Heterozygous knockout of the Bmi-1 gene causes an early onset of phenotypes associated with brain aging

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Materials and Methods

Morris water maze testing

Morris water maze testing was performed to assess hippocampus-dependent spatial learning and memory ability as previously described (Li et al. 2011). Briefly, a black circular pool (1.0 m in diameter, 0.5 m in height) was filled with water to a depth of 25 cm. One cm beneath the water's surface, a 10 cm dark cylindrical platform was fixed. The water was made opaque with black tempera paint (Shanghai Dyestuffs Research Institute Co. Ltd, China) and maintained at a temperature of 22 ± 2 °C. A digital video camera was positioned directly above the pool and connected to a computer controlled system (Beijing Sunny Instruments Co. Lt., China), enabling full collection of the swimming pattern, distance and speed.

For training, each mouse was placed in the water, facing the pool wall, at one of four pseudo-randomly chosen start positions. If the mouse failed to escape onto the platform within 60 s, it was guided to it and allowed to remain there for 15 s. Following each training exercise, the animals were placed in a clean cage and allowed a 30 min interval before the beginning of the next trial. Each mouse received four training trials on each of four consecutive days. Latency to escape from the water maze (the time to find the submerged platform) was calculated for each trial. The swimming distance and speed were also analyzed. On day 5, the probe test was carried out by removing the platform and allowing each mouse to swim freely for 60 s. The percentage of time spent in each quadrant was calculated.

Statistical analysis

All statistical analyses were performed using SPSS software, version 16.0 (SPSS Inc., USA). Group differences in the Morris water maze platform training data were analyzed by two-way ANOVA with repeated measures. Other data were analyzed with Student *t*-tests. Data are expressed as mean ± SEM. *P* value less than 0.05 was

considered statistically significant.

Results

Mild spatial memory decline in Bmi-$1^{+/-}$ mice

The escape latency progressively decreased over 4 days of training in both Bmi-$1^{+/-}$ and Bmi-$1^{+/+}$ mice [$F(3,78) = 31.43$, $P < 0.0001$]. The genotype and trial day × genotype interaction had no effect on escape latencies [($F(1,26) = 0.551$, $P = 0.464$ and $F(3,78) = 0.253$, $P = 0.859$, respectively]. Bmi-$1^{+/-}$ mice did not have a significantly increased escape latency to the submersed platform, compared with Bmi-$1^{+/+}$ group (Fig. S1a). Similarly, swimming distance was affected by training day [$F(3, 78) = 40.845$, $P < 0.0001$] but neither by genotype [$F(1, 26) = 0.424$, $P = 0.521$] nor by their interaction [$F(3, 78) = 0.646$, $P = 0.588$]. This data suggest that the spatial learning ability does not decline in Bmi-$1^{+/-}$ mice when compared with Bmi-$1^{+/+}$ controls.

In the probe trial, Bmi-$1^{+/-}$ mice showed a non-significant decrease of time spent in the target quadrant (33.21 ± 1.65% vs. 36.36 ± 2.05%; $P = 0.2434$), right adjacent quadrant (23.14 ± 1.14% vs. 25.43 ± 0.70%; $P = 0.1011$) or left adjacent quadrant (22.71 ± 0.93% vs. 24.79 ± 0.76%; $P = 0.0969$), but a significantly higher percentage of time in the opposite quadrant (20.94 ± 2.35% vs. 13.42 ± 1.54%; $P = 0.0127$), compared with Bmi-$1^{+/+}$ mice (Fig. S1b). These results suggest that Bmi-$1^{+/-}$ mice have mild spatial memory impairment which is a hallmark of the early stages of brain aging.

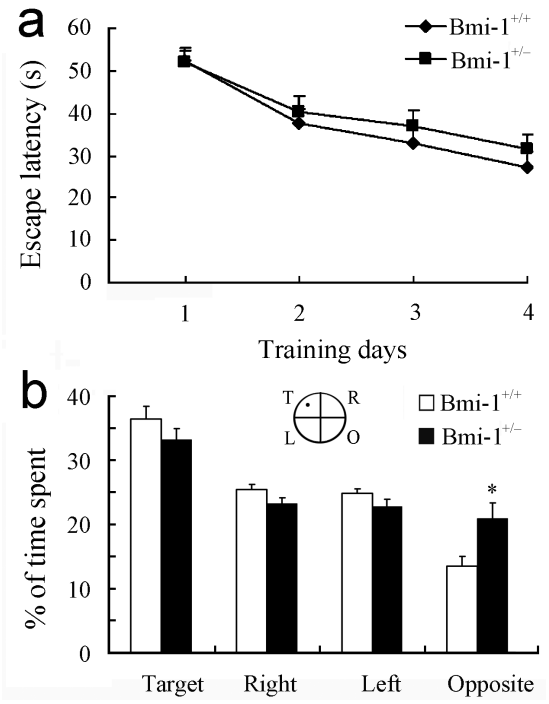

Fig. 1S Spatial learning and memory analysis of Bmi-$1^{+/-}$ mice. **a** Comparison of escape latency to the hidden platform of Bmi-$1^{+/+}$ mice and Bmi-$1^{+/-}$ mice during the Morris water maze training trails. **b** The percentage of time spent in each quadrant in the probe test. Data represent mean ± SEM from 14 mice per group performed in triplicate. *P<0.05 vs. Bmi-$1^{+/+}$ mice.

References

Li L, Ding J, Marshall C, Gao J, Hu G, Xiao M (2011) Pretraining affects Morris water maze performance with different patterns between control and ovariectomized plus D-galactose-injected mice. Behav Brain Res 217(1):244–247