Physiology & Behavior, Vol. 39, pp. 657–664. Copyright © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0031-9384/87 $3.00 + .00

BRIEF COMMUNICATION

Computer-Automated Method to Study Cardiac Conditioning to a Chemical Cue in Young Salmon[1]

PIERRE-PHILIPPE MORIN,*[2] JEAN-LOUIS VERRETTE,† JULIAN J. DODSON* AND FRANÇOIS Y. DORÉ‡

**Départements de Biologie et de †Génie Civil, Faculté des Sciences et de Génie*
Université Laval, Sainte-Foy, Québec, Canada, G1K 7P4
‡Ecole de Psychologie, Faculté des Sciences Sociales, Université Laval
Sainte-Foy, Québec, Canada, G1K 7P4

Received 19 September 1986

MORIN, P.-P., J.-L. VERRETTE, J. J. DODSON AND F. Y. DORÉ. *Computer-automated method to study cardiac conditioning to a chemical cue in young salmon.* PHYSIOL BEHAV 39(5) 657–664, 1987.—An apparatus was designed in which young Atlantic salmon (*Salmo salar*) are rapidly conditioned to reduce heart rate using a chemical-electric shock conditioning procedure. A chemical calibration system permitting efficient stimulus control is described as well as the electronic systems and computer software used to control all events of an experimental session and to quantify cardiac and chemical data. Efficient stimulus control and computer-automated recording techniques minimize inter-trial intervals and the time required for the measurement and analysis of cardiac responses. Data are presented showing that 15-month-old Atlantic salmon can be trained to reduce by 20% their heart rate to the synthetic chemical morpholine within 5 training trials whereas 10-month-old fish did not do so until 15 training trials.

Chemosensory perception Heart rate conditioning Atlantic salmon Computer control Chemical calibration system

SINCE the pioneering work of Malyukina and Marusov [8], only a small number of studies have used cardiac conditioning to investigate chemosensory perception in fish and few technical innovations have been reported that enhance the efficiency of the conditioning technique or the quality of chemical stimulation and cardiac data quantification. Investigators evaluate the dilution rate and the distribution pattern of a chemical substance, used as a conditional stimulus, by visually assessing the dispersion of dye from a distant source to the fish [2, 3, 7] or by measuring absorbance of dyed aliquots sampled near the fish head with a spectrophotometer [4–6]. Although calibration with a spectrophotometer is an obvious technical improvement over the visual dispersion-time method, no attempts have been made to provide a more precise and direct measurement of chemical dilution and distribution in a closed-circuit water system. In addition, slow flushing rate (between experimental trials) of the conditional stimulus as well as poor knowledge about its distribution pattern in test chambers have prevented investigators from using short inter-trial intervals (less than 3 min) which may be advantageous in certain experimental designs. Long inter-trial intervals ranging from 5 min [4, 5, 7] to 20 min [6] have been reported. The only known study to have used a shorter inter-trial interval (2.5 to 3.5 min) was that of Sandoval [12].

Before the first heart rate data are available for analysis in the context of an experiment, time-consuming effort is traditionally spent on surgically implanting electrodes and laboriously measuring inter-beat intervals recorded on pen physiographs [4–6, 12, 14]. The only known study on chemoreception using computer technology for cardiac data collection and treatment is that of Hirsch [3]. However, this investigator was unable to record reliable cardiac signals on computer without inserting electrodes subcutaneously in the cardiac region.

We have developed computer software and hardware as

[1]Contribution to the program of GIROQ (Groupe Interuniversitaire de Recherches Océanographiques du Québec).

[2]Requests for reprints should be addressed to Pierre-Philippe Morin, c/o Julian J. Dodson, Département de Biologie, Université Laval, Sainte-Foy, Québec, Canada, G1K 7P4.

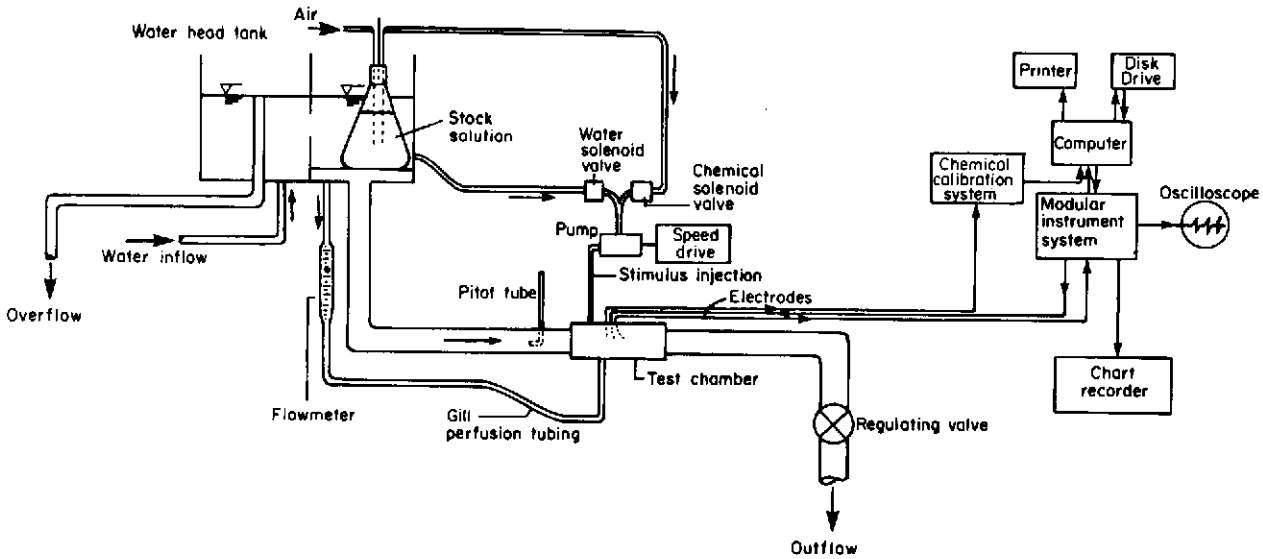

FIG. 1. Experimental layout.

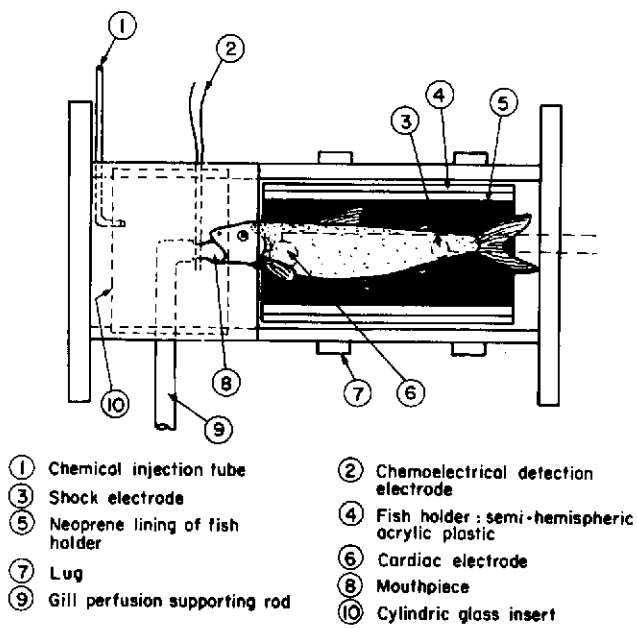

FIG. 2. View of test chamber with accessories.

well as electronic systems and devices to study chemosensory perception and learning in young salmon using a cardiac conditioning procedure. Our initial goal was to minimize inter-trial intervals and the time required for cardiac response measurement and analysis. In this paper, we describe (1) an apparatus with several components allowing stimulus control and data analysis; (2) a calibration test using a saline solution; and (3) two experiments on cardiac conditioning to a chemical substance.

APPARATUS

Figure 1 illustrates the experimental layout of apparatus. It is composed of a test chamber, a modular instrument system and a chemical calibration system all connected to a microcomputer. The test chamber receives three different water inputs. The main input is dechlorinated water drawn from the head tank (surface level 0.6 m above the test chamber) and flowing by gravity to a straight horizontal 1.5 m long PVC pipe (internal diameter 49 mm), which was long enough to reduce turbulence and to produce an established flow before reaching the test chamber. Flowing water is evacuated through a straight horizontal 0.5 m long PVC outlet pipe. Water velocity is measured by a Pitot tube just before the test chamber. It is kept constant (20.47 mm/sec) during an experimental session by a regulating valve situated at the extremity of the outlet pipe.

The second input to the test chamber delivers either water pumped from the head tank or a chemical substance used as a stimulus pumped from a 4-l erlenmeyer flask containing aerated stock solution. The erlenmeyer flask was placed in the head tank such that the stock solution and water in the head tank were maintained at the same temperature. A small silicone tube (internal diameter 3.175 mm) is connected to the head tank and a second tube is affixed to the stimulus stock solution to be tested. Each tube is attached to a pneumatic solenoid valve (Aro Inc., part number 5424-39-02 and 5427-39-02). They converge to a main tube allowing the injection of small quantities of either water or stock solution into the test chamber by a peristaltic pump (Cole Parmer Inc., part number 7553-00) equipped with a standard head (part number 7016-21) and controlled by a Masterflex variable speed drive (6 to 600 rpm). The third input to the test chamber is water running from the head tank through a medium-size silicone tube (internal diameter 6.477 mm) into the test chamber. It is used for perfusing the gills of fish. A small two-way valve and a Dumont flow meter (Cole-Parmer Inc., part number C3230-14) controls gill perfusion (300 ml/min).

The test chamber is equipped with stimulating electrodes for delivering conditioning shock and cardiac monitoring electrodes connected to a modular instrument system as well as electrodes used for chemoelectrical detection which are connected to a chemical calibration system. Both systems

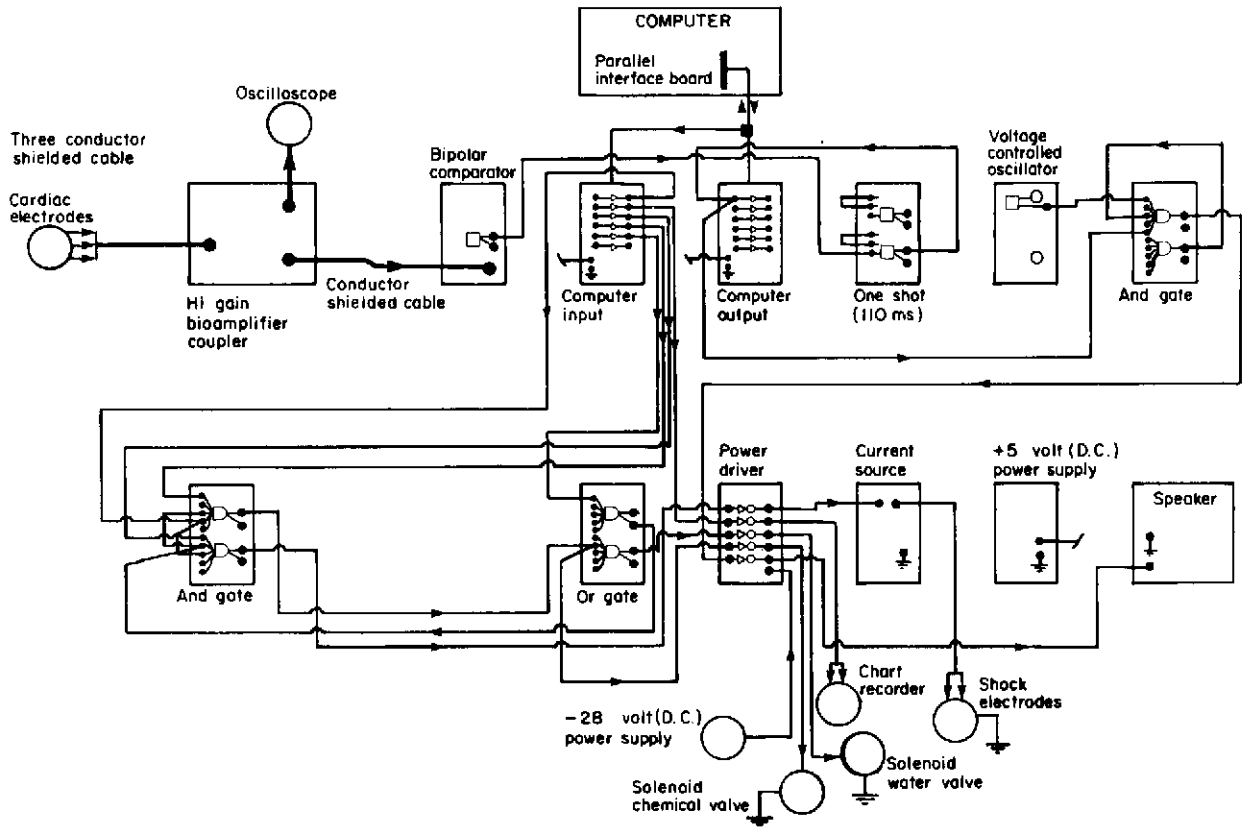

FIG. 3. Block diagram showing connections between modules of the Modular Instrument System. Arrows indicate direction of current, input source and output destination.

are used simultaneously although they are separately connected to the computer. A black box surrounds the test chamber eliminating any external visual disturbance which may stimulate the fish.

Test Chamber

The 205 mm long cylindrical test chamber is made of clear acrylic plastic (Fig. 2). It has an 8 mm wall with an inside diameter of 44 mm. The head section of the test chamber houses a cylindric glass insert which is pierced for the entry of a glass tube used for gill perfusion as well as an entry for a pair of stainless steel chemoelectrical detection electrodes. The head section also contains a glass tube for chemical injection. Glass was used instead of plastic to allow free flowing of the stimulus molecules which could otherwise stick to walls. Inside diameters of the chemical injection and gill perfusion tubes are 1.5 mm and 4 mm respectively. The distance between the injection tube and the fish snout is 40 mm. Gill perfusion tubes (Ace Glass Inc., part number 7585-02 and 7585-04) are a two-piece system including a removable inner mouthpiece and an outer supporting rod. Several mouthpieces of different diameters (7 to 18 mm) were blown in a workshop. They were made to fit jaws of different-size fish. The two chemoelectrical electrodes are made from 38 mm (25G) hypodermic needles with an insulating coat of varnish applied at one end, leaving an uncoated 6 mm tip at the fish's nostril level. The distance between these electrodes is 3 mm while the fish's snout is located 5 mm away.

The end section of the test chamber is used to hold the fish in position for adequate physiological monitoring and stimulation. It is composed of a rubber-sealed wall partition which can be removed for access to the chamber. The end section is also equipped with an immobilizing device adjustable from the outside by means of four teflon screws. This fish holder is made of acrylic plastic on which is glued 3 mm of neoprene (nylon on both sides). Small 6 mm and 10 mm gold-plated disc electrodes used for monitoring heart rate or for electrically stimulating the fish are embedded in small depressions made in the neoprene. These electrodes are placed in pairs on each side of the fish while the ground wire lies loose in water beyond the test chamber. When the test chamber is to be used for an experimental session, high vacuum grease (Corning Inc.) is first applied on each rubber seal. The wall-partition closes the chamber and two hose clamps seal it off. Finally, the test chamber is inserted in the main water line and secured with stainless steel bolts at both flanged ends.

Modular Instrument System

The modular instrument system (MIS) is a commercial electronic system (Coulbourn Inc.) that can be used for a variety of behavioral and physiological experiments. The MIS is composed of electronic modules connected to a solid

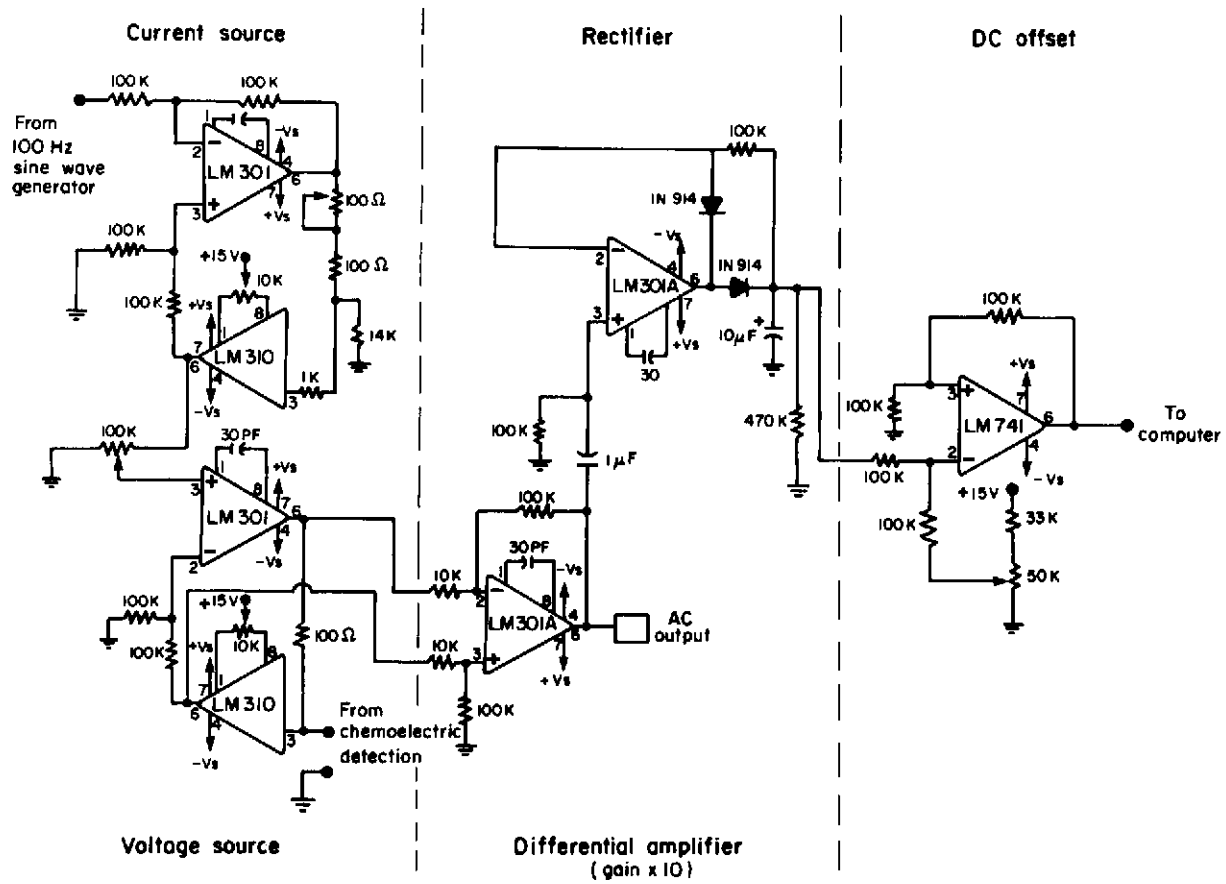

FIG. 4. Circuit diagram of the conductivity-monitoring device.

state power supply, all mounted on an equipment rack. Module functions and input/output signals are powered with +12 V, −12 V and −28 V DC. Connections between electronic modules of the MIS are shown in Fig. 3. The MIS is used for control functions and for data acquisition.

As a control system, the HI GAIN BIOAMPLIFIER/COUPLER module amplifies and filters cardiac analog signals which are converted to digital signals by a coupled BIPOLAR COMPARATOR module. Digital signals, the pulse duration of which is set at 100 msec by a ONE SHOT module v are sent to the microcomputer by a COMPUTER OUTPUT module. These signals initiate and drive a clock mechanism embedded in the computer to allow sequence programming. A COMPUTER INPUT module receives digital signals from the computer and sends them towards relays (AND GATE and OR GATE modules) or directly to a POWER DRIVER module to (a) produce electrical shocks; (b) activate solenoid valves; (c) send cardiac signals to a chart recorder; and (d) generate a sound converted cardiac signal.

Data acquisition using the MIS is quite simple. It occurs through the HI GAIN BIOAMPLIFIER/COUPLER, BIPOLAR COMPARATOR, ONE SHOT and COMPUTER OUTPUT modules before cardiac signals are digitized and processed in the computer software. Non-commercial modules are also included in the MIS. Fish receive shocks of variable intensities (0 to 100 mA) from a SHOCK STIMULATOR module while the cardiac rhythm can be heard from a SPEAKER module.

From the MIS, a ribbon cable is attached to a 16-pin socket of a parallel interface board (John Bell Engineering Inc., board number 79-295). The parallel board is plugged into slot number 2 of the microcomputer and was used to handle computer input as an interval timer counting heart beats or to control the MIS by sending output signals to the peripheral devices.

An external on/off switch controls a real time clock-interrupt language-machine program. An experimental session may be initiated or stopped with the interrupt switch. One wire from the switch was soldered to the nonmaskable interrupt line (NMI) located at the back of the parallel interface board while another wire was soldered to a small filled hole located on the main board of the computer. This tab is a 60 Hz vertical retrace pulse for the video circuitry. The NMI line prevents any access to the disk drive and the printer during an experimental session.

Computer software used for the MIS are three Applesoft BASIC programs (SAUMON, READSAUMON and TREATSAUMON), one of them (SAUMON) being completed with clock-interrupt driven routines written in 6502 assembly language. Besides controlling sequential events of an experimental session, SAUMON reads digital-converted heart rate signals and store data in memory while READSAUMON AND TREATSAUMON synthesize results before statistical analyses. Computer programs were developed for an Apple II microcomputer with 48K RAM memory and at least one disk drive (DOS 3.3).

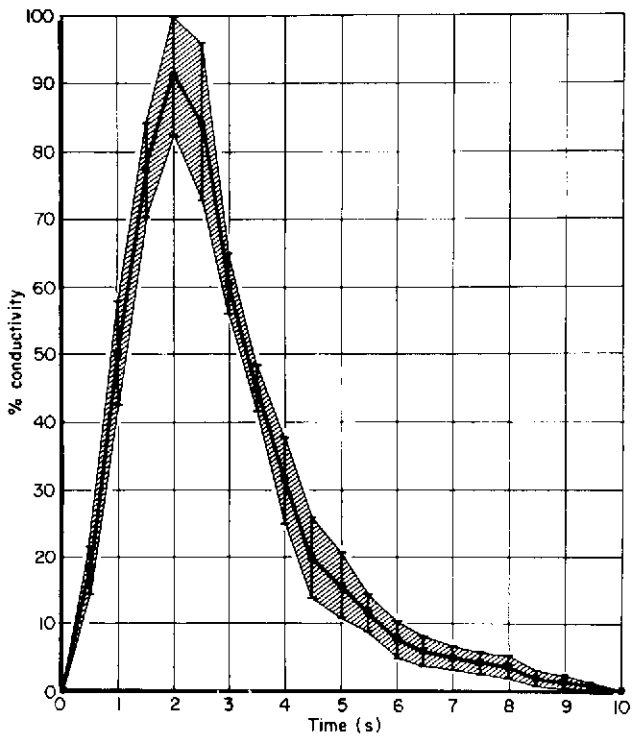

FIG. 5. Mean percentage (0.5 second interval) of maximum conductivity and standard deviations calculated from twenty calibration curves.

Chemical Calibration System

The chemical calibration system (CCS) picks up the electrical conductivity of a saline solution detected by a pair of stainless steel electrodes and sends an output signal to the computer where it is digitized. This electronic system is composed of an independent power supply (±15 V AC), a conductivity-monitoring device and a low frequency sine wave generator. The heart of the CCS is the conductivity-monitoring device (Fig. 4). A sine wave (100 Hz, 600 ohms) is generated and its amplitude is proportional to the electric potential generated when water conductivity is picked up by the chemoelectrical detection electrodes. As it flows through the circuitry, the potential acts as an AC signal which is amplified, transformed into a DC signal and finally adjusted to measure small variations in water conductivity.

The CCS output is wired to an analog-to-digital converter (John Bell Engineering Inc., interface board number 81-132) plugged in slot number 5 of the microcomputer. Wires are connected to pin number 1 (channel 1) and to pin number 15 (reference/ground) in the dual line socket of the analog-digital interface board. The input voltage range of the converter is from 0 to 5.12 V and the output conversion prints digit numbers ranging from 0 to 255 representing 20 mV increments. Careful voltage measurement was done before each chemical calibration test. A signal exceeding the 5.12 V level and sent to the analog-to-digital converter will permanently damage the interface board. Computer software for the CCS (CALCHIM and READCHIM) are used for reading channels of the analog-to-digital converter and for storing digitized data in memory.

CALIBRATION TEST WITH A SALINE SOLUTION

Success in inducing rapid and consistent heart rate conditioning to a chemical stimulus which dilutes through a mass of water relies mainly on well-designed stimulus control and monitoring techniques. The efficiency of the calibration chemical system (CCS) was verified over a calibration session with a live curarized fish in the test chamber. A 0.1% saline solution was injected into the chamber to study its dilution and dispersion pattern.

Preliminary adjustments of the pump speed and of the CCS were done to generate digit numbers, ranging from 0 to 5, in the computer memory. Percentages were eventually attributed to each digit so that results could be expressed in terms of percent of maximum conductivity detected by the chemoelectrical detection electrodes. These values were used to calculate an average curve from 20 individual calibration curves.

A trial begins with a 5 sec opening of the chemical valve (closing of the water valve) which feeds 7.3 ml of the saline solution towards the injection tube of the test chamber. The chemoelectrical detection electrodes detect the saline solution 8 sec following closure of the chemical valve. Chemical conductivity of the solution is recorded on computer and on the chart recorder for 20 sec following its initial detection. A fixed 60 sec inter-trial interval is used over a 20 trial session and the pump speed (109.4 rpm) is constant. After the 20 trial session, electric conductivity is directly measured from the 0.1% stock solution.

Results in Fig. 5 show an average calibration curve (means and standard deviations) calculated from 20 trials which indicates little variation between trials. When first detected, the saline solution quickly reaches its maximum concentration within roughly a two-second period. Then, the concentration decreases gradually towards a non-detectable level approximately 10 sec following initial detection of the chemical signal. In addition, a comparison of the maximum conductivity measured during calibration with that of the 0.1% stock solution revealed that the chemical stimulus is diluted by a factor of 15 when it reaches the chemoelectrical detection electrodes.

The time interval between the conditional and unconditional stimuli influences the rate at which experimental subjects acquire a conditioned response. Delayed conditioning, characterized by the presence of the conditional stimulus persisting until the onset of the unconditional stimulus, is the most efficient type of temporal relationship inducing rapid acquisition of a learned response [1]. In addition, a short delay between stimuli produces a more rapid acquisition of a learned response than does a long delay [9]. The control of the chemical stimulus (conditional stimulus) provided by the chemical calibration system permitted us to establish a short-interval delayed conditioning procedure that produced rapid and consistent learning over time. In addition, knowledge of the flushing rate of the chemical stimulus permitted us to minimize inter-trial intervals.

Our results on the dispersion pattern of a saline solution injected into a test chamber indicate that the chemical calibration curve is characterized by a rapid dispersion rate with little variation between trials. We assume that any chemical substance injected at a low concentration in the test chamber disperses in the same way as the 0.1% saline solution used for calibration. To insure that chemical stimuli were efficiently controlled in different conditioning situations, the chemical calibration procedure using a 0.1% saline solution

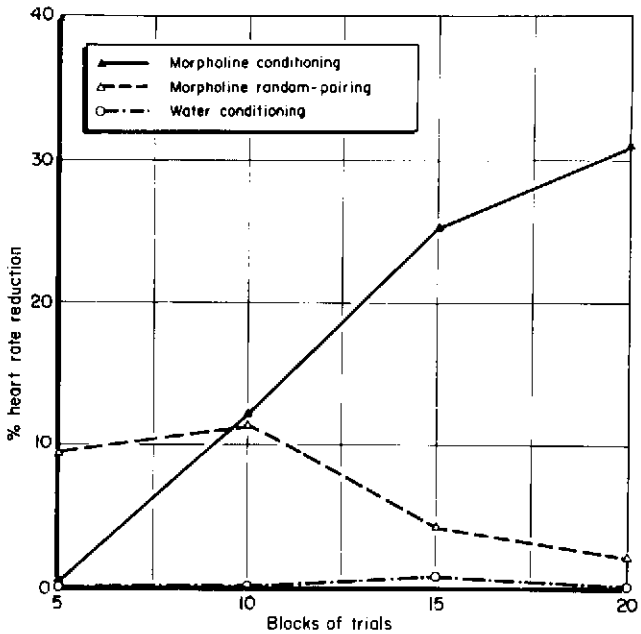

FIG. 6. Mean percentage in heart rate reduction per block of five trials in three groups of three 10-month-old Atlantic salmon tested under different conditioning situations.

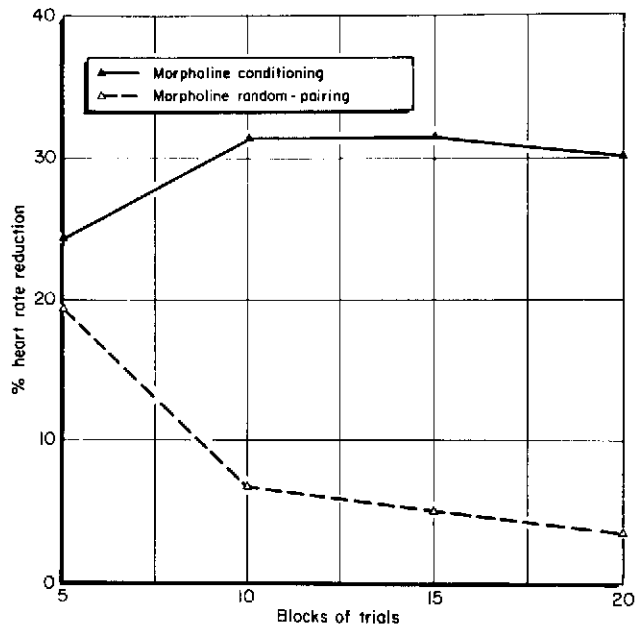

FIG. 7. Mean percentage in heart rate reduction per block of five trials in three groups of six 15-month-old Atlantic salmon tested under different conditioning situations.

was done weekly as well as before each experiment.

Best results for cardiac conditioning were obtained when the shock stimulus was initiated 8 sec after the initial detection of the chemical morpholine as its concentration declined to 3% (1.14×10^{-4} M) of its maximum value. This low concentration level is above the sensory threshold of one species of salmon, *Oncorhynchus kisutch*, (2.1×10^{-8} M) in heart rate conditioning experiments [3]. Preliminary experiments confirmed that a conditioned response was more difficult to obtain if the shock was applied before or after the 8-sec interval following chemodetection.

EXPERIMENT 1

In experiment 1, the rate of acquisition of a cardiac conditioning response to the synthetic chemical morpholine was examined in 10-month-old fish.

Method

Nine 10-month-old Atlantic salmon (10.1 to 11.2 cm, total length) were acquired from the Tadoussac salmon hatchery (Government of Quebec, Canada). The fish were housed in a closed-circuit 300 l aquarium one month prior to experimentation. Water (10 to 11 degrees C) was renewed weekly and fish were fed daily with a mixture of shrimps, crabmeat and scallops. The animals were maintained on an 8 light-16 dark photoperiod.

Each fish was anesthetized with 1% methyl pentynol for 2 to 4 min until gill movement ceased. The fish was quickly measured, weighed and injected in the dorsal musculature with d-tubocurarine chloride (Abbot Laboratories, Montreal, Canada), 0.002 mg/g body weight. Curare paralysis occurred after recovery from anesthesia and approximately 15 min after injection. The fish was placed in the test chamber with cardiac electrodes positioned under pectoral fins and with the mouthpiece adjusted for adequate gill perfusion.

Animals were allowed to habituate for a period of 35 min in the test chamber before learning trials commenced. A trial begins by injecting into the test chamber 7.3 ml of 5.7×10^{-2} M morpholine with a 5 sec opening of the chemical valve. Applying the dilution factor calculated during the chemical calibration test, we estimate that fish are exposed to a maximum concentration of 3.8×10^{-3} M. Sixteen sec after the closure of the chemical valve (see data on chemical calibration: 8 sec before initial detection of the chemical stimulus + 8 sec during detection), fish received a 2 sec mild electric shock stimulus. Intensities of each shock stimulation (28 to 46 mA) were adjusted during the first trials to reduce heart rate to an average of 10 beats per min.

Subjects were equally divided into three groups. Experimental subjects from the morpholine-conditioning group were presented with morpholine followed by a shock stimulus while the control water-conditioning group was presented with distilled water followed by a shock. A third group of subjects was used to control for pseudoconditioning. This random-pairing group was characterized by a complete randomized stimulus presentation [11]. Shock stimulation randomly preceded or followed chemical stimulation and the time interval separating both stimuli was also randomized. All fish were tested over 20 trials and the inter-trial interval (45 to 135 sec) was randomized to prevent inadvertent conditioning to temporal events.

For each training trial, inter-beat intervals (IBI) were recorded during an initial 8 sec period following closure of the chemical valve and before the chemical reached the fish (IBI before) and for the following 8-sec period during which the chemical was present in the vicinity of the fish nares (IBI

during). Percentage of cardiac deceleration was evaluated by comparing the largest IBI before with the largest IBI during chemical stimulation, according to the following formula:

$$100 - \frac{\text{IBI during} \times 100}{\text{IBI before}}$$

Results

Subjects from the morpholine-conditioning group decreased their heart rate over a 20-trial session whereas subjects from the random-pairing or the water-conditioning group did not (Fig. 6). Heart rate reduction between successive blocks of five trials was most often greater than 11% for the morpholine-conditioning group (block 1 to block 2: 11.7% and block 2 to block 3: 13.1%) but this reduction was always smaller than 3% for the random-pairing group (block 1 to block 2: 2.1%) and less than 1% for the water-conditioning group (block 2 to block 3: 0.6%).

Statistical analyses were performed on blocks of five trials. A two-way analysis of variance (groups × trials) with repeated measures on the second factor showed an interaction effect between factors, $F(6,18)=3.51, p<0.05$. A test for simple effects showed overall significant differences between groups of subjects at block 3, $F(2,24)=3.84, p<0.05$, and at block 4, $F(2,24)=6.42, p<0.01$. Paired comparisons between blocks with the Duncan's multiple-range test also revealed significant differences at block 3 and block 4 between the morpholine-conditioning and the morpholine random-pairing group ($p<0.05$) as well as between the morpholine-conditioning and the water-conditioning group ($p<0.05$).

One subject from the random-pairing group was particularly sensitive to morpholine with heart rate reductions reaching 53.7% within the first block of trials. Since there were only three subjects per group, the overactive subject influenced results obtained in the random-pairing group which reduced heart rate by 9.3% for the first block of trials. However, habituation occurred in this group as heart rate reduction became less evident towards the end of the session (block 3: 4.1% and block 4: 1.9%).

EXPERIMENT 2

In experiment 2, the rate of acquisition of the cardiac response to morpholine was examined in 15-month-old fish.

Method

Twelve 15-month-old Atlantic salmon (11.0 to 13.2 cm, total length) were equally divided into two groups: the morpholine-conditioning and the morpholine random-pairing group. The water-conditioning control group was deleted from the experiment as no evidence of sensitization or learning was detected in the previous experiment. Information on subjects and experimental treatments are identical to those described in experiment 1.

Results

Except for the first block of trials, subjects from the morpholine-conditioning group reduced their heart rate considerably more than subjects from the random-pairing group (Fig. 7). Heart-rate reduction for the morpholine-conditioning group ranged from 24.2% to 31.3% across blocks. Hear-rate reduction for the random-pairing group reached a maximum of 19.5% (block 1) before steadily decreasing below the 10% level for block 2 (6.8%), block 3 (5.0%) and block 4 (3.5%). A two-way analysis of variance (groups × trials) showed a main effect on groups, $F(1,10)=13.09, p<0.01$, and for the group-trial interaction $F(3,30)=4.35, p<0.05$. A test on simple effects revealed significant group differences for block 2, $F(1,40)=11.6, p<0.01$, block 3, $F(1,40)=13.5, p<0.01$, and block 4, $F(1,40)=13.7, p<0.01$.

GENERAL DISCUSSION

When trained to lower their heart rate to morpholine, 15-month-old fish (Fig. 7) reached a 20% level within the first five trials whereas 10-month-old fish (Fig. 6) did not do so until 15 training trials. Original responsiveness (first 5 trials) in older fish from the random-pairing group was twice that of younger fish although older fish took less time to habituate to morpholine (between 6 and 10 trials) than younger fish (between 11 and 15 trials). Our results are consistent with those of Walker [13] who found that Atlantic salmon yearlings (19-month-old) are more capable than underyearlings (7-month-old) of acquiring a conditioned motor activity in the presence of morpholine. Different rates of responding to a chemical stimulus between older and younger Atlantic salmon have led us to further investigate olfactory learning and imprinting mechanisms during early developmental stages (manuscript in preparation).

We have been successful with our methodological approach in demonstrating rapid and consistent conditioning in different age-group fish to a chemical cue efficiently monitored and controlled in the laboratory. This provides an opportunity to suggest further research projects on chemosensory perception in fish. Our method has already been proposed as a research tool in salmon restoration programs [10]. It can be used to identify the critical period for olfactory imprinting which is essential for successful homing of reproductive adults to their natal stream. An efficient utilization of the olfactory imprinting procedure would lead to improved stocking programs if the method allows a better understanding of the physiological, behavioral and environmental factors influencing the critical period. Finally, our automated and computerized approach to the study of chemosensory perception in fish could be extended to other research areas to include investigations of chemosensory sensitivity and threshold determination, sublethal effects of pollutants and of various learning phenomena.

ACKNOWLEDGEMENTS

The authors wish to acknowledge expert technical assistance and advice provided by Mr. M. Vinette on computer software and hardware and by Mrs. M. Lupien and J. Thibodeau on innovative programming. Appreciation is also expressed to the Ministère du Loisir, de la Chasse et de la Pêche (Quebec Government) for providing salmon and to Mr. P.-J. Paulhus, director of the Aquarium du Québec for the use of facilities at this institution. This study was supported by Canadian National Sportsmen's Fund and grants from NSERC of Canada to J. J. Dodson (A6951) and to F. Y. Doré (A7030).

REFERENCES

1. Doré, F. Y. *L'Apprentissage: Une Approche Psycho-Ethologique*. Paris: Maloine, 1983.
2. Hasler, A. D. and A. T. Scholz. *Olfactory Imprinting and Homing in Salmon*, Vol 14, edited by B. Heinrich, K. Johansen, H. Langer, G. Neuweiler and D. J. Randall. Berlin: Springer-Verlag, 1983.
3. Hirsch, P. J. Conditioning the heart rate of coho salmon (*Oncorhynchus kisutch*) to odors. Ph.D. dissertation, University of Wisconsin, Madison, 1977.
4. Holland, K. N. and J. H. Teeter. Behavioral and cardiac reflex assays of the chemosensory acuity of channel catfish to amino acids. *Physiol Behav* **27:** 699–707, 1981.
5. Johnstone, A. D. The detection of dissolved amino acids by the Atlantic cod, *Gadus morhua* L. *J Fish Biol* **17:** 219–230, 1980.
6. Johnstone, A. D., D. Whitford and A. D. Hawkins. The effects of an industrial waste (cryolite recovery sludge) upon the Atlantic salmon, *Salmo salar* (L.). *Water Res* **16:** 1529–1535, 1982.
7. Little, E. E. Conditioned cardiac responses to the olfactory stimuli of amino acids in the channel catfish, *Ictalurus punctatus*. *Physiol Behav* **27:** 691–697, 1981.
8. Malyukina, G. A. and Y. A. Marusov. Study of the olfaction of fishes by electrocardiography. *J Icthyol* **11:** 959–966, 1971.
9. Mazur, J. E. *Learning and Behavior*. Englewood Cliffs: Prentice-Hall, 1986.
10. Morin, P.-P., J. J. Dodson and F. Y. Doré. Olfaction, apprentissage d'une réponse cardiaque et restauration des rivières à saumons. In: *La Restauration des Rivières a Saumons, Actes du Colloque Franco-Québécois, Bergerac, France 1985*, edité par R. Billard et M. Thibault. Rennes: Institut National de la Recherche Agronomique, in press.
11. Rescorla, R. A. Pavlovian conditioning and its proper control procedures. *Psychol Rev* **74:** 71–80, 1967.
12. Sandoval, W. A. Odor detection by coho salmon (*Oncorhynchus kisutch*): a laboratory bioassay and genetic basis. M.S. thesis, Oregon State University, 1980.
13. Walker, J. H. Odour discrimination in relation to homing in Atlantic salmon, *Salmo salar*. M.S. thesis, University of New Brunswick, 1967.
14. Webbers, J. P. and E. E. Little. Multiple liquid stimulus dispenser. *Physiol Behav* **20:** 95–98, 1978.