

An automated swim alley for small animals: II. Training and procedures

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Experiments were conducted to establish training procedures and testing conditions that produce uniform and reliable swimming performance of rats in an automated swim alley. Data are given on the effect of water temperature and of platform rest interval on swimming speeds. In this system, rats will swim intermittently for as long as 24 h for a distance of more than 7 km.

Training

When a rat is placed in a straight swim alley, it does not necessarily swim spontaneously from one end to the other. Rats are sufficiently at ease in water to float easily (McArdle & Montoye, 1966) and to dive voluntarily (Dagg & Windsor, 1972). The behavior is similar to that in a straight runway; predictable movement from one end to the other, and improvements in speed occur when a motivating consequence is added. Escape from cold water, as low as 15°C, has been used to induce rapid directional swimming (Bättig, 1961, 1963, 1969; Birren & Kay, 1958; Kay & Birren, 1958). With an increase to 20°C, there is a decrement in swimming speed (Bättig, 1961), and at 23°C there is some loss of directivity (Birren & Kay, 1958). Casarett (1973) found that not all animals swim reliably in a two-platform swim alley at 22°C to 24°C and these results have been confirmed in our laboratory (King, Hunt, Castro, & Phillips, 1974).

We have developed procedures, based on avoidance conditioning, for training animals to swim reliably in water at 23°C or 24°C. The performance of animals trained by these methods in our automated swim alley (King et al., 1974) has been studied to determine the effects of varying water temperature and platform rest interval. From this information, we have established suitable conditions for maintaining and measuring swimming performance over many hours.

METHODS AND RESULTS

Subjects

Male rats of the Wistar strain obtained from Hilltop Laboratories at 45 days of age were used in all experiments. They were caged singly in a continuously lighted room maintained at about 24°C, and were provided food and water ad lib.

Apparatus

Two automated swim alleys (King et al., 1974) side by side, about 75 cm apart, were used. The room containing the alleys was darkened except for illumination from the cue lights above the raised platforms.

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Procedures

Untrained animals frequently float or swim in a circle, most often immediately after the platform has been lowered from under them. Continuous directional swimming was readily established, however, by a dunking procedure. When any floating or circling occurred, we submerged the animals momentarily by hand, faced them in the proper direction, and then released them under water. Only by swimming toward the raised platform or by being on it could they avoid being dunked.

Once swimming was established, most animals readily climbed onto the illuminated platform at the end of each traverse. The few that repeatedly failed to do so were manually placed on the platform. If performance did not improve, the animal was carried back toward the dark end of the alley, then submerged and released. This was repeated as often as necessary to induce the animal to swim voluntarily into the lighted area and climb onto the platform.

An animal occasionally would leave the platform before the end of the rest period. When this happened during training, the session was interrupted while the rat swam to the unlighted end, turned, swam toward and climbed back on the lighted platform. If such early departures occurred repeatedly, the rat was dunked as it reached the unlighted end. Few trained animals displayed premature departures from a platform during test sessions, and those only very early in the session.

More than 90% of the animals were trained by these methods. Those that could not be trained were eliminated from further performance testing. Additional training was attempted with a few failures; reliable swimming could be obtained by prodding the animal with a probe, such as a pencil point, when it failed to swim. It would appear that with patience and persistence virtually all animals can be successfully trained to swim in the apparatus.

Originally, all training sessions began by placing the animal on a platform and starting the rest-interval timer. Subsequently, it was found that more vigorous and more reliable swimming was obtained by abruptly dropping the animal into the water at the center of the alley at the beginning of the first session. The rat, apparently excited by this procedure, would then swim vigorously until it reached the raised platform. The session started when the animal settled down on the platform. The same procedure was used in the second and third sessions; thereafter, the animal was placed directly on the platform.

Four training sessions were conducted either daily or on alternate days. The water temperature was maintained at 23°C and the platform rest period for most experiments was 30 sec. The first swimming session consisted of 12 to 20 traverses and ended when an animal swam appropriately, or when it appeared to have become weakened by excessive dunking. The second session consisted of 26 traverses, the third session was extended to 50 traverses, and the fourth to 100.

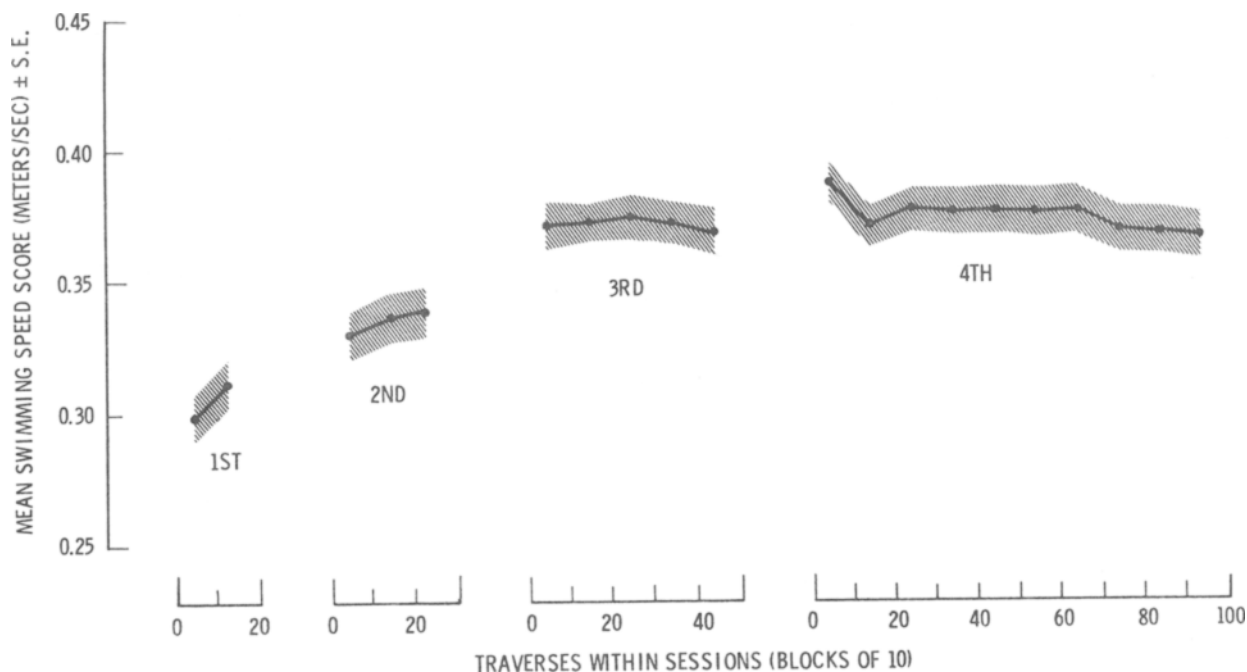


Figure 1. Effect of training on mean swimming-speed scores. Each individual swimming-speed score is the median speed in a block of 10 traverses, or of 6 traverses in the last block of the first and second sessions. The data points are the means and standard errors of the scores of 40 rats.

Results

Figure 1 illustrates swimming performance over the course of training. The 40 rats were 66 days of age and weighed 358 ± 2 g (mean \pm SE_M) at the start of training. The swimming-speed score of each animal was its median speed in each block of traverses (10 traverses per block in this case). The mean and standard error of these speed scores were computed for each block of traverses. A marked increase in swimming speed occurred after the first and second, but not the third, training sessions (see Figure 1). As training progressed, the animals learned to start promptly when the platform was lowered and to swim directly, without being dunked, to the other end of the alley. By the third session, they displayed a positive speed gradient as they approached the goal platform.

Effects of Platform Rest Interval

Methods

The rats were 82-90 days of age and weighed 474 ± 4 g when tested. A pretest followed the fourth training session by 4-8 days and preceded the test session by 1 day. Water temperature was raised from 23°C during training to 24°C for the pretest and test sessions. The platform rest time was 34 sec throughout the training and pretest sessions. Groups of four animals were assigned for testing with 34 or 17 sec of platform rest time, and three animals with 51 sec of rest (one animal lost in pretest due to equipment failure). The rats were assigned to the test groups so as to equilibrate speed scores from the fourth training session and rechecked for comparability on the basis of pretest scores. The pretest and test sessions each lasted 3 h.

Results

Swimming speed was highest for the 51-sec rest group, significantly lower for the 17-sec group, and intermediate for the 34-sec group (Figure 2). Because the platform rest periods accounted for a large portion of the total test period, the 51-sec group averaged only 158 ± 2 (mean \pm SE_M) traverses during 3 h. In the same period of time, the 34-sec group averaged 206 ± 5 , and the 17-sec group, 294 ± 5 . Deep colonic temperature was measured at the end of the 3-h test by means of a thermistor probe inserted rectally to a depth of 6 cm. The temperatures were $31.1 \pm .4$ °C for the 51-sec group, 28.8 ± 1.0 °C for the 34-sec group, and $28.5 \pm .4$ °C for the 17-sec animals.

Effects of Water Temperature

Methods

The 12 animals in this experiment were 57 to 61 days of age and weighed an average of 428 ± 6 g when tested. The pretest session occurred 4 to 6 days after the fourth training session and 1 day before the testing session. Both the pretest and test sessions were 3 h in duration. Throughout all sessions, the platform rest time was 30 sec. Water temperature was 23°C during training and 24°C in the pretest. Three groups of four animals each were tested at 24°C, 22°C, and 19°C, respectively. The animals were assigned to the test groups so as to equilibrate speed scores in the fourth training session and the comparability of groups was confirmed in the pretest.

Results

The animals tested at 24°C, the pretest temperature, exhibited a stable and uniform swimming performance

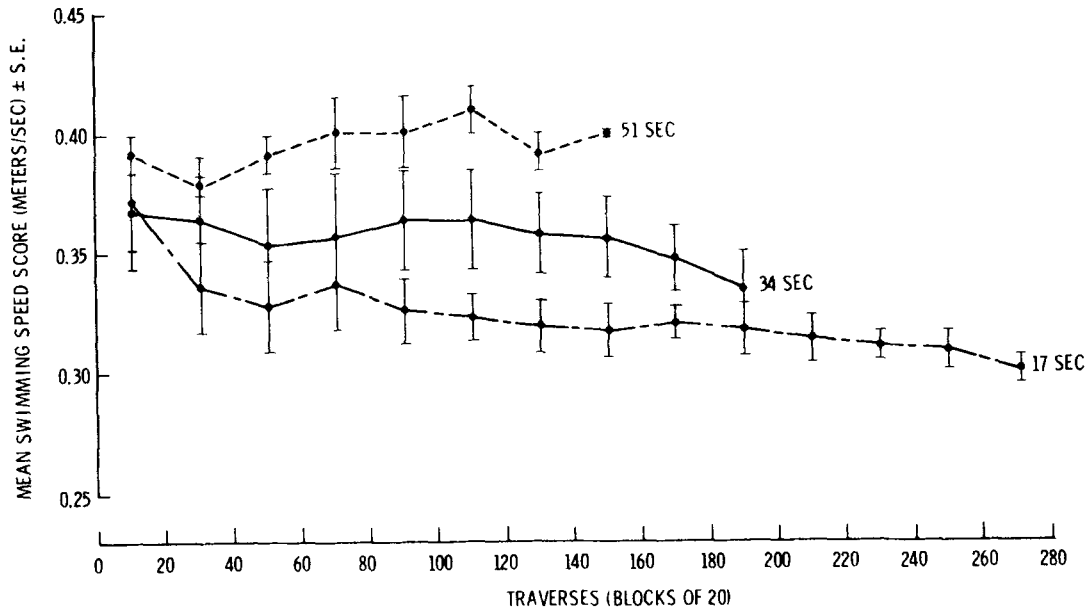


Figure 2. Effect of platform rest time on swimming-speed scores. The speed score here is the median speed in each block of 20 traverses. The number of animals in the 34- and 17-sec platform rest groups was four; in the 51-sec group, three.

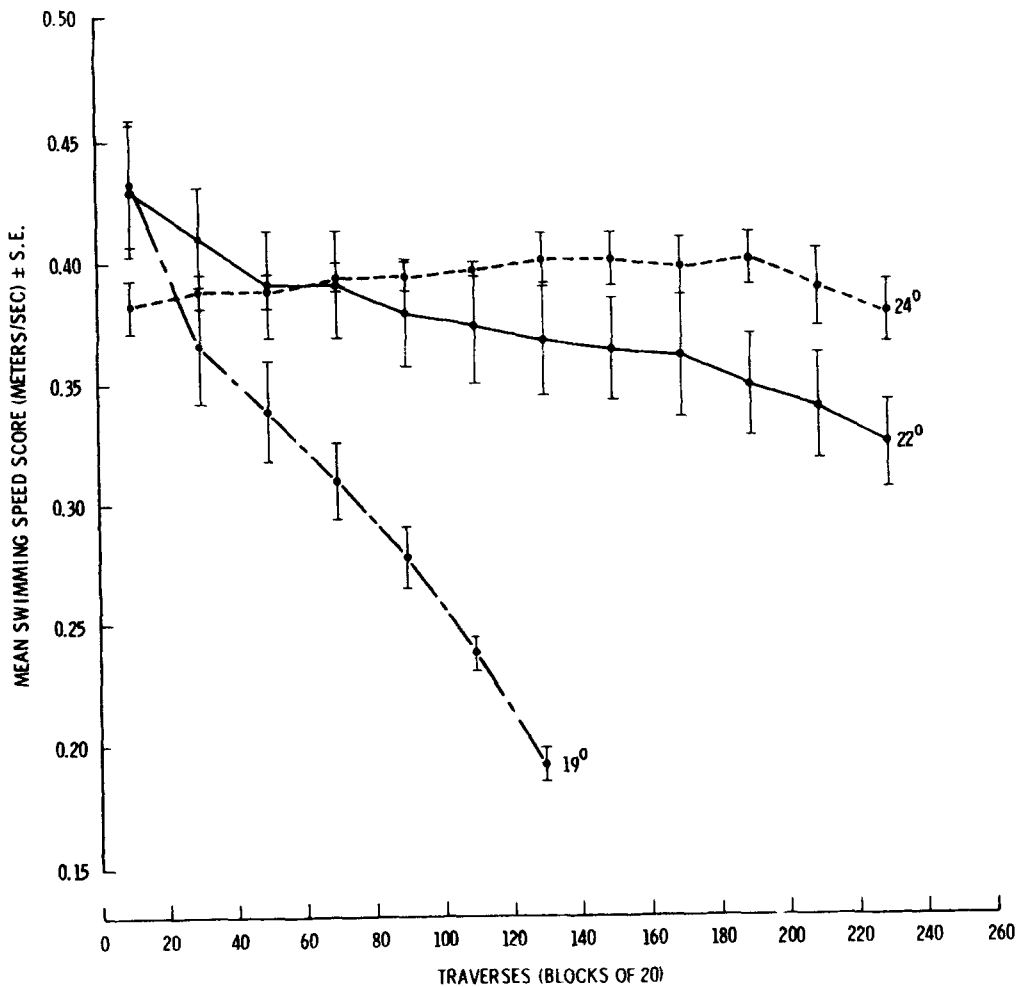


Figure 3. Effect of water temperature on swimming-speed scores. There were four rats in each group.

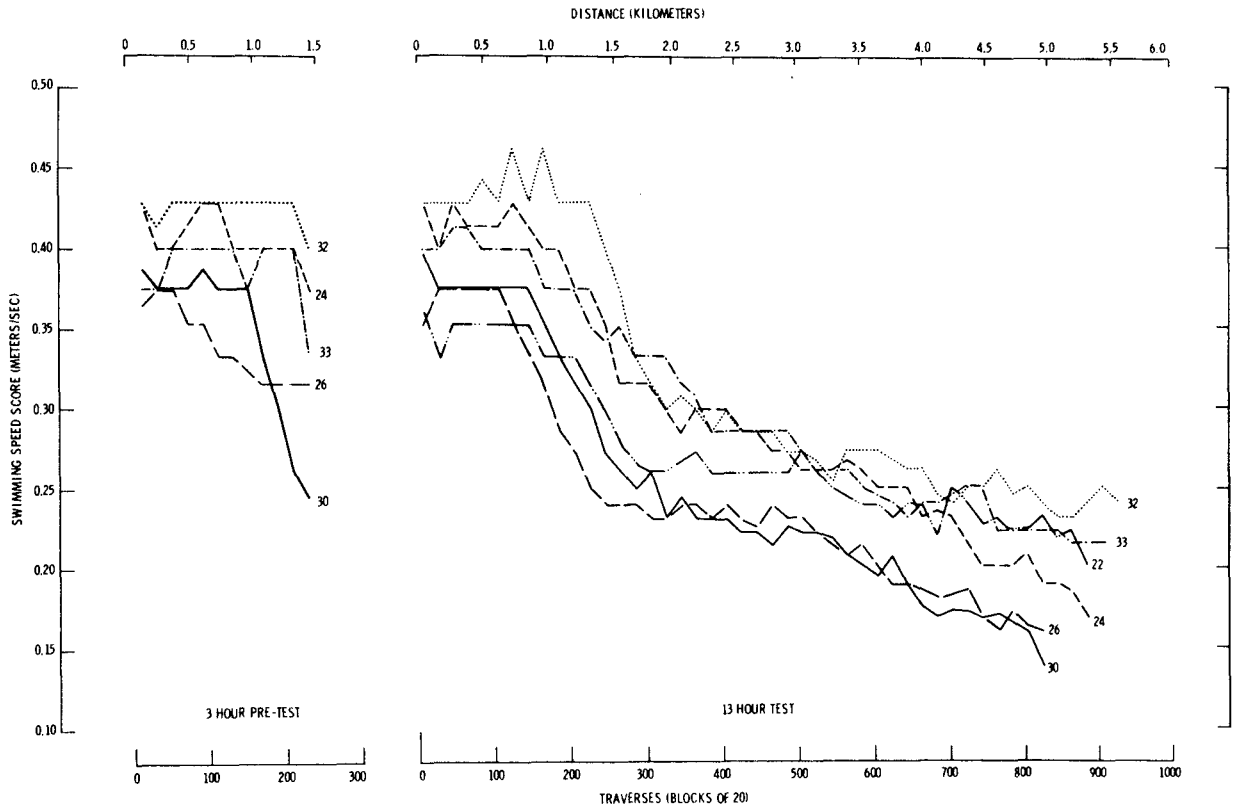


Figure 4. Individual swimming-speed scores in pretest and test sessions. The pretest scores for Rat 22 were not available because of a recorder malfunction.

throughout the test (Figure 3). These animals averaged 234 ± 2 traverses in the 3-h period. The average colonic temperature was $30.8^{\circ}\text{C} \pm .7^{\circ}\text{C}$ at the end of the session.

At the two lower temperatures, the animals initially swam faster than the 24°C group. For the 19°C group, however, the increased speed was quite transient, and progressive slowing was evident from the outset. None of these animals was able to complete the 3-h test. By about 140 traverses they were no longer swimming normally and appeared to be disoriented. They swam slowly, were canted to one side, and circled indiscriminately, although continuing to make swimming movements. Each animal was removed from the water when in this condition. Colonic temperature averaged $22.7 \pm .3^{\circ}\text{C}$ at the time of removal.

The animals that swam in 22°C water successfully completed the 3-h test but showed slowing throughout. By the end of the session, the scores of the 22°C group were well below those of the 24°C group. The 22°C animals averaged 233 ± 4 traverses; consequently, their total swimming distance was quite comparable to that of the 24°C animals. Colonic temperature at the time of removal was $27.8^{\circ}\text{C} \pm .2^{\circ}\text{C}$.

Long-Duration Testing

Swimming Performance

Figure 4 shows individual speed scores of control animals from one experiment in which a 13-h test session was employed. The animals were 70 to 90 days

of age and weighed 416 ± 4 g at the time of the 3-h pretest. The pretest followed the final training session by 3 to 7 days, and preceded the test session by 1 day. Platform rest time for both sessions was 30 sec, and the water temperature was 24°C .

The animals displayed no particular pattern in their swimming speeds during the pretest (Figure 4). In the test session, the swimming speeds of each animal either remained the same or increased slightly over a swimming distance of 600 m (100 traverses). The speeds decreased abruptly over the next 100 traverses and more slowly for the remainder of the test. An obvious correspondence between relative speed in the pretest and relative speed early in the test session is evident (see Figure 4).

A few animals were given an additional test session following a 5-h test and were able to swim for 24 h, attaining a total distance of more than 7 km. By the end of this period, although still swimming in a consistent, directional manner, they exhibited speed scores of less than .15 m/sec.

Body temperature

We did not monitor colonic temperatures continuously during a long test session, but final temperatures were measured immediately after all swim tests. Table 1 shows these data, obtained from a number of test or pretest sessions of varying length. The number of traverses completed during the session is also included. All animals had been similarly trained, swam in

Table 1
Colonic Temperatures of Rats Upon Completion
of Swimming Sessions

Duration of Swim	N	Traverses Completed	Colonic Temperature
0 min*	6	0	38.6 ± 0.2
30 min	4	47 ± 1	32.0 ± 0.7
1 h	4	86 ± 6	30.0 ± 0.5
1 h**	16	90 ± 2	30.2 ± 0.4
3 h	4	235 ± 2	30.8 ± 0.7
3 h**	24	234 ± 2	28.6 ± 0.2
3 h†	4	206 ± 5	28.8 ± 1.0
5 h	7	368 ± 5	27.6 ± 0.3
7 h	6	506 ± 4	27.5 ± 0.3
13 h*	6	883 ± 18	26.4 ± 0.2
24 h††	1	1245	26.0
24 h††	1	1584	26.3

Note—The water was 24° and the rest intervals in all but one case were 30 sec. (Values, where not singular, are means ± SE_M.)

*same group

**pretest session

†34-sec rest interval

††posttest session

24°C water, and had platform rest intervals of 30 or, in one case, 34 sec. Colonic temperature fell at a decelerating rate (see Table 1), stabilizing at about 2°C above the 24°C water temperature in the latter half of a 24-h period. Swimming performance did not simply parallel the temperature change. While the temperature initially fell rapidly, swimming speed (cf. Figure 4) was maintained for an hour or more. Subsequently, the swimming speed underwent an abrupt decline followed by a lower, steady loss during the period when the colonic temperature was seemingly stabilizing.

DISCUSSION

A uniform swimming performance by rats in the automated swim alley is critically dependent on prior training. The level of performance depends on water temperature and platform rest interval.

The training methods we employed entailed punishing the animals for any response other than swimming directly to the raised platform. With such training, virtually all animals swam consistently and reliably in long-duration tests; without it, many did not.

Animals trained with 34 sec of platform rest performed adequately over a 3-h test session with rest intervals as short as 17 sec. Speed of swimming was positively correlated with length of rest interval. This would be expected, since a longer rest interval should lead to greater recovery from exertion and also should be a more effective reinforcer for completing a traverse. Longer rest intervals also reduced the total amount of time available for swimming, so fewer traverses were completed within a given period of time. Shorter rest intervals produced slower swimming but a larger number of traverses in a given time. This is an example of a frequently encountered problem; maintaining maximum motivation in an exercise task does not necessarily provide the best conditions for testing endurance.

Swimming speed was initially greater with water temperatures below 24°C, consistent with reports from straight alley swimming (Bättig, 1961; Birren & Kay, 1958). Endurance, however, was decreased at the lower temperatures, consistent with reports from tank swimming (Dawson, Nadel, & Horvath, 1968; Tan, & Richter, 1954; Tucker & Horvath, 1971). Tan et al. (1954) reported that rats have greatest endurance in tank immersion when the water is at normal body temperature (about

37°C). Whether our trained animals could be kept swimming steadily in water above 24°C is an unexplored question.

The swimming performance of individual animals in water at 24°C changes in a predictable manner during long-duration sessions, and can be related in part both to motivational and endurance factors. Under our standard test conditions, the performance in the 13-h test, as shown in Figure 4, was typical. Note the sharp inflection in the swimming curves after a hundred or more traverses, followed by a slower but continuing decrement in swimming speed. Before the inflection, the high level of performance was most probably dependent on the motivation of the animals, induced by training experiences and test conditions. After the inflection, the deterioration in performance probably reflected a reduction in the animal's capacity, due to fatigue or to body temperature falling beyond a critical point or a combination of both factors. Swimming performance in water at 24°C is undoubtedly related to hypothermia, but not in any simple way. For example, colonic temperature fell most rapidly in the first half hour, and decreased about 8°C by the end of the first hour (Table 1), when the animals were still swimming strongly (Figure 4). As the animals' temperatures apparently stabilized in the latter half of a 24-h session, swimming speeds continued to decline.

Animals swimming in 19°C water provide evidence that some sort of critical body temperature operates at a value higher than the 18°C body temperature associated with lethargic or stuporous hypothermia (Folks, 1966, p. 128; Popovic, 1959). These animals became disoriented in 2 to 2.5 h when their colonic temperatures were between 22°C and 23°C. Animals swimming in 24°C water, however, were still swimming in an organized and directed fashion when their colonic temperatures were around 26°C, even though they had been swimming as long as 24 h.

The testing of swimming performance of trained rats in the automated swim alley provides both the means for testing endurance, available previously only with the tank-swimming method, and the means for continuously scoring performance, available previously only with the standard straight alley. Thus, both immediate and prolonged effects on swimming speed and endurance can be studied within a single experimental arrangement. This combination allows a fuller understanding of effects without extraneous variables being introduced by changes in experimental procedures.

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