

Quantification of swim patterns in the Morris water maze

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Spatial learning and memory in rodents is most often assessed in the Morris water maze. Neurobiologists have to distinguish behavioral patterns to unravel underlying neuronal systems. We analyzed swim patterns of mice videotaped before and after training with a multitrial procedure in the water maze. In addition to traditional parameters, the animals' position in relation to trained and other possible platform locations was estimated five times per second by an image analysis system. This parameter, *cumulative distance to platform*, was correlated with time spent in the platform quadrant but not with latency to and crossings of the platform location. We detected a subgroup of animals with concentric patterns within the group of spatial/persistent patterns. Random patterns were classified as well. Swim patterns before training were not predictive for the one after training. In summary, image analysis systems have made it very convenient to quantify behavior. Using their capacity, we have further improved the analysis of swim patterns, revealing animals' different approaches to solve a problem.

Behavioral biologists are confronted with the problem of how learning and memory can reliably be quantified using behavioral measures. Automated video-tracking and image analysis systems have made it very convenient to quantify the performance of animals in behavioral tasks and have made it possible to calculate many parameters. One of the most frequently used tasks to measure spatial learning and memory of rodents is the Morris water maze (Morris, 1984). Various procedures (e.g., multitrial, reversal, delayed matching to place) have been developed to examine the neurobiological basis of learning and memory (Brandeis, Brandys, & Yehuda, 1989; Poucet & Benhamou, 1997). Since the creation of mutant mice, the multitrial procedure in the water maze has become the standard and most widely used behavioral task (Wolfer, Stagljjar-Bozicevic, Errington, & Lipp, 1998). Specifically, the analysis and interpretation of this multitrial water maze procedure requires refinement.

Briefly, in a pool of warm water, animals are trained to locate a platform in a fixed position just below the water surface. During several days, a number of trials are given (i.e., multitrial procedure). The animal's ability to find it most efficiently depends on the use of a configuration of cues surrounding the pool. Allowing the animal to swim for a fixed time without the platform (i.e., free swim, probe, or transfer trial) reveals the search strategy. Learning is defined by shorter latencies and by decreased path length to the platform. Because the path to the platform is in no way constrained, different strategies are possible,

each of which may result in successively shorter escape latencies. For example, animals that direct their search predominantly to the area of the maze where the platform was located during training can have the same latency to the platform as animals that use a circular strategy, but do not show direct search toward the platform area (Schenk & Morris, 1985). In order to classify swim patterns as persistent (i.e., focused toward the trained platform location) or random, several parameters have to be consulted—total distance swum, velocity, latency to platform, time spent in quadrants, and crossings of platform location. Gallagher, Burwell, and Burchinal (1993) forwarded another quantitative measure: cumulative distance to zone. In the water maze task, this parameter is called the cumulative distance to platform, which is the distance between the position of the swimming animal and the location of the platform calculated several times per second.

The aim of the present study was to identify and distinguish between different search strategies of animals using the cumulative distance to platform parameter. Conventional parameters were calculated as well. Furthermore, we were interested to see whether explorative behavior during a free swim trial before training might be of predictive value for memory and strategy observed after spatial training. In our sample of swim patterns, we characterized three different types: spatial/persistent, concentric, and random swim. The swim pattern before training did not predict the strategy exhibited after training.

METHOD

Behavioral Task

Prior to the study, the swim patterns of 24 mice (4–5 months of age) were videotaped. The animals had participated in earlier experiments to assess spatial learning and memory in the water maze. Briefly, in a pool (white sidewall; 140-cm diameter) filled with warm water ($26 \pm 1^\circ\text{C}$) made opaque by the addition of chalk, a

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Table 1
Path Length in Meters and Velocity During the
Two Free Swim Trials in Absence of the Platform

Search Pattern	Path Length (Meters)				Velocity (Centimeters/Second)			
	FS 1		FS 2		FS 1		FS 2	
	<i>M</i>	<i>SE</i>	<i>M</i>	<i>SE</i>	<i>M</i>	<i>SE</i>	<i>M</i>	<i>SE</i>
Persistent	11.88	0.38	12.35	0.42	19.89	0.63	20.66	0.71
Concentric	11.64	0.58	14.35*	0.58	19.50	0.99	23.99*	0.97
Random	11.07	0.81	12.37	0.34	19.22	1.21	20.68	0.57

Note—FS 1, before training; FS 2, 5 min after Training Trial 15. Data represent means \pm SE. **p* < .05 concentric versus persistent and random in FS 2, and versus concentric in FS 1.

platform (8.5-cm diameter) was submerged 5 mm below the surface of the water, invisible to the animal. The ratio between the surface area of the pool and the platform was 270:1. Thus, the chance to locate the platform by accident was quite small. The pool was divided arbitrarily into four quadrants with the platform in a fixed position in the middle of one quadrant during the training trials. For each trial, the mouse was placed in the water at one of four different locations. A maximum of 60 sec was allowed, during which the mouse had to find the platform and climb onto it. If the animal failed to find the platform, it was guided with a sieve to the platform. On 3 consecutive days, 15 training trials were given with inter-trial intervals of 5 or 60 min (Table 1). In Free Swim Trial 1 (FS1), which occurred prior to training, and Free Swim Trial 2 (FS2), which occurred 5 min after the last trial, the platform was absent and animals were allowed to swim freely for 60 sec.

The swim patterns of these two free swim trials were subjected to further analysis. FS 1 yielded information on the behavioral response to this novel situation; FS 2 yielded information on learned behavior—that is, the strategy used.

Data Analysis

The videotapes of the mice during FS 1 and FS 2 were analyzed by the image analysis system EthoVision 1.7, Video Tracking, Motion Analysis and Behavior Recognition System (Noldus Information Technology BV, Wageningen, The Netherlands). The position of the animals was registered five times per second. The following traditional parameters were chosen: latency to find the platform, time spent and distance swum within the quadrants, number of exact crossings of the platform position (and comparable positions in the three other quadrants), velocity, and total distance swum (path length).

The cumulative distance to platform parameter is the sum of distances between the platform in the water maze and each sample of the animals' positions collected by the image analysis system. Thus, if an animal swims in the vicinity of the learned platform location, the cumulative distance will be short. To address the specificity for the learned location, the same calculations were done for arbitrary platform positions in the other three quadrants (i.e., the adjacent quadrants left and right, and opposite to platform position).

To allow comparison between experiments with different sampling steps or maze sizes, we presented cumulative distance to platform as a percentage. This was done easily on the basis of the following calculation: Cumulative distance to platform divided by the sum of the cumulative distance to left, right, opposite, and the platform location, multiplied by 100, results in percentage of cumulative distance to platform.

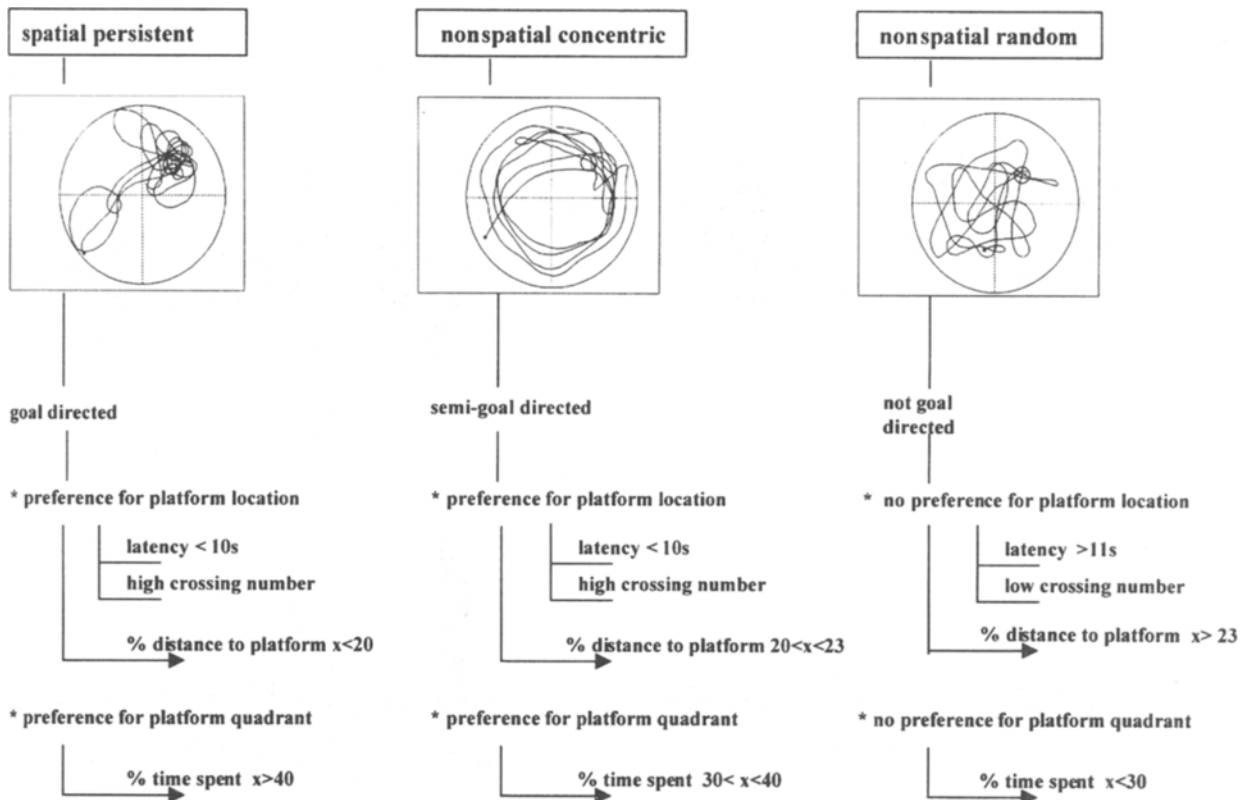


Figure 1. Flowchart demonstrating the classification of the three swim patterns: spatial/persistent, concentric, and random, with representative examples of the swim path during the free swim trials after training.

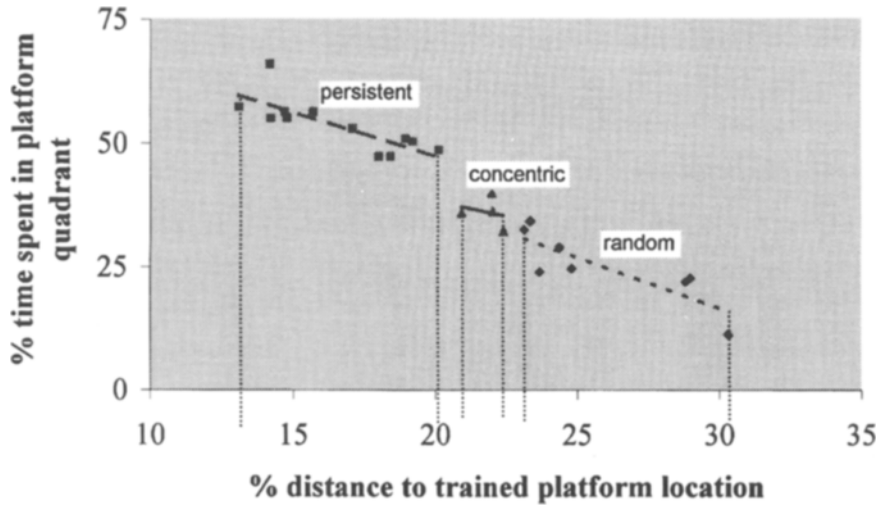


Figure 2. Percentage of cumulative distance to platform and time spent in the platform quadrant per animal. Vertical lines indicate the borders of classification.

Data Preparation

To circumvent possible interference with general activity, we chose swim patterns of 24 mice with similar path lengths during FS 1. According to traditional criteria, individual swim patterns were labeled nonspatial/random and spatial/persistent. A nonspatial/random pattern was scored as such when no preference for the platform location relative to the other three possible locations could be observed (i.e., $\leq 25\%$ time spent in platform quadrant, latency > 15 sec, not more crossings than other possible platform loca-

tions). This is a common swim pattern seen in a free swim trial before training. A spatial/persistent swim pattern is characterized by a clear preference for the platform position (i.e., $> 30\%$ time spent in platform quadrant and many crossings, and a short latency to the platform location). The next step was to integrate the cumulative distance to platform parameter.

After classification, data were subjected to an analysis of variance (ANOVA), followed by Tukey tests when appropriate. Pearson's correlation coefficient was used. Alpha level was set at $p < .05$.

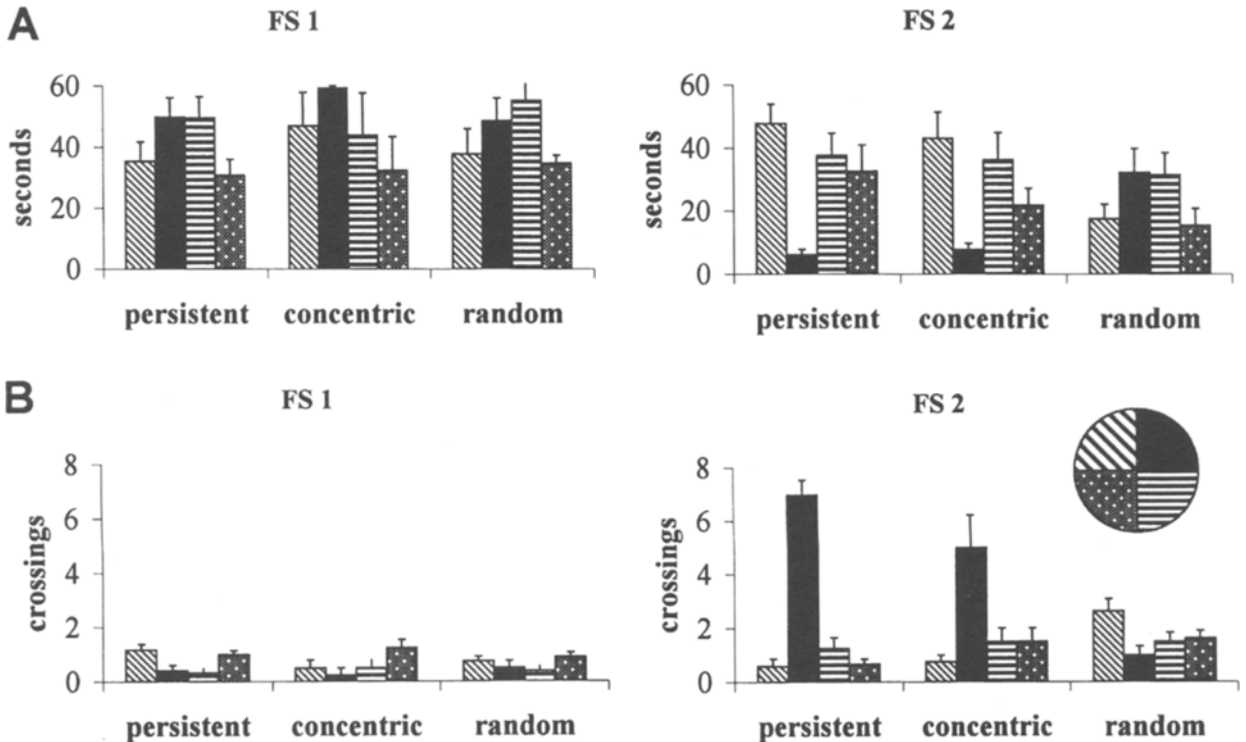


Figure 3. (A) Latency to and (B) crossings of (possible) platform positions of animals with persistent, concentric, and random swim patterns during the free swim trials before (FS 1) and after (FS 2) training. Black bars denote the platform location; other patterns refer to possible locations adjacent or opposite to the platform (see circular insert). The pool was divided into four quadrants; the black area is the platform location. Note that before training, the groups were comparable. Data are presented as means $\pm SE$.

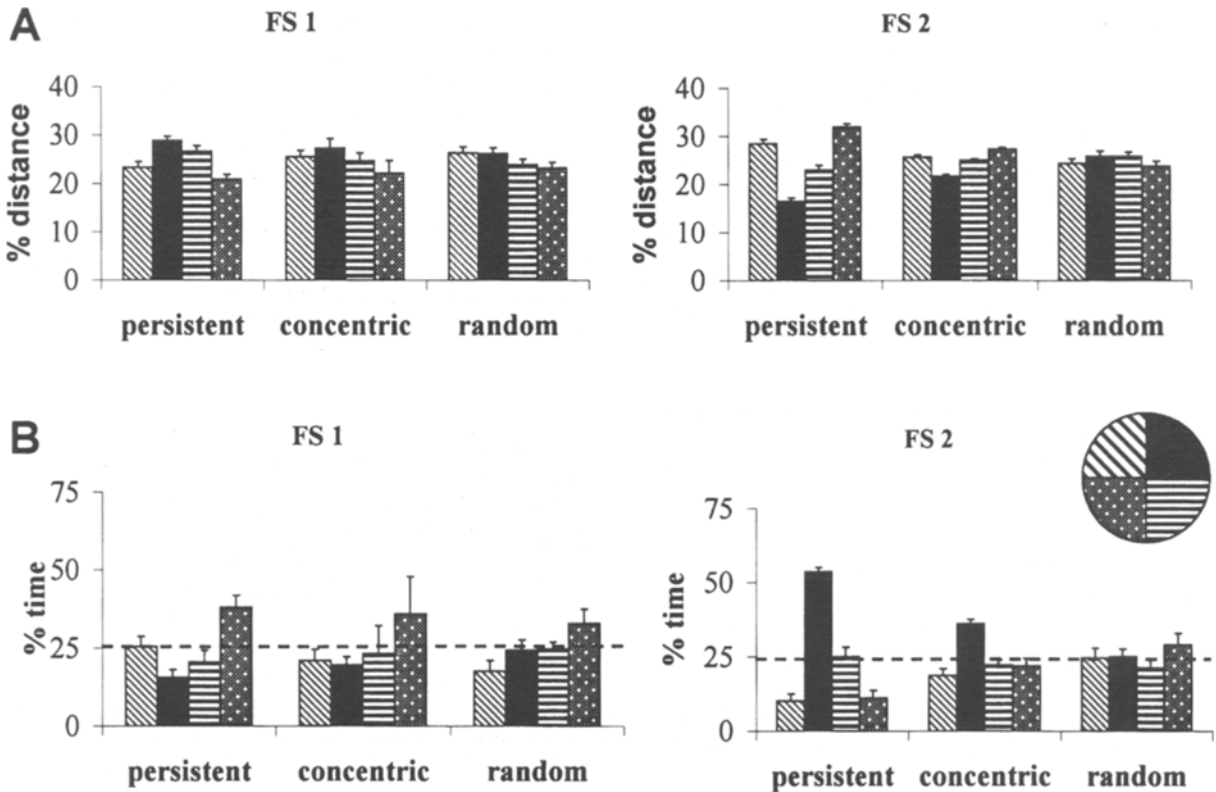


Figure 4. (A) Percentage of cumulative distance to and (B) percentage of time spent in quadrants of animals with persistent, concentric, and random swim patterns during the free swim trial before (FS 1) and after (FS 2) training. The circular insert represents the division of the pool into four quadrants, with the black area, the platform location. Black bars denote the platform (quadrant); other patterns refer to locations adjacent or opposite to the platform (see insert). The horizontal line indicates chance level. No differences were found between the groups in FS 1. Training significantly increased the time in platform quadrant in the persistent and concentric groups. Cumulative distance to platform was significantly reduced in the persistent group only. Data are presented as means \pm SE.

RESULTS

Using the traditional parameters, we classified two distinctly different patterns: a persistent strategy and a random strategy in FS 2. Incorporation of cumulative distance to zone revealed a third pattern that lay hidden within the persistent group: a concentric strategy (Figure 1). These animals had a short latency to the platform but swam in large loops keeping a certain distance from the sidewall. However, they did have a certain bias to the platform quadrant—the time in the platform quadrant was high as well as the number of crossings of the trained platform location. Figure 1 depicts representative samples of the three swim patterns. The concentric pattern is different from the one seen in animals with hippocampal lesions (Logue, Paylor, & Wehner, 1997; Morris, Garrud, Rawlins, & O’Keefe, 1982; Schenk & Morris, 1985) and glucocorticoid receptor deficient mice (Oitzl, de Kloet, Joels, Schmid, & Cole, 1997), which also swim in large circles at a certain distance from the wall. The other characteristic of this pattern, which was *not* seen in the present experiment, is short latencies, many crossings of all (possible) platform locations, but the time spent in the platform quadrant does not exceed chance level. The

flowcharts presented in Figure 1 demonstrate the decision steps for defining the three patterns. Thus, on the basis of cumulative distance to platform, combined with time in quadrant, three groups were discernible: spatial/persistent ($n = 12$), concentric ($n = 4$), and random ($n = 8$).

For the spatial/persistent mice, a significant negative correlation was found between cumulative distance to platform and time spent in platform quadrant ($r = -.717$, $p < .01$; i.e., low cumulative distance to platform and much time in quadrant; see also Figure 2). The more time animals spent in quadrant, the higher the number of crossings of the platform location ($r = .628$, $p < .05$). Remarkably, latency was not correlated with cumulative distance to platform. For the concentric group, no significant correlations were found. Random patterns revealed significant correlations between cumulative distance to platform and the actual length of the swim path in the platform quadrant ($r = .953$, $p < .001$) as well as time spent in platform quadrant ($r = -.838$, $p < .01$). Path length in the platform quadrant was also correlated with time spent in this quadrant ($r = -.870$, $p < .001$).

In addition to the correlation between cumulative distance to platform and time spent in platform quadrant,

Table 2
Latency to Find the Platform During Training Trials
(With Platform) for Animals With Spatial/Persistent,
Concentric, and Random Swim Patterns

Search Pattern	Day 1		Day 2				Day 3	
	Trials 1–3		Trials 4–7		Trials 8–11		Trials 12–15	
	<i>M</i>	<i>SE</i>	<i>M</i>	<i>SE</i>	<i>M</i>	<i>SE</i>	<i>M</i>	<i>SE</i>
Persistent	51.62	2.11	32.34	4.45	25.59	5.51	15.22	2.94
Concentric	54.48	5.52	37.70	5.07	19.50	1.44	18.70	11.64
Random	47.26	3.91	33.39	4.88	23.39	4.36	22.66	3.96

Note—Day 1 started with a free swim trial, followed by three training trials (intertrial interval [ITI] = 60 min). On Day 2, four trials were given with an ITI of 5 min, followed by another block of four trials after 60 min. On Day 3, a block of four trials was followed by Free Swim Trial 2 (ITI = 5 min). During the ITI, mice were placed under a red warming lamp. Performance improved over trials [$F(3,63) = 30.530$, $p < .001$], but was not significantly different between the groups [$F(2,21) = 0.061$, $p = .941$].

Figure 2 also illustrates the borders for the three definitions of swim patterns.

Table 2 depicts that total path length and velocity were similar for the three groups before training (FS 1), but increased significantly ($p < .05$) in animals showing a concentric pattern after training (FS 2). Training did not influence these two parameters in the spatial/persistent or the random group. Figure 3 presents the parameters latency to and crossings of platform positions, and Figure 4 shows cumulative distance to platform and time in quadrant as assessed for the two free swim trials. In FS 1, the groups showed a similar pattern of these four measures. However, as we detected in FS 2, training differentially affected the animals' swim pattern. Statistical values are shown in Table 3. All animals learned something (see Table 1 for latency during training trials), even the random group, which had shorter latencies to possible platform locations and more crossings in FS 2 than in FS 1. Apparently, these mice knew that there is a platform somewhere in the pool at a certain distance from the wall. There was no bias for a certain quadrant. The pattern of this group differed significantly from that of the spatial/persistent and concentric groups. The persistent mice did not differ from the concentric mice in terms of latency to and crossings of platform, but the time spent and cumulative distance to platform were significantly different. Only in the persistent group was cumulative distance to platform

Table 3
Statistics on the Swim Patterns Defined by
Cumulative Distance to Platform and Time in Platform
Quadrant, Crossings of and Latency to Platform
Location After Training in Free Swim Trial 2

Swim Pattern	Distance ^a	Time ^b	Latency ^c	Crossings ^d
Persistent vs. random	0.000*	0.000*	0.000*	0.001*
Random vs. concentric	0.038*	0.013*	0.014*	0.003*
Persistent vs. concentric	0.012*	0.000*	0.977	0.141

^a $F(2,23) = 29.269$, $p < .001$; ^b $F(2,23) = 60.483$, $p < .001$; ^c $F(2,23) = 10.686$, $p < .001$; ^d $F(2,23) = 28.438$, $p < .001$. *Significance by post hoc Tukey HSD.

significantly lower relative to the three other possible platform locations (Wilcoxon $p < .05$). In contrast, latency, time, and crossings showed significant effects for the concentric group as well.

DISCUSSION

The present study revealed that the rarely used parameter cumulative distance to platform in the analysis of water maze behavior allows a more refined distinction between swim patterns than traditional approaches. In a group of mice, preselected on the basis of swim path length before training, three different swim patterns were detected: spatial/persistent, concentric, and random. Criteria for differentiation were given on the basis of cumulative distance to platform and time in platform quadrant.

Swim patterns indicate the strategy that animals use to solve the task. Different patterns reflect different ways of information processing and, thus, underlying neuronal substrates. Therefore, a fine-tuned method of classifying behavior is required. When an animal moves toward a goal, such as the submerged platform in the water maze, it may do so using only distal cues or by performing a previously learned motor routine like swimming concentric circles some distance from the sidewall, or by combining these two methods. These methods can be efficient in a number of ways, but do not require spatial information processing. The spatial strategy requires the processing of complex stimuli configurations. Animals focus their search to the trained platform location. The concentric strategy, which was found to be hidden in the spatial/persistent group in the present study, could be detected only by the use of the cumulative distance to platform parameter. Otherwise, these animals would have been falsely classified as capable of spatial learning.

Cumulative distance to platform can be calculated only by an image analysis system, and we should use the advantages of automated, computerized techniques to quantify behavior. Of course, we are confronted with a large amount of data, but the flexibility of the systems makes it possible to extract the essentials in an efficient way.

Originally, cumulative distance to zone was presented as a measure to quantify and compare search patterns of old and young rats (Gallagher et al., 1993). Our study shows that combining the cumulative distance to platform with the time spent in platform quadrant measure further improved the analysis of swim patterns and thus allowed the identification of more subtle differences between swim strategies.

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