Supporting Information

Western Diet Accelerates the Impairment of Odor-Related Learning and Olfactory Memory in the Mouse

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OLFACTORY TESTS

Buried Pellet Test

Preparations: Two weeks prior to testing, mice were introduced to a new “treat” (piece of a protein bar), to overcome food neophobia. Before the test, mice were fasted for 10 hrs during the night phase and all olfactory tests were performed during the light phase. During all testing days, mice were habituated in the test room, in their home cages without feeder bin for 1 hr. For each tested animal, a clean cage was filled with clean bedding (~3cm) evenly distributed throughout the cage, and the pellet was buried ~0.5cm below it.

Testing procedure: The mouse was placed in the center of the test cage and timer and recording camera set for 5 min. After that time, the mouse was returned to its cage with the original cagemates. For each mouse, the test was performed twice with an interval of ~1 hr. During each trial, the pellet was buried in a different spot in the cage.

Analyzed parameters: The mean time for the mouse to uncover and start eating the treat. If mouse did not find it within 5 min., the trial was ended, and a score 300 sec. noted. Differences in the time needed to uncover by mice the hidden food, based exclusively on olfactory clues, allowed to test odour detection ability.

Block Test and Habituation Test

Preparations: Before experiment mice were housed individually (having free access to food and water) with 4 wooden blocks: F1-F4 for 48 hrs. Before the olfactory tests, animals were habituated in their home cages without feeder bin and blocks for 1 hr. Blocks along with a handful of bedding were placed in a clean plastic bag labelled with mice identification.

Testing procedure:
Day1: During 5 trials, blocks F1-F3 (with the scent from the animal’s own cage) + block N1 (i.e. F4 block from another mouse’s cage =1st social scent) were placed in the middle of the cage and mice behavior recorded with a camera for 2 min. After that time, recording was stopped, blocks removed from the cage and placed back into the plastic bag. The interval between trials lasted approx. 5 minutes.

Day2: Next day, after habituation, blocks N1 (from another mouse’s cage, used in the test the day before =1st social scent) and N2 (from another mouse’s cage =2nd social scent) were placed in the experimental cage and animal’s behavior was recorded for 2 min. After performing the olfactory test, mice were placed in a new clean cage with their original cagemates.

Analyzed parameters: time spent by mouse sniffing each block (sniffing defined as nasal contact with the block) during testing day1 and day2. Difference in sniffing time between blocks covered with familiar (F1-F3) and new social scent (N1) allowed to assess odour discrimination ability. Mean time of sniffing blocks with the same scent (N1) during consecutive trials (T1-T5) allowed for recording habituation/testing non-associative learning. Percent of time spend by mice from each group sniffing block N2 allowed to test novel odour recognition. Comparison of the sniffing time between block N1 and N2 during second testing day, allowed to test long-term recognition memory.

To avoid spatial preference effect, positions of the blocks (F1-F3, N1 and/or N2) were changed during each trial/day. Additionally, different social scents were used during the three timepoints of olfactory testing. No signs of aggression were observed during performing the block/habituation test.

Figure 1S Obesogenic diets do not affect the time to approach the scented block (A) and total movement activity (B) during the block test. Bars represent means ± SD from trials T1-T5 for each mouse.
