Journal of Neuroscience Methods, 15 (1985) 63–72
Elsevier

NSM 00517

On-demand platform improves accuracy of the Morris water maze procedure

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(Received April 20th, 1984)
(Revised June 13th, 1985)
(Accepted June 13th, 1985)

Key words: water maze – spatial memory – video-tracking – rat

In order to prevent chance finding of the hidden target in the Morris water tank task, the rigid underwater platform is replaced with a collapsible platform, resting at the bottom of the pool. A computerized videosystem tracks the rat's movement across the pool and raises the platform when the animal has stayed in the target area for a predetermined time. Acquisition of the task with the collapsible platform proceeds at a similar rate as with the rigid platform when the criterion conditions are easy (target distance 15 cm, target time 2.5 s), but gradually deteriorates when the target time increases to 10.0 s. Successful solution of the modified task requires accurate localization of the target under open loop conditions and is thus well suited for investigation of the fine structure of the cognitive maps and of their changes induced by lesions or drugs.

Introduction

Examination of spatial memory of rats has recently been greatly improved by the introduction of the water maze technique (Morris, 1981). Although the method can be used for conventional visual discrimination learning (Morris, 1984), it is mainly intended for place learning (O'Keefe and Nadel, 1978), i.e. for testing the capability of rats to find an invisible target according to its relationship to remote extramaze cues. In the original version of the task a rat is lowered into a large pool (130 cm in diameter) of opaque water containing an invisible small platform (10 cm in diameter, 1 cm below water surface). The animal rapidly learns to find the platform and after a few trials can reach it from any point of the pool within a few seconds. Correct solution of this navigation task requires representation of the pool and of the surrounding environment in the brain of the animal. The cognitive map, containing also the location of the target and the actual position of the rat, allows the animal to

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follow the shortest path to the target. The rat can find the target also using a less efficient search strategy. In the latter case, the animal does not know the exact position of the target, but by systematically visiting possible target locations in the pool, it eventually hits the underwater platform and escapes. The time required for an experienced animal to find a randomly located platform is about 20 s on the average (Morris, 1981; Sutherland and Dyck, 1984), with highly variable escape latencies ranging from a few seconds (when the animal hits the platform by chance shortly after start) to the 60 s cut-off (when the animal has missed the target and continues the search). Distinguishing the mapping and search strategies is impossible in individual trials, however, since a short latency escape can be produced by chance by a randomly searching rat. In order to determine more reliably that the rat is using a mapping strategy, Morris (1981, 1984) suggested to test the trained animal in a pool with the platform removed and to measure the time it spends in the target area or in the pool quadrant containing the target. The disadvantage of this procedure is that the test is an extinction trial and that the failure to find the platform in its usual place may encourage the animal to use search strategy again. The present paper describes a modification of the water maze task which makes it possible to eliminate the search strategy and to examine the mapping strategy without the confounding effect of random hits. The method uses a collapsible platform which rests at the bottom of the pool beyond the reach of the swimming rat. After the rat has stayed in the target area for a predetermined time the platform is raised and available for escape. The whole experiment is monitored and controlled by a computerized videosystem.

Method

Animals

Twenty 3-month-old male hooded rats (Long–Evans strain) were obtained from the breeding colony of the Institute. They were housed 5 per cage in an animal room with natural light and had free access to food and water.

Water maze

The circular pool is 130 cm in diameter and 55 cm high with a white painted wall. It is filled 30 cm deep with water made opaque by addition of non-toxic white paint. Escape is provided by the collapsible platform (Fig. 1) which consists of a heavy base (*1*) with bearings (*2*) for two vertically arranged parallel rods (*3,4*) the far ends of which are connected with a vertical plate (*5*) carrying the white circular platform (*6*). A horizontal bearing in the upper rod (*7*) is connected to a pneumatically driven piston (*8*) moving in a cylinder (*9*) the closed end of which turns in another horizontal bearing (*10*) fixed to the base. The cylinder is connected by vacuum tubing (*11*) to a solenoid operated valve and compressed air outlet. Under resting conditions the platform is in the low position 7 cm above the tank floor. After activation of the solenoid the piston lifts the parallel rods and the platform raises to a level of 29 cm, i.e. 1 cm below water surface.

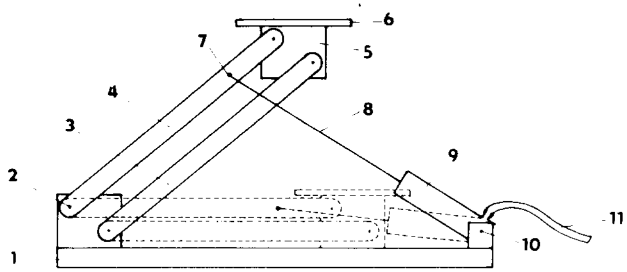

Fig. 1. Scheme of the collapsible platform. Full lines, platform raised; interrupted lines, platform collapsed. For description see text.

Computerized videosystem

The videosystem automatically tracks the rat swimming in the pool, evaluates the parameters of the trajectory and controls platform movement. The tracking system resembles that described by Morris (1984) but the position of the rat is represented

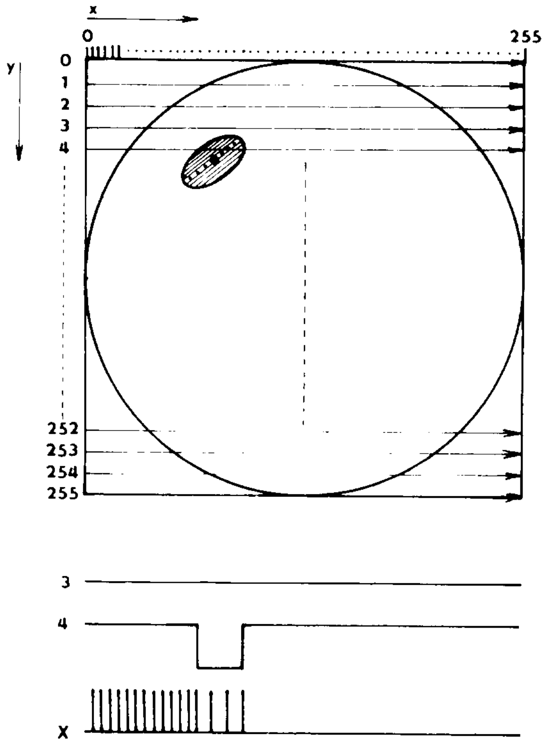

Fig. 2. Scheme of the function of the camera-computer preprocessing interface. Above: scanning of the pool by the TV-camera raster. Below: 3, no black object was detected in line 3; 4, videosignal corresponding to the black object intercepted by line 4; X, corresponding clock pulse output. Note that the clock rate is halved as long as the videosignal deflection is on. For details see text.

by the estimated center of its head and back rather than by the point of the first intercept of the rat's body with the linear scanning raster.

The videosystem consists of a TV camera (TFK 500, GDR), a 16 bit microcomputer (LSI-11, DEC USA), a camera-computer preprocessing interface module connected with a universal parallel I/O unit DRV-11 belonging to the standard cards of the microcomputer. The set-up contains moreover a graphical video-RAM (MATROX 256^2), a teletype, a line recorder and two TV monitors–graphical displays. The TV camera is fixed above the center of the pool which it sees through a circular opening in a white screen masking out all but the water surface. The videosignal deflection corresponding to an object (e.g. the black head and back of the swimming rat) is detected by a comparator in the interface module. The coordinates of the estimated center of the object (line number as ordinate and distance from the line onset as abscissa) is measured as follows (Fig. 2). The center of the intercept of the object is obtained for each scanning line. This is accomplished by counting clock pulses (7 MHz) from the beginning of the line till the left margin of the object. Thereafter the clock frequency is halved to 3.5 MHz. The clock pulses are inhibited at the right margin of the object. Readings of all 256 lines of a half-frame are stored in the main memory of the computer from which the center ordinate of the object is evaluated by software during the next, non-analyzed half-frame. The x and y coordinates of the object center are stored in the main memory as well as in the video RAM with a frequency of 25 samples/s.

The distance between the current position of the rat and the target is computed in each evaluated half-frame, i.e. at 40 ms intervals. When this value remains below a preset limit D continuously in the span of N consecutive samples the computer activates the solenoid and the platform is raised. The rat–target distance can exceed the value D within a criterion run for a preset maximum percentage number of samples E. The rat–target distances are averaged over 8 samples, i.e. at 320 ms intervals are displayed continuously at the left margin of the TV monitor together with the lines indicating the criterion distance D. This curve can be plotted after termination of the experiment with a line recorder connected to a 6-bit DAC interfaced by the parallel I/O unit DRV-11 of the microcomputer. The TV monitor controlled by the video RAM also displays center of the pool marked by a cross, position of the target and borders of the area corresponding to the critical distance D (Fig. 3–5). The trajectory can be optionally smoothed by moving average of 8 successive points after preliminary rejection of spurious readings falling far from the main trajectory.

Procedure

The rats are first trained with the raised platform placed on the midpoint of one of the cardinal radii of the pool. The rat is lowered into water facing the wall of the pool at one of the remaining radii and forced to swim. If the platform is not found during 1 min, the animal is placed upon it by hand and left there for 30 s. The same time on the platform is also allowed after spontaneous escape reactions. The animal is removed from the platform by hand, and started again from another point of the pool while the target position remains unchanged. After two trials the platform is collapsed and its position is introduced into the computer memory. Criterion parameters (critical distance D, minimum number N of consecutive samples within this distance, i.e. the target time, and the percentage of samples E which can exceed this range) are set to make the task relatively easy, e.g. D = 15 cm, N = 64 ≐ 2.5 s, E = 12.5%. The animal is started as usual and allowed to explore the target area. When the criterion is met, the platform raises and the animal is allowed to climb upon it and to spend 30 s on it before being started again. The to-criterion-time is recorded automatically, the time when the animal actually reaches the platform is entered by an experimenter-controlled key. When the criterion is not reached during 1 min the platform raises automatically and the rat is placed upon it by hand.

After termination of each trial the computer prints the trial identification code, escape latency (s), to criterion time (s), length of the trajectory (arbitrary units) and the time spent in the 4 quadrants of the pool (s). The trajectory can be photographed from the screen of the monitor together with the record of the search expressed by the changing rat–target distance.

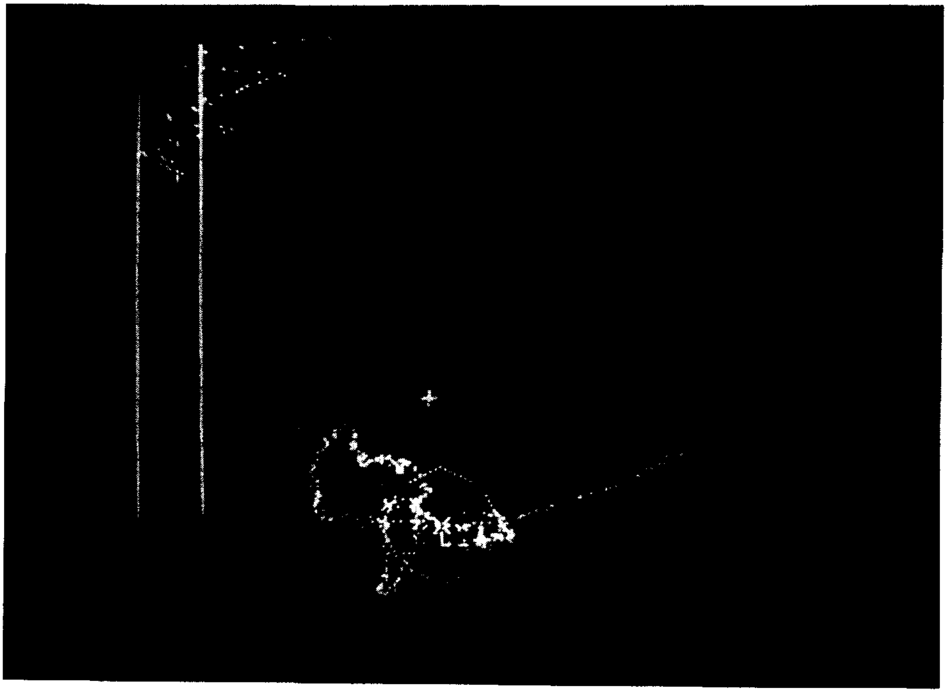

Fig. 3. An example of the rat's trajectory (plotted at 40 ms intervals) at an early stage of training. +, center of the pool; X, center of the target; dotted line, limits of the target area; two vertical lines at the left hand margin, time axis (1 min) and criterion distance level for plotting the succession of rat-target distances (at 320 msec intervals). Criterion parameters: D, 15 cm, N, 64 (≐ 2.5 s), E = 12.5% are defined in text.

Twelve trials are given per day. After the performance of the animal has stabilized, criterion can be made more difficult either by reducing the critical distance or by increasing the target time from 2.5 to 5.0, 7.5 or 10.0 s, while the permitted number of erroneous samples remains 12.5% of N.

Results

Rats learn navigation to the collapsible platform at a similar rate to escaping to the rigid platform, but they use a somewhat different strategy. When the rat swims too fast across the target area, the criterion is not met and the platform is not raised. On other occasions the criterion is attained just when the animal is leaving the target area. Due to inertia of the mechanical system the platform raises about 1 s later, when the rat is already some distance away from the target. A record of an illustrative trial is shown in Fig. 3. The rat started from the east, first traversed the target area in an east–west direction during 1.6 s, than from north to south during 1.9 s, and finally reached the 2.5 s criterion time after entering the target area from the west. The new strategy emerging after one or two sessions with the easy criterion

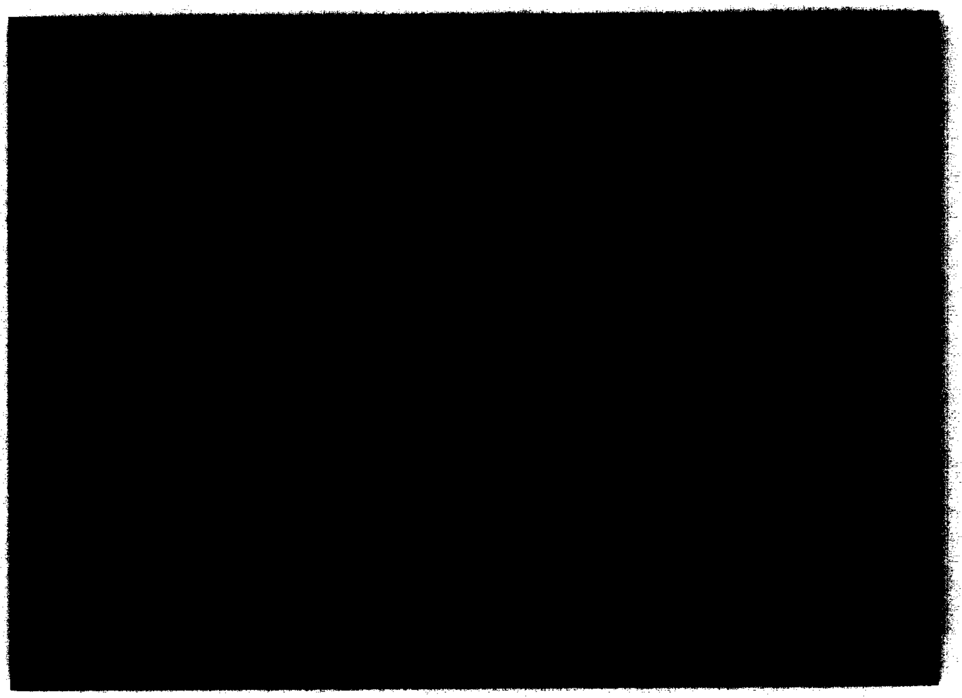

Fig. 4. An example of the rat's trajectory at a later stage of training. For description see Fig. 3. Note the abortive attempt to find the platform to the west from the target which was immediatelly followed by correct target location.

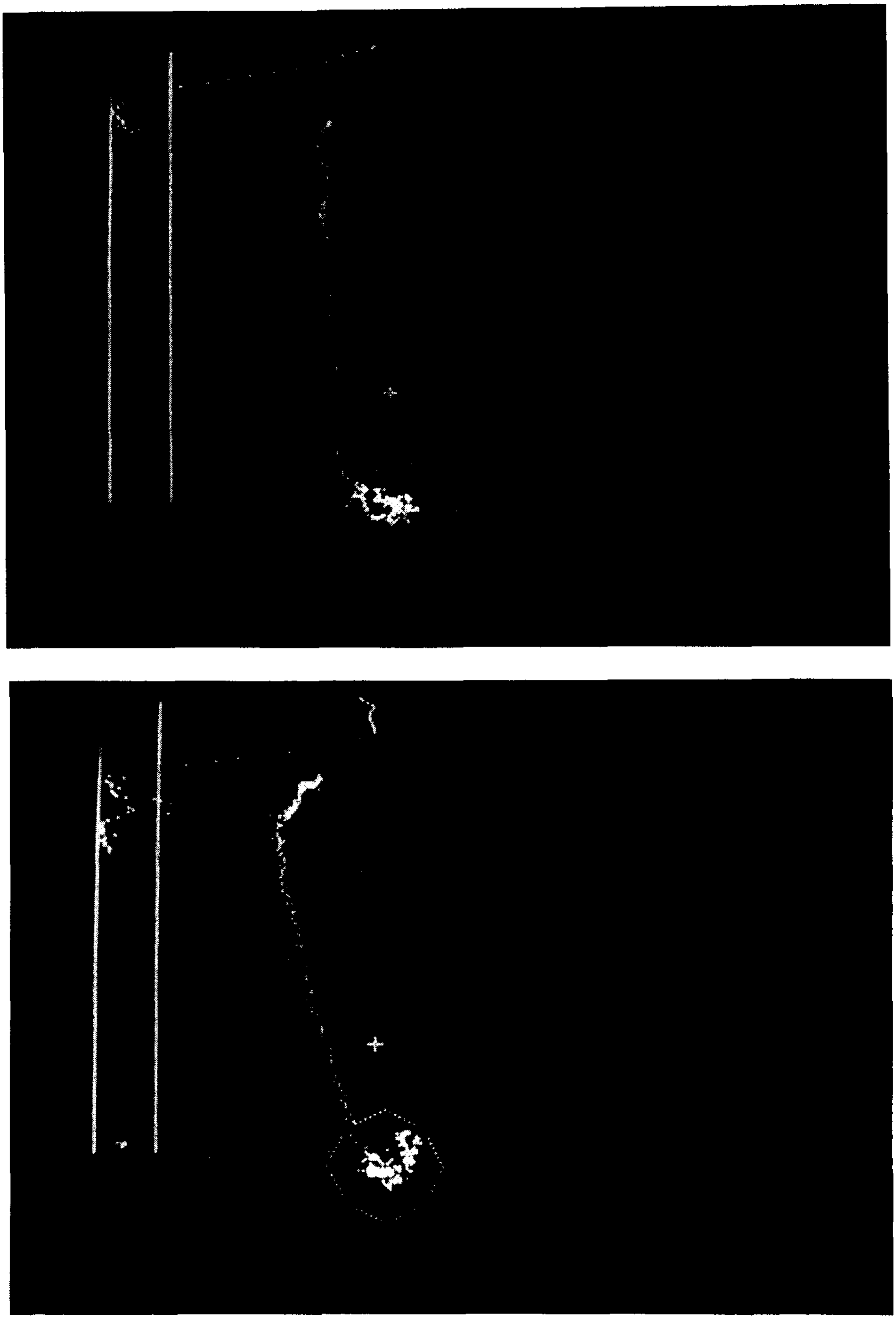

Fig. 5. Examples of the swimming trajectories corresponding to target times 5 s (A) and 10 s (B). Other descriptions as in Fig. 3.

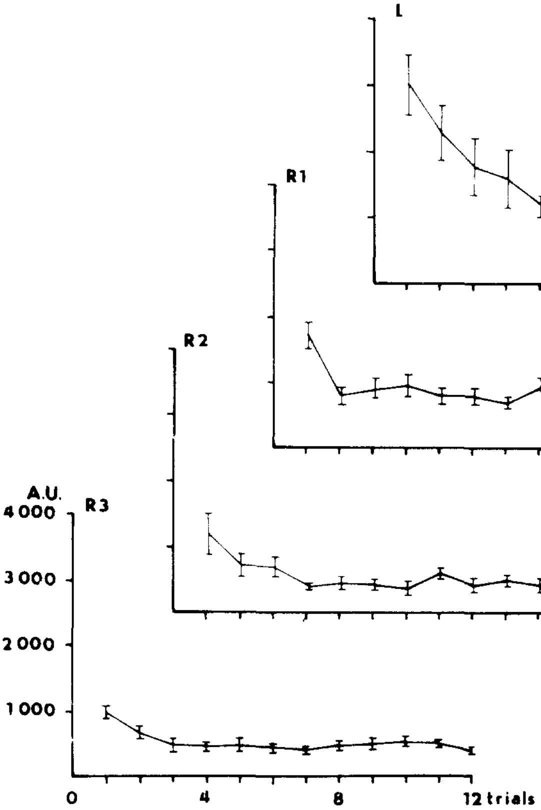

Fig. 6. Performance of a group of 20 rats during acquisition (L) of the navigation task and during subsequent 3 retention sessions (R1 to R3). Ordinate: average length of the trajectory in arbitrary units (A.U.) ±S.E.M. (100 A.U. ≙ 20 cm). Abscissa, ordinal number of the trial. Note that performance becomes asymptotic from the third session.

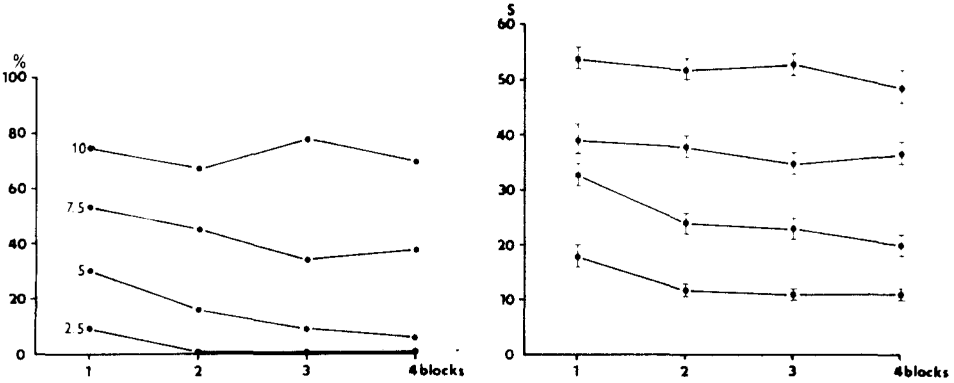

Fig. 7. The effect of increasing the criterion time from 2.5 s to 10 s on navigation performance of a group of 20 rats. D = 15 cm, E = 12.5%. A: percentage of trials in which the rat failed to find the platform during 1 min. B: average escape latency ± S.E.M. Abscissa in both A and B: blocks of 3 trials.

conditions (D = 15 cm, N = 64) consists in rapid swimming to the border of the target area, abruptly slowing the movement and treading water while slowly rotating around the presumed target (Fig. 4). Once the animal shows this behavior it is possible to increase the target time N or to reduce the critical distance D. Fig. 5A, B shows the trajectories obtained with target times N = 128 (= 5 s) and N = 256 (= 10 s) for the critical radius D = 15 cm.

When the criterion is made too difficult and the rat fails to reach it soon enough, it may resume search of the platform in other areas of the pool for the rest of the trial. The 30 s interval on the platform is usually sufficient for reassuring the animal that the platform location has not changed. The performance of individual rats can be arbitrarily expressed by the criterion values which can support target location in half of the 1-min trials in a session. In a group of 20 rats overtrained with the same platform position during 4 sessions (Fig. 6) the incidence of successful platform locations dropped from 97.7% with the 2.5 s target time to 27.5% with the 10 s target time (Fig. 7). 10% rats failed to solve the task in half of the trials with the 5 s target time and these numbers increased to 40% and 80% with target times of 7.5 and 10.0 s, respectively. Every rat which failed with a short target time also failed with longer target times.

Discussion

The use of the collapsible platform adds to the navigation task a new feature – accurate memory retrieval under open loop conditions. Whereas rats trained with the rigid platform learn to swim across the target area in rather wide sweeps intersecting over the platform, training with the collapsible platform requires pin-point localization of the target. This is clearly demonstrated by comparison of the trajectories of rats overtrained with the rigid platform after the latter has been removed (Morris, 1981, 1984) and of rats overtrained with the collapsible platform at long target times (e.g. Fig. 5): the rat's movement covers a much wider area around the target in the former than in the latter case. The rigid platform can be successfully located when the rat follows a definite trajectory and finds the platform somewhere along it. Failure to find the platform is interpreted as a navigation error and attempts to correct it may lead the animal away from the target. Collapsible platform training with the inherent delay between correct target location and escape opportunity precludes an immediate feedback control of the memory readout. This requires a more accurate location of the target which must be maintained for considerable time in absence of confirmatory feedback signals. The rat must not only find the target but also demonstrate a remarkable confidence in the correctness of the choice. This form of the navigation task is well suited for establishing the accuracy of the cognitive map (dimension of the smallest reliably located target in cm or in percentages of the pool diameter, the smallest discernible angular distance between remote cues). The technique is particularly important for assessing interventions which eliminate accurate platform location but do not interfere with efficient search strategies, e.g. retrohippocampal lesions (Schenk and Morris, 1985) or systemic application of atropine (Sutherland et al., 1982). At the same time the task provides an unusual method for testing the capability of the animal to assert a memory image during a relatively long response–reinforcement interval.

The proficiency of rats in this modification of the water maze task indicates that within definite limits finding hidden targets is not deteriorated by their delayed availability. Many natural mapping-based behaviours include a delay interval; a dog digging at a particular spot of the garden to recover a hidden bone or a bird retrieving hoarded nuts from a cachet under snow (Shettleworth, 1983) must show considerable persistence in their accurately aimed efforts before they receive the reward. Cognitive mapping implies expectancies about what is to be found at different locations. Additional time is usually required to find a small specific target in the large mapping-determined area or to wait there for appearance of periodically or randomly available targets. The latter case is commonly encountered in predatory animals who use mapping in order to find places where to expect appearance of the prey. The trade-off between reliance on mapping and duration of the subsequent search or wait is an important factor, which determines how long the animal continues the search before giving up and cancelling the corresponding map entry.

With increasing difficulty of the criterion requirements many rats failing to solve the task in the 1 min interval abandon the attempts to find the target altogether and prefer to swim close to the wall of the pool during the entire 1-min interval. This behavior, resembling the so called 'learned helplessness' (Seligman and Beagley, 1975) suggests that the failure to find the platform in the usual place reinstates the highly prepared species-specific defense reaction to escape from water by climbing the walls of the pool (Barraco et al., 1978). A necessary prerequisite for the successful use of the collapsible platform technique is that it must allow the animal to effectively reduce the total swimming time (at least by 50%). This factor must be carefully considered when testing the limits of the navigation task.

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