Cardiac Responses to a Natural Odorant as Evidence of a Sensitive Period for Olfactory Imprinting in Young Atlantic Salmon, *Salmo salar*[1]

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Olfactory imprinting was assessed in young Atlantic salmon, *Salmo salar*, undergoing smoltification (parr–smolt transition) by measuring their cardiac responses to a natural odorant, L-cysteine. Condition factor and body coloration were used for characterizing the degree of smoltification. In Experiment 1, heart rate conditioning to L-cysteine was used to compare olfactory learning between fish from different age groups of smoltification. In Experiments 2 and 3, other fish from the same age groups of smoltification were exposed to L-cysteine and their long-term olfactory memory was assessed by measuring their unconditioned cardiac responses to L-cysteine after smoltification. In Experiment 2, the time from the end of odor exposure to testing for olfactory recognition was kept constant for all age groups of smoltification whereas in Experiment 3, the age of fish tested for olfactory recognition was kept constant. Greater conditioning (heart rate reduction) to L-cysteine occurred in age-groups 3 (612–619 d since birth) and 6 (642–649 d) as compared with any other age group of smoltification. Fish tested for odor recognition exhibited a greater unconditioned response (cardiac deceleration) to L-cysteine if they belonged to age-group 3 than to any other age group of smoltification. Our results demonstrated the existence of a sensitive period for olfactory imprinting in Atlantic salmon that occurred between 21 and 28 d after the onset of smoltification induced in the laboratory.

Nous avons évalué l'empreinte olfactive chez des jeunes saumons atlantiques, *Salmo salar*, en smoltification (transition tacon-saumoneau) en mesurant leur réponse cardiaque à une substance odorante naturelle, la L-cystéine. Le facteur de condition et la coloration corporelle sont utilisés pour caractériser le degré de smoltification. Dans l'Expérience 1, le conditionnement de la fréquence cardiaque à la L-cystéine est utilisé pour comparer l'apprentissage olfactif entre des poissons de différents groupes d'âge en smoltification. Dans les Expériences 2 et 3, d'autres poissons des mêmes groupes d'âge en smoltification sont exposés à la L-cystéine et leur mémoire olfactive à long terme est évaluée en mesurant leur réponse cardiaque inconditionnelle à la L-cystéine après la smoltification. Dans l'Expérience 2, l'intervalle de temps entre la fin de l'exposition à l'odeur et le test de la reconnaissance olfactive était maintenu constant pour tous les groupes d'âge à la smoltification alors que dans l'Expérience 3, l'âge des poissons testés pour la reconnaissance olfactive était maintenu constant. Un conditionnement (réduction de la fréquence cardiaque) plus marqué est apparu chez les groupes d'âge 3 (612–619 d depuis la naissance) et 6 (642–649 d) par rapport à n'importe quel autre groupe d'âge de la smoltification. Les poissons testés pour la reconnaissance olfactive et appartenant au groupe d'âge 3 ont manifesté une plus forte résponse inconditionnelle (décélération cardiaque) à la L-cystéine que n'importe quel autre groupe d'âge de la smoltification. Nos résultats démontrent l'existence d'une période sensible d'empreinte olfactive chez le saumon atlantique qui s'est produite entre 21 et 28 d après le début de la smoltification induite en laboratoire.

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Imprinting is a well-known phenomenon in higher vertebrates, especially in precocial birds (Hess 1973). In the few hours after hatching or birth, offspring acquire a following response to their parents or to artificial stimuli resembling them. This response, which is the basis of a strong attachment bond with the imprinting stimulus, results from a learning process that takes place during a restricted period in an individual's life known as the sensitive period. Imprinting results in relatively long-lasting memory and may be completed at a time when the appropriate reaction is itself not yet performed (Immelmann and Suomi 1981). Although the concept of imprinting and the validity of some of the aforementioned criteria are controversial

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issues, in all phenomena that have been characterized as imprinting, some learning processes are favored during specific, usually early, periods of the individual's life time (Immelmann and Suomi 1981).

Attempts have been made to identify various kinds of imprinting in lower vertebrates such as turtles (e.g. Grassman et al. 1984) and fish (e.g. Russock 1986) but the sensitive period has generally proved difficult to define. In anadromous salmonids, homing behavior has been attributed to an olfactory imprinting mechanism. Adult salmon would be able to return to their spawning grounds by detecting and orienting toward the natal stream odor that they imprinted to as young fish.

Supporting evidence for a long-term olfactory memory in salmon comes from artificial imprinting studies. Prior to seaward migration, young coho salmon, *Oncorhynchus kisutch*, exposed to the synthetic chemical morpholine were subsequently decoyed as adults into streams scented with this same artificial chemical (Hasler and Scholz 1983). Although these studies have established the olfactory hypothesis as the leading explanation for homestream recognition by homing salmon, an important feature of true imprinting has still to be demonstrated: the existence, onset, and duration of a sensitive period for olfactory imprinting (SPOI) (Hara 1970; Hess 1973; McCleave et al. 1984; Stabell 1984). At this point, it is worth noting that imprinting necessarily implies a long-term retention of what is learned during the sensitive period but any long-term memory phenomenon is not always the result of an imprinting process.

Classical experiments on imprinting in birds have determined the temporal limits of the sensitive period by exposing groups of young birds to natural or to artificial stimuli during identical short time periods during different developmental phases (Hess 1973). This experimental approach has not been used in any study of salmon imprinting. However, attempts have been made to define the SPOI by exposing groups of young salmon to artificial odorous chemicals during smoltification. The parr–smolt transition is generally believed to be the most probable developmental stage for the SPOI even though a sensitive period occurring during earlier life history stages (including the egg stage) is not excluded (Horrall 1981; Quinn 1985).

A period ranging from 4 to 9 wk, coincident with smoltification, has been used for imprinting salmonids to an artificial chemical (Dizon et al. 1973; Madison et al. 1973; Scholz et al. 1973; Cooper and Hasler 1973, 1974, 1976; Cooper and Scholz 1976; Cooper et al. 1976; Scholz et al. 1976; Scholz et al. 1978a, 1978b; Johnsen and Hasler 1980). Time periods of less than 4 d have occasionally been used for imprinting (Cooper et al. 1976; Sutterlin et al. 1982). In one study (Cooper et al. 1976), a group of coho salmon undergoing smoltification was exposed to morpholine during 44 d while another group was exposed to the same chemical for 2 d. They were released in Lake Michigan and later caught during two consecutive years in a stream scented with morpholine. Results revealed, quite surprisingly, that more fish from the 2-d exposure group (n = 78) were captured in the morpholine-scented stream than fish from the 44-d exposure group (n = 46). Similar inconsistencies between morpholine exposure time and frequency of migrant returns have been found in other imprinting studies (e.g. Madison et al. 1973; Sutterlin et al. 1982).

Although they suggested that olfactory learning during smoltification is a relatively rapid process, previous investigations have failed to accurately define the SPOI. It has only been roughly estimated from a small number of adults returning to an artificially scented stream. Therefore, any link between the classical phenomenon of filial imprinting in precocial birds and long-term olfactory memory in salmonids is still speculative and must be substantiated.

Fish are known to decelerate heart rate readily to various novel environmental stimuli (Laming and Savage 1980) including sound (Hawkins and Johnstone 1978), light (Kawamura et al. 1981), temperature (Nagai and Iriki 1977), and chemical substances (Malyukina and Martem'yanov 1981). In young salmonids, cardiac deceleration has been used as an unconditioned response to assess olfactory memory (Muzi, cited in Hasler and Scholz 1983) and also as a conditioned response (associative learning) to assess their ability for detecting odors (Hirsch 1977; Sandoval 1980; Morin et al. 1987a, 1987b).

The purpose of this study is to verify, in young Atlantic salmon, *Salmo salar*, undergoing smoltification, the validity of the imprinting theory as an explanation of long-term olfactory memory in homing adult salmon. Three experiments were conducted to define when the sensitive period occurs during smoltification and to reveal that a sensitive period had occurred by its effects on olfactory recognition following smoltification. In the first experiment, the magnitude of a conditioned cardiac deceleration to a natural odorant, L-cysteine, was measured in fish from different age groups of smoltification. The sensitive period for imprinting begins when a stimulus becomes conspicuous to an animal (Bateson 1964). This should facilitate the acquisition of a conditioned response. We therefore postulated that the ability to learn an odor should be optimal during a sensitive period and that the magnitude of the conditioned cardiac deceleration would significantly increase at such times.

In the second and third experiments, retention of an olfactory memory was evaluated by simply exposing fish to L-cysteine at different time intervals during smoltification and measuring their unconditioned cardiac deceleration to the odor at different intervals after smoltification. Fish decelerate heart rate in response to a recognized odor. Young coho salmon decelerated heart rate in the presence of an imprinted odor (morpholine or phenethyl alcohol) 85–100% of the time as compared with 0–10% in the presence of a novel odor (Hasler and Scholz 1983). We therefore postulated that the magnitude of unconditioned cardiac deceleration would be significantly greater among fish exposed to an odor during a sensitive period. In Experiment 2, the time from the end of odor exposure to testing for olfactory recognition was kept constant for all age groups of smoltification whereas in Experiment 3, the age of fish tested for olfactory recognition was kept constant. This was done to avoid confounding the influence of the age of testing and time between exposure and testing (Bateson and Hinde 1987).

Materials and Methods

Fish and Maintenance

Two hundred and forty 2-yr-old Atlantic salmon (mean fork length = 15.7 cm ± 0.8 SD) were used in the experiments. They were hatchery-raised offspring of a wild stock obtained from the Ste-Anne River, located on the Quebec north shore 370 km to the northeast of Quebec city. Fish were transported to the Aquarium du Québec and housed in a 3000-L (226.1 × 112.4 × 101.6 cm) concrete holding tank supplied with dechlorinated water (4 ± 0.5°C) under a constant 16 h dark : 8 h light photoperiod regime. The tank was completely covered and the only light sources were four incandescent 7-W bulbs situated 35 cm above the water's surface. A 300-L tank (157.5

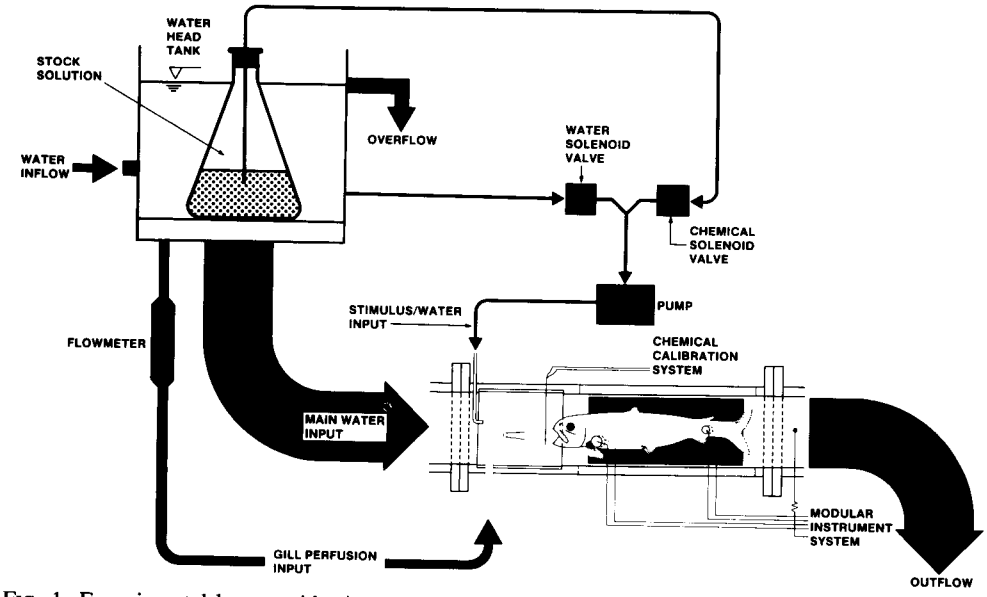

FIG. 1. Experimental layout with view of test chamber. This diagram also illustrates stimulating and cardiac monitoring electrodes attached to the fish and communicating to the modular instrument system.

× 50.8 × 35.6 cm) was used for exposing small groups of fish to an odorous substance, L-cysteine. Anosmic fish show no conditioned cardiac response to L-cysteine (unpubl. data). Photoperiod and water conditions in the 300-L tank were similar to those maintained in the 3000-L tank. All fish were fed daily with a mixture of shrimps, crab meat, and scallops. After 20 d of acclimation, 120 fish were randomly selected for odor-recognition tests. They were divided equally into six floating cages (68.6 × 34.3 × 30.5 cm) located in the holding tank. Cages were made of polyester material with "Beehive" 0.75 × 0.25 cm mesh (Gerrard Mills Inc.) and small PVC piping. The remaining fish were left to swim freely in the holding tank.

Smoltification was induced in fish by acclimating them to 11 ± 0.5°C and a long and constant 8 h dark : 16 h light photoperiod by 1° and 1-h daily increments between 23 and 29 December 1985. Natural smoltification occurs in about 30% of 2-yr-old salmon native to the Ste-Anne River (Anonymous 1987). In the laboratory, parr–smolt transition began on 30 December and was completed on 25 February for a total smoltification period of 58 d.

Apparatus

Heart rate responses of fish to an odorous chemical were assessed in a test chamber connected to different water lines and computer-assisted electronic systems (Fig. 1). This procedure is fully described in Morin et al. (1987b). The main water line contained dechlorinated water flowing at a constant velocity (20.5 mm/s) from the head tank to the test chamber before its evacuation through an outlet pipe. The stimulus-input line to the test chamber delivered either water from the head tank or a chemical stimulus from an erlenmeyer flask containing the stimulus solution placed in the head tank. Water temperature in the head tank as well as in the test chamber remained constant (10.5 ± 0.5°C) for the entire study. A peristaltic pump was used for stimulus injection which was controlled by a pair of solenoid valves. The gill-perfusion line brought water from the head tank directly to the gills of fish (300 mL/min).

The head section of the test chamber housed stainless steel and glass parts to allow free flowing of odorous molecules which could otherwise stick to walls. The test chamber was also equipped with immobilizing devices to hold the fish in position for adequate monitoring and stimulation. Electrodes used for delivering the conditioning shock to the fish or for monitoring cardiac rhythm were connected to a modular instrument system, while those used for chemodetection were connected to a chemical calibration system. Both electronic systems interacted with a microcomputer which controlled all events of an experimental session. The test chamber was located in a black box for eliminating any external visual disturbance.

Experiment 1

The magnitude of conditioned cardiac deceleration of L-cysteine was measured in fish during six successive 8-d intervals of smoltification. The age since birth of each age group was as follows: age-group 1: 592–599 d, age-group 2: 602–609 d, age-group 3: 612–619 d, age-group 4: 622–629 d, age-group 5: 632–639 d, age-group 6: 642–649 d.

Twenty fish of each age group were conditioned. As it took about 3 h to test each fish over a period of 2 d, an 8-d interval was the minimum time required to test 20 fish. A 2-d off interval separated each age group.

Parr coloration and condition factor (weight·fork length^{-3}) were used as indexes of smoltification. Parr coloration was assessed by attributing a score to fish on the presence of body marks characteristic of parr and the absence of marks characteristic of smolts. The marks and their respective scores were as follows: presence of vertical parr marks (2 points), presence of contrasting dark back and yellowish belly (1 point), presence of red spots near the lateral line (1 point), absence of blackened pectoral fins (1 point) and caudal fin (1 point), and absence of a pectoral black spot on either side of the fish (1 point). Individual fish were scored prior to conditioning and scores were averaged to generate a mean parr coloration index for each age group of smoltification.

Each fish was anesthetized (1% methyl pentynol), injected in the dorsal musculature with *d*-tubocurarine chloride (0.002 mg/g body weight), and placed in the test chamber. Injection of small quantities of tubocurarine does not interfere with learn-

ing or with odor sensitivity (Morin et al. 1987a). An experimental session commenced with a 35-min acclimatization period during which we measured resting heart rate. This was followed by a trial during which 7.3 mL of L-cysteine (5.7×10^{-3} M) was injected into the test chamber. When the stimulus reached the fish, it was diluted to a concentration of 3.8×10^{-4} M (Morin et al. 1987b). Eight seconds later, a 2-s continuous shock stimulus was delivered to fish. Intensity of stimulation (30–46 mA) was adjusted at the beginning of the session to produce strong bradycardia averaging 10 beats/min. Fish were trained over two sessions (intersession interval = 24 h) of 20 trials each, separated by short intertrial intervals of random duration (45–135 s).

Each age group of 20 fish was divided equally in two groups, each of which was treated differently on the basis of stimulus presentation. Fish from the conditioning groups were presented repeatedly with the systematic pairing of an odorous stimulus shortly followed by an electric shock. Since the odor signals the presence of the shock, this perfect correlation between the stimuli typically induces an increase in the magnitude of the conditioned response (Weisman and Litner 1969). Fish from the random pairing group were trained with a random presentation of the odor–shock sequence and were used as a control for pseudoconditioning or sensitization (Rescorla 1967). In this group, the conditioned response does not occur, since the presence of the odor is uncorrelated with that of the shock. This treatment therefore represents a good control procedure with which to assess excitatory conditioning (Mackintosh 1983).

Heart rate reduction to an odor cue typically occurs in the context of a single interbeat interval (IBI) and the phenomenon is masked when IBIs measured during the entire odor–stimulation interval are averaged. For this reason, the largest IBI recorded 8 s before odor presentation was compared with the largest IBI recorded 8 s during odor stimulation, and the percentage of heart rate reduction was calculated according to the following formula:

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

Learning occurred if the percentage of heart rate reduction of conditioned fish increased significantly between blocks of trials and if cardiac deceleration of conditioned fish was significantly greater than that of fish tested under the random pairing procedure (Morin et al. 1987a, 1988).

Experiments 2 and 3

In these experiments, long-term olfactory memory was assessed by measuring unconditioned cardiac responses to L-cysteine after fish from six different age groups had been exposed to an odorous chemical during smoltification. These age groups were identical to those of Experiment 1. They were exposed to L-cysteine at the same time as the subjects of Experiment 1 were conditioned to L-cysteine. Smoltification was induced in all fish as previously described.

On the first day of odor exposure, fish were transferred to a tank in which L-cysteine was diluted to obtain a concentration of 10^{-4} M. Water was renewed completely the following day (day 2). L-cysteine was added to water on days 3, 5, and 7 and water renewal occurred on days 4, 6, and 8. Fish were transferred back to their holding tank on day 8. Before exposing fish from another age group of smoltification, water in the odor–exposure tank was renewed on days 9 and 10.

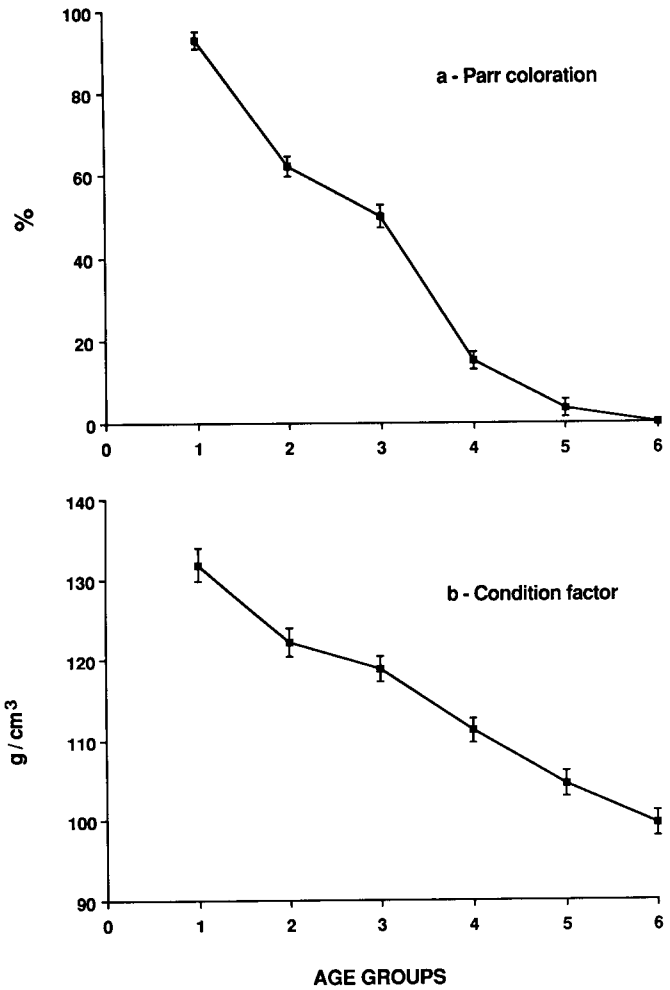

FIG. 2. Morphological changes during smoltification in six different age groups of 20 fish. (a) Parr coloration: mean percentages of body marks characterizing a parr; (b) condition factor: weight·fork length^{-3} ($\times 10^{-4}$). The vertical bars represent the mean ± SE.

Olfactory recognition was assessed in fish that had been exposed to L-cysteine during smoltification by measuring their unconditioned cardiac responses to L-cysteine at different times during the postsmoltification period. Fish from each age group of smoltification were divided equally in two groups (n = 10) to be tested either under a fixed- or a variable-interval treatment. Under the fixed-interval treatment (Experiment 2), fish from each age group were tested 55 d after the end of their initial exposure to L-cysteine. In these fish, the time from the end of odor exposure to testing for olfactory recognition was kept constant. Under the variable-interval treatment (Experiment 3), age-groups 1–6 were tested 85, 75, 65, 55, 45, and 35 d, respectively, after the end of their initial odor exposure. In this experiment, the age of fish tested for olfactory recognition was kept constant.

Fish were anesthetized (1% methyl pentynol), injected in the dorsal musculature with *d*-tubocurarine chloride (0.002 mg/g body weight) and placed in the test chamber for a period of 35 min before exposure trials commenced. A trial consisted of injecting 7.3 mL of L-cysteine (5.7×10^{-3}M) into the test chamber and measuring heart rate 8 s before and 8 s during odor stimulation. Applying a dilution factor calculated during a chemical calibration test (Morin et al. 1987b), we estimated that fish were exposed to a concentration of 3.8×10^{-4}M. Fish

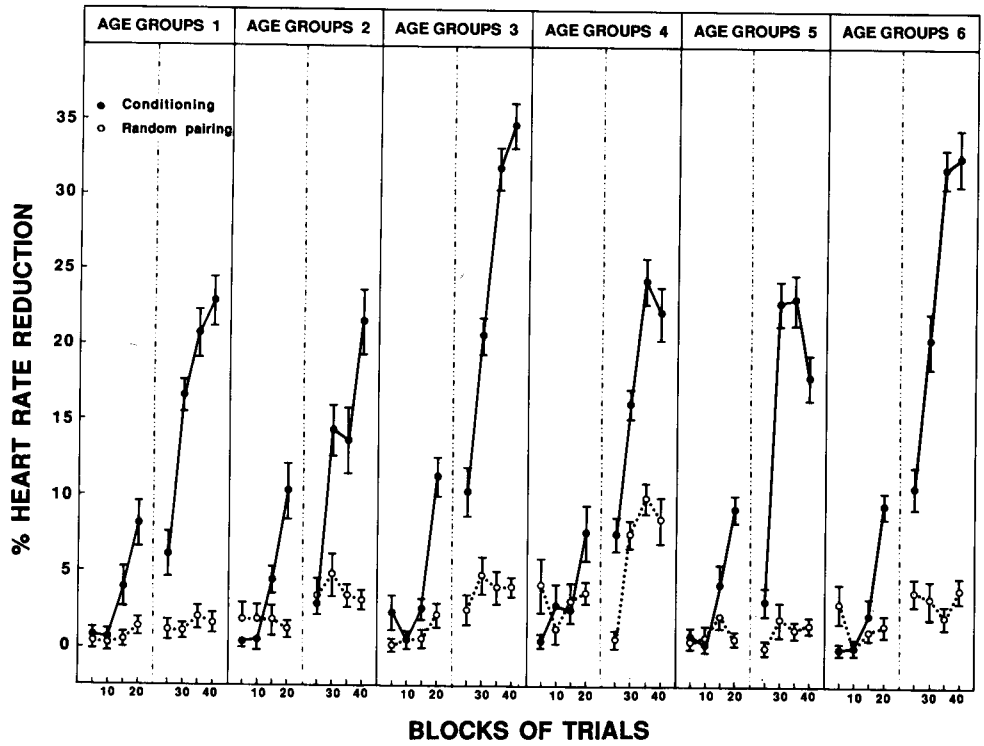

FIG. 3. Mean percentage of heart rate reduction, per block of five trials, in 2-yr-old Atlantic salmon tested in the presence of L-cysteine (3.8×10^{-4} M). During smoltification, six different age groups of 10 fish were conditioned to L-cysteine followed by a shock stimulus and six age groups of 10 fish were exposed to a random odor–shock presentation. The vertical bars represent the mean ± SE.

were tested over a single experimental session of 10 trials separated by short intertrial intervals of random duration (45–135 s). Initial cardiac rhythm and heart rate reductions to L-cysteine were assessed in the same manner as in Experiment 1.

Results

Morphological Changes during Smoltification

Changes in parr coloration during smoltification (Fig. 2) were highly correlated with changes in condition factor ($r = 0.82$). Overall differences in parr coloration ($F = 313.05$; df = 5,114; $P < 0.01$) and in condition factor ($F = 74.97$; df = 5,114; $P < 0.01$) were significant between the age groups of smoltification as both indexes declined from age-groups 1–6. The importance of these changes was also examined between successive age groups. All fish from age-group 1 could be distinctively identified as parr. Significant changes ($P < 0.01$) occurred in parr coloration between all age groups except between age-groups 5 and 6 (Duncan's multiple range test). Fish from age-group 5 could clearly be identified as smolts, as they had little parr coloration. Significant changes in condition factor ($P < 0.01$) occurred between all age groups except between 2 and 3 (Duncan's multiple range test).

Experiment 1: Olfactory Learning during Smoltification

Resting heart rate (beats per minute) increased continuously from age-group 1 (70.5 beats/min) to age-group 6 (81.4 beats/min) (further details in Morin et al. 1989). This could have influenced subsequent cardiac deceleration in the context of cardiac conditioning to L-cysteine. However, since there were no significant correlations between initial heart rate during

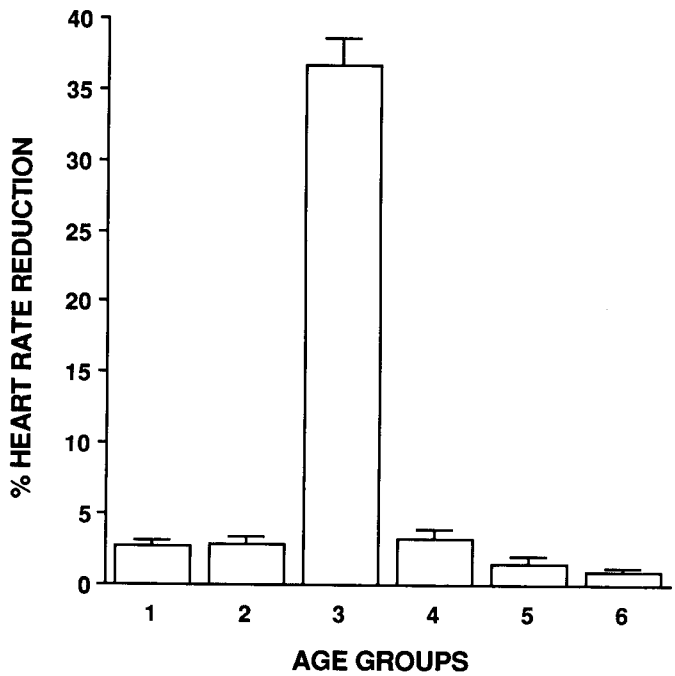

FIG. 4. Mean percentage of heart rate reduction in 2-yr-old Atlantic salmon exposed to L-cysteine (3.8×10^{-4} M) during a 10-trial session. Six age groups of 10 fish had been preexposed to L-cysteine (10^{-4} M) during an 8-d interval of the smoltification period (age-groups 1–6: first to sixth age group from the beginning of smoltification) and were tested at a fixed-time interval after the end of an initial odor exposure. In each age group, testing began 55 d after the end of odor exposure. The vertical bars represent the mean ± SE.

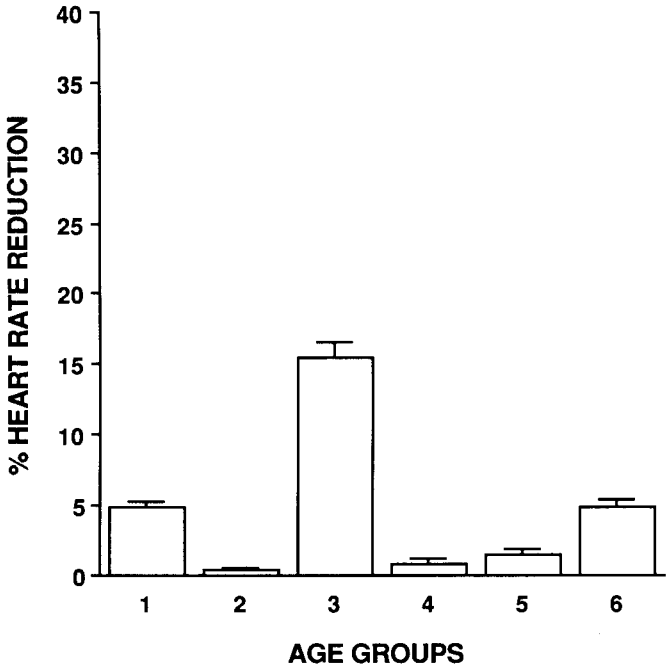

FIG. 5. Mean percentage of heart rate reduction in 2-yr-old Atlantic salmon exposed to L-cysteine (3.8×10^{-4} M) during a 10-trial session. Six age groups of 10 fish had been preexposed to L-cysteine (10^{-4} M) during an 8-d interval of the smoltification period (age-groups 1–6: first to sixth age-group from the beginning of smoltification) and were tested at the same age following initial odor exposure. In age-groups 1–6, testing began 85, 75, 65, 55, 45, and 35 d, respectively, after the end of odor exposure. The vertical bars represent the mean ± SE.

acclimatization and subsequent heart rate reductions at each block of trials (ranging from $r = 0.02$ to 0.22; $P > 0.05$), it was not necessary to conduct repeated measures analysis of covariance (Pedhazur 1982).

A three-way multiple analysis of variance (age groups × treatment groups × blocks of trials) with repeated measures on blocks of trials was used to reveal main effects on cardiac response to L-cysteine (Fig. 3) (Harris 1975; SPSS-X 2.1). Blocks of trials differed from each other ($F = 62.29$; df = 7,102; $P < 0.01$) but interaction effects were also found for age groups × blocks ($F = 1.76$; df = 35,431; $P < 0.01$), treatment groups × blocks ($F = 38.87$; df = 7,102; $P < 0.01$), and for age groups × treatment groups × blocks ($F = 2.16$; df = 35,431; $P < 0.01$). A univariate analysis of variance was used for analyzing simple main effects of age groups and treatment groups at different blocks of trials. At blocks 6, 7, and 8, heart rate reduction in conditioned fish of all age groups was greater than that of fish tested under a random-pairing situation (F values varied from 9.20 to 96.08 and were all significant at $P < 0.01$).

Another way to decompose interaction effects was to perform an analysis of simple effects between age groups within each treatment group. It showed overall differences in heart rate reduction to L-cysteine between age groups of conditioned fish ($F = 5.37$; df = 5,108; $P < 0.01$) but none between age groups of control fish tested under the random pairing situation. Since most conditioned fish (age-groups 1, 2, 3, 4, and 6) reached their best performance at the end of the second experimental session, we examined the relative importance of their heart rate reduction at block 8 by comparing the age groups of smoltification. Overall differences were found at block 8 ($F = 8.31$, df = 5,108; $P < 0.01$) and the Duncan's multiple range test revealed greater heart rate reduction in conditioned fish from age-groups 3 and 6 than in conditioned fish from any other age group of smoltification ($P < 0.05$). Heart rate reduction for age-group 3 (34.8%) was similar to that of age-group 6 (32.8%) whereas heart rate reduction for age-groups 1 (22.8%), 2 (21.6%), 4 (22.3%), and 5 (18.2%) did not differ.

Experiments 2 and 3: Odor Preexposure and Recognition

When tested for odor recognition, some fish that had been exposed to L-cysteine during smoltification reduced their heart rate in the presence of the same odorant. Overall differences in heart rate responses to L-cysteine were found between fish of different age groups of smoltification ($F = 63.41$; df = 5,54; $P < 0.01$) when they were all tested 55 d after the end of their initial odor exposure (Fig. 4). Cardiac deceleration reached 37% in fish from age-group 3, and according to a Duncan's multiple range test, this value was higher than that of any other age group of smoltification ($P < 0.01$). Heart rate reduction was similar in age-groups 1, 2, 4, 5, and 6 and did not exceed 5%. Significant differences were found in fish from different age groups of smoltification ($F = 19.93$; df = 5,54; $P < 0.01$) when they were tested at a different time interval following odor exposure (Fig. 5). When tested 65 d after the end of initial odor exposure, heart rate reduction to L-cysteine reached 15% in age-group 3 and this performance was more pronounced than that of any other age group of smoltification ($P < 0.01$) in which the interval between exposure and testing was longer (age-group 1: 85 d; age-group 2: 75 d) or shorter (age-group 4: 55 d; age-group 5: 45 d; age-group 6: 35 d). Cardiac deceleration in age-groups 1, 2, 4, 5, and 6 did not differ and never exceeded 5%.

Discussion

Imprinting is characterized by two interdependent events: (1) a greater ability for learning discrete environmental cues during a relatively short time interval and (2) a capacity to retain this information in long-term memory to fulfill a biological function. When both characteristics are present, the short interval during which learning ability and the capacity for long-term memory is optimal is called the sensitive period for imprinting. They remain valuable criteria of imprinting despite a considerable broadening of the original concept (Immelmann and Suomi 1981). The following discussion specifically addresses the question: are the aforementioned criteria of imprinting distinguishable in the present series of experiments designed to verify the existence of olfactory imprinting in Atlantic salmon?

The first criterion of imprinting refers to a heightened learning ability during a relatively short time interval. This was achieved in fish from age-groups 3 and 6 conditioned to L-cysteine during smoltification. A greater ability for olfactory learning prevailed in these fish as compared with any other age group of smoltification, as they exhibited significantly greater heart rate reductions to L-cysteine.

The second criterion of imprinting deals with a long-term retention of the information learned during a short time interval. Smolts previously exposed to L-cysteine as age-group 3 reduced heart rate to L-cysteine significantly more than smolts preexposed to L-cysteine during any other interval of smoltification. These fish were able to recognize the odor of their preexposure experience, and we conclude that fish of age-group 3 reached

the second criterion of imprinting. This recognition ability persisted irrespective of the length of time from the end of odor exposure to testing (Experiment 2) or the age of testing (Experiment 3). However, heart rate reduction to L-cysteine in smolts preexposed during age-group 3 of smoltification decreased when measured 65 d (Experiment 3) as compared with 55 d (Experiment 2) after the end of odor exposure. Because subjects remained in freshwater after smoltification and for several months prior to testing, we propose that the weakening of the cardiac response may have been due to the process of desmoltification (Evropeytseva 1963). A reappearance of parr characteristics (e.g. contrasting dark back and yellowish belly) was observed in fish tested 65 d after the end of odor exposure.

Both criteria of imprinting, a greater ability for learning during a relatively short time interval and a long-term memory of the information learned during this interval, were present only in fish from age-group 3 of smoltification. Clearly, the existence of a SPOI was demonstrated in these fish. Fish from age-group 6 were less able to recognize their preexposed odor, since heart rate reduction in these fish was relatively small. Thus, contrary to fish from age-group 3, fish from age-group 6 failed to reach the second criterion of imprinting.

Our results indicate that there is only one SPOI during smoltification (age-group 3) even though there appears to be two distinct optimal periods for olfactory learning (age-groups 3 and 6). What could have caused a greater ability for olfactory learning in fish from age-group 6 if it was not associated with an imprinting process as in fish from age-group 3? Morin et al. (1989) proposed that thyroid hormones facilitate olfactory learning during smoltification and found significant positive correlations between histological indexes of thyroid activity and heart rate conditioning to L-cysteine during smoltification. Thyroid activity as indicated by histological indexes was greatest in fish from age-groups 3 and 6. Morin et al. (1989) also proposed that thyroid hormones facilitate olfactory imprinting. As evidence, they reported apparently different plasma–tissue fluxes of thyroxine between age-groups 3 and 6, suggesting the existence of a thyroid–brain mechanism for olfactory imprinting in fish from age-group 3 but not in fish from age-group 6. Thus, thyroid hormones seem to exert a general facilitative effect on olfactory learning (age-groups 3 and 6) and a more specific one on olfactory imprinting (age-group 3).

Although we have demonstrated a SPOI in Atlantic salmon, this must not be taken to imply that olfactory imprinting is an all-or-nothing affair. In accordance with current views of imprinting, we have used the expression "sensitive period" without the restrictive connotation of the expression "critical period." During a sensitive period for imprinting, the animal's capacity to learn and store information in memory is optimal, implying that some of this capacity may persist beyond the sensitive period. Conversely, none of these capacities for learning and memory would occur beyond a "critical period" for imprinting. The concept of a critical period for imprinting appears untenable. In birds, the irreversibility of filial imprinting (i.e. an incapacity to learn beyond the critical period) and its influence on later sexual preferences are controversial issues (Bateson 1966). Beyond the so-called critical period, birds can still develop social attachments providing they are sufficiently familiar with the imprinted object (Hoffman 1987). In the present study, some imprinting may have occurred in more than one age group of smoltification. Conditioned fish from all six age groups of smoltification reduced their heart rate to L-cysteine suggesting that learning ability was not unique to fish from age-group 3 or 6. In addition, all smolts preexposed during smoltification reduced to some extent their heart rate in the presence of L-cysteine. For example, heart rate reduction to L-cysteine reached 3% (Experiment 2) or 5% (Experiment 3) in fish from age-group 1. In other experiments (unpubl. data), we have shown that unconditioned heart rate reduction to L-cysteine reached 0.64% ($\pm$0.44 SD) in fish with no prior exposure to L-cysteine and tested at the same time as fish from age-group 3 (Experiment 2). Thus, some imprinting may have occurred beyond the optimal SPOI even though olfactory imprinting was clearly most pronounced in fish from age-group 3.

It could be argued that greater heart rate reductions in some fish conditioned to L-cysteine during smoltification were due to an increase in the general reactivity of fish to environmental cues instead of a heightened ability for olfactory learning. However, the magnitude of heart rate reductions did not change between trials or between different age groups of smoltification in fish trained under a random presentation of the odor–shock sequence. This suggests no change in the reactivity of fish to L-cysteine, during the course of the study. In addition, a greater learning ability during smoltification did not occur because of a greater reactivity to the shock as its intensity was adjusted, at the beginning of each training session, to induce bradycardia identical for every fish used in the experiment.

Although not measured in the present study, olfactory sensitivity may increase concomitantly with an olfactory learning ability during smoltification to facilitate olfactory imprinting in anadromous salmonids. The possibility that greater olfactory sensitivity during smoltification plays a role in the imprinting process was investigated by Rehnberg et al. (1985). In their study, the olfactory sensitivity of juvenile cohos was assessed by measuring avoidance responses to an amino acid (L-serine or L,D-alanine) presented before, during, and after smoltification. Threshold concentrations were lower before (10^{-7} M) than during smoltification (10^{-5} or 10^{-6} M). These results demonstrated no increase in sensitivity during smoltification and apparently indicate that olfactory sensitivity plays no role in the imprinting process. However, the possibility of heightened olfactory sensitivity during smoltification cannot be rejected, as Rehnberg et al. (1985) did not specify exactly when fish were tested during smoltification and apparently pooled data obtained throughout the period of smoltification. Under such circumstances, a brief period of heightened olfactory sensitivity would have been difficult to detect.

In the present study, we used an autonomic response (heart rate) instead of motor responses (following behavior) to identify an imprinting process. In juvenile salmon, unconditioned heart rate reduction to an odor previously experienced during smoltification may be regarded as an orientation response to an imprinted stimulus. In classical examples of filial imprinting, orienting movements of newly hatched birds (e.g. stretching of the neck) towards a previously experienced decoy are generally associated with a preference for that imprinted stimulus or a tendency to follow it. For this reason, orienting responses are reliable measures of the imprinting process in animal studies (Hess 1973).

In other fish studies, the expression "arousal response" is used to describe an unconditioned cardiac deceleration to a novel stimulus (Laming and Savage 1980; Laming and Ennis 1982; Rooney and Laming 1988). In contrast, we use the expression "orientation response" to designate an unconditioned cardiac deceleration elicited by a familiar stimulus. In the present study, we have shown in smolts that an uncondi-

tioned cardiac deceleration occurs in the presence of a previously experienced odor, L-cysteine (3.8×10^{-4} M), because of its recognition in the context of olfactory imprinting. Smolts of the same age do not decelerate heart rate to L-cysteine at a concentration of 3.8×10^{-4} M if they have not been preexposed to that stimulus (unpubl. data).

Our results suggest that olfactory imprinting in anadromous salmonids is controlled by two different mechanisms which seem to interact with each other in a specific manner. The first mechanism may act to increase the general capacity of fish for learning olfactory cues. Thyroid hormones may facilitate olfactory learning by increasing the excitability of fishes' nervous system during smoltification (Morin et al. 1989). Such learning capacity, however, may not in itself be sufficient for olfactory imprinting to occur. Accordingly, long-term olfactory memory was not demonstrated in one of our high-learning age groups of smoltification (age-group 6). Thus, a second mechanism may be involved in the consolidation of the odor information into long-term memory.

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