tein determined by the streptomycin-resistant locus is P_{10} and is different from the so-called K-protein (*14*), which is P_5 according to our nomenclature (*11*). Thus, the protein determined by the spectinomycin locus is different from that determined by the streptomycin locus or from the K-protein.

The function of the spectinomycin protein (P_4) has been studied previously (*8*). Omission of this protein from a reconstituted 30*S* subunit caused only a partial decrease in incorporation activity, assayed with both synthetic and natural messenger RNA (mRNA). However, omission of the streptomycin protein (P_{10}) during reconstitution gives a particle that shows a drastic decrease in incorporation activity when assayed with natural mRNA, but only a weak decrease with a synthetic mRNA (*11*). Thus, the functions of these two proteins (P_4 and P_{10}) appear to be different.

However, the function of the P_4 protein was studied with a partial reconstitution system and the possibility of contamination of "P_4-deficient particles" by P_4 could not be excluded (*8*). The reconstitution system now available, which allows 30*S* subunit formation from 16*S* RNA and each of the purified ribosomal protein components, should give more definite information as to the functional role of the protein controlled by the spectinomycin locus.

A. BOLLEN
J. DAVIES
Biochemistry Department, University of Wisconsin, Madison 53706
M. OZAKI
S. MIZUSHIMA
Laboratory of Genetics, University of Wisconsin

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Memory-Blocking Agents: Effects on Olfactory Discrimination in Homing Salmon

Abstract. *Homing salmon were injected intracranially with puromycin, actinomycin D, or cycloheximide. From 4 to 7 hours after such treatment these agents markedly inhibited olfactory bulbar discrimination between home water and other natural waters, including spawning sites for other groups of salmon. At longer intervals after treatment there was a partial restoration of olfactory memory-based discrimination. The dosages of the inhibitors used could be shown to have depressed incorporation of H^3-leucine into protein by 78 percent or of H^3-uridine into RNA by 41 percent in the salmon brains 4 hours after intracranial injection. These findings suggest that acute blockage of RNA synthesis or protein synthesis can interfere with long-term olfactory memory in anadromous salmon, at least as this function can be analyzed by electrophysiological methods. This implies that long-term olfactory memory depends upon continued metabolism of RNA and continued protein synthesis.*

A rapidly growing literature (*1*) indicates a relation between memory and cellular-genetic and protein-synthetic activity in the central nervous system. Much of the research in this field has utilized the goldfish (*2*) and, more specifically, relates DNA-dependent RNA synthesis [but not DNA synthesis (*3*)] to establishment of long-term memory. Actinomycin D and puromycin block establishment of long-term memory (*4*); short-term memory apparently is not dependent on this mechanism (*2*). Although there seems to be agreement that RNA synthesis is part of a memory-establishing mechanism, there is no information concerning the character of the residual memory process. Transfer of learned behavior in goldfish and rats through preparations of brain RNA (*1, 5*) would suggest persistence of long-lived RNA or de-

Table 1. Effects of puromycin, actinomycin D, or cycloheximide on electroencephalographic (EEG) olfactory discrimination in Chinook salmon that had homed to the University of Washington College of Fisheries pond. The mean evoked response is expressed as arbitrary values obtained by planimetric measurement of the integrated olfactory bulbar response. For further explanation see Fig. 1. Soos refers to Soos Creek; U.W. to the University of Washington College of Fisheries.

Treatment	Dosage	No. of fish	Integrated bulbar EEG response to test water: Mean evoked response to pond water*	Mean ratio of responses × 100 (%): Soos/U.W. fisheries	Mean ratio of responses × 100 (%): Skykomish/U.W. fisheries
	4 to 7 hours after intracranial injection				
Saline	0.05 ml	12	1.44 ± 0.06	49.8 ± 3.9	82.1 ± 4.8
Puromycin HCl	500 μg	8	0.95 ± .13†	94.8 ± 7.9‡	115.4 ± 8.2§
	1000 μg	2	.58 ± .02‡	110.3 ± 0.5‡	110.0 ± 0.7
Actinomycin D	100 μg	9	.99 ± .11‖	84.0 ± 4.3‡	121.3 ± 7.6‡
	200 μg	4	1.03 ± .17	87.0 ± 4.8	120.0 ± 11.8‖
Cycloheximide	1000 μg	5	1.07 ± .09§	95.4 ± 3.6‡	105.6 ± 7.8†
	9 to 28 hours after intracranial injection				
Saline	0.05 ml	4	1.28 ± 0.09	45.8 ± 8.5	74.5 ± 8.5
Puromycin HCl	500 μg	4	1.18 ± .15	68.8 ± 9.7	78.0 ± 8.6
Actinomycin D	100 μg	8	1.13 ± .08	74.1 ± 8.3	86.1 ± 4.1
Cycloheximide	1000 μg	4	0.92 ± .09	70.7 ± 2.1†	96.0 ± 6.0

* University of Washington fisheries pond. † $P < .025$. ‡ $P < .001$. § $P < .005$. ‖ $P < .01$.

rivative protein, or both. On the other hand, Rappoport and Daginawala (*6*) recently showed that each olfactory experience in catfish leads to production of new and characteristic RNA's (as judged by changes in base ratio). Other evidence of rapid qualitative changes in neuronal RNA's comes from work such as Edström's (*7*) which concerns the changes in the Mauthner nerve fiber of goldfish after spinal cord transection.

In an attempt to reconcile evidence of rapid changes in nervous RNA with the seemingly long-lived RNA-dependent mechanisms on which memory may be based, we conducted experiments on long-term olfactory memory in spawning salmon. We tested the effects of blocking agents for RNA or protein synthesis on the discrimination of various natural waters by homing salmon.

Homing salmon have been found to be able to distinguish, electrophysiologically, "home water" from other natural waters (*8*). Since, in such tests, discrimination always occurs between home water and waters in which the salmon has never been, the discrimination can be taken as an expression of long-term olfactory memory; such memory, in normal guidance of upstream migration, must persist over a period of 3 to 4 years (*9*).

A total of 60 small male ("jacks") Chinook salmon (*Oncorhynchus tshawytscha*) were taken from the pond into which they had migrated to spawn on the University of Washington campus. Upon removal from the spawning pond, the salmon were injected intracranially with an antimetabolite (actinomycin D, puromycin HCl, or cycloheximide; see detailed protocol in Table 1) or with an equivalent volume of physiological saline solution. All fish were kept in the same large, round concrete tank for 4 to 28 hours after injection.

The methods for exposure of the brain, stimulating the olfactory bulb by infusing tested waters into the nostril, and recording the electrical changes evoked in the olfactory bulb have been described (*8, 10*) earlier. Tested waters were taken from three spawning areas: the University of Washington College of Fisheries pond, the Green River State Hatchery (near Auburn, Washington), and the Skykomish State Hatchery, 60 miles (100 km) northeast of Seattle. Samples were obtained on the same day and frozen samples were thawed on the day of testing.

To test the adequacy of the doses of actinomycin D and puromycin, 15 salmon were used in a separate experiment. These tests showed that 500 μg of puromycin produced a depression of 78 percent in the incorporation of H^3-leucine into protein in the brain [method of Lowry *et al.* (*11*)], a 30-minute pulse-labeling period being used. The 100-μg dose of actinomycin D produced a 41 percent depression in the incorporation of H^3-uridine into

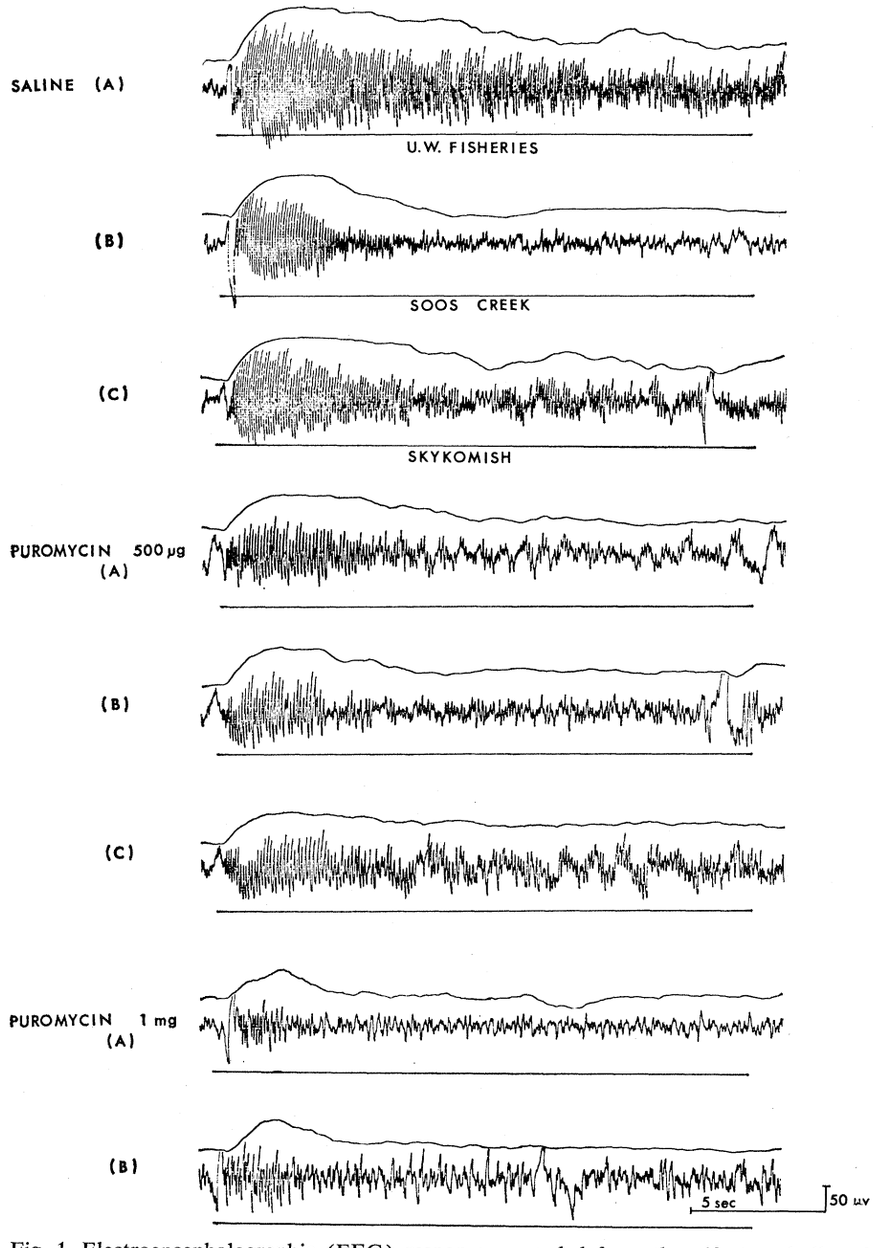

Fig. 1. Electroencephalographic (EEG) responses recorded from the olfactory bulbs of three Chinook salmon (which had homed to the University of Washington campus) during infusion a natural waters through the nostril into the olfactory organ. In each instance, (A) is the home water response; (B) the response to water from the Green River State Hatchery, on Soos Creek near Auburn; and (C) the response to water from the Skykomish State Hatchery. Of the three lines representing each response, the lowest shows the duration of the infusion of each tested water, the middle one is the actual EEG record, and the upper one an electronically integrated summation of the EEG response. The area under the first 10 seconds of the integrated response was measured with a planimeter to give a quantitative expression of the magnitude of each response and these values are summarized in Table 1. The upper group of responses are from a control salmon; the two lower groups are from salmon given puromycin at two indicated dosage levels 4 hours before testing.

total RNA [method of Scherrer *et al.* (*12*) which also employed a 30-minute pulse-labeling period].

The results as outlined in Table 1 showed that all three antimetabolites that we used depressed the mean recorded magnitudes of the olfactory responses to home water, particularly the higher dose of puromycin. However, it is even more significant that the ability of the olfactory bulbar response to differentially respond to home versus nonhome waters was strongly impaired by all of the three antimetabolities. Generally, olfactory discrimination was more strongly inhibited a short time (4 to 7 hours) after antimetabolite administration than it was for a longer period (9 to 28 hours). Thus, blockage of olfactory long-term memory by blockage of RNA and protein synthesis appears to be a temporary phenomenon, and recovery is already well advanced in the 9- to 28-hour interval. With the dosages used, there was relatively little dosage effect upon olfactory discrimination, since such discrimination was almost completely lost at the 4- to 7-hour interval.

The tentative conclusion that may be drawn from these data is that expression of long-term olfactory memory in homing salmon, as defined under our experimental conditions, requires continuous protein or RNA synthesis, or both. If this thesis should be sustained by further research, it would indicate that long-term memory is a continuous metabolic process, not merely a stamping out of long-lived residual template RNA or protein. Since the inhibition was only temporary and long-term olfactory memory was partially restored about 1 day later, it would appear that a residual basis for olfactory memory function outlasted the interruption in RNA and protein synthesis. The nature of this residual factor cannot, of course, be deduced from these experiments. Such a hypothesis adds a new aspect to the study of the chemical basis of memory and it deserves further active investigation.

KIYOSHI OSHIMA
AUBREY GORBMAN
HIRAKU SHIMADA

Department of Zoology, University of Washington, Seattle 98105

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Optics of Arthropod Compound Eye

Abstract. *The extent to which light can escape from one ommatidium into its neighbors in the compound eye has been examined by recording from single receptors during stimulation of single facets. In the "apposition" eye of the drone honeybee and locust, optical interaction is extremely small. In the "superposition" eye of the crayfish, more than half the light captured by the average cell gets in through neighboring facets, even when screening pigments are in the fully light-adapted position.*

Each optical subunit of the typical arthropod compound eye consists of a corneal lenslet of moderate thickness, followed by a crystalline tract: this latter is short in many diurnal animals, but can be extremely long in nocturnal Crustacea and Lepidoptera. Exner (*1*) classified the extreme types with long optics as "superposition" eyes, in the belief that light rays entering many facets could constructively combine to form one erect image on the receptor layer behind, at least in the dark-adapted eye. Upon light exposure, a pigment screen moves to partly surround each optical element in some eyes, supposedly eliminating the superposition process. Exner classified eyes with short optics as "apposition" eyes, these usually having an intervening pigment screen between ommatidia in all conditions of adaptation. The optics and rhabdoms of apposition ommatidia were supposed to act as individual light-guides, so that light entering one facet was confined to that ommatidium by internal reflection (*1*).

Two other variants on the superposition theme exist. First, it has been proposed (*2*) that some kind of usable image may result from the interference of diffraction patterns, supposedly produced by successive facets. Second, Parker (*3*) thought that Exner's idea of cooperative imaging in superposition eyes was implausible, but still believed it possible for light to scatter from one ommatidium to the next, without images being formed.

Each of these concepts shares the feature that light entering one facet can escape into neighboring ommatidia. This was widely believed to be so for the eye of the firefly (*1, 4–6*), but has recently been disputed (*7*). Similarly, Parker's claim (*3*) to have detected the spread of light between ommatidia of dark-adapted crayfish eyes has since been suggested unlikely (*5*) and could not be confirmed (*4*). Finally, apparent receptor sensitivity changes have been reported during migration of screening-pigment in nocturnal moth eyes (*8*), and these changes might well be explained partly by a corresponding change in optical interaction between ommatidia. But because of the gross recording and stimulating techniques used, these effects might equally well be interpreted as showing alterations in either size of the receptive field or the light-attenuating power of individual ommatidia; in similar moth eyes, the crystalline tracts are said to act as light guides (*9*).

It is difficult to evaluate these conflicting observations in the absence of any objective measurement of the extent of light spread between superposition ommatidia (*10*). Such measurements are discussed here, made with electrical recordings from single photoreceptors in response to light flashes delivered to single facets of the eye. The apposition eyes of the bee and locust are compared with the superposition eye of the crayfish.

Individual retinula cells were penetrated with micropipettes filled with KCl,