AN EVALUATION OF THE USE OF THE EEG TECHNIQUE TO DETERMINE CHEMICAL CONSTITUENTS IN HOMESTREAM WATER

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INTRODUCTION

A large body of literature now exists that points to olfaction in migratory fish as the important sense in orientation near and in the homestream (Collins *et al.*, 1962; Fagerlund *et al.*, 1963; Groves *et al.*, 1968; Hasler, 1960a, 1960b, and 1966; Hasler and Wisby, 1951). It is hypothesized that salmon can store odor information about the homestream and use these cues upon the homing migration.

The chemical or chemicals involved in homestream cues are probably of a very low concentration and perhaps a complex mixture. Efforts to determine these chemicals have met with some success. Fagerlund *et al.* (1963) found that a portion of these chemicals is volatile, although the non-volatile portion may play some part in orientation for the fish. Hasler (1966) reported that the active fraction was organic, heat labile and volatile at 25 C. Idler *et al.* (1961) concluded that the material was neutral, dialyzable and heat labile.

Hara *et al.* in 1965 reported that homestream water perfused through the nares of salmon produced a characteristic high amplitude wave in an electroencephalographic (or EEG) recording from the olfactory bulb. The EEG, they suggested, might be used to study on a physiological basis the olfactory hypothesis.

Since the EEG had been looked on as a possible bioassay for individual homestream recognition (Oshima *et al.*, 1960a and b; Ueda *et al.*, 1967), it was felt that the EEG might provide useful information about homestream chemicals that were needed for homing. Because the technique is quite recent, this problem was first approached by repeating earlier experiments by Fagerlund *et al.*, Hasler, and Idler *et al.* Although the active fractions de-

scribed by these workers may not be the same as the stimulants in the EEG experiments, it was felt that there should be some correspondence in the groups of chemicals described in the two types of experiments. This paper reports on an investigation designed to evaluate the feasibility of using EEG as a means of detecting the chemicals present in homestream water that are responsible for the homing of coho salmon in a Wisconsin stream bordering on Lake Michigan.

METHODS

Adult spawning coho salmon *(Oncorhynchus kisutch)* that had homed to a tributary of the Ahnapee River, Algoma, Wisconsin in the fall, 1970, were used in these studies. They were trapped in the stream on the same day as the experiments and brought back to a temporary laboratory, where they were held in city tap water.

The testing procedure was similar to that of Dizon *et al.* (1973). The fish were anesthetized with tricain methanesulphonate (MS222, 0.01) and immobilized with gallamine triethiodide (flaxedil, 2 mg/kg body weight). A portion of the brain was exposed by means of a dental drill; a platinum coated stainless steel electrode (Transidyne General) was inserted in the olfactory bulb. EEG responses evoked by test samples were amplified with a Bioelectric Instruments (model DS2c) and recorded on a Hewlett Packard model 7712B oscillagraph. Figure 1 presents a typical recorder trace showing the background and stimulus response. A second channel of the oscillagraph was equipped with an integrating preamplifier, so that the integration of the EEG could be recorded. The integrator sums the voltages in the positive part of the wave form. The slope of the line obtained from the integrator can be used to quantify the EEG records. To standardize the response, the slope of the integration of a response to each sample was divided by the slope of the integration of the response to home-

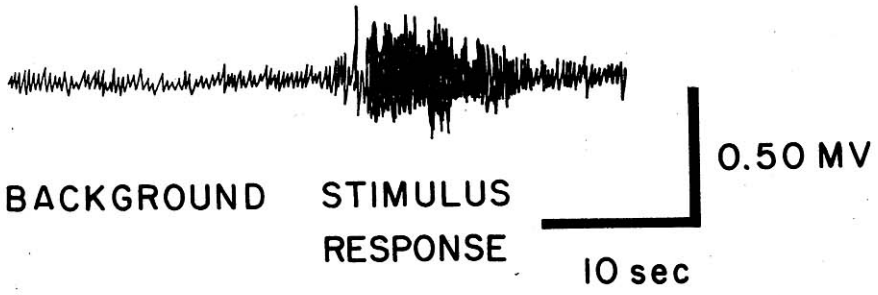

FIGURE 1. Example of electroencephalographic output (hand wash).

stream water. An F-test was used to test the significance of the responses. Each sample was introduced in a random order one time each trial. Trials were repeated 4 times. Algoma city tap water was used to rinse the nares between trials.

Water samples from the homestream were fractionated in a variety of ways. Factors considered were: the variability of the response of a fish to a given sample and the pH, molecular weight, and volatility of the sample. Additional studies on the chemical nature of the homestream dealing with carbon filtration, dialysis, and Sephadex chromotography were not conclusive and will not be reported here. Experiments with the ionic strength and concentration of the homestream water will not be reported for the same reason. For further details consult Cooper (1971).

In the first experiment, two samples of stream water, one taken directly from the stream and one stored at 5 C for one day, were used to test two fish for their variability in response.

In the second experiment, a group of samples of different pH were prepared by adding sodium bicarbonate (0.01M) to Algoma city tap water and adjusting the pH of the solutions with sulfuric acid or sodium hydroxide, as needed, to pH 5, 6, 7, 8, and 9. One fish was used in this experiment.

In a third experiment, homestream water was filtered through a glass fiber filter and 0.45 micron and 0.22 micron pore size Millipore filters. Four fish were used in this experiment.

Finally, in a fourth experiment, homestream water was fractionated by means of a vacuum distillation apparatus at 6 mm Hg pressure at 20 C. This equipment consisted of a Snyder-ball column and a water-jacketed condensor. A thermometer was positioned at the top of the column to observe the temperature of the distillate. A dry ice acetone bath was used to trap the distillate. It took 3.5 hours to reduce 750 ml to 250 ml under these conditions. Two fish for each of 2 distillations were used in this experiment.

For the experiments reported here, a total of 10 fish and 15 water samples were used.

RESULTS

The results of the first experiment (Figure 2 and Table 1) indicate that the variability in fish response as reflected in the standard deviation was roughly 25%. Data from different animals cannot be compared directly, i.e. the data from coho 144 and 146 cannot be pooled, although the ranking for samples for each fish can be compared.

In the second experiment, filtration through glass fiber filter, 0.45 micron or 0.22 micron pore size Millipore filters did not seem

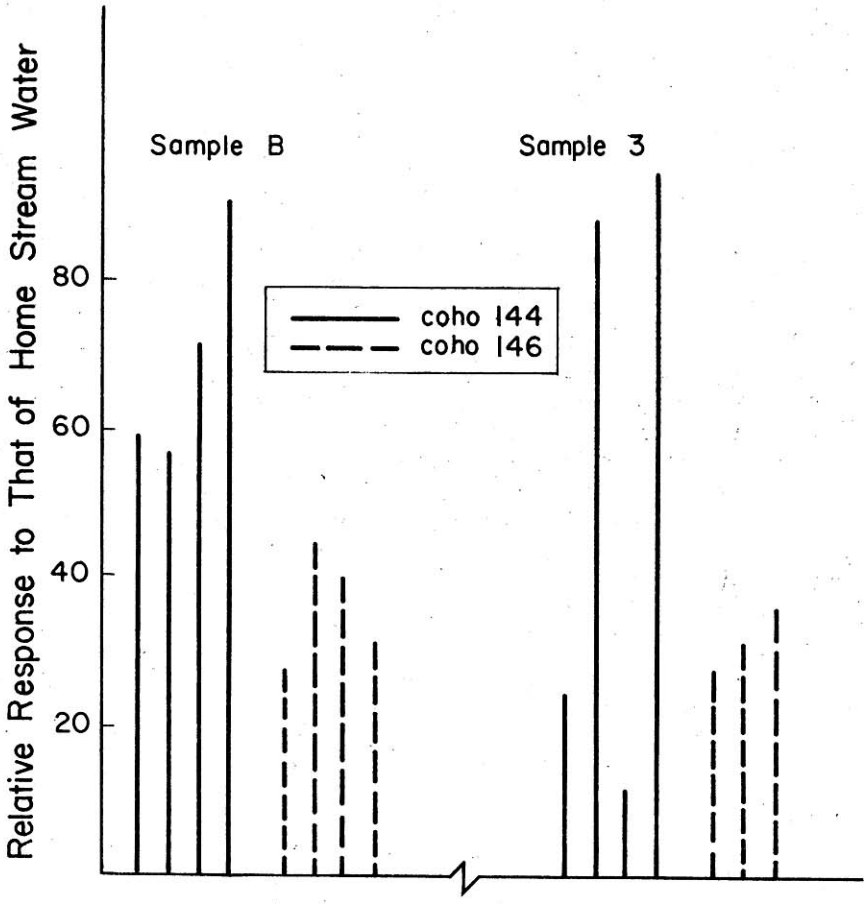

FIGURE 2. Replicates of raw stream water (B) and water stored for one day at 5 C (3).

TABLE 1. REPLICATE SAMPLES OF RAW STREAM WATER AND STREAM WATER STORED AT 5 C

(Mean and standard deviation of the integration of response to the stimuli expressed as the per cent of the integration of the response of a reference sample of stream water.)

	Raw	Sample 5 C
Coho 144	66 ± 9	50 ± 25
Coho 146	46 ± 10	32 ± 4

to affect the samples. The amplitude of responses to these samples was roughly the same before or after filtration (Table 2).

The salmon responded most strongly to acid pH (4 and 5). They responded less strongly to basic pH (8 and 9) than to neutral pH (7) (Figure 3).

In the fourth experiment, it is clear that there is a higher amplitude response to the non-volatile portion than to the volatile portion of the water distilled at 20 C (Table 3).

TABLE 2. EEG RESPONSES TO WATER FILTERED THROUGH GLASS FIBER FILTER, 0.45 AND 0.22 MICRON PORE SIZE MILLIPORE FILTERS

	Coho	Coho	Coho	Coho
Water	144	146	147	150
Homestream	69 ± 20	36 ± 11	37 ± 6	80 ± 14
Glass fiber	65 ± 30	31 ± 24	17 ± 1	141 ± 51
0.45 μ	80 ± 50	—	7 ± 7	88 ± 15
0.22 μ	80 ± 50	55 ± 25	36 ± 20	80 ± 12

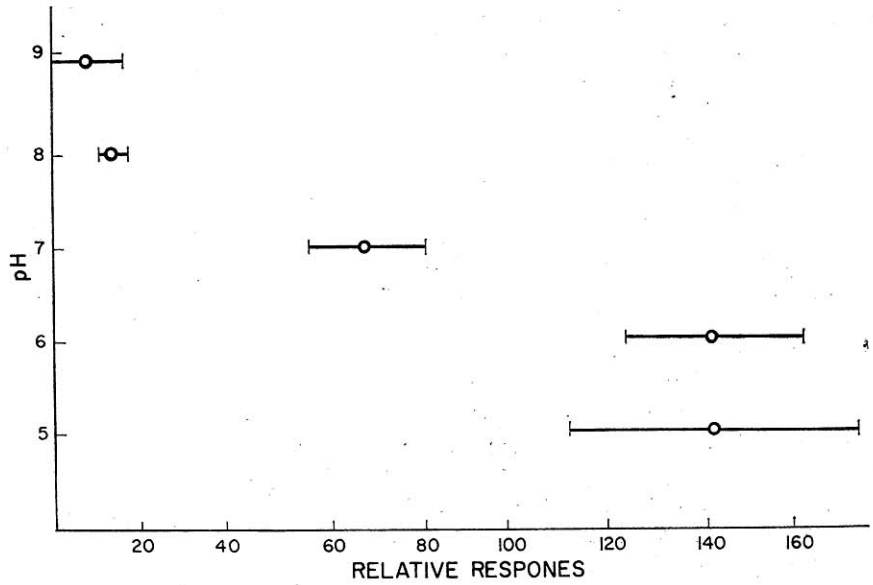

FIGURE 3. EEG response to homestream water adjusted to pH, 5, 6, 7, 8, and 9 (mean and standard deviation).

TABLE 3. VACUUM DISTILLATION OF HOMESTREAM WATER. DUPLICATE RUNS ON EACH FISH FOR TWO SETS OF DISTILLATION SAMPLES

(Mean and standard deviation of the integration of the response to the stimuli expressed as the per cent of the integration of the response of a reference sample of stream water.)

	Samples			
	Distillate 1	Residue 1	Distillate 2	Residue 2
Coho 131	58 ± 25	97 ± 11	—	—
Coho 132	30 ± 3	70 ± 1	—	—
Coho 144	—	—	23 ± 15	114 ± 47
Coho 146	—	—	0 ± 0	47 ± 12

DISCUSSION

One problem in interpreting the data is that one cannot make inter-fish comparison. This may be due to difference in handling of the fish. Coho salmon were taken out of the trap at one time on the day of the experiment. It is possible that fish had been in the trap for different lengths of time for a few minutes to several days. Ueda *et al.* (1967) have pointed out that fish kept in a holding tank for a week became unresponsive to their homestream water; this may be due to an advance stage of sexual "Ripening" (as Fagerlund *et al.*, 1963 suggests) or a gradual acclimation of the fish to the homestream water odor.

The results of the third experiment show that the homestream odor has a molecular weight under one million, the approximate size of material retained on a 0.22 micron pore size Millipore filter. Since none of these filters seems to add an odor to the homestream (or subtract one from the water), they are a useful technique for cleaning up the extraneous matter in the water; there was a large quantity of material left on the filters after the experiments. The final experimental results indicate that the stimulatory portion of the water is non-volatile at 20 C, since there is a higher response to the residue than to the distillate.

The second and fourth experiments mentioned above leave little doubt that the EEG technique can be used to detect difference in water samples; it is possible to determine whether the character of the water sample has been changed by the experimental procedures. However, since the standard deviation in response is quite large, the technique may not be useful in detecting subtle differences between samples.

A second underlying problem that may limit the usefulness of the EEG technique, is that it is not possible to distinguish between

two kinds of responses from the fish. A sample that represents a danger or avoidance reaction, such as handwash (a sample in which the hand has been dipped) will give the same amplitude response, as far as one can tell from the EEG, as a sample that is stimulatory, such as the homestream water. Therefore, it is impossible to tell whether the distillation experiment, for instance, produced residues important in homing or whether merely concentrated "avoidance" fractions were formed. Indeed, the stimulatory fractions observed with the EEG technique may not be related in any simple manner to either the stimulatory fraction found in behavioral work or those fractions actually necessary for homing. Perhaps in the future, any experimental results obtained with the EEG technique should be confirmed with a behavioral bioassay.

Until these problems are overcome, any future work that attempts to utilize the EEG to determine homestream constituents may be of limited value.

ACKNOWLEDGEMENT

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