Developments of a water-maze procedure for studying spatial learning in the rat

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Developments of an open-field water-maze procedure in which rats learn to escape from opaque water onto a hidden platform are described. These include a procedure (A) for automatically tracking the spatial location of a hooded rat without the use of attached light-emitting diodes; (B) for studying different aspects of spatial memory (e.g., working memory); and (C) for studying non-spatial discrimination learning. The speed with which rats learn these tasks suggests that they may lend themselves to a variety of behavioral investigations, including pharmacological work and studies of cerebral function.

Introduction

The purpose of this paper is to describe developments of a new behavioral procedure designed initially to study spatial localisation in the rat (Morris, 1981). The procedure was devised in response to theoretical controversies about the neural basis of spatial and working memory (O'Keefe and Nadel, 1978; Olton et al., 1979) and ideas that learning about spatial relations may be fundamentally different from other forms of associative learning.

The essential feature of the technique is as follows: rats are placed into a large circular pool of water from which they can escape onto a hidden platform. The platform is hidden, first by arranging that its top surface is just beneath the water surface, and second by rendering the water opaque so that the platform is invisible. Thus the platform offers no local cues to guide escape behaviour. In principle, rats could escape from the wafer by swimming randomly, or in unsystematic search paths throughout the pool; but in practice, normal rats very quickly learn to swim directly
towards the platform from any starting position at the circumference of the pool. The accurate directionality of their escape behaviour (and other measures of performance) provide evidence that the rats escape by learning the spatial position of the platform relative to distal cues. Comparison of the performance of normal and brain-damaged rats (Morris et al., 1982; Sutherland et al., 1983), and of rats given drugs (Sutherland et al., 1982) or cerebral neurotoxins (Hagan et al., 1983) offers a new way of examining the neurobiology of spatial learning.

The technique of using escape from water to motivate learning has been used for many years (Glaser, 1910; Wever, 1932; Waller et al., 1960). Water temperature, and increases in water temperature when rats swim from one compartment into another at a higher temperature (Woods et al., 1964) affect learning rate in simple escape tasks. Swimming 'ability' is also related to learning rate (Woods and Holland, 1961). In these and the present procedures, the animals do not need to be deprived of food to motivate learning, or given extensive pretraining. The use of electric shock to motivate escape behaviour is obviated. Rats are natural swimmers and 'take to' water-escape tasks easily, even if they have sustained telencephalic brain lesions of various kinds. With respect to the present task, the water provides an intramaze environment which controls for olfactory cues and, as will be described, allows for a variety of training procedures to dissociate learning and performance effects.

This paper describes several new developments of the 'water-maze' procedure including: (1) a technique for tracking the paths taken by rats during swimming; (2) variations in the training schedules to examine different aspects of spatial memory; and (3) procedures for examining non-spatial discrimination learning.

Materials and Methods

Subjects

The results of several experiments using both male and female rats will be described. The rats are maintained on ad libitum food and water, and housed singly or in groups of two rats per cage.

Apparatus

Two different pools have been constructed (Fig. 1). The first was 1.32 m in diameter × 0.60 m high (as used in Morris, 1981). The second, currently in use, is 2.14 m in diameter × 0.40 m height. Both were constructed from hardboard, attached to a baseboard, and coated with fibreglass and resin. The first was then painted white, but this coating 'flaked' and had to be frequently repainted; the larger pool is coated with permanent white gelcoat. Each pool is filled with water (26 ± 1°C) to depths of 0.40 m and 0.25 m, respectively, and rendered opaque by the addition of a small quantity of milk (pure or powdered). The second pool has been fully plumbed into the laboratory for ease of filling and draining, and mounted on a frame so that the water is at waist height. Four points around the circumference of the pool are arbitrarily designated North, South, East, or West and, on this basis, the pool area divided into 4 quadrants (NW, SW, etc).
Escape from the water is provided by means of circular rigid platforms (9 cm diameter). Two platforms were made from sections of perspex tubing filled with stones, they were sealed at the top to provide the platform and at the bottom to form a baseboard. The height of these platforms is arranged to be 1 cm below, or 1 cm above the water level; thus one platform is hidden, the other visible. A third platform was made from a short (3 cm high) section of perspex tubing filled with expanded polystyrene. This platform is anchored by means of nylon threads to a metal base (section of heavy galvanised caging) which sits on the bottom of the pool. Each platform can be moved to any position in the pool, generally at the centre of one of the 4 arbitrary quadrants. These platforms are painted white, grey or with black and white stripes and used in different procedures designed as ‘hidden platform’, ‘cueing procedure’ and ‘two-platform task’ (Fig. 2) as described below.

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Fig. 1. Cross-sectional diagram of pool, and its position in the laboratory. Note support frame to ensure water level is at roughly waist height, and perspex disc attached to laboratory ceiling to limit field of view of TV camera.

Fig. 2. Cross-sectional diagram of pool showing 3 different arrangements of escape platforms as described in text.
Tracking techniques

The simplest measure of performance in the pool is the latency to escape from the water. As rats swim at a fairly constant speed for a given water temperature, latency is quite sensitive to the amount that the rat has learned about the task. The original procedure was to monitor the rat's behaviour via a video-camera placed above the pool, relaying the output to a video-recorder and monitor. The paths taken in escaping were then transcribed onto charts, slowing down the replay of the tapes when necessary to achieve accuracy of transcription. From these charts, path-length is measured using a map-measuring device. Path-directionality, defined as deviation from the correct heading angle towards the platform, is measured at various distances along the path with a protractor. These transcribing and measuring procedures have been adequate but suffer from inevitable inaccuracies. A tracking system has therefore been adopted to allow for objective analysis.

The tracking system consists of an image-analyser (HVS Ltd, VP110) coupled to a small BBC Model B Microcomputer. The image-analyser transforms the conventional video-image into an image of high-contrast edges. A threshold adjustment allows the operator to monitor various degrees of contrast and the image-analyser provides, by way of output, the x and y coordinate of the first high-contrast edge found in tracking down the horizontal lines of the video picture. The x and y coordinate is displayed and also expressed as two analogue voltage signals for relay to the computer. These signals are sampled at the rate of 10 Hz.

With suitable reflected lighting, this system can be used to track the position of a black hooded rat by adjusting the contrast sensitivity until the black head of the rat is picked out against the white surfaces of the water and of the remainder of the animal's body. Thus the image-analyser monitors the exact x and y coordinate of the rat's head. No light-emitting diodes attached to the animal are necessary.

The image-analyser will also operate from the composite video output of a video-recorder such as the SONY C9 Betamax recorder which provides a high signal to noise ratio, particularly with CrO₂ tape. The animals may then either be tracked 'on-line' or from videotape. The SONY C9 also has remote infrared controls so that a single experimenter may start and stop the videotape while handling the rat.

One complication is that the HVS image-analyser samples from an operator-defined 'sampling space' shaped in the form of a square. As the pool is round, this sampling space must be equal on both sides to the diameter of the pool leaving 4 'sectors' outside its perimeter, at the corners of the sampling square. To ensure that these do not result in high-contrast edges 'trapping' the x, y coordinate monitoring system, a perspex sheet which a circular disc cut from it is placed high above the pool beneath the video-camera, positioned to delimit the view of the camera to the full water surface only. This sheet is back-illuminated by lights powered via a rheostat which can be adjusted until any contrast edge between the disc and the perimeter of the opaque water surface is eliminated. The only high-contrast edge remaining is then around the black head of the rat (Fig. 3).

Computing programs

A suite of computer programs has been written for (a) sampling, (b) processing
and (c) displaying the paths taken by the animal, taking advantage of the full colour graphics of the BBC Model B computer. The x, y coordinates are sampled at 10 Hz between start and stop signals indicating entry into the pool and arrival at the platform, respectively. The data coordinates are stored on diskettes. The first stage of data-processing involves a ‘smoothing’ algorithm which rejects false x, y coordinates. If the lighting arrangements are set properly, the level of artifact is very low, generally less than 1%. However, false coordinates do arise due to occasional ‘flashes’ of high-contrast on the water-surface, or due to artifacts during replay from the video-recorder. The algorithm operates according to the principle that the rat can only swim at certain speeds and thus any x, y coordinate pair greater than a certain distance from a preceding coordinate pair must be false. False coordinates are replaced with the average values of preceding and succeeding permissible coordinates. The ‘smoothed’ data are then used to calculate path-lengths, directionality, position in pool and other measures of performance as described below. All programs were written in BBC Basic software, in consultation with a professional software house (Computer One Ltd.).

**Behavioural procedures**

1. *Acquisition and reversal*

   The simplest experimental procedure involves training rats to find the hidden platform at a fixed location in space over a series of trials. Fig. 4A shows the results of an experiment in which 16 male rats were trained to find the platform in the small pool over 20 trials spread across 3 consecutive days (from Morris, 1981). For one group, the platform was placed in one quadrant for all 20 trials (different quadrants for different rats) and then moved on trial 21 to the opposite quadrant (e.g. to SE from NW). For a second group, platform position varied from trial to trial.
randomly. The initial decline in latency (e.g. trials 1–8) is due to two factors: (1) the rats learning to swim away from the side walls and thus increasing their likelihood of contacting the platform by chance; (2) true spatial learning about the location of the platform. For Group Place, escape latency stabilised between 5–8 s as the rats learned to take relatively direct paths to the hidden platform; Group Random, on the other hand, shows only the initial decline in latency associated with overcoming thigmotaxis. These findings show that the effective performance of Group Place is not due to any local visual cue associated with the platform, reflected waves or other non-spatial cues.

On trial 21, the escape latency increased for Group Place because, initially, the rat searched near its former platform location (Fig. 4A, B). Relearning is generally very rapid. Although moving the platform to the opposite quadrant is most easily described as a ‘reversal’ procedure, this description is inexact. The reason is that the allocentric spatial relationship of the distal room cues remains invariant when the platform is moved. The rat needs only to learn the new position of the platform, not that of the entire array of cues upon which effective escape performance is reliant (cf. transposition procedures of Suzuki et al., 1980). Work by Sutherland et al., (1983) has shown that moving the platform to a new place is accompanied by a disinhibition of exploratory rearing on the platform which habituates over the course of initial training.

2. Path-directionality
Rats which have learned the position of the platform head towards it from a distance (Fig. 5A). Thus the angle subtended between a tangent to the rat’s swim-path and a line drawn directly towards the platform declines as accuracy improves. The angles recorded for different rats may be collated using circular statistics (Gaile and Burt, 1980) and plotted on polar diagrams. Fig. 5B shows
path-directionality data on trial 28 of training from a group of hippocampal and cortical lesioned rats (from Morris et al., 1982). The length of the vector drawn from the centre of the polar diagrams is a measure of the tendency for a group of rats to be heading in the ‘correct’ direction.

3. Use of cueing procedure

Impairments in performance in the hidden platform task after brain lesions or drugs may arise because these lesions affect the motivation to escape, or sensorimotor factors rather than spatial learning per se. The cueing procedure, in which the escape platform protrudes above the water surface provides a control for this. In one experiment (Morris and Schenk, 1983) rats with entorhinal cortex lesions were given 28 trials of hidden platform training, during which a lesion deficit was observed, followed by 20 trials of the cueing procedure. They were then returned to the hidden platform condition for 4 further trials. Fig. 6 shows the mean performance over the last 4 trials of the cueing procedure and the succeeding 4 trials. There are two main findings in these results: (1) the cueing procedure abolished the lesion-induced deficit in escape latency; but (2) the succeeding hidden platform procedure caused the latency deficit to reappear. Thus, it seems that the entorhinal cortex lesion rats are suffering a genuine spatial deficit, rather than an impairment induced by motivational or sensorimotor factors.

4. Transfer tests

The spatially distributed swimming pattern on ‘reversal’ trials suggested an alternative type of test; namely, removing the platform from the apparatus entirely. The rat is placed into the water for a limited period (e.g. 60 s) and his ‘spatial bias’
Fig. 6. Mean escape latencies of a group of rats given radiofrequency lesions of entorhinal cortex (n = 11) and a combined group of sham lesion and unoperated control rats (n = 12). Note increased latency on reintroduction of hidden platform procedure is restricted to EC group showing that their impairment is not due to motivational or reinforcement aspects of the task.

Fig. 7. Paths taken during successive 60 s and 24 h transfer tests of a single typical rat given only limited training, traced from the graphics printer output from the image analysis program. Right-hand columns show time spent and annulus crossings for Adjacent-left quadrant (in this case NW), Training, Adjacent-right, and Opposite quadrants.
measured. One measure records the time spent searching in each of the 4 quadrants of the pool. A second measure records the number of crossings of regions of the pool marking the exact position and surface area of the former platform. The rationale behind these measures is that rats trained to (say) NE should spend relatively more time in NE than in the other 3 quadrants; and that to the extent that their searching for the now absent platform is accurate, they will cross a small circular area inside the NE quadrant (where the platform used to be) more often than the corresponding areas of the other 3 quadrants.

Originally, both measures were recorded by hand with the aid of electronic timers and counters; lines were marked on the video-monitor to indicate the 4 quadrants and 4 'annuli' marked to note the positions of the platform within each quadrant. However, the tracking analysis program is now used to compute both measures automatically. Fig. 7 shows an example from an experiment in which rats were given only very limited training (13 trials) prior to transfer tests 60 s and 24 h after the final trial of training. The results from one typical rat are presented. Note that he spends over 50% of his time in the training quadrant during the first transfer test, but only 36% during the second test. Furthermore, he crosses the former platform position relatively more often during the first than the second test.

5. Effects of repeated transfer tests

Unpublished pilot experiments have shown that the absolute magnitude and rate of decline of the 'spatial bias' revealed in transfer tests is dependent upon two main factors: (1) the total number of training trials; and (2) the spacing of trials. In one experiment in which only one trial was given per day (Heatlie, 1983) excellent transfer test performance was obtained after only 10 training trials (60% time spent in training quadrant). The decline in spatial bias with repeated testing may be due to (1) true forgetting; or (2) extinction effects (the platform no longer being present). A study was therefore conducted to compare these factors. Three groups of 7 rats were trained for 20 trials and then given transfer tests as follows: Group 1 was given transfer tests 4 h, 48 h and 336 h after training; Group 2 at 48 h and 336 h; and Group 3 at 336 h only. To provide a single index of performance for each group at each retention interval, the number of crossings of the training annulus position has been plotted as a percentage of the total number of platform annulus crossings. This 'spatial bias' measure would be 25% in the event of no spatial bias. The results plotted in Fig. 8 show (1) that at each interval, the group which was repeatedly tested (Group 1) performs at least as well (if not better) than the groups only given one or two transfer tests; and therefore (2) that the function relating spatial bias to retention interval is relatively insensitive to extinction effects. More thorough studies concerning the relationship between training parameters and retention are planned.

6. Spatial 'matching to sample' procedure

A series of experiments are currently underway to compare spatial and working-memory theories of hippocampal function. Full details will be reported elsewhere, but the necessary prerequisite of these experiments was the development of 'matching to sample' procedures in the pool. The simplest strategy is to run two trials per
day, per rat. On trial 1, the platform is placed in any of several positions in the pool (e.g. SW, NW, NE or SE). The rat is allowed to swim to find the platform and then left on it for 30–60 s. On trial 2, the platform is again hidden in the same position. The question at issue is whether the rats can benefit on trial 2 from information about the platform's position gained during trial 1. If so, the escape latency on trial 2, should be faster than on trial 1. The results of one experiment using this procedure in the small pool (Morris, 1983) showed significantly faster escape on trial 2. However, before accepting this improvement as evidence of spatial 'matching to sample', an alternative possibility must be considered. Trials 1 and 2 differ in other respects, e.g. on trial 1, the rat is dry; on trial 2, he is wet. Thus, the improvement in latency may be a non-specific or motivational difference. Accordingly, a further group of rats was trained in which platform position on trial 2 was also random with respect to position, as on trial 1. The results for this group showed the following pattern of results: on that subset of trials for which the platform was in a different position on trial 2 than it had been on trial 1, escape latency on trial 2 was no different than that shown during trial 1. However, for the 25% of trials for which the platform was in the same position on trial 2 as it had been on trial 1 (required by the random schedule of four possible platform positions), escape latency on trial 2 was faster than on trial 1. These findings imply that the animals are showing a 'win-stay' tendency in the pool irrespective of training schedule. More extensive results are presented in Morris (1983). A replication of this experiment using a 'two-platform' procedure (see below—section 7) is currently being tested to provide a measure of choice accuracy of spatial 'matching to sample'.

Fig. 8. Retention functions based on proportion of training annulus crossings relative to total crossings at 3 post-training intervals. These are pilot data based on n = 7 rats per group only.
7. **Spatial and non-spatial discrimination learning**

The hidden platform procedures do not provide any index of spatial discriminability. A ‘two-platform’ procedure has therefore been developed (Fig. 2), which can be used to study both spatial and non-spatial discrimination learning. Both platforms are visible, 1 cm above the water. One platform is rigid; the other is the floating platform as described previously. As the floating platform sinks beneath the rat when he attempts to climb onto it, the animal soon learns to avoid it and to approach the rigid one preferentially. Training can be in two forms: *spatial discrimination* training, in which the rigid platform is defined by its position in space; or *visual discrimination* training, in which it is defined by its brightness or pattern (e.g. grey positive; black and white stripes negative). In the latter case, heavy black curtains suspended from rails attached to the laboratory ceiling are drawn around the pool to exclude extra-maze cues. In one experiment in which these procedures have been used, rats learned the spatial task to a criterion of 17/20 correct in 49 ± 10 trials (inclusive of criterion); the visual discrimination task took 120 ± 13 trials (n = 9). A ‘correct’ response was defined as approach to the rigid platform without touching the floating platform. This procedure is presently being used to examine the effects of hippocampal lesions (with modifications to examine reference and working-memory, Olton et al., 1979) and the effects of anticholinergic drugs (Hagan and Tweedie, in prep).

The surrounding black curtains can also be used to exclude extra-maze cues in rats trained with the curtains open to localise a platform at a fixed location. As shown in Fig. 9, pulling the curtains around the training quadrant section of the pool caused a partial drop in performance; pulling them around the entire pool caused performance to drop to chance. These findings imply that extra-maze cues are essential for effective spatial localisation performance. However, they also imply that the cues at or nearest the training quadrant are not the only cues of importance.

![Fig. 9. Percent correct rigid platform approaches in 10 trial test blocks with curtains open, drawn around training quadrant only, or completely enclosing pool. See text for discussion of implications.](image-url)
That performance drops to chance when all extra-maze cues are excluded implies, as an incidental but not unimportant finding, that the rats are unable to distinguish the floating and rigid platforms on the basis of local cues alone.

Discussion

The primary purpose of this paper has been to describe several developments of the water-maze procedure. The automatic tracking provides a level of objectivity usually only possible in fully automated experiments. The matching-to-sample technique shows that contrary to the situation in foraging tasks, rats may show 'win-stay' strategies in escape behaviour irrespective of the schedule imposed upon them. And the two-platform technique shows how non-spatial learning theory may also be studied using the same general paradigm.

One conceptual and several practical aspects of the task deserve further comment. The use of the hidden platform requires that the rat approach a position in space which it cannot hear, see or smell. Extra-maze cues must be used for accurate localization. Thus the absolute levels of performance reflect the 'acuity' of spatial memory and its disruption by drugs or lesions. In T-mazes, by comparison, rats make only a binary decision between going left or right, to one part of the apparatus or to another. Once that decision is made, the design of the maze arms leads the animal to reward; no further demands are placed upon the spatial localisation system. In the water-maze, on the other hand, accurate directionality requires continual monitoring of the animal's position in relation to extra-maze cues. This may involve 'cognitive mapping', as proposed by O'Keefe and Nadel (1978); or some more parsimonious 'snapshot' procedure for homing in on the correct location (Cartwright and Collett, 1982).

From a practical point of view, the general procedures may prove useful for several different kinds of neurobiological study. Already, the technique has been exploited in studies of cortical function (Kolb et al., 1983a, b) sex differences and hemispheric asymmetry (Therrien et al., 1982; Dennenberg, pers. comm.) superior collicular lesions (Milner and Lines, 1983), nutritional deficiency (Heatlie, 1983) and in studies of catecholamine depletion (Hagan et al., 1983). The latter study revealed intact spatial learning in animals that were ataxic after striatal dopamine depletion, the water serving to 'activate' the animals. Studies of 'latent learning' (Sutherland and Linggard, 1982) and 'overshadowing' and 'blocking' of spatial and non-spatial learning (Morris and Heatlie, in prep.) are also underway, using controlled cues inside the curtaining, as well as pharmacological studies, some using osmotic minipumps. Gage (pers. comm.) is currently using the procedure to investigate recovery of function after septo-hippocampal transplants.

The main virtue of the procedure is the speed of training. Few of the experiments conducted in St. Andrews have lasted more than two weeks, although there is no reason why more extensive training could not be examined. The disadvantages of the technique are (1) an inability to vary motivational level or reinforcement magnitude; (2) that experiments must be run by hand, which is more tedious than using fully
automated equipment; (3) that immersion into water may cause endocrinological or other stress effects which, while not of primary interest, may nevertheless interact with lesion or pharmacological manipulations in uncontrolled ways.

Equipment

The image analysing equipment may be obtained from HVS IMAGE ANALYSING, 22 Cromwell Road, Kingston KT2 6RE, UK. The BBC Model B Computer is widely available, but we have found that TWILLSTAR COMPUTERS LTD., 17 Regina Road, Southall, Middlesex, UB2 5BL, UK. can usually supply within 48 hours. Fibreglass and resin are available from yacht chandlers.

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