

The capacity to cope with environmental challenges is a fundamental characteristic of almost all living species. The adaptive response involves behavioral, physiological and neuroendocrine adjustments to the situation. The nature of the response may vary between individuals, but research has documented the existence of basically two different ways in coping with these challenges, an active and a passive coping style. In rodents there is evidence that individual differences in aggression reflect both alternating coping styles. In our laboratory, we found a correlation between the amount of the neuropeptide vasopressin and coping styles in wild house mice (*Mus musculus domesticus*) as well as in wild-type rats (*Rattus norvegicus*). This may well be one of the neurobiological characteristics of different coping styles (Everts, 1997).

#### **LEARNING, SWIMMING AND THE MORRIS WATER MAZE**

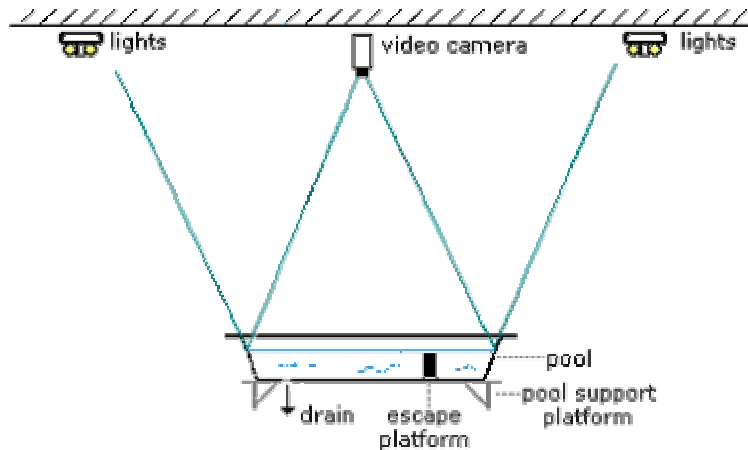
Since vasopressin in the brain is known for its role in learning and memory, we wondered whether the difference in vasopressin, as found in wild-type rats, also correlates with differences in learning behavior. One of the most commonly used tasks to study learning is the Morris water maze, introduced some 15 years ago by Richard Morris (1984). This task is not about learning to swim, since rats are born swimmers, but the animals have to learn to locate a platform placed stationary just below the surface of the water in a swimming pool. Because the platform is hidden, animals have to use extra-maze cues to navigate to the correct position.

#### **THE USE OF ETHOVISION**

Classically, Morris maze testing involved measuring the latency time to reach the platform and counting the number of visits to (imaginary) quadrants in the pool. But when studying coping styles, one wants to collect as much objective observational data as possible. For instance, the latency time to reach the platform may be the same in both styles, but the way in which the animal solves this task may be different; one type may swim slowly, orient itself and then go straight to the platform, whereas the other may swim around fast and find the platform by accident. By using EthoVision we were able to monitor several parameters, like latency to the platform, swimming distance, average speed, and time and distances in quadrants, giving an almost complete 'description' of the behavior of each animal. In addition, it provides reliable data for the statistical validation of the results.

#### **EXPERIMENTAL APPROACH**

Our Morris maze experience started with a drive to a local garden center to purchase a black, circular polyester pool (diameter: 140 cm, height: 35 cm). The pool was placed on a platform some 30 cm above the ground with a drain attached to the bottom of the pool (see figure 1). In the experimental setting we tried to stay as close as possible to the natural situation. Because rats are nocturnal animals, we reversed their day-light cycle and tested them under dim-light conditions. Since rats have impaired vision under red-light conditions, the normal tubes were replaced by red light tubes. A small 15 W light bulb was positioned near the observer and computer equipment to provide some dim light.



*Schematic overview of our Morris water maze setup. By using spatial orientation, animals have to find the escape platform which is submerged 2 cm below the water surface, invisible to the rats.*

### **PRACTICAL ISSUES**

Although the water maze itself is not deep, there may be some unexpected pitfalls when using EthoVision to monitor the animal's behavior in the water. In our settings, the experimenter and equipment were in the same room as the maze itself. The computer and video monitors were light-attenuated and computer beeps were omitted from EthoVision. This way animals were not conditioned by the computer beeps at the beginning or end of a trial. To avoid as much stress as possible, each animal was lowered very gently into the water (27 °C) and rubbed dry with a towel after each trial. A wireless mouse or infrared remote control is recommended, so that you can start observing as soon as the animal is in the water.

With any water maze, preventing the light from reflecting in the water is an important issue. Reflections cause disturbances in the background, thereby obstructing EthoVision to 'see' the rat in the pool. One solution is to provide indirect light from the ground surrounding the pool. However, if you want to prevent yourself from tripping over these lights, the following solution may be more practical. Position the red light tubes on the ceiling in such a way that they are well outside the 'reflection zone' of the camera hanging over the pool (see figure 1). There may be some occasional reflections because of the waves the animals make during their stay in the pool, but this is hardly a problem anymore.

During our experiments, we also tested wild-type rats which are light brown, agouty colored. As mentioned in many publications, it is possible to use milk powder to render the water opaque. Again, because of the red light, the contrast between the brown colored rat and the milky background is large enough for EthoVision to produce reliable results. The powder, say 400 g, is best dissolved in 2 liters of warm water first (this may take some time!) and then added to the water in the pool. Don't forget to clean the pool after each testing day; you don't want any fungus to swim around in your pool unasked for! Polypropylene pellets are often used as an inexpensive substitute for milk powder (Cain *et al.*, 1993). However, the cohesion between the separate pellets in water is high and the gap, which is formed behind the rat when swimming, does not close. This causes a trace, disturbing the background image EthoVision uses to detect the animal. Also, it is more difficult to remove (with a skimmer for instance) all the inevitable feces, floating around after several trials, from these synthetic pellets.

### **CONCLUDING REMARKS**

The experiments showed that wild-type rats were able to learn the task in 7 trials (2 trials per day). On the seventh trial they needed an average of 6 seconds to locate and climb on the hidden platform. Although we did not find any significant difference in the behavior of the wild-type rat, at least not in the Morris maze, the data collected with

EthoVision are of such quality that we can rely on the results. That the results did not match our expectations is also part of science.

#### **REFERENCES**

1. Morris, R.G.M. (1984). Development of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, **11**, 47-60.
2. Everts, H.G.J. (1997). *Individual Variation in Lateral Septal Vasopressin: Behavioural Consequences*. Ph.D. Thesis, University of Groningen.
3. Everts, H.G.J.; Koolhaas, J.M. (1999). Differential modulation of lateral septal vasopressin receptor blockade in spatial learning, social recognition, and anxiety-related behaviors in rats. *Behavioral Brain Research*, in press.

#### **CONTACT INFORMATION**

Henk Everts and Jaap Koolhaas. Department of Animal Physiology, University of Groningen, Haren, the Netherlands  
E-mail: j.m.koolhaas@biol.rug.nl