INSTRUMENTATION & TECHNIQUES

An automated swim alley for small animals: I. Instrumentation

NANCY W. KING, EDWARD L. HUNT, RICHARD D. CASTRO, and RICHARD D. PHILLIPS Biology Department, Battelle. Pacific Northwest Laboratories, Richland, Washington 99352

A swim alley for testing the long-term performance capacity of rats is described. Rats swim back and forth in an alley, in temperature-controlled water, between platforms that are alternately and automatically raised and lowered. At the end of each traverse, an animal is allowed to rest on a raised platform for a period of time before the platform is lowered, which initiates the next traverse. Continuous measurement is made of speed of movement between platforms.

Swimming behavior is frequently used to test the fatigability and physical capacity of small animals. The behavior has been employed both as an independent and as a dependent variable. As an independent variable, swimming has been studied for its effects on metabolism (Dawson, Roemer, & Horvath, 1970; Lustinec, 1958; McArdle, 1967; Tucker & Horvath, 1971), cardiac output (Dawson, Nadel, & Horvath, 1968), and the lethality of ionizing radiation (Kimeldorf & Jones, 1951). As a dependent variable, swimming has been used to assess the response to hypothermia (Dawson, Nadel, & Horvath, 1968; Dawson, Roemer, & Horvath, 1970; Tan, Hanson, & Richter, 1954), hypoxia (Tucker & Horvath, 1971), ionizing radiation (Casarett, 1973; Kimeldorf, Jones, & Castanera, 1953), amphetamine (Bättig, 1963; Kay & Birren, 1958), nicotine (Bättig, 1968, 1969; Hrubes & Bättig, 1970), and age and sex (Birren & Kay, 1958).

Two types of swimming apparatus, the straight alley and the water tank, are commonly employed for measures of physical capacity; the tank has been used more frequently (Bättig, 1963, 1968; Dawson et al., 1968, 1970; Hrubes & Bättig, 1970; Kimeldorf & Jones, 1951; Kimeldorf et al., 1953; Lustinec, 1958; McArdle, 1967; McArdle & Montoye, 1966; Tan et al., 1954; Tucker & Horvath, 1971). Because of the various ways an animal can stay alive in a deep pool of water (e.g., by floating or treading water rather than by swimming) the performance is highly variable (McArdle & Montoye, 1966). Also, when animals are tested repeatedly to some criterion of "exhaustion," they learn to exhibit the behavior that leads to their removal, thus invalidating the test (Hrubes & Bättig, 1970).

A less frequently used apparatus is the straight alley.

Escape from cold water can provide the incentive for swimming rapidly to the goal end of the alley (Bättig, 1961, 1963, 1969; Birren & Kay, 1958; Kay & Birren, 1958). Performance can be scored in terms of swimming time or speed. The alley procedure has the disadvantage of being extremely time consuming and requires handling of the animal between trials. Consequently, fatigue testing over a long series of trials has rarely been attempted.

A variant on the straight alley has been devised by Casarett (1973) to study the effects of X-rays on the performance capability of rats. In her system, the animal swims back and forth the length of a 3.15-m alley between two platforms; at the end of each traverse, the animal is allowed to rest briefly before the platform is lowered and the next traverse started. The Casarett apparatus has the advantage that the swimming procedure is more standardized than with trial-to-trial handling of the animal by the experimenter. Swimming behavior is maintained in test sessions as long as 26 min in still water that varies from 22° C to 24° C.

We have used Casarett's basic idea in constructing an automated apparatus with improvements designed to enhance its versatility and make it more suitable for long-duration repetitive swimming. The improvements are: (1) an increase in alley length with movable end platforms that allow for use of shorter swimming distances when desired; (2) unobstructed top access to the platform and swimming areas to allow for handling during training and, if desired, attachment of connectors to the performing animal; (3) placement of cue lights above the platforms to provide directional information to the animal when in the dark (to minimize extra-alley cues); (4) continuous water replacement to flush out animal wastes during long-duration swimming sessions; and (5) accurate temperature regulation of replacement water to provide reliable temperature control, with close tolerances, of the immersion water.

This research was supported by the Office of Naval Research, Contract No. N00014-70-C-0332, with funds provided by the Naval Bureau of Medicine and Surgery.

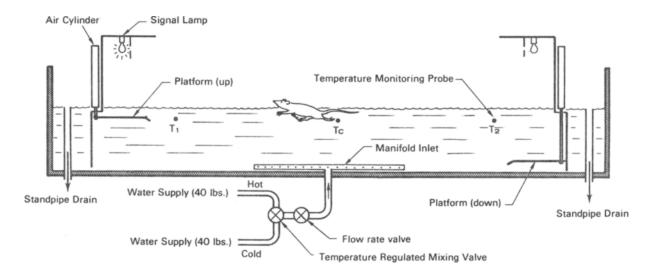


Figure 1. Schematic drawing of swim alley, not drawn to scale.

METHOD

Apparatus

Figure 1 is a schematic drawing of the automated swim alley. The alley is made of plywood lined with fiberglass, and is 30.5 cm wide and 38 cm high. The distance between the platforms was set at 6 m. The height of the standpipe drains determines the depth of the water, which we set at 27 cm. Water is supplied to the system through a mixing valve (Series 9600, Model PX3, ITT Lawler, Mount Vernon, N. Y.) which regulates the water temperature. The alley is filled through a large-capacity valve (not shown) which is then closed and a fixed rate of continuous replacement with fresh water is provided by a low-capacity flow-rate valve (Colorflow No. 8F-200 B, Manitrol Co., Cleveland, Ohio). The water enters the alley through an inlet manifold which distributes the water over a large area. Our input water contains air bubbles which distract the animals, so an air-venting chamber is used to remove bubbles from the water just before entry to the alley. This device (not shown) exhausts accumulated air automatically from the top of the chamber through a float-activated valve (Auto-Vent No. 7, Mist-O-Maid, Chicago, Illinois). Thermistor probes $(T_c, T_1, T_2, in Figure 1)$ attached through one wall of the alley (20 cm above its floor) are used to monitor temperature of the water. With a flow rate of about one replacement of water every 2 h and a temperature at the center (T_c) of 24°C, the temperature gradient from evaporative cooling to the end locations $(T_1 \text{ and } T_2)$ did not exceed .3°C when the room temperature was 22°C to 22.5°C. With a greater difference between room air and inlet water temperatures, the gradient would obviously increase. Signal lamps located above each platform are lighted above only the raised platform to provide a directional cue.

Solid state electronic and electromechanical equipment is used to switch, time, and record events in the alley. The sequence of events, controlled jointly by the rats' behavior and timers, is as follows: (1) The animal swims to and climbs upon the rest platform; this stops the swim-time clock and starts the rest-interval timer. (2) When the rest interval ends, the swim time is printed, and simultaneously the signal light above the animal turns off, the light at the far end turns on, and the far platform begins to rise. When the far platform is fully raised, the platform on which the animal is sitting lowers. (3) When the platform reaches its lowest point, the swim-time clock begins to run, and the animal begins a new traverse. A view of one end of the swim alley and the platform mechanisms is shown in Figure 2. The platform device is mounted in a large transparent (Lucite) canopy which confines the rat to the alley. This canopy is suspended from the sidewalls of the alley with a bottom clearance sufficient for water to circulate to the standpipe drain. The entire unit may be removed for servicing and can be located anywhere along the length of the alley to set shorter or longer swimming distances.

The platform is made of transparent plastic (6.35 mm thick) perforated with (15, 1-cm-diam) holes to reduce its impedence to movement through the water. The plastic is covered by a piece of stainless steel mesh which is bordered by a bead of silicone rubber to protect the animal from the sharp metal edges. The platform mechanism and canopy are constructed so as to eliminate any projections, edges, or holes that might provide the animal with a foothold when the platform is in the lowered position.

Each platform is attached to the plunger rod of a bidirectional air cylinder (Tom Thumb, AVB-P-D, $3/4 \times 8$, P9, PHD, Inc., Fort Wayne, Ind.) with a 20-cm throw. The plunger is operated by 27.6 newtons/cm² line pressure (40 p.s.i.g.) and is controlled remotely by a four-way electromechanical air valve (MAC 1801-7-76, MAC, Inc., Wixon, Mich.). Pressure applied through the bottom port of the cylinder raises the platform to 4 cm below the surface of the water and, when applied through the top port, lowers the platform 20 cm. When the platform reaches its upper limit, it operates the pressure switch (two-way valve, MAV-2, Clippard Instrument Laboratory, Inc., Cincinnati, Ohio) shown in Figure 2 to supply the air pressure for lowering the platform at the other end of the alley. In this manner, the movements of the two platforms are interlocked pneumatically. The throw and return speeds of the cylinders are adjustable.

The platform is hinged, as shown in Figure 2, to form a lever arm that raises an actuator pin and operates Switch A when an animal climbs onto the raised platform. Depression of the platform by the animal's weight is limited to approximately 5 mm by a stop (not shown) located under the platform. An adjustable leaf spring attached to the stop is used to set platform sensitivity so it will operate with the addition of a minimum weight (150 g).

Operation of Switch A at the end of a traverse stops the swim-time clock and starts the rest-time clock. At the end of the rest interval, the control circuit operates a digital recorder, printing the data for the prior traverse, and resets the system for

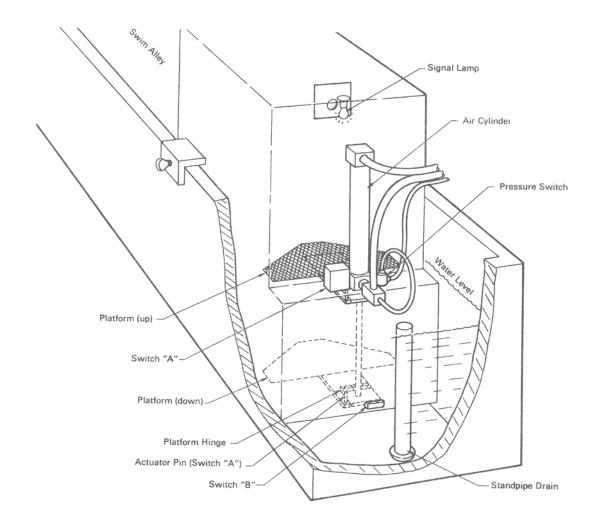


Figure 2. Cut-away section view of the platform mechanisms and one end of the swim alley.

the next traverse. Air pressure is switched to raise the other platform and, through the interlock system, to lower the platform from under the animal. When the rest platform is lowered and the animal is forced to begin the next traverse, a magnet attached to the underside of the platform operates a reed switch (VI-F, Reed Switch Development Co., Greenwich, Conn.), Switch B, and this starts the swim-time clock. The traverse ends with arrival of the rat at the raised platform. At the end of the rest period, the recording and reset operations recur.

OPERATIONAL TESTING AND DISCUSSION

Flow-rate and temperature of the water, noises and vibratory cues associated with operation of the platforms, and the speed of platform movement were equalized at the two ends of the alley. Two alleys were operated simultaneously for these experiments, and operational characteristics of both were made the same. During swimming sessions, the room was darkened and care was taken to ensure that the light from the signal lamps of one alley did not directly illuminate the other alley. Surfaces were painted or taped in such a way as to ensure that there were no distracting reflections.

Empirical tests were made of both alleys to determine if swimming speeds of rats were equal in both directions and both alleys. No systematic differences in swimming performance were observed. The automated swim-alley apparatus worked reliably and was found to be suitable for use in extended swimming sessions with trained rats (King, Hunt, Castro, and Phillips, 1974).

The swimming behavior of our rats in the automated swim alley was consistent with descriptions of this behavior for adults of this species (Dagg & Windsor, 1972). The animals propelled themselves primarily by use of the hind legs (moved alternately), especially after the first few traverses, and swam at an angle of about 25 deg to the surface, with the tail used as a rudder. Some rats readily floated and/or voluntarily dived underwater. Such behavior was not compatible with swimming directly to, climbing upon, and remaining on the raised platform. About 25%-50% of our animals would swim consistently in our initial tests of the apparatus. Casarett (1973) solved problems of performance stability by selecting only those animals that would swim consistently after two or three exposures to her apparatus. While it is possible that our losses were higher than hers, perhaps because we used a different strain of rat, we concluded that use of such a selection procedure was inappropriate and developed training procedures based on avoidance conditioning to obtain uniform performances (King, Hunt, Castro, & Phillips, 1974).

REFERENCES

- Bättig, K. Das Schwimmen von Ratten durch einen Wasserkanal. Methodische und pharmacologische Einflüsse auf Leistung und Ermüdung. Helvetica Physiologica Acta, 1961, 19, 384-398.
- Bättig, K. Die Wirkung von Training und Amphetamin auf Ausdauer und Geschwindigkeit der Schwimmleistung der Ratte. Psychopharmacologia, 1963, 4, 15-27.
- Bättig, K. Die Wirkung von Nikotin auf die Schwimmausdauer testgewöhnter Ratten. Zeitschrift Praeventimedizin, 1968, 13, 111-131.
- Bättig, K. The effect of nicotine on the swimming speed of pre-trained rats through a water alley. Psychopharmacologia, 1969, 15, 19-27.
- Birren, J. E., & Kay, H. Swimming speed of the albino rat: I. Age and sex differences. Journal of Gerontology, 1958, 13, 374-377.
- Casarett, A. P. Swim-tank measurement of radiation-induced behavioral incapacitation. Psychological Reports, 1973, 33, 731-736.
- Dagg, A. I., & Windsor, D. E. Swimming in northern terrestrial mammals. Canadian Journal of Zoology, 1972, 50, 117-130. Dawson, C. A., Nadel, E. R., & Horvath, S. M. Cardiac output of

the cold-stressed swimming rat. American Journal of Physiology, 1968, 214, 320-325.

- Dawson, C. A., Roemer, R. B., & Horvath, S. M. Body temperature and oxygen uptake in warm- and cold-adapted rats during swimming. Journal of Applied Physiology, 1970, 29, 150-154.
- Hrubes, V., & Bättig, K. Effect of inhaled cigarette smoke on swimming endurance in the rat. Archives of Environmental Health, 1970, 21, 20-24.
- Kay, H., & Birren, J. E. Swimming speed of the albino rat: II. Fatigue, practice, and drug effects on age and sex differences. Journal of Gerontology, 1958, 13, 378-385.
- Kimedorf, D. J., & Jones, D. C. The relationship of radiation dose to lethality among exercised animals exposed to roentgen rays. American Journal of Physiology, 1951, 167, 626-632.
- Kimeldorf, D. J., Jones, D. C., & Castanera, T. J. Effect of X-irradiation upon the performance of daily exhaustive exercise by the rat. American Journal of Physiology, 1953, 174, 331-335.
- King, N. W., Hunt, E. L., Castro, R. D., & Phillips, R. D. An automated swim alley for small animals: II. Training and procedures. Behavior Research Methods & Instrumentation. 1974, 6, 535-540.
- Lustnec, K. Uxygen consumption of rats during swimming. Physiologia Bohemoslovaca, 1958, 7, 208-215.
- McArdle, W. D. Metabolic stress of endurance swimming in the laboratory rat. Journal of Applied Physiology, 1967, 22, 50-54.
- McArdle, W. D., & Montoye, H. J. Reliability of exhaustive swimming in the laboratory rat. Journal of Applied Physiology, 1966, 21, 1431-1434.
- Tan, E. M., Hanson, M. E., & Richter, C. P. Swimming time of rats with relation to water temperature. Federation Proceedings, 1954, 13, 150-151 (Abstract).
- Tucker, A., & Horvath, S. M. Metabolic responses to normoxia and hypoxia in the altitude-adapted rat during swimming. Journal of Applied Physiology, 1971, 31, 760-765.

(Received for publication May 20, 1974; revision received August 24, 1974.)