Behavior Patterns in Cod Released by Electrical Stimulation of Olfactory Tract Bundlets

Abstract. *The olfactory tracts in the cod (*Gadus morhua *L.) consist of four bundlets. Three were cut symmetrically on both sides leaving only one of the four bundlets intact. Weak electrical pulses given to the uncut bundlets elicited, for each, a different behavior pattern in free-swimming cod. The results support a spatial basis for olfactory discrimination and open new experimental possibilities linking the neuroanatomical substrate and fish behavior.*

In fishes, the olfactory sense constitutes an information pathway important for a variety of life processes, such as feeding, social interaction, and migration (*1*). The actual repertoire of behavior patterns in fishes is limited in number compared with that of higher vertebrates, yet it is virtually unknown which specific action patterns are released via the olfactory system. Our experiments show behavior patterns in cod that can be induced through this system. Electrical stimulation of each of the four discrete olfactory tract bundlets elicits, for each, a different pattern.

The first experiments demonstrating that electrical stimulation of the olfactory system can induce a specific behavior pattern were carried out by Grimm (*2*). He showed that by stimulating the olfactory tract, which conveys information from the bulb to the brain proper, he could induce feeding behavior in goldfish. The long olfactory tract in gadoids, cyprinids, and silurids can be divided into several bundlets. Each enters different brain nuclei, which would reasonably imply a different function (*3*). The compound action potential of each bundlet reveals at least two components that can be correlated with the fiber spectra of its axons (*4*).

The olfactory system of the cod (*Gadus morhua* L.) has the same characteristic elements as that of other vertebrates. The receptors are situated on the leaves of the olfactory rosette, and their axons form the short olfactory nerve. In the olfactory bulb, the large number of axons terminate in discrete structures where they make synaptic contact with secondary neurons. The axons of the secondary neurons make up the majority of the fibers in the olfactory tract. The central aspect of this long olfactory tract in cod can be divided into four distinct bundlets, which can be separated under the microscope. Our results were obtained by cutting three of the four bundlets and leaving the fourth intact. The remaining bundlets on both sides were hooked on bipolar stimulating electrodes of platinum wire, which were cemented to the skull. These electrodes were connected by thin leads to a pulse generator. During surgery the fish were anesthetized with tricaine (MS-222 Sandoz). They recovered within half an hour in observation tanks (1.2 by 0.5 by 0.6 m) and were free to move about with the leads attached. The electrical shocks were between 0.3 and 6.0 μA and 0.3 msec long. These stimulus intensities were lower than those used for studying responses of fish to stimulation of central brain regions (*2, 5*).

Fish in which the lateral part of the lateral tract was left intact took a position in the water with head down; usually the barbel and first rays of the pelvic fins touched the bottom (Fig. 1). Electrical stimulation at low intensities made the fish regain the head-down position even when it had not previously been in this position. The stimulated fish descended to the bottom and then moved backward using the pectoral fins and tail. Increasing the shock intensity resulted in more rapid adjustment to the head-down position and also in a greater angle between the fish and the bottom. Higher shock intensities induced rapid turns. The behavior could be induced by shocks of frequency as low as 4 to 8 Hz, but it occurred more rapidly when the frequency was increased as far as 40 Hz. Above this no further change appeared. Normally a frequency of 20 Hz was used. In this and other behavior patterns, males and females reacted alike.

The medial part of the lateral olfactory tract is fanlike. When only this bundlet was intact, the fish swam restlessly and more rapidly than normal. Electrical stimulation of this part of the tract in fish at rest induced immediate swimming. Increasing the shock intensity above a certain level induced such rapid snapping of the jaws that it could not properly be studied on film (24 frames per second). When the duration of the volley of shocks was long, only one snap at the beginning of each pulse train was induced.

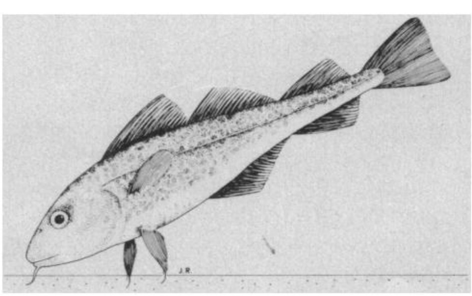

Fig. 1. Position taken by a cod in which only the lateral bundlets of the lateral olfactory tracts were intact.

The lateral part of the medial tract contains a large number of thin unmyelinated and some myelinated fibers (*4*). Fish that had only this bundlet intact swam slowly around in a head-up position with their snouts usually above water. Electrical stimulation of this bundlet induced this posture in a fish irrespective of previous behavior. At high shock intensity, the fish quivered and rapid muscle contractions could be seen on the sides of the body while the body of the fish remained in a straight position.

Fish with only the medial part of the medial olfactory tract intact lay quietly on the bottom with the pelvic and anal fins touching the gravel bottom. Electrical stimulation of the tract made them lift their pelvic fins backward, pressing the heart region to the bottom. Several trains of shocks in a row, each of which induced this behavior, made the fish change coloration. White spots appeared on the dorsal part of the body, and contrast stripes appeared on the sides. Increasing the strength of the stimulus made the fish move backward in close contact with the bottom. A still higher intensity induced a rapid snapping, followed by retreat movements as described above.

It is tempting to associate the different behavior patterns with various action patterns that can be seen in normal fishes. These behavior patterns certainly have survival value. In this discussion, we shall rely heavily on the description of cod behavior given by Brawn (*6*). The head-down and backing movement seen when the lateral part of the olfactory tract is stimulated resembles the behavior described in observations of feeding cod (*6, 7*). The head-down and backward movement can be attributed to a fixed action pattern adjusted to finding benthic prey. The sensory organs on the barbel and pelvic fins would give the fish additional information on the prey's location as these structures are dragged along the bottom.

The restless swimming seen in the fish with the medial part of the lateral olfactory tract intact also seems to fit into a food-searching pattern. Because the sense of smell detects no vector information, the orientation reaction induced by olfactory stimuli is necessary. When there is no other stimulus present to indicate direction, the orientation reaction

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will be a random search. When there is a directional cue, like the presence of a stream that the fish can sense, the animal will move upstream when it smells prey. The snapping movement seems to be an attempt to catch the prey. A high stimulus intensity was needed to induce snapping. These shocks presumably recruited nerve impulses in the thin axons of the olfactory tract. The results indicate that a certain concentration or certain odors must be present to elicit this behavior.

The head-up position taken by the fish that had only the lateral part of the medial olfactory tract intact is more difficult to associate with any particular behavior. It might be pertinent that, in her description of the cod's reproductive behavior, Brawn (*6*) noted that upward swimming is an invitation to the female from the male to spawn. If the female is receptive, she follows the male to the surface, or the thermocline, where spawning takes place. Spawning includes a horizontal swim with a stiff body and quivering movements. Although quivering movements were seen in our experiments, they were not associated with horizontal swimming. Our experiments were performed too late in the season to see if spawning was actually induced by electrical stimulation of this or other tract bundlets.

When the cod is afraid, it assumes a skin coloration with white spots on the dorsal part of the body, while dark and pale vertical areas appear on the sides (*7*). If the fish does not find a hiding place when afraid (as it could not in the observation tank), it "freezes" to the bottom. The detailed manner in which this occurs has not yet been fully described. The pressing of the body against the gravel and the slow movements of the fins and tail, together with slow respiratory movements, may indicate fear behavior. The rapid snapping, similar to that observed with stimulation of the medial part of the lateral tract, with the retreat movement following immediately afterward may in this situation be a defense mechanism.

The distinct modification of behavior induced by eliminating the input from three out of four olfactory tract bundlets in fish opens a new possibility for investigating the neuroanatomical substrate for certain behavior patterns. The origin of fibers in a specific bundlet can be traced back to their cell somata in the olfactory bulb, and the position of these neurons can be determined. Experiments to determine the bulbar position of the neurons composing the different bundlets are still needed. Finger has described the central termination of the olfactory tract fibers in catfish, dividing the tract into two bundles (*3*). A further subdivision of the olfactory tract might provide information about the exact location of the regions associated with a specific behavior pattern.

It is reasonable to hypothesize that each specific behavior described here can be evoked by a distinct odorant or group of odorants. If such is the case, it would imply that the coding of olfactory information has a spatial basis. The results of recent studies on the organization of the olfactory bulb in salmonid fish show that the medial part of the olfactory bulb responds to bile salt while the lateral part responds to amino acids (*8*). Neurons situated in the medial part of the bulb project mostly to the medial tract, and neurons in the lateral part to the lateral tract (9). Thus, we suggest that behavior associated with food search may be elicited by substances including and resembling amino acids, whereas other behaviors may be induced by bile salts or analogs. Feeding behavior has been reported to be induced in fish by amino acids and related substances (*10*).

The experiments needed to uncover the substances that induce feeding behavior or other action patterns are often difficult to perform and time-consuming. The results of our experiments have shown that specific changes in behavior are released when the cod receives olfactory information. Such specific behavior patterns are easy to identify in a bioassay and will thus facilitate the search for the substances that induce them in cod.

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References and Notes

1. H. Kleerekoper, *Olfaction in Fishes* (Indiana Univ. Press, Bloomington, 1969), p. 222; J. E. Bardach and T. Villars, in *Chemoreception in Marine Organisms*, P. T. Grant and A. M. Mackie, Eds. (Academic Press, New York, 1974), p. 49; J. Atema, in *Olfaction and Taste*, F. LeMagnen and P. Macheod, Eds. (Information Retrieval, London, 1977), vol. 6, p. 165.
2. R. J. Grimm, *Science* **131**, 162 (1960).
3. R. E. Sheldon, *J. Comp. Neurol.* **22**, 177 (1912); T. E. Finger, *ibid.* **161**, 125 (1975); S. O. E. Ebbesson, *Comp. Biochem. Physiol.* **42A**, 121 (1972).
4. K. B. Døving, and G. Gemne, *J. Neurophysiol.* **28**, 139 (1965); R. A. Westerman and J. A. F. Wilson, *Z. Zellforsch. Mikrosk. Anat.* **91**, 186 (1968).
5. L. S. Demski, D. H. Bauer, J. W. Gerald, *J. Exp. Zool.* **191**, 215 (1975); G. E. Savage and M. G. Roberts, *Brain Behav. Evol.* **12**, 42 (1975).
6. V. M. Brawn, *Behaviour* **18**, 107 (1961); *ibid.*, p. 177; *J. Fish Res. Board Can.* **26**, 583 (1969).
7. M. G. Pawson, *Comp. Biochem. Physiol.* **56A**, 129 (1977).
8. G. Thommesen, *Acta Physiol. Scand.* **102**, 205 (1978); K. B. Døving, R. Selset, G. Thommesen, paper presented at the 3rd Congress of the European Chemoreception Research Organization, Pavia, Italy, 10 to 14 September 1978; *Acta Physiol. Scand.*, in press.
9. K. B. Døving and M. Dubois-Dauphin, unpublished observations.
10. A. W. Sangster, S. E. Thomas, N. L. Tingling, *Tetrahedron* **31**, 1135 (1975); A. M. Sutterlin, *J. Fish. Res. Board Can.* **32**, 729 (1975).
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