THE JOURNAL OF EXPERIMENTAL ZOOLOGY 216:327-329 (1981)

RAPID COMMUNICATION

Induced Locomotion by Midbrain Stimulation in Restrained Skipjack Tuna, *Katsuwonus pelamis*, L.

S.M. KASHIN, R.W. BRILL, W.N. IKEHARA, AND A.E. DIZON
Pacific Gamefish Foundation, Honolulu, Hawaii 96825

ABSTRACT We show that electrical stimulation of the midbrain of restrained and sedated skipjack tuna (*Katsuwonus pelamis*) produces coordinated locomotory activity. Therefore, our preparation is a viable alternative for creating normal swimming movements which can be closely controlled by the experimentalist, and demonstrates that a midbrain "locomotory center" is present in skipjack tuna, as in other teleosts. *Key words:* induced locomotion, midbrain stimulation, skipjack tuna, *Katsuwonus pelamis*

Because tunas are both highly active and physiologically very delicate, it is difficult to successfully force them to swim in a water tunnel. Most die immediately or survive for only a few hours under such conditions. Although the electromyograms (EMG) of swimming skipjack tuna (*Katsuwonus pelamis*) have been successfully recorded (Brill and Dizon, '79), only one simplest instrumentation can be employed on tuna to be swum in a water tunnel because the fish must be transferred from their holding tank to the swimming chamber quickly, or they will not regain equilibrium.

Normal locomotory movement can be induced by electrical stimulation of the brain (Kashin et al., '74) and spinal cord (Grillner, '74) of fishes. Such preparations allow better control of experimental conditions and thus greatly facilitate investigations of the parameters of locomotion. We therefore decided to investigate the feasibility of inducing coordinated locomotory movements in skipjack tuna by selectively stimulating regions of the brain shown to effect locomotion in other teleost species.

Our goals were (1) to locate the "locomotory center" in the brain of tuna, and (2) to produce a preparation where rhythmic swimming movements could be induced and controlled by electrical stimulation of the "locomotory center" in restrained tuna.

MATERIALS AND METHODS

We used four skipjack tuna, ranging in fork length from 39.4 to 40.0 cm and in weight from 849 to 915 gm. The fish were selected from stocks maintained at the Kewalo Basin Laboratory of the National Marine Fisheries Service (Honolulu, Hawaii). Nakamura ('72) provides details on the capture and handling of tuna at this facility.

Each fish was dip-netted, injected intramuscularly with 37.5 mg of anaesthetic (sodium thiopental), and returned to its holding tank.

When the fish could no longer swim (within 5 to 10 minutes), it was removed from its holding tank and placed in a plexiglass restraining trough. The head was clamped firmly to a stainless-steel seawater delivery tube that was placed in the fish's mouth for irrigation of the gills. The anterior half of the fish's body was held by foam rubber and plastic foam packed between the fish and the sides and the bottom of the restraining trough. The posterior portion of the fish, from the anterior margin of the anal fin to the caudal fin, was thus suspended in air.

The cranium roof above the cerebellum was removed. In some preparations, the forebrain was also removed in an attempt to prevent spontaneous locomotion. However, removal of the forebrain had no discernible effect on either the occasional bouts of spontaneous locomotory movements or on those induced by brain stimulation. Using a micromanipulator, monopolar or bipolar stainless-steel stimulating electrodes were pushed through the portion of the cerebellum that overlay the visual

Send all correspondence and reprint requests to R.W. Brill, Pacific Gamefish Foundation, P.O. Box 25115, Honolulu, Hawaii 96825.

S.M. Kashin's present address is Institute of Oceanology, Academy of Sciences, Moscow (USSR).

W.N. Ikehara's present address is Department of Zoology, University of Hawaii, Honolulu, Hawaii 96822.

A.E. Dizon's present address is National Marine Fisheries Service, P.O. Box 3830, Honolulu, Hawaii 96812.

0022-104X/81/2162-0327$01.50

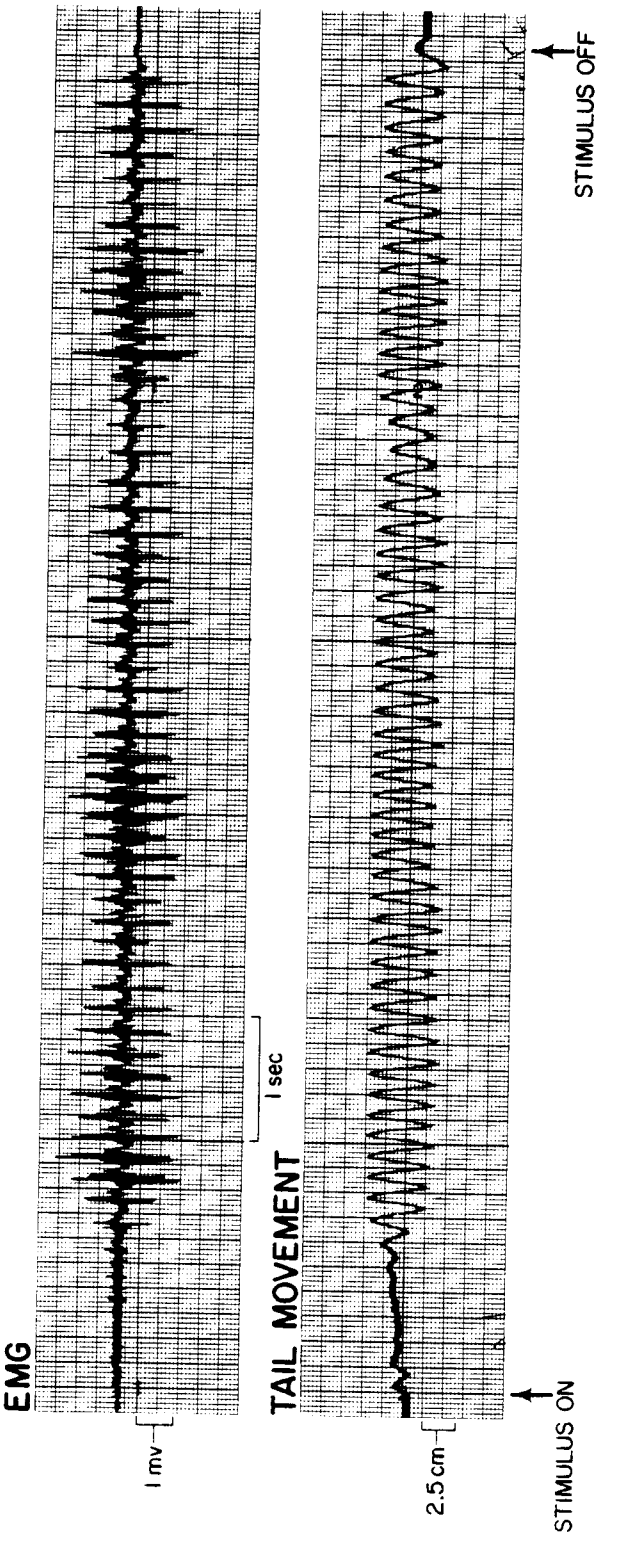

Fig. 1 EMG and tail movement recorded during brain stimulation. The EMG electrode was inserted into the red muscle portion of the myotomes on the right side of the fish; upward pen movement indicates tail flexure to the right.

lobes and into the midbrain. The electrodes were insulated needles with the uninsulated tips measuring 1-2 mm. The ends of the electrodes were positioned approximately 9 mm below the surface of the cerebellum. Both monopolar and bipolar square-wave stimulation were applied. The duration of the stimuli was 1.5-5 msec, the frequency 80-120 Hz, and the applied voltage 0.1-5 volts.

Muscle activity was monitored by EMG electrodes inserted into the lateral red muscle behind the dorsal fin. Tail movements were measured by a rotary potentiometer that was suspended directly above the caudal fin, and attached to the caudal peduncle by an aluminum rod. EMG and tail movements were recorded simultaneously on a two-channel pen recorder.

Additional intramuscular doses of sodium thiopental (25 or 37.5 mg) were administered occasionally to keep the fish quiet.

RESULTS

We found that skipjack tuna have an area in the midbrain, the so-called "locomotory center," which produces symmetrical rhythmic swimming motions when stimulated. The best point of stimulation is along the midline of the brain in the caudal part of the optic lobes, 9-11 mm under the surface of the cerebellum. (In tunas, the corpus cerebelli is elevated forward, covering the optic lobes.) Swimming movements started shortly after the stimulation began, continued as long as stimulation was applied, and ceased as soon as stimulation stopped (Fig 1). The threshold of stimulation which evoked locomotory movements varied from 0.1 to 0.3 V for different fish and for both monopolar and bipolar stimulation.

When the electrodes were 1-2 mm lateral to the midline, rhythmic movements occurred with the body tonically bent toward the side being stimulated.

DISCUSSION

Our results show it is possible to produce locomotory movements in skipjack tuna by the electrical stimulation of the midbrain. We feel that these evoked locomotory movements are "natural" for the following reasons. One, based on the position of the electrodes, it is highly unlikely that we are directly stimulating neurones within the spinal cord. Therefore, the activated neurones (or axons) within the "locomotory center" must be giving the command to swim to the spinal cord, which is the normal situation in teleosts (Grillner and Kashin, '76). Two, the induced tail-beat frequency was generally 5-6 Hz, which would result in a swimming speed of approximately three to four body-lengths per second in free-swimming skipjack tuna (Brill and Dizon, '79). These speeds are often maintained by free-swimming skipjack tuna (Magnuson, '78). Three, it is improbable that under our experimental conditions tuna can learn new unusual ways of swimming.

As was shown in previous work (Kashin et al., '74), the induced swimming movements of *Hexagnammus octogrammus* with implanted chrome electrodes were identical to those of intact fish. Ideally, to prove our hypothesis, we should show that midbrain stimulation is able to induce normal locomotion in free-swimming fish. This is not possible with skipjack tuna, because they must swim continuously to ram-ventilate their gills; and they quickly become hypoxic when their forward locomotion is interrupted.

We feel we have shown that midbrain stimulation in skipjack tuna is a viable alternative for creating normal locomotory activity that can be controlled by the experimentalist. Moreover, the fish used in this study survived and responded to brain stimulation for up to 6 hours. Our technique should therefore allow the biomechanics and energetics of tuna locomotion to be studied in a more controllable situation than is possible in a freely behaving fish.

ACKNOWLEDGMENTS

This work was partially supported by NSF grant ENG-77-21236-A01 to Dr. Theodore Y. Wu (California Institute of Technology), the National Marine Fisheries Service (NOAA) Honolulu Laboratory, and the Pacific Gamefish Foundation (Honolulu, Hawaii).

LITERATURE CITED

Brill, R.W., and A.E. Dizon (1979) Red and white muscle fibre activity in swimming skipjack tuna, *Katsuwonus pelamis* (L.). J. Fish. Biol., *15:* 679-686.

Grillner, S. (1974) On the generation of locomotion in the spinal dogfish. Exp. Brain Res., *20:* 459-470.

Grillner, S., and S. Kashin, (1976) On the generation and performance of swimming fish. In: Neural Control of Locomotion: Advances in Behavioral Biology. R.M. Herman, S. Grillner, P.S.G. Stein, and D.G. Stuart, eds. Plenum Press, New York, Vol. 18, pp. 181-201.

Kashin, S.M., A.G., Feldman, and G.N. Orlovosky (1974) Locomotion of fish evoked by electrical stimulation of the brain. Brain Res., *82:* 41-47.

Magnuson, J.J. (1978) Locomotion by scombroid fishes: Hydromechanics, morphology and behavior. In: Fish Physiology Vol. VII. W.S. Hoar and D.J. Randall, eds. Academic Press Inc., New York, pp. 239-313.

Nakamura, E.L. (1972) Development and uses of facilities for studying tuna behavior. In: Behavior of Marine Animals Vol. 2. H.E. Win and B.L. Olla, eds. Plenum Press, New York, pp. 245-277.