303.4	-2.10	0.17
4	Trained APPtg versus trained C57BL/6J	236.5	1.59	0.39
	Main effect on Day 1- % age time spent on TQ: [F(3,35) = 4.16, p = **0.01**]			
1	Overtrained APPtg versus Overtrained C57BL/6J	2.95	0.52	0.95
2	Overtrained APPtg versus trained APPtg	17.31	3.02	**0.02**
3	Overtrained C57BL/6J versus trained C57BL/6J	9.19	1.74	0.31
4	Trained APPtg versus trained C57BL/6J	-5.17	-0.95	0.77

Supplementary Table 3. Summary table showing linear mixed model followed by post hoc Tukey's comparison on WM relearning task on 7-month-old mice. Latency, track length, and % age time spent on target quadrant were used as measures of learning and memory. Red-bold values are $p < 0.05$; TQ, target quadrant.

	Main effect on overall training trials- Latency: [F(3,35) = 7.28, p = **0.0006**]			
	Group	**Estimate**	**t-ratio**	**p**
1	Overtrained APPtg versus Overtrained C57BL/6J	1.59	0.30	0.98
2	Overtrained APPtg versus trained APPtg	-19.43	-3.66	**0.004**
3	Overtrained C57BL/6J versus trained C57BL/6J	-6.70	-1.37	0.52
4	Trained APPtg versus trained C57BL/6J	14.32	2.86	**0.03**
	Main effect on overall training trials- Track length: [F(3,35) = 7.48, p = **0.0005**]			
1	Overtrained APPtg versus Overtrained C57BL/6J	18.3	0.19	0.99
2	Overtrained APPtg versus trained APPtg	-358.4	-3.77	**0.003**
3	Overtrained C57BL/6J versus trained C57BL/6J	-103.5	-1.18	0.64
4	Trained APPtg versus trained C57BL/6J	273.2	3.04	**0.02**
	Main effect on overall training trials- % age time spent on TQ: [F(3,35) = 7.45, p = **0.0006**]			
1	Overtrained APPtg versus Overtrained C57BL/6J	-0.41	-0.11	0.99
2	Overtrained APPtg versus trained APPtg	14.45	3.83	**0.002**
3	Overtrained C57BL/6J versus trained C57BL/6J	5.33	1.53	0.42
4	Trained APPtg versus trained C57BL/6J	-9.53	-2.68	0.05

Supplementary Table 4. Summary table showing one-way ANOVA followed post hoc Tukey's comparison for different groups during probe trials on days 4, 6, and 9. Proximity was used as a measure of spatial memory. Overtrained C57BL/6J - N=10 for probe trial - day 4 and N=11 for probe trials - day 6 and day 9; Overtrained APPtg - N=8; Trained C57BL/6J- N=10; and trained APPtg - N=10. Red-bold values are $p < 0.05$.

	Main effect: Probe trial - day 4 [$F(3,34) = 4.48$, $p =$ **0.009**]			
	Group	**Estimate**	**t-ratio**	**p**
1	Overtrained APPtg versus Overtrained C57BL/6J	-0.91	-0.25	0.99
2	Overtrained APPtg versus trained APPtg	-9.14	-2.49	0.07
3	Overtrained C57BL/6J versus trained C57BL/6J	-9.28	-2.68	**0.05**
4	Trained APPtg versus trained C57BL/6J	-1.05	-0.30	0.99
	Main effect: Probe trial - day 6 [$F(3,35) = 11.13$, $p =$ **0.00002**]			
1	Overtrained APPtg versus Overtrained C57BL/6J	-1.40	-0.49	0.960
2	Overtrained APPtg versus trained APPtg	-14.25	-4.89	**0.0001**
3	Overtrained C57BL/6J versus trained C57BL/6J	-6.99	-2.60	0.06
4	Trained APPtg versus trained C57BL/6J	5.86	2.13	0.16
	Main effect: Probe trial - day 9 [$F(3,35) = 2.66$, $p =$ **0.06**]			
1	Overtrained APPtg versus Overtrained C57BL/6J	3.12	1.03	0.74
2	Overtrained APPtg versus trained APPtg	-4.85	-1.55	0.41
3	Overtrained C57BL/6J versus trained C57BL/6J	-4.92	-1.70	0.33
4	Trained APPtg versus trained C57BL/6J	3.05	1.03	0.73

Supplementary Table 5. Summary table showing one-way ANOVA followed post hoc Tukey's comparison for different training groups during probe trials on days 4, 6, and 9, respectively. % age time spent on target quadrant was used as a measure of spatial memory. Overtrained C57BL/6J - N=10 for probe trial - day 4 and N=11 for probe trials - day 6 and day 9; Overtrained APPtg - N=8; Trained C57BL/6J - N=10; and trained APPtg - N=10. Red-bold values are $p < 0.05$.

	Main effect: Probe trial - day 4 [F(3,34) = 3.40, p = **0.02**]			
	Group	**Estimate**	**t-ratio**	**p**
1	Overtrained APPtg versus Overtrained C57BL/6J	-3.34	-0.51	0.95
2	Overtrained APPtg versus trained APPtg	10.53	1.62	0.37
3	Overtrained C57BL/6J versus trained C57BL/6J	16.70	2.74	**0.04**
4	Trained APPtg versus trained C57BL/6J	2.84	0.46	0.96
	Main effect: Probe trial - day 6 [F(3,35) = 8.89, p = **0.0001**]			
1	Overtrained APPtg versus Overtrained C57BL/6J	1.88	0.32	0.98
2	Overtrained APPtg versus trained APPtg	24.04	4.11	**0.001**
3	Overtrained C57BL/6J versus trained C57BL/6J	15.94	2.96	**0.02**
4	Trained APPtg versus trained C57BL/6J	-6.22	-1.12	0.67
	Main effect: Probe trial - day 9 [F(3,35) = 1.63, p = 0.19]			
1	Overtrained APPtg versus Overtrained C57BL/6J	-5.18	-0.69	0.89
2	Overtrained APPtg versus trained APPtg	8.12	1.06	0.71
3	Overtrained C57BL/6J versus trained C57BL/6J	12.80	1.81	0.28
4	Trained APPtg versus trained C57BL/6J	-0.50	-0.07	0.99