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4. These studies were conducted under contract HSM-42-71-95 between the Center for Studies of Narcotic and Drug Abuse of the Division of Narcotic Addiction and Drug Abuse, NIMH, and the Research Triangle Institute. We thank Drs. Monique Braude and Stephen Szara, Center for Studies of Narcotic and Drug Abuse, NIMH, for interest and encouragement. We thank Daynise Skeen for technical assistance.

3 April 1972

Synthetic Scotophobin in Goldfish: Specificity and Effect on Learning

Abstract. *Synthetic rat scotophobin was injected intracranially into common goldfish* (Carassius auratus) *which were then trained to avoid light or dark. The substance interacts with the learning process in goldfish in an apparently specific way, facilitating the acquisition of dark avoidance, a task homologous with that acquired by rats from which the natural peptide was isolated, while inhibiting acquisition of light avoidance.*

The isolation and characterization of scotophobin (*1*, *2*), a molecule formed in the brains of rats learning to avoid a darkened compartment, and its synthesis (*3*) and reported activity in mice (*4*) raise the question of the activity of this learning-linked rat peptide in other vertebrates.

We have studied the effect of synthetic scotophobin (*5*) in *Carassius auratus*, the common goldfish. In addition to our work with the goldfish (*6*), evidence that scotophobin may be active in goldfish has been reported by Guttman *et al.* (*7*), who found in fish screened for dark preference that scotophobin depressed a high preinjection level of time in the dark in a procedure not involving learning. Our work bears on the question of the specificity of the scotophobin effect in goldfish, using a procedure in which fish injected with scotophobin were trained to actively avoid light or dark. In this procedure, we have used normal fish rather than animals selected for dark preference, as in other reports (*1*, *7*). We have found that scotophobin appears to interact with the learning process in goldfish in a specific way, facilitating the acquisition of dark avoidance, a task homologous with that acquired by the rats from which the natural peptide was isolated, while inhibiting acquisition when the cues are reversed.

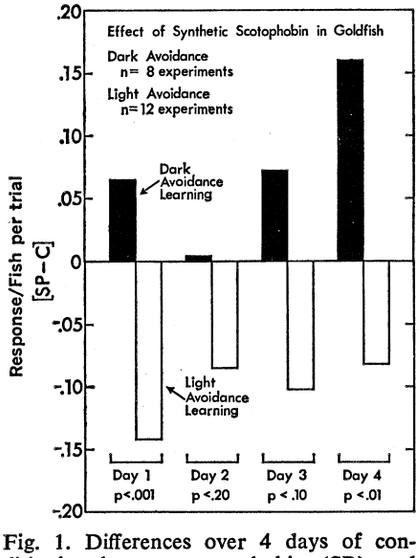

Fig. 1. Differences over 4 days of conditioning between scotophobin (SP) and control (C) fish in acquisition of active dark-avoidance responding (black bars), and between SP and C fish in acquisition of active light-avoidance responding (white bars). Positive values indicate faster learning in SP fish; negative values indicate slower learning in SP fish. Measure is the response per fish per trial.

Common goldfish 8 to 10 cm long were, before use, kept in the laboratory at least 1 week after arrival from Ozark Fisheries, Stoutland, Missouri. Fish were maintained in shallow home tanks under constant illumination. In groups of five to ten, fish were trained to avoid light or to avoid darkness in a large "fish shuttlebox" (*8*). The tank was divided by an opaque partition allowing a 4-cm clearance underneath. Electric current could be applied, as described by Fjerdingstad (*9*), across either end of the tank. Two clear stimulus lamps were mounted on the ends of the tank to permit either compartment to be lighted while the other was dark. The entire tank was under a lightproof cover.

Synthetic scotophobin [peptide D (*4*)] was injected in various nominal doses ranging from 12.5 to 120 ng. In all cases the intracranial route was used with an injection volume of 10 μl (*10*). Control fish received injections of the vehicle (*11*). All glassware was siliconized.

Training was begun, one session of ten trials per day, 48 hours after injection and was continued at least 4 days. The learning of scotophobin recipients was evaluated with respect to controls trained concurrently to avoid light or to avoid darkness. A trial for light-avoidance conditioning consisted of a 15-second presentation of the stimulus lamp in one end of the tank, followed by the addition of shock (in that end only) for 45 seconds, after which the lamp came on in the other end, beginning the next trial. The number of light-avoidance responses for a trial was recorded as the number of fish observed to swim under the barrier away from the light before the onset of shock. Only net avoidances were counted; if a fish swam away from the light but returned before the onset of shock, it was not counted as avoiding. Dark-avoidance training was identical with light-avoidance training except for the reversal of cue significance: to avoid shock the fish were required to swim out of the darkened compartment, not into it as in light-avoidance training (*12*).

Figure 1 presents the results of 20 experiments involving a total of 313 fish trained for 4 days to avoid light or to avoid dark (*13*). Negative values indicate slower learning in scotophobin

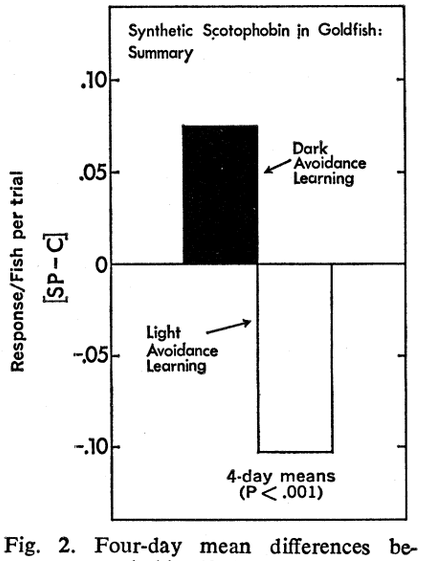

Fig. 2. Four-day mean differences between scotophobin (SP) and control (C) fish in acquisition of dark-avoidance responding (black bar) and light-avoidance responding (white bar).

(SP) than control (C) fish (SP < C), whereas positive values denote faster learning in SP fish (SP > C) (*14*). A differential effect of scotophobin may be seen on light-avoidance and dark-avoidance learning (*15*). This differential inhibition or facilitation varies over the period of conditioning. When averaged across days (Fig. 2), the effect may also be seen.

It is unlikely that the effect of scotophobin should be equally strong at all dosage levels used; however, we have not considered it appropriate in this report to omit any doses or experiments in which the effect was diminished or absent (*16*). Differential effects were observed with the lowest nominal dose (12.5 ng), and these effects were generally absent with the highest dose (120 ng). The optimum dose for facilitating dark-avoidance learning appears to be lower than the optimum dose for inhibiting light-avoidance learning.

Although we worked with the synthetic substance, we cannot of course say that the entire sequence of 15 amino acid residues is required for the activity we observed.

Caution is particularly indicated in "reading into," interpreting, or extrapolating from these data. We regard the work as evidence that scotophobin does have behavioral activity in fish; that this activity is at least consistent with that reported for mice; and that it interacts with learning in goldfish in a somewhat specific manner, either facilitating or inhibiting learning, depending on the task itself. Although a variety of mechanisms has been proposed whereby such an effect might occur (*17*), we do not at this time propose a mechanism mediating the effect.

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5. We thank G. Ungar for samples of synthetic scotophobin. These samples were synthesized by W. Parr and purified by G. Ungar.
6. The activity of synthetic scotophobin in goldfish was described at the 1971 annual meeting of the Society for Neuroscience [(R. C. Bryant, *Society for Neuroscience Abstracts* 1971)].
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11. In the first two experiments, both experimental and control injections contained approximately 0.1 percent (v/v) methanol, since the scotophobin sample obtained was in an aqueous stock solution containing methanol. In all other experiments, the vehicle was distilled water.
12. In this procedure, level of correct responding for the first day was typically 30 percent for light-avoidance controls and near zero for dark-avoidance controls; on the fourth day, the level for light-avoidance controls was approximately 80 percent and that for dark-avoidance controls was 50 percent.
13. Total scotophobin recipients: light-avoidance (LA) fish, 120; dark-avoidance (DA) fish, 91. Controls: LA, 60; DA, 42.
14. The measure is (Response/Fish)/(Trial). For example, if on a given session (day), a total of 17 avoidance responses was recorded for a single group of seven fish during ten training trials, the measure would be (17 Responses/7 Fish)/(10 Trials) = .243 (R/F)/(T).
15. Probabilities in all cases calculated by the two-tailed t statistic, with 18 degrees of freedom (light-avoidance experiments, 12; dark-avoidance experiments, 8). The values on which Figs. 1 and 2 are based were calculated from means of 20 separate experiments, not 20 individual fish.
16. Nominal doses used were 12.5, 25, 30, 50, 60, and 120 ng. Whether the full dose was administered in each case is not assured, since there is some uncertainty as to the degree of stability or nature of degradation of synthetic scotophobin (G. Ungar, R. Bowman, personal communications).
17. See W. L. Byrne, Ed. *Molecular Approaches to Learning and Memory* (Academic Press, New York, 1970).
18. Partially supported by the Tennessee Department of Mental Health and the U.S. Public Health Service. We thank Dr. L. A. Kepner, Dr. B. M. Kulig, Dr. F. Petty, and Dr. O. L. Wolthuis for criticism and discussion.

27 April 1972

Capillary Suction Test of the Pressure Gradient Theory of Amoeboid Motion

A contraction-hydraulic (rear contraction) theory has been proposed to explain the amoeboid movement of *Amoeba proteus* (*1, 2*), *Pelomyxa palustris* (*3*), and *Endamoeba invadens* (*4*), as well as certain other amoebae. In the contraction-hydraulic theory a positive pressure gradient is responsible for protoplasmic flow. Recently, Allen *et al.* (*5*) sucked the posterior ends of specimens of *Chaos carolinensis* and *Amoeba proteus* into capillary micropipettes subjected to a pressure reduction of 30 to 35 cm of water. When this pressure was applied, protoplasm flowed backward into the pipettes, but the pseudopodal flow continued in the opposite direction. Allen *et al.* assumed that only endoplasm was flowing into the pipette and that lack of reversal of pseudopodal flow ruled out the presence of a contraction-hydraulic mechanism in these pseudopods. The frontal contraction model (*6*) was proposed as an alternative mechanism. In support of their theory Allen *et al.* also cite the observation of Kanno (*7*) that sucking more than half the cytoplasm from the tail region of a proteus-like amoeba that is moving in a forward direction in a capillary still allows forward movement to continue.

A contraction-hydraulic mechanism explains the data of Allen *et al.*, since their pipettes must have withdrawn mostly or only ectoplasm (gel). They made the assumption that the pipette withdrew mostly or only endoplasm (sol) from the amoeba. The diameter of their pipettes prohibited insertion into the amoeba, and also prohibited the withdrawal of only endoplasm. As the ectoplasm was sucked into the pipette (arrow 1 in Fig. 1), gel was drawn in from the sides (arrows 2 and 3). Because of the structural viscosity (*8*) of the gel and its glutinosity, ropiness, and elasticity (*9*), gel should be drawn in from the sides. This will squeeze the sol and force it forward (arrows 4, 5, and 6). In this way a positive and not

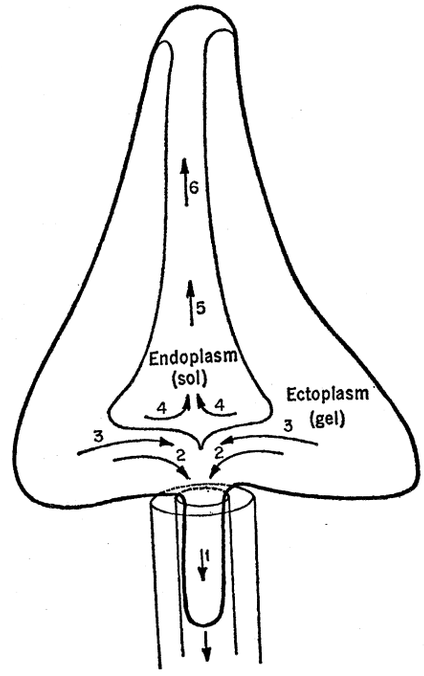

Fig. 1. Flow of ectoplasm and endoplasm according to the contraction-hydraulic theory.