Supporting Information

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Fig. S1. The 0.5 h massed and spaced training (Fig. 1). (*A*) Relative horizontal optokinetic response (HOKR) gain was similar for all training protocols with 1 h and 0.5-h training (P < 0.05; one-way ANOVA). (*B*) Representative eye traces from individual animals at the start (black), at the end (blue), and on the second day (red) after 0.5 h massed (M, M0.5) or spaced training (S_{60} , S_{60} 0.5). (*C*) Pooled data on the first and second days after M0.5 (n = 5; blue) and S_{60} 0.5 (n = 6; red) training also show similar acquisition but significantly lower retention of HOKR gain by massed training compared with spaced training. *P < 0.05 vs. end of training on the first day, paired *t* test.



Fig. S2. No change in the volume of the floccular molecular layer after S_{60} training (Fig. 4). (A) Nissl-stained serial sections of the flocculus (FI), which were used to measure the total volume including the molecular layer (ML), the granule cell layer (GL), and the white matter. (Scale bar, 200 μ m.) (B) No change was detected in the total volume or the molecular layer of the FI on the second day after S_{60} training compared with the untrained control.



Fig. S3. An increase in HOKR gain on the fifth day after massed training was observed, and this increase was similar with or without a second day of HOKR recording (Fig. 5). The HOKR increase due to M training (red) on the fifth day was not different from that of the M1 group (black), for which the second day of recording was skipped. These data show that the HOKR gain increase on the fifth day was not affected by 1-min recording of HOKR and is a true representation of the slow development of long-lasting memory induced by massed training.

Table S1.	Mean HOKR ga	in (SEM) at 0.25	Hz, 17° (pe	eak-to-peak)
screen oso	illation			

Training protocols	Start of training	End of training	Second day
$S_{10} (n = 6)$	0.40 (0.02)	0.81 (0.03)	0.60 (0.07)
S ₂₀ (n = 9)	0.41 (0.03)	0.84 (0.07)	0.69 (0.06)
S ₄₀ (n = 6)	0.41 (0.02)	0.81 (0.05)	0.76 (0.05)
S ₆₀ (n = 9)	0.42 (0.02)	0.82 (0.05)	0.90 (0.06)
M (<i>n</i> = 10)	0.42 (0.02)	0.83 (0.07	0.67 (0.06)
S ₆₀ 0.5 (<i>n</i> = 6)	0.33 (0.02)	0.44 (0.04	0.49 (0.04)
M0.5 (<i>n</i> = 5)	0.38 (0.05)	0.53 (0.07)	0.43 (0.05)

Related to Fig. 1.



Movie S1. HOKR system (Fig. 1). The mouse was restrained in an up-sloping cylinder and mounted via a head-attached screw on the stage surrounded by a checked-pattern screen that was driven by a motor. The screen was illuminated with 80-100 lx. The frontal view of the right eye, under the illumination of four infrared light-emitting diodes, was captured using a CCD camera placed at an angle of 60° laterally from the midline and 30° down from the horizontal plane and output into a personal computer for image processing and online calculation. The image was converted from gray (upper right inset) to binary (upper left inset) format to increase the contrast for calculating the right pupil position.

Movie S1