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Electroencephalographic Evidence for Retention of Olfactory Cues in Homing Coho Salmon

Abstract. *Differences were observed in the magnitude of the evoked electroencephalographic response to 1 percent morpholine for homing coho salmon* (Oncorhynchus kisutch) *exposed to morpholine as fingerlings 1 month before smolting as compared to salmon not exposed to morpholine as fingerlings. These results indicate that olfactory information has been retained for 18 months, the period between smolting and the homeward migration.*

Since the studies of Hara *et al.* (*1*) suggested that the electroencephalographic (EEG) technique might be used as a bioassay for home stream recognition in migrating salmon, many physiological (*2*) and behavioral experiments (*3–6*) have been performed to interpret the significance of the EEG home stream responses. Much of this work has been summarized by Hara (*7*).

Despite early reports that the EEG technique was specific in that only the home stream water elicits a characteristic evoked potential (*1, 3*), more recent work (*4–6*) has failed to find such a specificity. In addition, analysis of the EEG technique is complicated because other factors such as *p*H, ionic strength, and nonspecific stimulatory products also affect the EEG response.

We used the EEG technique to determine whether coho salmon fingerlings can be imprinted by exposure to an artificial chemical, morpholine (*8*), and to see if they can retain the information from this chemical until the adult spawning migration 18 months later. Dizon *et al.* (*5*) showed a significant difference in the magnitude of the evoked potentials to morpholine for a group of coho salmon exposed to morpholine as fingerlings 9 months earlier as compared to an unexposed group. Since the salmon were held in a hatchery after chemical exposure and were not sexually mature when tested, we felt it necessary to see whether adult salmon also showed this response. A longer paper on this work appears elsewhere (*9*).

Two groups of 8,000 coho salmon fingerlings were held in large tanks at Oak Creek, South Milwaukee, Wisconsin, in April 1971 and supplied with water (*10*) pumped from Lake Michigan. Both groups were marked by different fin clips. One group was exposed to morpholine at 5×10^{-5} mg/liter; the other was not. The 5-week exposure period, starting 3 weeks before smolting and ending 2 weeks afterward, was chosen because it was viewed as adequate for imprinting (*11*). The salmon were then released at the mouth of Oak Creek. During the spawning migration in the fall of 1972, morpholine was released into Oak Creek at a stream concentration of about 1×10^{-4} mg/liter. It was hypothesized that if the salmon had imprinted to morpholine as the home stream chemical, then only the exposed group of fish would recognize Oak Creek as the home stream since it contained morpholine.

Salmon were captured in the mouth of Oak Creek upon their return as adults, paralyzed with Flaxedil (2 mg per kilogram of body weight), and restrained in a holding box. Their gills were flushed with city tap water saturated with oxygen. A portion of the skull over the forebrain was removed with a dental drill to permit the insertion of an electrode (*12*) into the olfactory bulb. The EEG recordings were made with a Grass Instruments polygraph equipped with a model 7P5 preamplifier, and the signals were integrated with a Grass model 7P10 integrator (which has an infinite time constant). Heartbeat monitored by electrocardiography (EKG) indicated the condition of the fish. Both EEG and EKG signals were recorded on magnetic tape with an FM tape recorder.

Fourteen chemical solutions and water samples were tested by introduction into the nares. Morpholine at 1, 0.1, and 0.01 percent was used to see if the fish had "remembered" the imprinting chemical. Three other morpholine solutions at these concentrations buffered with sodium bicarbonate (0.01*M*, *p*H 7.5) served to control for *p*H, which varies in the unbuffered solutions and is a factor in the EEG response (*5, 9*). Two buffer solutions were tried, one at *p*H 9.5 to control for *p*H and another at *p*H 7.5 to control for ionic strength. Phenethyl alcohol at 0.1 and 0.01 percent was used to determine whether an organic compound other than morpholine would elicit evoked potentials. Lake Michigan and Oak Creek waters were chosen to test for the influence of water that the fish had recently experienced and to determine the presence of nonspecific stimulatory products. Sodium chloride (0.06*M*) served as an internal standard. Finally, South Milwaukee city tap water was used to rinse the fishes' nares between samples and to prepare test solutions. Approximately 10 ml of each sample were delivered in a random sequence through a Pasteur pipette to the nares at an approximate rate of 1 ml/sec.

The experiment was standardized by dividing the integration of the reaction to each test solution by the integration of the NaCl record (*13*). Differences in means for all fish were evaluated for significance by the Mann-Whitney *U* test (*14*); responses separated by less than 0.03 were considered the same rank.

Eleven imprinted and nine nonimprinted fish were tested with the EEG technique. The responses of these two groups to 1 percent morpholine were significantly different ($U = 12$, $P \leq .01$) (Table 1). If only the salmon tested three or more times are compared (eight imprinted and six nonimprinted salmon), the differences between the two groups are also significant ($U = 3$, $P \leq .001$). No fish responded to concentrations of 0.1 and 0.01 percent morpholine. The magnitude of the evoked potentials to morpholine for the 11 imprinted fish was roughly correlated with the period in the migration at which they were tested. The fish at the start and the end of the season gave

approximately equal responses of a lower amplitude, while those tested at the peak of the season showed the highest amplitude. No fish responded to the phenethyl alcohol solutions.

Buffer at *p*H 7.5 elicited a significant response as judged by the signed rank test (*15*) ($N = 12$, $P = .0003$), but buffered morpholine solutions did not. Thus, two stimuli (morpholine and *p*H 7.5 buffer), each of which produced strong reactions when used alone, together elicited no response. Therefore, we hypothesized that the two stimuli had counteracting effects. Although the *p*H of 1 percent morpholine is higher than 9.5, while that of buffer is 7.5, these results cannot be explained on the basis of *p*H alone. Low potentials in response to buffer at *p*H 9.5 had been reported (*5*) and were confirmed in our experiments. There were no significant differences in the magnitude of responses to the buffer solutions for the morpholine-exposed and control groups ($U = 37$, $P \leq .05$). However, morpholine-exposed salmon showed an increasing responsiveness to buffer during the season, as they did to morpholine. Thus, this sensitization was specific not only to morpholine (the imprinting odor) but to other stimuli as well.

Three qualitative differences appeared in the evoked potentials to morpholine as compared to the responses to other substances (Fig. 1). First, there seems to be a delay in neural reaction to morpholine as compared to buffer (1.9 ± 1.6 seconds as compared to 0.5 ± 0.5 second) or handwash (not shown). This lag may be a low-amplitude buildup to the evoked potential. Second, the adaptation to morpholine is slow, with a clear EEG display lasting more than 30 seconds. This long adaptation is similar to that of rainbow trout to amino acids (*16*). Third, responses to morpholine cannot be eliminated readily by rinsing the nares with city water, as is possible with other stimuli.

Although fish were imprinted to morpholine at 1×10^{-5} mg/liter, evoked potentials occurred only at concentrations higher than 0.01 percent (10^2 mg/liter) for Dizon *et al.* (*5*) and at 1 percent (10^4 mg/liter) in our work. [An attempt to elicit EEG responses with morpholine at 0.9 mg/liter was unsuccessful (*17*).] In contrast, a threshold of 10^{-6} mg/liter for behavioral response to morpholine has been reported (*18*). That the EEG technique is less sensitive than behavioral responses is borne out by data for other substances. For L-serine, the behavioral threshold is 10^{-4} mg/liter (*19*) and the EEG threshold is 10^{-1} mg/liter (*20*); for phenethyl alcohol, these respective thresholds are 10^{-3} mg/liter (*21*) and 10 mg/liter (as reported here). The EEG technique is less sensitive; hence, higher morpholine concentrations were used for EEG testing than were needed for the initial imprinting and for decoying the fish back to Oak Creek.

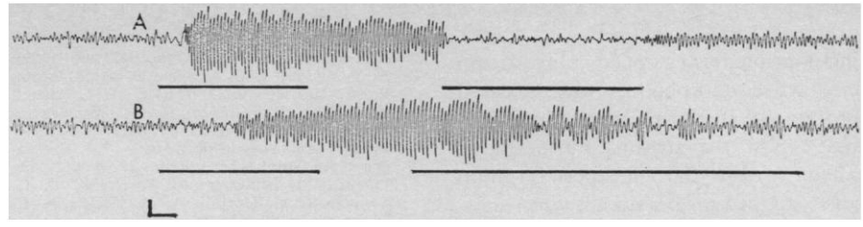

Fig. 1. Electroencephalographic records of a response to *p*H 7.5 buffer (A) and to 1 percent morpholine (B). In each case the first stimulus marker (heavy line beneath record) indicates the sample, and the second indicates a tap water rinse. The calibration mark shows 1 second and 200 μv. The morpholine response is characterized by a longer delay period and a continuation of the response after a tap water rinse.

In experiments by Oshima *et al.* (*6*), salmon that had previously shown no response to water from the University of Washington College Fisheries exhibited evoked potentials when they were kept in this water for 3 days. This suggested that fish respond to water to which they have been most recently exposed.

We found that spawning coho salmon in the imprinted and nonimprinted groups held for 1 week in Oak Creek water did not respond to this water, nor did fish held in Lake Michigan water react to Lake Michigan water. Moreover, nonimprinted fish caught in Oak Creek did not react to morpholine. Thus, our studies suggest that recent exposure is not an important factor during the central part of the spawning migration.

Results obtained with the EEG technique are supported by those in other phases of our study (*9*, *22*). In a census of salmon returning to the stream during the spawning season, the ratio of exposed to unexposed captured salmon was 8 : 1 (216 exposed and 28 unexposed), although a ratio of 1 : 1 would be expected if morpholine had no effect on the experimental group. In a second group of experiments (*22*), displaced salmon were tracked past a stream north of Oak Creek by means of an ultrasonic transmitter. Morpho-

Table 1. Electroencephalographic responses to morpholine and *p*H 7.5 buffer of the morpholine-exposed (M) and unexposed (C) groups of salmon. All responses are for three or more trials, except as indicated. Response data are the integration of the evoked potential for test solutions divided by the integration of the response to 0.06*M* NaCl. Ranks are those determined by the Mann-Whitney *U*-test. The two groups differ statistically.

Date	Group	Morpholine				Buffer			
		Response		Rank		Response		Rank	
		M	C	M	C	M	C	M	C
16 October	M*	0.54		1		1.00		5	
16 October	C*		0.79		2		0.72		1
26 October	C*		1.21		9		1.00		5
31 October	M	1.33		10		1.00		5	
31 October	M	1.38		12.5		0.95		2	
2 November	C		1.02		5		1.27		12
2 November	M	1.41		12.5		1.26		12	
4 November	C		1.00		5		1.54		16
4 November	M	1.51		15		1.63		17.5	
9 November	M	1.73		16.5		1.16		10	
14 November	C		1.02		5		1.03		5
14 November	C		1.11		8		1.09		8.5
14 November	M†	2.45		19		1.63		17.5	
15 November	M	1.39		12.5		2.28		19	
15 November	M	6.54		20		7.88		20	
16 November	C*		1.00		5		1.10		8.5
19 November	C		1.03		5		1.29		12
21 November	M	2.19		18		1.00		5	
27 November	M*	1.70		16.5		1.50		15	
28 November	C		1.40		12.5		1.33		14

* Two trials. † One trial.

line-imprinted fish stopped at this stream only when morpholine was released into it; at all other times they continued past the stream. Furthermore, salmon from the unexposed group never stopped at this stream when morpholine was present. The EEG studies reported here, together with census and ultrasonic tracking information, indicate that the exposed group of salmon had been imprinted to morpholine and had retained this information for 18 months (*23*).

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13. The slopes of the integration line were used. For a constant Y distance (such as one period for a reset of the integrator) of the line segment, this analysis is linearly related to the area under the integration curve.
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23. Data obtained during the 1973 research season tend to support the results reported here. In addition, current information indicates that we are dealing with an olfactory memory. However, the possibility that responses to morpholine are also by means of taste or a general chemical sense cannot be eliminated.
24. We thank A. Scholz, S. Drzeweicki, and R. Smith for extensive assistance in the field and A. Dizon, R. Horrall, and D. Madison for advice in these studies. We also thank J. Hildreth, E. Mueller, and R. Poff. Supported by training grant T900192 with the Federal Water Quality Administration; National Science Foundation grants GB7616 and GB343; University of Wisconsin Sea Grant, Department of Commerce, NOAA 2-35209; the Wisconsin Department of Natural Resources; and the city of South Milwaukee.

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Near Identity of Cognitive Structure in Two Ethnic Groups

Abstract. *As part of a large-scale family study in Hawaii, Americans of either Japanese or European ancestry were administered a battery of 15 cognitive tests. Principal component analyses (varimax rotations) yielded the same four major cognitive factors for each of the two ethnic groups, and these factors are defined by strikingly similar factor loadings.*

A large-scale family study is currently in progress in Hawaii, with the primary objective of assessing genetic and environmental bases of performance on various tests of cognitive ability. The project is a collaborative effort between investigators at the University of Hawaii and the University of Colorado, with administrative headquarters in the Behavioral Biology Laboratory, University of Hawaii. Data are being obtained on 15 cognitive variables (*1*), various environmental indices, blood group and enzyme systems, and dermatoglyphics. During the initial year of the project, data were obtained on 262 Americans of Japanese ancestry (AJA) and 782 Americans of European ancestry (AEA) (*2*). Although this represents only a small fraction of the subjects we plan to test, one highly stable relationship is beginning to emerge from the partial data set. In view of the current controversy about the heritable nature of group differences in intellectual functioning, we are presenting these initial results now. In brief, we find a highly similar cognitive structure in AJA and AEA subjects.

Guttman and Guttman (*3*) called attention to the desirability of employing intercorrelation patterns, rather than means or variances, in cross-ethnic studies of mental traits because the lat-

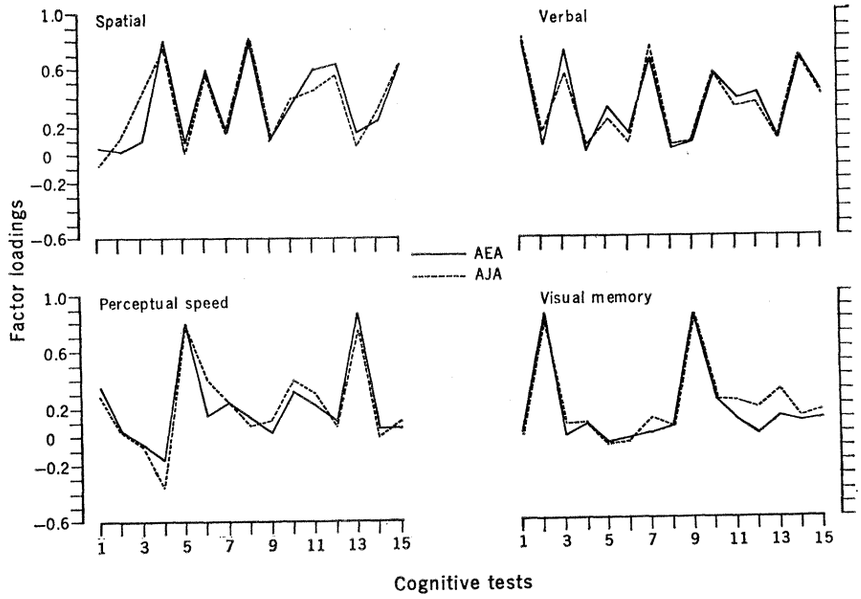

Fig. 1. Loadings of 15 cognitive tests on four factors in Americans of Japanese (*AJA*) and of European (*AEA*) ancestry.