TECHNICAL NOTE

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The Atlantis Platform: A New Design and Further Developments of Buresova's On-demand Platform for the Water Maze

Introduction

The water maze is a widely used spatial learning task (Morris 1981, 1984) in which rodents are trained to escape from water onto a hidden platform located within a large, usually circular, pool. Over a series of trials, rats (or mice) will rapidly learn to take relatively direct paths to the platform, indicating that they may have learned its spatial location. Transfer tests, sometimes called probe tests, in which the platform is removed from the pool, offer further evidence of true spatial learning, as trained animals display a search pattern focusing on the platform location in the training quadrant. Performance in this task has provided a simple and convenient assay of the effects of drugs or brain lesions on cognitive function in rats (for review, see Brandeis et al. 1989; McNamara and Skelton 1993), and its use with mice has revealed covariation between spatial learning and strain-specific differences in neuroanatomical circuitry (Schwegler et al. 1988) and the effects of targeted deletion of genes putatively involved in synaptic plasticity (Grant et al. 1992, Silva et al. 1992).

Despite its efficacy, the task as it is usually run has a number of limitations. First, animals may develop strategies for finding the platform, such as circling at roughly the appropriate distance from the sidewall, which do not actually require them to learn where the platform is located. Such strategies can, nevertheless, lead to a steady decline in escape latency that may be falsely interpreted as spatial learning. Second, during a transfer test, animals may initially search the training quadrant but, failing to locate the now-removed platform, move elsewhere in the pool. If this tendency to move elsewhere differs across treatment groups, an analysis of time (or distance) spent in the training quadrant throughout the entire transfer test will be a confounded index of the extent of spatial learning. Animals that go directly to the platform area and then search elsewhere because they fail to find the now-removed platform may be scored incorrectly as poor learners. Third, because transfer tests, as conventionally run are extinction trials (i.e., no escape is possible), the use of multiple transfer tests may compromise the asymptote reached in learning or even, because learning is so rapid, encourage animals to search outside the trained area when they notice that the platform is absent. Thus, performance with multiple transfer tests, while desirable in pharmacological work, may underestimate the true level of learning.

Buresova et al. (1985) designed an ingenious on-demand platform which, they suggested, would distinguish true spatial mapping from other

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search strategies and eliminate extinction in transfer tests. This consists of a collapsible cantilevered platform that is raised near to the water surface when the rat dwells within a defined area for a set period of time (dwell time). Illustrative trajectories were presented suggesting that rats swam to the border of the target area and then “trode water” around the presumed target. The quantitative data showed that escape latency increased at longer dwell times but did not, however, establish that the on-demand platform definitively improved spatial localization performance over that obtained with a conventional escape platform as measured by a transfer test with the platform absent.

In this paper we outline a new design for an on-demand, or “Atlantis, platform,” involving electromagnetic control. We also describe a new method of tracking the swimming animal, using video-frame grabbing, which enables the raising of the platform to be entirely under software control. The transfer test performance of rats trained with this new platform is compared, for the first time, with that of animals trained with a conventional hidden platform. We find that training with the Atlantis platform produces significantly more focused search at the correct target location at short dwell times despite this procedure resulting, as Buresova et al. (1985) found, in longer escape latencies during training.

Materials and Methods

SUBJECTS AND APPARATUS

The rats (Lister hooded) were trained in a standard 2-meter open-field water maze as described previously (Morris 1984). To track the paths taken by the swimming rats, a video camera was mounted directly above the pool, and the signal led via a video recorder to a frame store (Wild-Vision, Hawk V12) mounted in an Acorn A5000 RISC computer. The software running on the computer identified the location of the head of the swimming rat and plotted the animal's path on the computer monitor in real time; data analyses were performed later by the same program. The software also controlled the raising of the Atlantis platform.

The water maze was set within a large laboratory room containing prominent extra-pool cues (curtain, posters on walls, a section of primate caging, etc.). This room was lit by diffuse reflected light from four exterior floodlights mounted on platforms outside the pool and positioned below the exterior rim of water maze. The water was maintained at $25 \pm 1^\circ\text{C}$ and made opaque by the addition of a quantity of powdered milk. The pool was filled and drained daily by means of electrically operated valves controlled by a time clock. The regular platform consisted of a section of drain pipe (diam. 11 cm) filled with stones and sealed at the top; the Atlantis platform is described below.

ATLANTIS PLATFORM DESIGN

The Atlantis platform (Fig. 1) consists of a cylinder of robust expanded foam (150 mm diam., 182 mm height) designed to provide 2 kg buoyancy. This cylinder can move vertically along a central guide pin, (180 mm height), by virtue of a PTFE (polytetrafluoroethylene) bush. The guide pin is attached to the chrome plated mild steel base plate by a stainless steel screw. The base plate was chrome plated to avoid corrosion when immersed in water; it has a circular groove machined into it, into which a copper coil had been inserted and potted using an epoxy resin. When the coil is energized using a 12 V DC power supply,

Figure 1: The Atlantis platform: An engineering drawing of the electromagnetically controlled rising platform. Units are in millimeters.

the base plate becomes an electromagnet. The cable from the power supply to the electromagnet is led via a vertical pipe mounted on the outside of the pool to an entrance port in the side of the pool. A chrome-plated steel armature is attached to the lower surface of the moving cylinder. When the electromagnet is activated, it acts upon the steel armature to maintain the cylinder in the lowered position. Upon inactivation of the electromagnet, the cylinder is released and floats under its own buoyancy to its uppermost position on the guide pin. A damper built into the center of the cylinder slows the rate at which the cylinder rises, and this, coupled with water channels machined into the cylinder, allows the cylinder to rise with a minimum of disturbance to the surface of the pool. The 2 kg buoyancy provides a stable platform for the rats (typical maximum weight, 600 grams).

COMPUTER SOFTWARE

The software used to track the animals by frame-grabbing has several advantages over electronic tracking apparatus. The new software finds the rat by examining each point in the video picture with a complete scan of the screen, searching for a target lighter (e.g., albino animals) or darker (e.g., hooded rats) than the surrounding water. Starting from the last known location, the software arranges for the pixels to be examined in a spiral with a gross scan (i.e., jumping neighboring pixels) followed, after a possible target has been located, by a fine scan (i.e., pixel by pixel). Figure 2 illustrates the principle of the gross and fine scan. The fact that the scanning software “knows” where the rat was last located, and uses this information to determine in what area to search, reduces the requirement for computer time and allows for more frequent and, therefore, more accurate, tracking. The A5000 RISC architecture machine is sufficiently fast for this frame-grabbing and analysis to be conducted in real time at 10 Hz. To avoid tracking points other than the rat, the system is able to ignore defined areas of the video image, using a mask

Figure 2: Finding the target using software image analysis: The video screen consists of pixels of varying levels of brightness. The target (e.g., the black head of a hooded rat) is defined as being within a certain range of absolute brightness and also relatively darker than the surrounding pixels. Using these two thresholds, the software scans a region of the picture starting from where the rat was last located ($1/10$ sec. earlier, broken line drawing of rat), jumping every 2 (as shown) or more pixels until a possible target is found. It then initiates a fine scan (every pixel) to optimize the exact location of the head of the rat. Rat not drawn to scale.

frame, such as those outside the pool. In addition, to be tracked successfully, a target must lie within a defined range of both absolute and relative brightness. The brightness of every point in the video image is recorded before a trial commences and in the absence of the target animal (pretrial image). To be tracked, a point on the animal (e.g., the head of a hooded rat) must be within a given range of luminance (absolute threshold) and also darker than the recorded brightness of the pretrial image at that location (relative threshold). This gives excellent tracking across the full surface area of the pool irrespective of any uneven lighting and without the necessity of attaching anything to the animal.

The raising of the Atlantis platform is controlled by three parameters: inclusion radius, dwell-time, and raise-regardless time (Fig. 3). For the platform to be raised, the rat must remain continuously within an area defined by the inclusion radius (typically 20 cm) centered around the platform for a period defined by the dwell time. Both parameters can be altered during an experiment, for example, the dwell time may be gradually increased (0, 1, 3 sec, etc.). The dwell-time clock is reset to zero if the rat swims, even momentarily, outside the defined inclusion radius. If the rat fails to stay in this region for long enough at any period during a trial, the platform is raised automatically after the raise-regardless time (typically 60 sec).

EXPERIMENTAL PROCEDURE

Ten Lister hooded rats were trained (Atlantis platform $n = 8$; regular platform $n = 8$) using the following protocol.

Figure 3: The parameters controlling the Atlantis platform: The inclusive radius is an area centered around the platform. The dwell time is the duration for which the animal must remain continuously within the inclusive radius for the platform to rise. The raise-regardless time is the time after which the platform will rise anyway if the animal fails to find the platform (during training) and/or it has deliberately not been raised at an earlier point in the trial. Two possible trial outcomes are shown to illustrate these parameters: a successful (*top*) and unsuccessful (*bottom*) trial.

Day 0: All animals were first given six trials *pretraining* in the water maze using the regular hidden platform but with curtains drawn around the pool to occlude extra-maze cues. The curtains were then removed for all remaining procedures. *Day 1:* A transfer test (A) was then conducted (platform absent, swimming in the pool for 60 sec, extra-maze cues visible), followed immediately by three trials of place-navigation training with either the Atlantis or regular hidden platform. For the Atlantis platform, the inclusion-radius was set at 20 cm and the dwell time at 1 sec. (*Note:* For the purposes of this experiment, the Atlantis platform was *not* "raised regardless" at the end of a transfer test, as this would have biased the results in favor of the Atlantis group by virtue of avoiding any extinction effect that transfer tests incur; doing so may improve performance yet further than that shown below). *Day 2:* A second transfer test (B) was then given followed immediately by three trials of training on this and 2 subsequent days (days 2–4). The rats were placed into the pool at the sidewalls and the time taken to complete their escape onto either type of platform was recorded. *Day 5:* Transfer test (C) was then given followed by three trials with an increased dwell-time of 3 sec. This training continued for three more days (days 6–8). *Day 9:* Transfer test (D) was then conducted, completing the experiment.

Results

Animals trained with the Atlantis platform learned to swim to the vicinity of the platform location and localize their search in this place. They

seemed unperturbed by the rising of the platform, which typically took ~ 1 sec; they climbed onto it and otherwise treated it as a normal platform. There was some indication that the rising of the platform, and perhaps the slight wave action associated with it, may have attracted the rats toward the correct area.

The data were analyzed with respect to both latency to escape onto the platform (inclusive of dwell time) during training trials and time spent in the various quadrants of the pool during transfer tests. A plot of escape latency over days is shown in Figure 4. This shows that over days 1–4, during which the dwell time was maintained at 1 sec, escape latency declined comparably in both the regular and Atlantis groups. When the dwell time was increased to 3 sec (days 5–8), the total escape latency of the Atlantis group showed a sharp increase while the group trained with the regular platform continued its gradual decline in escape latency. An Anovar of escape latencies in which groups ($n = 2$), phases ($n = 2$) and days ($n = 4$) were factors revealed a significant group effect ($F = 20.49$, $df\ 1/14$, $P < 0.0005$). Figure 4 shows a graph of escape latency against day. The Atlantis animals perform less well than those trained to the normal platform in the initial trials of the first phase of the experiment because of the additional complexity of raising the platform. In the second phase, they also show longer latencies than the control animals throughout the training period following the increase in the dwell time.

Performance during the series of four transfer tests is shown in Figure 5A–D. Prior to any place–navigation training (but after the pretraining), both groups swam throughout the area of the pool and neither showed a bias to any quadrant (Fig. 5A: group $\times$ quadrant interaction $F = 1.33$, $df\ 2/42$, $P > 0.27$; numerator degrees of freedom reduced by 1 as quadrant percentages necessarily add to 100%). After one day's training (day 1 in Fig. 4), there was already a clear indication of preferential searching in the training quadrant (Fig. 5B: $F = 24.97$, $df\ 2/42$, $P < 0.001$) but no significant difference between groups ($F < 1$). After the full 4 days training

Figure 4: Escape latency across days (not including pretraining): Phase 1 (dwell time = 1 sec for the Atlantis group) occurs over days 1–4; phase 2 (dwell time = 3 sec) occurs over days 5–8. (●) Regular platform; (■) Atlantis platform. Mean ± 1 S.E.M.; $n = 8$ per group.

Figure 5: The series of transfer tests during the course of training. (A) Prior to any spatial training; (B) after one day of training; (C) after phase 1 of training; (D) after phase 2 of training. Note the striking spatial bias to the training quadrant by the Atlantis group after phases 1 and 2.

with a 1 sec dwell time (phase 1), the group trained with the Atlantis platform showed significantly more time in training quadrant (Fig. 5C: group $\times$ quadrant interaction $F = 6.01$, $df\ 2/42$, $P < 0.01$). Finally, after a further 4 days training with a dwell time of 3 sec (phase 2), the Atlantis group continued to spend a greater mean time searching in the training quadrant than the regular group, although the group $\times$ quadrant interaction did not quite reach significance (Fig. 5D: group $\times$ quadrant interaction $F = 2.49$, $df\ 2/42$, $0.10 > P > 0.05$). This trend was probably the result of the Atlantis animals reaching a performance ceiling at $80.5 \pm 3.0\%$ time spent in the correct quadrant to which the rats trained with the standard platform are gradually approaching $69.0 \pm 5.4\%$ (chance performance = 25%). We did, however, observe highly focused searching *within* the training quadrant by animals trained to the Atlantis platform relative to the controls; the strikingly localized search pattern of a representative animal in this group is shown in Figure 6 and compared with that of an animal trained with the regular platform.

Rat 3319 - Regular Platform

Rat 3324 - Atlantis Platform

Figure 6: Plot of paths taken by illustrative rats of each group during the final transfer test after phase 2. Note the particularly accurate spatial localization of the Atlantis rat and its persistent searching at this single location. The rat trained with the regular platform does well, but the searching is less focused.

Discussion

The purpose of this paper has been to describe a new design for an on-demand (Buresova et al. 1985) or Atlantis platform, operating under the control of a new software-based image-analysis system. We also sought to examine whether its use would lead to more focused searching during transfer tests.

Our experience of the platform is that it is highly (100%) reliable and that its gradual rise to a position just below the water surface causes minimal behavioral disruption. The rats treat it as an escape platform in exactly the same way as the regular platform. Its construction is robust and we experienced no difficulties in having an electromagnetic coil energized beneath the water surface; leading the connecting wires via piping mounted outside the pool also ensured no interference with the animals. The software frame-grabbing technique also works well, maintaining continuous tracking of the target. Clearly the continuity of tracking is a prerequisite for the appropriate control of the Atlantis platform.

Our experimental results quantitatively confirm the qualitative assertions of Buresova et al. (1985) in showing significantly improved localization of rats trained to the Atlantis platform compared with those trained to the regular hidden platform. After sufficient training, the animals appeared to exercise their minimum turning distance to remain in the area of the platform and, at times, to tread water above it. Rats given 4 days of training (three trials per day) to the Atlantis platform, with an inclusion radius of 20 cm and a dwell time of 1 sec, showed significantly more time in the training quadrant than animals trained to the fixed submerged platform. This trend continued after a further 4 days training at a dwell time of 3 sec, although a ceiling effect was apparently reached with the Atlantis platform at a level of ~80% time spent in the trained quadrant. Examination of the paths taken by animals in individual transfer tests further illustrated the exceptionally focused searching to the exact area of the platform. These results provide clear evidence that rats can learn the spatial location of the platform, and remember to stay there, rather than merely developing effective nonspatial search strategies, which can so easily happen with the standard platform. This improved spatial localization occurs despite substantially longer escape latencies during training.

The Atlantis platform offers a number of advantages in the investigation of the effects of pharmacological, surgical, and genetic manipulations on spatial learning. The improved localization demonstrated here should increase the discriminative capacity of the water maze procedure, avoiding type I statistical errors. This improvement may arise for a number of reasons: First, this type of platform forces the animal to learn the location of the platform, as other nonspatial search strategies will not be successful in rapidly raising the platform. Second, the animals are rewarded for remaining persistently in a fixed location over this site. Thus, in transfer tests, trained animals show a much decreased tendency to move elsewhere in the pool despite an initial lack of success in locating the now-removed (or now-unresponsive) platform. This improved performance could be particularly helpful in studies of remote memory and/or the consolidation of long-term memory (cf. Zola-Morgan and Squire 1990) as it may then be possible to study retention over very long time periods (e.g., 6 months). It should, however, be noted that the requirement to stay at a specific place for a prescribed period of time

may add an episodic component to an otherwise strictly spatial task. That is, not only does the animal need to learn where the platform is located (a fact), it also needs to remember that it has to stay in this spot once it gets there (which is more in the nature of remembering an event). This may complicate the analysis of experiments on remote memory in theoretically interesting ways because some theorists maintain that retrograde amnesia is more extensive for event-related information than for factual information (e.g., McCarthy and Warrington 1990), while others have argued that both these types of declarative information are impaired equally and subject to equivalent consolidation over time (Squire 1993). Finally, in experiments conducted solely with the Atlantis platform, a further advantage may be obtained from the reduction of any extinction effect in transfer tests, the platform is raised at the end of successive transfer tests. This may be advantageous in longitudinal studies of the effects of aging on cognitive function in which repeated within-subject transfer tests are typically scheduled (M. Gallagher, pers. comm.).

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