INVESTIGATING THE TWO-TRIAL Y-MAZE AS A PERFORMANCE ASSAY FOR SHORT-DURATION SLEEP DEPRIVATION STUDIES IN C57BL/6 MICE

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INTRODUCTION

The two-trial Y-maze is a simple recognition task that measures spatial recognition memory in rodents[1,2]. It is based on the innate preference of rodents to explore novelty and does not require the use of appetitive, aversive or complex associative conditioning[1,2]. As such, it provides a quick and relatively easy assessment of spatial recognition memory. The present study evaluated the usefulness of the two-trial Y-maze for short-duration (6 h) sleep deprivation (SD) studies in mice. Specifically, we investigated whether Y-maze performance reliably reflects the impairment of waking functions associated with SD[3,4].

METHODS

N=21 male C57BL/6 mice were used; twelve were 11 weeks old and nine were 21 weeks old. They were housed under standard vivarium conditions (22° C; 12-12 h light-dark schedule) with food and water available ad libitum. All experiments were conducted during the light phase.

The Y-maze consisted of three arms (width x length x height: 8.5 x 35.5 x 15.0 cm), with a 120° angle between each arm. One arm was designated as the 'Start' arm, in which the animals began the trials; the other two arms were randomly assigned to be the 'Open' and 'Novel' arms. Pictures of geometric shapes were placed on the walls furthest from center of the Open and Novel arms as visual cues. The influence of distal visual cues and the presence of the observer was minimized with a black drape surround. The floor of the maze was covered with corn cob rodent bedding, which was renewed after each trial to minimize olfactory-based traces from the previous mouse.

The Y-maze experiment consisted of two trials separated by a 6 h inter-trial interval. During the first trial, the mice were allowed to explore the Start and Open arms for 10 min; the Novel arm was blocked. Following the first trial, seven 11 week-old mice and five 21 week-old mice were subjected to 6 h SD, in their home cage, through gentle handling. The other five 11 week-old mice and four 21 week-old mice were allowed to sleep uninterrupted for 6 h.

After the 6 h inter-trial interval, the second trial was conducted, during which the mice were allowed to explore all three arms for 5 min (experiment 1). After 2 weeks, the Y-maze experiment was repeated with the same mice assigned to the same conditions to determine the reproducibility of the assay (experiment 2).

The percentages of duration and number of entries in the Open and Novel arm during the second trial were calculated and analyzed by 2×2 mixed-effects ANOVA with a between-subject factor of condition (SD vs. control), a within-subject factor of arm (Novel vs. Open), and both age and the age by arm interaction as covariates. Planned contrasts were computed to further examine significant effects and interactions.

To analyze the performance of individual mice, the difference in duration and number of entries between the Novel and Open arms in the second trial, expressed as the percentage of the total for the Open arm subtracted from the percentage of the total for the Novel arm, was calculated for each experiment.

RESULTS AND DISCUSSION

Fig. 1A shows duration spent in the Open and Novel arms expressed as a percentage of total time in the Y-maze during the second trial of the first experiment. Fig. 1B shows number of entries in each arm expressed as a percentage of total number of entries in the first experiment. Statistically significant differences between the SD and control groups are indicated in the figures.

In the first experiment, when comparing duration in the SD group vs. the control group, there was no significant condition by arm interaction ($F[1,18]<0.1$, $P=0.98$). Both the SD group ($t[18]=2.5$, $P=0.023$) and the control group ($t[18]=2.2$, $P=0.042$) spent more time during the second trial in the Novel arm than in the Open arm. The age by arm interaction covariate was statistically significant ($F[1,18]=5.0$, $P=0.038$). Further analysis showed that the 11 week-old mice spent more time in the Novel arm than the Open arm ($t[18]=4.2$, $P<0.001$), whereas the 21 week-old mice did not ($t[18]=0.7$, $P=0.49$).

Number of entries likewise showed no significant condition by arm interaction ($F[1,18]=2.2$, $P=0.16$) in the first experiment. Both the SD group ($t[18]=2.6$, $P=0.020$) and the control group ($t[18]=4.2$, $P<0.001$) entered the Novel arm significantly more often than the Open arm in the second trial.

Fig. 1C shows duration spent in each arm expressed as a percentage of total time in the Y-maze during the second trial of the second experiment. Fig. 1D shows number of entries in each arm expressed as a percentage of total number of entries in the second experiment. Statistically significant differences between the SD and control groups are indicated in the figures.

In the second experiment, when comparing duration in the SD group vs. the control group, there was no significant condition by arm interaction ($F[1,18]=0.1$, $P=0.72$). Neither the SD group ($t[18]=1.7$, $P=0.11$) nor the control group ($t[18]=1.0$, $P=0.34$) spent more time in the Novel arm than in the Open arm.

With regard to number of entries, there was again no significant condition by arm interaction ($F[1,18]=1.4$, $P=0.26$). The SD group exhibited a trend of greater preference for the Novel arm ($t[18]=1.8$, $P=0.081$), and for the control group this effect was significant ($t[18]=3.2$, $P=0.005$).

Figs. 2A and 2B show the difference scores for duration of the individual mice in the SD and control groups, respectively. Figs. 2C and 2D show the difference scores for number of entries. Notice the inconsistencies between the two experiments. Although group performance appeared to be similar between the two experiments, 75% of the mice showed inconsistent performance with respect to duration and/or number of visits in the second experiment compared to the first experiment. It is unlikely that this can be attributed to a learning effect, as the 2-week interval between experiments is believed to exceed the Y-maze retention capability of rats[1]. Moreover, at the individual level, increases as well as decreases in performance were observed.

When specifically comparing the performance of the 11 weeks old mice in the SD condition between experiments 1 and 2, a uniform decline in performance (i.e., a decrease in the extra time spent in the Novel arm relative to the Open arm) was observed for duration (see Fig. 2A). This resembles a finding in humans of sensitization to SD following repeated exposure[5]. Why it was not observed for the 21 weeks old mice is unclear; larger samples would need to be studied to further examine age-related differences.

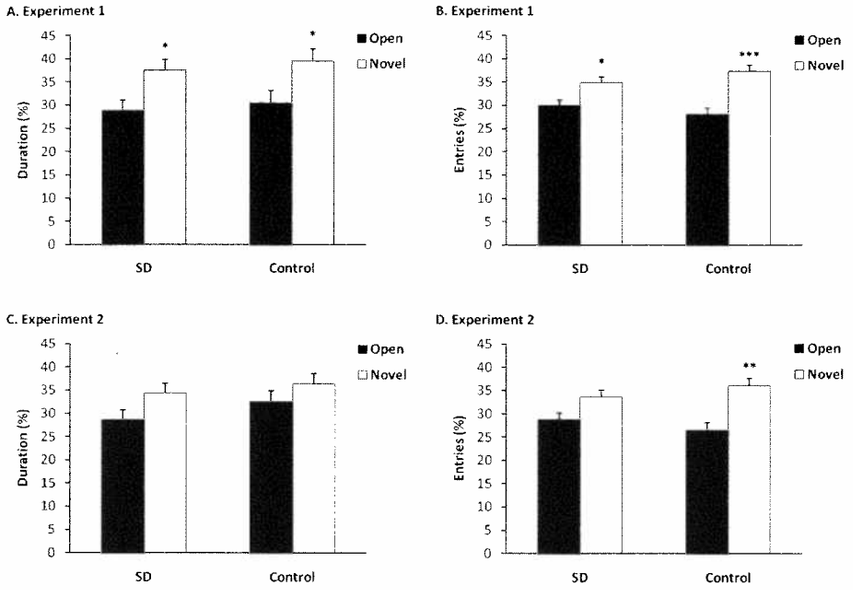

Figure 1. Effects of sleep deprivation (SD) on spatial recognition memory in the two-trial Y-maze. **A.** Duration of visits to each arm (mean and standard error) during the second trial of the first experiment. **B.** Number of entries in each arm (mean and standard error) during the second trial of the first experiment. **C.** Duration of visits to each arm during the second trial of the second experiment. **D.** Number of entries in each arm during the second trial of the second experiment. Comparisons between Novel and Open arms: (*)P<0.1, *P<0.05, **P<0.01, ***P<0.001.

CONCLUSIONS

In our study, C57BL/6 mice showed the expected preference for the Novel arm in the second trial of the two-trial Y-maze assay, particularly in the first experiment. In an earlier report[6], 12 h SD decreased this exploratory behavior significantly, whereas 6 h SD did not. In agreement with that study, we found that Y-maze performance was not significantly affected by 6 h SD compared to control. Furthermore, we observed that performance changes from the first to the second trial were inconsistent between mice as well as within mice between the two experiments. As such, the two-trial Y-maze did not prove to be a reliable assay of spatial recognition memory for short-duration SD studies in C57BL/6 mice. Whether this finding generalizes to other strains with different baseline performance on the two-trial Y-maze remains to be examined.

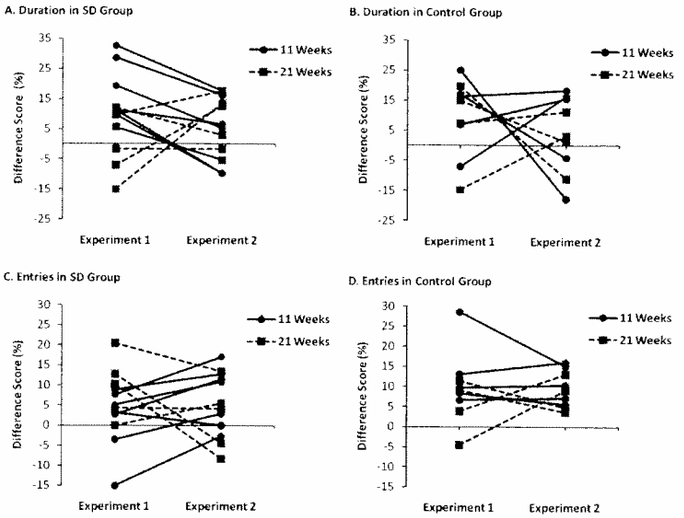

Figure 2. Difference between the Novel and Open arms in the individual mice. **A.** Difference in duration of visits between the Novel and Open arms for experiments 1 and 2 in the sleep deprivation (SD) group. **B.** Difference in duration of visits between the Novel and Open arms for experiments 1 and 2 in the control group. **C.** Difference in number of entries between the Novel and Open arms for experiments 1 and 2 in the SD group. **D.** Difference in number of entries between the Novel and Open arms for experiments 1 and 2 in the control group.

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