Supplementary Material

A Chronic Increase in Blood-Brain Barrier Permeability Facilitates Intraneuronal Deposition of Exogenous Bloodborne Amyloid-Beta1-42 Peptide in the Brain and Leads to Alzheimer's Disease-Relevant Cognitive Changes in a Mouse Model

Odor discrimination apparatus and procedure

A black Plexiglas 60 cm square field with 30 cm high walls was located in a dimly lit (10 fc) testing room with a high ventilation rate (3 min volume exchange). Three 4 x 4 x 2.0 cm (l, w, h) aluminum food cups were placed in three corners of the field. A food reinforcer (30 mg portions of chocolate flavored puffed rice) was placed in a 1.6 cm deep, 1 cm diameter depression in the center of each cup. The food in two of the cups was covered (1.0 cm below the surface of the cup) with a wire mesh so that it was not accessible to the animal, while in the third cup (the "target" cup), the food could be retrieved and consumed.

A cotton-tipped laboratory swab, located between the center and rear corner of each cup, extended vertically 3 cm from the cups' surface. Immediately prior to each trial, fresh swabs were loaded with 25 µl of either lemon, almond, or mint odorants (McCormick flavor extracts). The mint odor was always associated with the target food cup. (It should be noted that in pilot studies, the odor associated with food was counterbalanced across animals, and no discernible differences in the rate of learning could be detected in response to the different odors.)

On the acclimation day (Day 1), each food-deprived animal was placed in the field for 20 min with no food cups present. At the end of that day's light cycle, three pieces of chocolate flavored puffed rice that would serve as the reinforcer in this task were placed in each animal's home cage to acquaint them with the reinforcer. On the subsequent test day, animals received four training trials in the field with three food cups present. On each trial, an animal was placed in the empty corner of the field. On Trial 1, the reinforcing food (rice) was available to the animal in the cup marked by mint odor. An additional portion of food was placed on the top surface of the same cup for the first trial only. The trial continued until the animal retrieved and consumed the food from the target cup, after which the animal was left in the chamber for an additional 20 s and then returned to its home cage to begin a 5-min intertrial interval (ITI). On

Trials 2-4, the location of the food cups was re-arranged, but the baited cup remained consistently marked by the mint odor. Both the corner location of the mint odor and its position relative to the remaining odors was changed on each trial. On each trial, the latency to retrieve the food and errors were recorded. An error was recorded any time that an animal contacted an incorrect cup, or its nose crossed a plane parallel to the perimeter of an incorrect cup. Similarly, an error was recorded when an animal sampled (as above) the target cup but did not retrieve the available food.

Passive avoidance apparatus and procedure

A chamber illuminated by dim (< 5 fc) red light was used for training and testing. Animals were confined to a circular ("safe") chamber (10 cm diameter, 8 cm high). The walls and floor of this chamber were white, and the ceiling was translucent orange. The floor was comprised of plastic rods (2 mm diameter) arranged to form a pattern of 1 cm square grids. A clear exit door (3 CM square) was flush with the floor of the safe compartment, and the door could slide horizontally to open or close the compartment.

The bottom of the exit door was located 4 cm above the floor of a second circular chamber (20 cm diameter, 12 cm high). This "unsafe" chamber had a clear ceiling and a floor comprised of 4 mm wide aluminum planks that formed a pattern of 1.5 cm square grids oriented at a 45^{o} angle relative to the grids in the safe compartment. When an animal stepped from the safe compartment through the exit door onto the floor of the unsafe compartment, the compound aversive stimulus comprised of a bright (550 Lux) white light and "siren" (an oscillating tone that peaked at 60 dB above the 50 dB background) was initiated.

Animals were placed on the platform behind the exit blocked by the Plexiglas door. After 4 min of confinement, the door was retracted and the latency of the animal to leave the platform and make contact with the grid floor was recorded. These initial step-down latencies typically range from 8-20 s. Upon contact with the floor, the door to the platform was lowered and the aversive stimulus (light, noise, and vibration) was presented for 4 s, at which time the animals could return to the safe platform, where they were again confined for 4 min. At the end of this interval, the door was opened and the latency of the animal to exit the platform and step onto the grid floor (with no aversive stimulation) was recorded.

Spatial water maze apparatus and procedure

In our protocol, animals were confined in a clear Plexiglas cylinder on the safe platform for 5 min on the day prior to training. Second, a considerably longer ITI (10 min) was used than is typical (c.f., 90 s). Lastly, the maze, surroundings, and water were black; visual cues were constructed of patterns of lights.

A round black pool (140 cm diameter, 56 cm deep) was filled to within 24 cm of the top with water made opaque by the addition of a nontoxic, water soluble, white paint. A hidden 11 cm diameter perforated white platform was in a fixed location 1.5 cm below the surface of the water midway between the center and perimeter of the pool. The pool was enclosed in a ceiling-high black curtain on which five different shapes (landmark cues) were variously positioned at heights (relative to water surface) ranging from 24-150 cm. Four of these shapes were constructed of strings of white LEDs (spaced at 2.5 cm intervals) and included an "X" (66 cm arms crossing at angles 40^{o} from the pool surface), a vertical "spiral" (80 cm long, 7 cm diameter, 11 cm revolutions), a vertical line (31 cm) and a horizontal line (31 cm). The fifth cue was constructed of two adjacent 7 W light bulbs (each 4 cm diameter). A video camera was mounted 180 cm above the center of the water surface. These cues provide the only illumination of the maze, totaling 16 fc at the water's surface.

On the day prior to training, each animal was confined to the escape platform for 360 s. Training was conducted on the two subsequent days. On Day 1 of training, animals were started from a unique location on each of five trials. (The pool was virtually divided into four quadrants, and two starting points were located in each of the three quadrants that did not contain the fixed escape platform. The starting point on each trial alternated between the three available quadrants.) An animal was judged to have escaped from the water (i.e., located the platform) at the moment at which four paws were situated on the platform, provided that the animal remained on the platform for at least 5 s. Each animal was left on the platform for a total of 10 s, after which the trial was terminated. Trials were spaced at 10 min intervals, during which time the animals were held in a warmed (27.5^{o}C) opaque (5 Lux) box lined with wood shavings. On each trial, a 90-s limit on swimming was imposed, at which time any animal that had not located the escape platform was placed by the experimenter onto the platform, where it remained for 10 s.

Animals were observed from a remote (outside of the pool's enclosure) video monitor, and animals' performance was recorded on video tape for subsequent analysis. Day 2 of training proceeded as did Day 1.

Lashley Maze apparatus and procedure

The Lashley III maze consists of a start box, four interconnected alleys, and a goal box containing a food reward. Over the trials, the latency of rats to locate the goal box decreases, as do their errors (i.e., wrong turns or retracing). Here, the Lashley III maze was scaled for mice, and parameters have been developed that support rapid acquisition. The maze was constructed of black Plexiglas. A 2 cm wide x 0.1 cm deep white cup was located in the rear portion of the goal box, and 45 mg BioServe (rodent grain) pellets served as reinforcers. Illumination was 80 Lux at the floor of the maze. The maze is isolated behind a shield of white Plexiglas to prevent the use of extra-maze landmark cues.

Food-deprived animals were acclimated and trained on two successive days. On the day prior to acclimation, all animals were provided with three food pellets (i.e., those that would serve as reinforcers) in their home cages to familiarize them with the novel reinforcer. On the acclimation day (Day 1), each mouse was placed in the four alleys of the maze, but the openings between the alleys were blocked so that the animals could not navigate the maze. Each animal was confined to the start and subsequent two alleys for 4 min, and for 6 min in the last (goal) alley, where three food pellets were present in the food cup. This acclimation period promotes stable and high levels of activity on the subsequent training day. On the training day (Day 2), each animal was placed in the start box and allowed to traverse the maze until it reached the goal box and consumed the single food pellet present in the cup. Upon consuming the food, the animal was returned to its home cage for a 25 min ITI, during which the apparatus was cleaned. After the ITI, the mouse was returned to the start box to begin the next trial, and the sequence was repeated for a total of five trials. Both the latency and errors (i.e., a turn in an incorrect direction, including those which result in path retracing) to enter the goal box were recorded on each trial.

To estimate capacity for long-term retention in this task, following a 30-day

retention interval from completion of training, animals were placed in the start box and allowed to traverse the maze again for two trials. The ratio of errors on the trials following the retention interval to average errors on the last two trials during acquisition training was calculated for each animal and served to index long-term retention in this task.

Selective attention apparatus and procedure

For this task, animals were trained and tested in two contexts. In one context, a search for a food pellet was guided by odor cues; in the other context by visual cues. The first context (odor discrimination) was the same black Plexiglas box used in the odor-guided discrimination task described above. The second context (visual discrimination) was identical in all respects except for the addition of 3 cm wide white vertical stripes that were placed on the inside walls (thus distinguishing the Odor and Visual Discrimination contexts). Each of these boxes was equipped with 10 cm wide flat panels (constructed of black Plexiglas) placed behind the food cup and that spanned each corner. These flat surfaces could be backlit by a single white LED and had 3 mm holes (spaced at 3 mm intervals) drilled into them which, when illuminated, formed one of three 6 cm shapes: a circle (O), an X or two horizontal lines (=). These patterns of light served as visual cues (which produced a 30 Lux above background illumination, measured from the center of the test box). The food cups at the bottom of each panel contained cotton wadding (into which odorants could be loaded) covered by a metal screen. All parameters such as lighting conditions, ITI, and deprivation schedule were identical to those used in odor discrimination. In the box previously used for odor discrimination, the cotton wadding in the food cups was loaded with the previously described odors (almond, lemon, or the target odor, mint) and mice were trained to associate the mint odor with accessible food in the previously described manner. The remaining cup also contained food, but it was not accessible to the animals (i.e., it was located under the screen in the bottom of the cup). During odor discrimination training, the lights behind the flat surfaces were not illuminated, and the unlit visual stimuli were randomly cycled between the odors, insuring that in this odor discrimination box, these stimuli had no discriminative role in localizing the food. Animals received eight days of

training (five trials/day) in this task, at which time, no animal committed more than three errors across the final four trials.

After completion of the initial odor discrimination training, mice began training for the visual discrimination in the box with white stripes. Here, the unique visual pattern on each flat panel was illuminated, creating visual cues for the mice to follow. There were no odorants on the cotton in the food cups. A food pellet was accessible to the animal in the cup located under the circle (O) visual cue; the two remaining cups contained inaccessible food pellets. Training proceeded in exactly the manner used for training in the odor discrimination box. Animals received eight days of training (four trials/day) in this task. Upon completion, animals were required to meet the training criteria of no more than three errors across the final four trials in order to continue on in this task (one animal from each group was terminated from this task as a result of failing to meet these criteria). Following completion of initial training in the odor and visual discriminations, animals were "overtrained". For this, and to adapt animals to alternating between the two tasks, mice began alternating between days (for six days, six trials/day) in the two tasks, followed by four days of training where they performed one of the discrimination tasks (four trials) during the first half of the light cycle and one discrimination during the second half of the light cycle. The order of the discrimination tasks within a day alternated across days.

Following completion of training, animals were subjected to the test of selective attention, where they were first placed in the odor discrimination box with both odor cues (the relevant targets) and illuminated visual cues (the task-relevant distracters) present. For this task, the target odor cue (mint) was always present in a cup other than the one marked by the distracting visual cue [O]). To perform effectively in the task, animals were required to inhibit their approach to visual cues and selectively attend to odor cues to efficiently locate the accessible food. Fifteen minutes after the initial test of selective attention, a test of the animals' ability to disengage from the tendency to approach the odor cue was assessed. For this test, animals were placed in the visual discrimination box with both visual cues (the relevant targets) and odor cues (the task-relevant distracters) present. For this task, the target visual cue (O) was always present in a cup other than the one marked by the relevant odor cue (mint). To perform effectively in the task, animals

were required to inhibit their approach to odor cues and selectively attend to visual cues in order to find food. Animals then continued to alternate between boxes and were tested in this manner until they had been tested for performance in each box two times (for a total of four test trials). The number of errors accumulated over the eight test trials served as the index of selective attention and the animals' ability to shift between relevant cues (i.e., to disengage from one type of cue and focus attention on the alternate type of cue). Errors were scored in the same manner described above for the Odor Discrimination task.