Devillers[5] in *Salmo* and *Esox*, and Kastomarova[6] in *Misgurnus*.

Methods. Eggs of *Brachydanio rerio* are relieved from chorion by means of fine dissecting needles and brought into sterile, lid-covered glass vessels filled with Holtfreter's solution. The following methods of experimental yolk removal have proved appropiate.

1. Partial yolk removal by the aid of a pipette. Half of the yolk is removed by pipettation at the 64-cell stage and at blastula stages. The pipettes are made of thin glass tubes of an outer diameter of 3 mm and a wall-thickness of 0.5 mm. The diameter of the pipette opening is 50 µm. A smaller diameter proves inefficient when sucking up the relatively large yolk granules. The pipette, inserted into the vegetative pole of the egg, sucks up the yolk by capillary attraction. After the pipette has been carefully removed from the yolk, the wound is immediately closed by the yolk membrane, which prevents a further loss of yolk.

This method turned out to be unsuitable for total yolk removal, because a removal in this way is impossible without severely damaging the blastoderm. Moreover, young cleavage stages can only be treated with a great loss of material.

2. Total yolk removal by bursting. Total yolk removal is possible by utilizing the surface tension of the rearing solution. The egg, freed from chorion, is brought to the surface of the liquid by sucking it slightly into a Pasteur pipette. Once in contact with the surface of the liquid, the surface tension tears the yolk membrane, which makes the yolk float out. The blastodisk rounds off and sinks to the bottom of the glass.

This method represents a rather simple way of removing the yolk totally. But in contrast to method 1 the yolk membrane is also removed. That, however, does not prevent a further development. Unfortunately this method is only applicable to blastula stages, because the cells of the blastodisk of young cleavage stages are damaged by the surface tension of the liquid.

3. Yolk removal by cutting it off with a scalpel. Yolk removal by cutting it off with a scalpel can be carried out on blastula stages as well as on young stages. The yolk is cut off below the margin of the blastodisk. The relatively large wound closes quickly. As the blastodisk lies like a cap on the yolk sphere, often the total yolk cannot be removed by 1 cut, which results in the remaining of considerable yolk quantities in the egg. After a 2nd cut, however, the wound is often so large that the egg dies.

This method of yolk removal can certainly be carried out quickly, but it is accompanied by a considerable waste of material.

Results. If the yolk is totally removed before reaching the 8-cell stage, there arise germs with irregular groups of cells, which cannot be related to a certain germ layer. If the yolk is removed completely from the 8-cell stage, an irregularly shaped germ develops, which has a chorda, nerve-tissue and somites. Entodermal derivatives cannot be observed. The tissues do not show any topological order and are to be found among cells, which remained undifferentiated.

Germs, developing without yolk from the 64-cell stage on, grow for about 50 h without retardation into almost normal embryos. After 70 h a further development cannot be observed; 30–40 h later the germs dissolve.

If only half the yolk is removed from the 64-cell stage, for 150–200 h the egg develops without retardation into an embryo whose trunk-tail-region is bound dorsally. The head is well developed. But oedema is to be found in all tissues.

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Computer-compatible system for continuous volumetric measurement of water consumption[1]

W. Classen, J.R. Martin and K. Bättig

Institut für Verhaltenswissenschaft, Eidgenössische Technische Hochschule, Turnerstrasse 1, CH-8092 Zürich (Switzerland), 19 April 1979

Summary. An inexpensive drinkometer system is described that is compatible with most data acquisition systems and can be used for continuously monitoring the drinking pattern of laboratory animals. The change in electrical inductance resulting from the descent of a free-floating ferrite unit through a drinking tube, surrounded by a double coil, is converted to a signal proportional to the volume of fluid consumed.

A number of diverse techniques have been devised to continuously monitor water consumption. Typically, the number of licks has been measured[2,3]. However, such systems accurately indicate the volume of fluid consumed only when the lick volume remains relatively constant. Although it has been reported that the volume consumed per lick is invariable[4], other studies have noted considerable variation between animals[5]. Furthermore, alterations in the feeding schedule can affect lick volume[6] and the volume per lick has been found to vary by as much as 50–70% in individual rats from one day to another during the acquisition of schedule-induced polydipsia in this laboratory[7]. It is also questionable whether the constancy of lick parameters extends to brain-damaged or drug-treated animals exhibiting impaired motor coordination or stereotypic responses. In addition, those lickometer circuits that are

Drinking parameters for vagotomized and control rats

Parameter	Control (n = 4) Night	Day	Vagotomized (n = 6) Night	Day
12-h intake (ml)	30.7 ± 2.4	2.6 ± 0.7	8.8 ± 4.2**	8.5 ± 4.3*
Bout size	1.7 ± 0.4	1.4 ± 1.1	2.4 ± 1.7	2.0 ± 0.9
Number of bouts	18.7 ± 3.2	1.7 ± 0.6	5.2 ± 3.9**	4.0 ± 1.1**

Note: all values are mean ± 1 SD. ** $p < 0.01$, * $p < 0.05$; compared to control group with nondirectional Student t-test.

activated by electrical current flow from drinking spout to animal may provide added reinforcement[8] and have been reported to alter taste preference[9]. One different approach would involve the use of a weight-transducer system to periodically weight the water bottles, but this would be expensive. Another alternative technique has been described that involves the measurement of the changes in electrical resistance in a cobalt wire inserted into a tube containing the water[10]. The submerged portion has a lower temperature and thus a lower resistance. However, the potential effect of external temperature variations and corrosion of the submerged wire are disadvantages. The present paper describes an inexpensive drinkometer system that directly measures the volume of fluid in a drinking tube, but avoids the problems previously discussed. This is accomplished by monitoring changes in electrical inductance as a floating ferrite core descends through a coil with the water level. This system is readily interfaced with a microprocessor, a computer, or the output can be recorded with a polygraphic instrument.

Apparatus. Figure 1 presents a diagram of the drinkometer system and figure 2 shows the circuits used in its construction. The plexiglass drinking tube (21 mm inner diameter, 25 mm outer diameter, 50 cm long) contains a ferrite rod encased in a free-floating sealed plexiglass tube (12 mm o.d., 19 cm long). Surrounding the drinking tube is a primary coil (700 turns, 17 cm long) of insulated copper wire (0.25 mm diameter) and an identical secondary coil overlying the primary coil. Both coils are mounted on an outer plexiglass tube (25.5 mm i.d., 30 mm o.d., 19 cm long). This outer tube can be moved up or down after loosening a screw, so as to select the starting output value. The primary coil is supplied by a 20 kHz sine wave current of constant voltage produced by an audiofrequency generator (LAG 26, Leader Electronics, Japan). The induced ac current is converted to dc current. A capacitor further flattens the converted current which is then measured with a high resistance voltage meter. The drinkometer circuit thus provides a signal proportional to the volume of water consumed by an animal. Accuracy of approximately 0.2 ml is obtained for measurements obtained within the linear portion of the calibration curve. Use of several separate drinkometer circuits simultaneously necessitated the addition of a simple integrated operational amplifier to increase the power of the audiofrequency output. The secondary coil of each drinkometer is connected to a high resistance digital voltage meter via a multiplexer switch. In this laboratory, the output of each drinkometer circuit is monitored every 24 sec and the voltage meter output (BCD coded) transmitted to a PDP-11 computer for storage and subsequent analysis.

Experimental method. In evaluating the performance of the previously described drinkometer system, daily water intake of bilateral subdiaphragmatically vagotomized (n=6) and sham operated rats (n=4) was measured. Following recovery from surgery, the Wistar-derived RHA/Verh rats were adapted to the test cages where both powdered lab chow and tap water were continuously available. Water intake was then measured for a 24-h period, beginning at the start of the 12-h night portion of the light-dark cycle.

Results and discussion. The accompanying table shows the total water intake, bout size and number of bouts for the 2 groups during both day and night portions of the light-dark cycle. Individual drinking bouts were defined on the basis

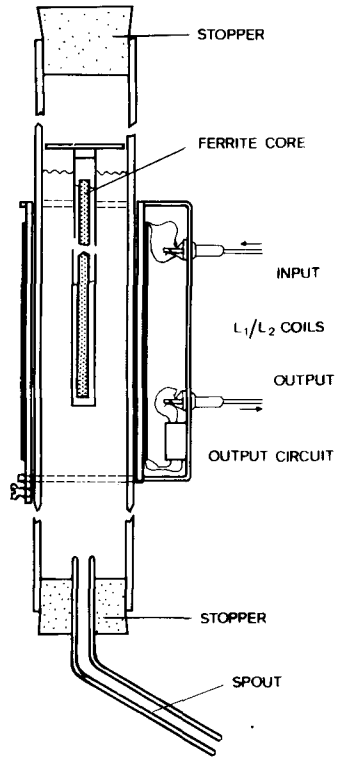

Fig. 1. Diagram of the drinkometer system.

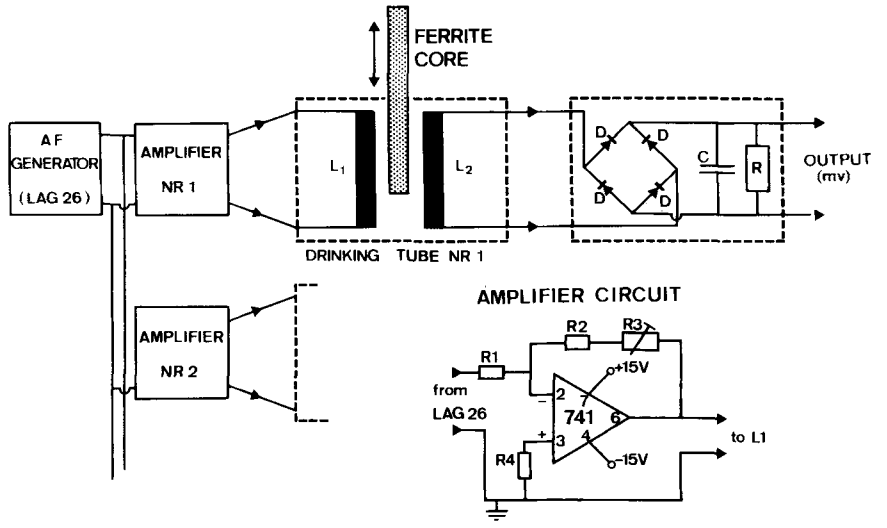

Fig. 2. Circuit diagram of the drinkometer system. L_1: primary coil, L_2: secondary coil, D: diode AA119, C: 1 µF capacitor, R: 680 Ω resistor, R_1: 12 kΩ resistor, R_2: 15 kΩ resistor, R_3: 0–10 kΩ variable resistor, R_4: 1 kΩ resistor, 741: IC 741 operational amplifier. All resistors are 0.25 W, 5%.

of an interbout interval of at least 5 min before a separate bout could occur. The vagotomized rats drank less than controls during the night, but more during the day. The vagotomized animals also had fewer drinking bouts during the night, but more than control rats during the day. Bout size was comparable for the 2 groups. On the basis of data collected in this laboratory, this drinkometer system has been found to provide a sensitive method of continuously monitoring drinking while avoiding the difficulties inherent in alternative systems.

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