Impact of preoperative environmental enrichment on prevention of development of cognitive impairment following abdominal surgery in a rat model

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Supporting information 1: Replicated study

As current behavioral cognitive experiment was an exploratory study, no previous data available and no sample size was calculated. However, due to the relatively low sample size (n=8 in each group), this remains the issue of replicability of the results. Therefore, to ensure the reliability and validity of our results, we replicated our *in vivo* behavioral experiment using the same experimental protocol described in original manuscript.

Results of replicated study

There were no differences between groups in the habituatory pattern or total locomotor counts during a repeated open-field test. In addition, all physiological parameter were comparable between groups during isoflurane anesthesia (supporting Table 1), and all animals recovered from anesthesia and surgery uneventfully.

During the training phase in novel object recognition task, there was no

difference in baseline exploratory preference among all groups in both young (supporting Fig. 1A, $p = 0.28$) and aged (Fig. 1B, $p = 0.37$) rats. Total exploration time during the training phase were comparable within each age group (supporting Table 2, young; $p = 0.87$, aged; $p = 0.90$, Kruskal-Wallis test), as well as between young and aged group (main effect for group, $F_{(1, 56)} = 2.24$, $p = 0.14$, two-way ANOVA).

During the testing phase, in the young groups, there was no difference in novel object preference between groups (sedentary/non-surgery group; 78.6±11.0%, PEE/non-surgery group; 77.3±7.8%, sedentary/surgery group; 76.7±10.7%, PEE/surgery group; 78.3±7.0%, $p = 0.94$, Kruskal-Wallis test). On the other hand, the sedentary/surgical rats in the aged group exhibited significantly impaired novel object recognition performance (novel object preference of 56.1±11.9% *vs.* 80.4±8.8% in sedentary/non-surgical group, $p < 0.05$, Wilcoxon-Mann-Whitney test with Bonferroni correction). However, such impairment was not observed in the PEE/surgery group (novel object preference of 72.2±11.9% *vs.* 75.6±11.1% in PEE /non-surgical group, $p = 0.54$, Wilcoxon-Mann-Whitney test with Bonferroni correction). Specifically, the novel object preference in the PEE/surgery group was significantly higher than that in the sedentary/surgery group ($p < 0.05$, Wilcoxon-Mann-Whitney test with Bonferroni correction).

All these results of the replicated study are sufficiently statistically similar to the original results, lending support to the replicability of our findings.

Supporting Table 1. Physiological parameters during isoflurane anesthesia

Group		Mean arterial pressure (mmHg)		Pulse rate (beats/min)		Oxygen saturation (%)		Body temperature (°C)	
		Time 1	Time 2	Time 1	Time 2	Time 1	Time 2	Time 1	Time 2
Young	Sedentary/Non-surgery	96.1 ± 8.3	97.7 ± 7.6	366.2 ± 20.5	369.2 ± 27.5	98.2 ± 1.1	98.5 ± 1.5	36.5 ± 0.7	36.7 ± 0.6
	Sedentary/Laparotomy	98.0 ± 9.5	98.1 ± 9.5	378.0 ± 25.6	372.3 ± 25.3	98.0 ± 1.7	98.0 ± 1.6	36.8 ± 0.5	36.9 ± 0.4
	PEE/Non-surgery	100.6 ± 10.4	102.0 ± 11.7	351.7 ± 27.2	364.0 ± 21.7	98.5 ± 1.6	98.2 ± 1.2	37.1 ± 0.8	36.8 ± 0.6
	PEE /Laparotomy	102.6 ± 9.7	100.5 ± 8.6	367.5 ± 23.0	360.8 ± 22.0	98.8 ± 1.6	99.1 ± 1.2	36.7 ± 0.5	36.8 ± 0.4
Aged	Sedentary/Non-surgery	99.7 ± 12.0	98.3 ± 9.6	388.9 ± 26.4	378.5 ± 23.5	99.0 ± 1.2	98.7 ± 1.5	36.9 ± 0.5	37.0 ± 0.6
	Sedentary/Laparotomy	103.2 ± 10.4	102.7 ± 7.5	370.7 ± 21.5	368.9 ± 27.7	99.2 ± 1.5	98.2 ± 1.1	36.5 ± 0.7	36.8 ± 0.5
	PEE /Non-surgery	102.4 ± 9.3	101.7 ± 7.2	361.2 ± 24.7	362.4 ± 25.5	98.8 ± 1.5	98.5 ± 1.2	37.0 ± 0.5	37.1 ± 0.6
	PEE /Laparotomy	101.7 ± 10.6	99.7 ± 9.0	383.3 ± 29.0	379.0 ± 27.4	98.4 ± 1.0	99.2 ± 0.8	36.9 ± 0.5	36.5 ± 0.7

Each parameter was recorded at Time 1 – after induction of anesthesia, before procedure, and at Time 2 – immediately after procedure, before termination of anesthesia. Data were expressed as the mean ± standard deviation. Each group consisted of 8 animals.

PEE: preoperative environmental enrichment

Supporting Table 2. Total exploration time during the training phase of novel object recognition test

	Sedentary		**PEE**	
	Non-surgery	Surgery	Non-surgery	Surgery
Young	48.9 ± 10.7	49.4 ± 15.4	52.3 ± 12.6	48.0 ± 11.6
Aged	44.4 ± 12.9	45.6 ± 11.1	43.5 ± 12.2	46.9 ± 9.5

Total time spent exploring the two objects in each group is expressed as mean ± SD in seconds. Each group consisted of 8 animals. PEE: preoperative environmental enrichment

Supporting Figure 1

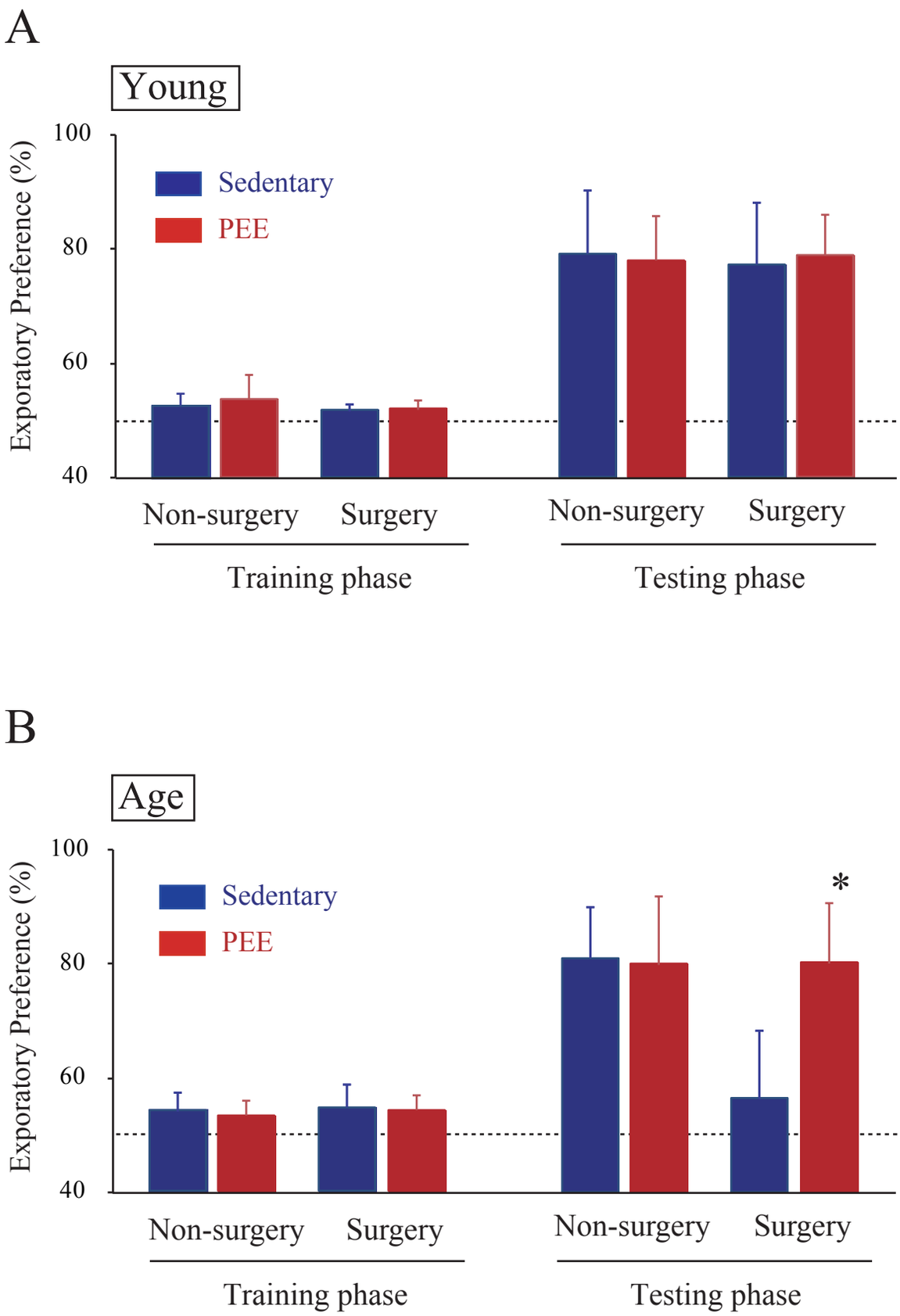

Figure legend

Supporting Figure 1. **Effects of preoperative environmental enrichment (PEE) on cognitive function assessed by a novel object recognition test in young (A) and aged (B) rats.** Percentage of preference between 2 objects in the training phase and testing phase of the novel object recognition test performed 7 days after non-surgery or surgery in sedentary or PEE rats is shown. Each vertical bar represents the mean ± SD (n = 8 in each group). *$p < 0.05$ *vs.* sedentary/surgery group, Kruskal–Wallis ANOVA followed by Wilcoxon-Mann-Whitney test with Bonferroni correction.