Reciprocal Inhibition as Indicated by a Differential Staining Reaction

Abstract. Neurohistological and neurophysiological studies have shown that the bilaterally represented Mauthner's cells in teleosts are related both structurally and functionally. The VIIIth nerve afferents, as well as the axoaxonal collaterals, display a distribution pattern which supports the concept of polar function of the neuron. Inasmuch as it is possible to alter the staining reaction of both the Mauthner's cells by unilateral stimulation of the entering VIIIth nerve roots, it is proposed that the synaptic endings serve principally as activators and that neuronal excitation or inhibition is determined by the chemical state of the dendrites, the cell body, and the axon hillock region.

In an attempt to relate the structure of the neuron with its function, extensive histological and neurophysiological studies have been made on the bilaterally represented pair of Mauthner's cells which lie in the floor of the medulla oblongata of the teleost. In an earlier report, a differential staining reaction of the various parts of this neuron (dendrites, cell body, and axon hillock region) was described (*1*). At that time it was suggested that this might be indicative of specific intracellular chemical changes characterizing the phenomenon of excitation and inhibition of the neuron.

A new series of studies involving 15 bullheads (ten experimental fish and five controls) has shown that it is possible to induce a differential staining reaction of the two Mauthner's cells. The procedure involved the exposure and stimulation of roots of the VIIIth nerve after craniotomy. Hypothermal anesthesia was used. The VIIIth nerve roots on the right side of five experimental fish were stimulated at threshold level (ten stimulations per second for 1.0 second); the same type of stimulus was applied on the left side in the other five fish. The response to this stimulus was a rapid unilateral flexion of the tail. The brain was removed and processed by a freeze-dehydration technique; this was followed by staining with activated Protargol (*1*). The control fish were treated in the same manner except for the nerve stimulation.

The staining reaction of the Mauthner's cell on the same side as the applied VIIIth nerve stimulation was characterized by deeply stained dendrites and a somewhat lighter cell body, while the axon hillock and the proximal portion of the axon appeared to be an almost translucent lavender. In contrast, the Mauthner's cell contralateral to the applied stimulus stained in an opposite manner, with the den-

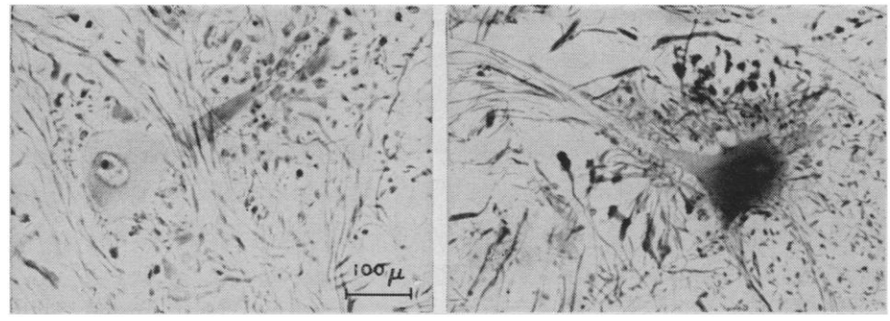

Fig. 1. Photomicrograph of the bilaterally represented Mauthner's cells, showing the differential staining reaction produced by unilateral stimulation of the VIIIth nerve afferents. The cell on the side of the applied stimulus (right) shows deeply stained dendrites and cell body and a lightly colored axonal region. The dendrites and cell body of the contralateral cell (left) stain a light color, while the axonal region is colored a deep purple. The orientation of the photographs is the same as that of the cells in the medulla oblongata of the bullhead. The axon of each cell is directed toward the center and somewhat upward.

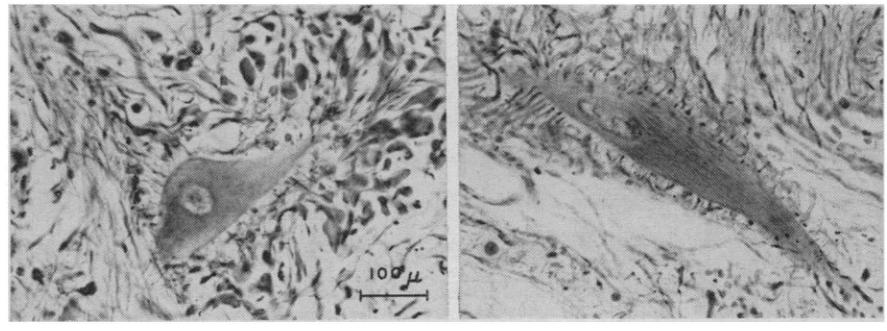

Fig. 2. Photomicrograph of the Mauthner's cells in the control fish. Note the uniformity of the staining reaction of the cytoplasm in contrast to the staining seen in the experimental bullheads. The orientation is the same as in Fig. 1.

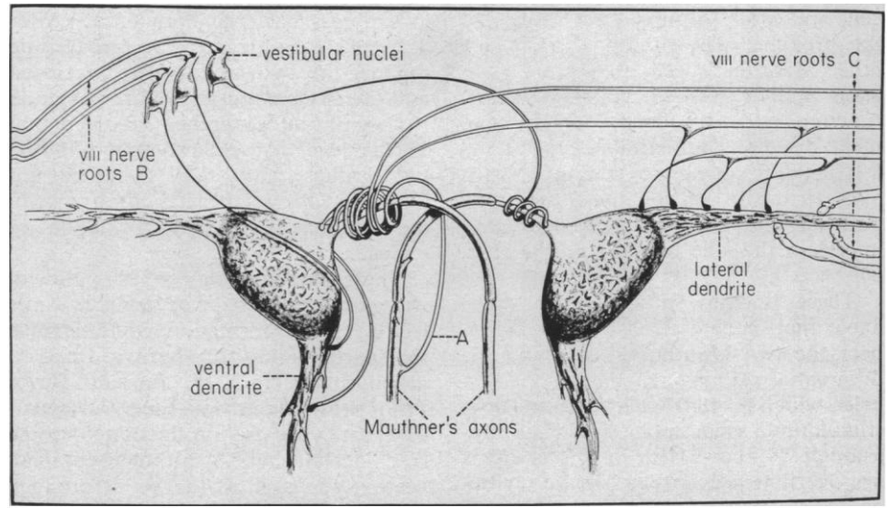

Fig. 3. Schematic representation of VIIIth nerve-Mauthner's cell in a teleost. (*A*) Axon collateral extending from the axon of the "excited" Mauthner's cell to the axon pole of the "inhibited" cell. (*B*) Indirect VIIIth nerve afferents to the vestibular nuclei; these in turn make synaptic contact with the two Mauthner's cells. (*C*) Direct VIIIth nerve afferents which end on the dendrite and cell body of the homolateral Mauthner's cell as well as on the axon pole of the contralateral cell.

drites and cell body colored lavender and the axonal pole of the neuron a deep purple (Fig. 1). Both of the Mauthner's cells in the nonstimulated fish had a rather homogeneous lavender coloration (Fig. 2). In all fish, both experimental and controls, the neuronal elements such as the nucleus appeared to be essentially normal. The surface of the dendrites, the cell body, and the axon hillock region were literally covered with synaptic endings.

In previous neurophysiological experiments it was shown that the observed response of the fish to unilateral stimulation of roots of the VIIIth nerve was a powerful flexion of the tail to one side only, in a one-to-one stimulus-response pattern (*2*, *3*). Also, it was found that bilateral simultaneous stimulation of roots of the VIIIth nerve could not induce the two cells to discharge at the same instant, as was evidenced by recording the evoked potentials from the two Mauthner's axons (*3*).

Histological studies have shown that the distribution of the VIIIth nerve synaptic endings is to the dendrites and cell body of the homolateral Mauthner's cell and to the axon pole of the contralateral cell. This pattern is in accord with the concept of polar function of the neuron, in which it was postulated that the afferent fibers which end on the dendrites and the cell body function to excite neuronal discharge, while those ending on or near the axon hillock serve to inhibit neuronal discharge (*1*, *3*).

A system of axoaxonal collaterals has been found to interconnect the two Mauthner's cells. These branchings arise from the axon of one Mauthner's cell, and after forming a spiral around the proximal part of the axon of the other Mauthner's cell, they end on its axon hillock. These collaterals may function as a feedback loop to augment the direct inhibitory effect of the VIIIth nerve synaptic endings, which also terminate on or near the axon pole of the Mauthner's cell contralateral to the side of their origin (Fig. 3).

These findings of the existence of axoaxonal collaterals which interconnect the two Mauthner's cells and the differential staining reaction of the two cells, which is alterable by afferent fiber stimulation, seem most significant. Inasmuch as the VIIIth nerve afferents are distributed to rather specific regions on the two cells and since it has not been possible to induce both cells to discharge simultaneously even though the entering roots of the VIIIth nerve of both sides are stimulated at the same time, it seems likely that the distinctive cell coloration is indicative of neuronal excitation and inhibition.

It is proposed that in this VIIIth nerve Mauthner's cell system the two cells function as reciprocating units and that the synaptic effect is to alter the intracellular chemical state which results in excitation or inhibition as the case may be. This, of course, implies that the dendrites, the cell body, and the axon pole are of prime functional importance in nervous integration, while the synaptic endings serve as activators rather than as specific excitors or inhibitors (*4*).

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References and Notes

1. E. Retzlaff, *J. Comp. Neurol.* **101**, 407 (1954).
2. ———, *Federation Proc.* **14** (1955).
3. ———, *J. Comp. Neurol.* **107**, 209 (1957).
4. This report was presented in part at the 21st International Congress of Physiological Sciences, Buenos Aires, Argentina, 9–15 Aug. 1959, by Chauncey D. Leake. In the discussion which followed he suggested that the Cl^- ion shift mechanism might be responsible for the differential staining reaction described. It should be noted that G. J. Romanes [*J. Anat.* **84**, 104 (1950)] proposed that Cl^- ions are essential for the effective staining of neurons by the reduced silver method.

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Orientation of Migratory Restlessness in the White-Crowned Sparrow

Abstract. Individuals of two migratory races of white-crowned sparrows (*Zonotrichia leucophrys*) caged under an open sky showed a pronounced orientation in their night restlessness during normal periods of migration for the species. In August and September 1958 most birds showed a southerly orientation at night; daytime activity was random to somewhat northerly. In April and May 1959 most birds showed a strong northerly orientation at night; daytime activity was random to somewhat southerly (*1*).

Several species of caged passerine birds, which normally migrate at night, exhibit night restlessness (*Zugunruhe*) during the season of migration (*2*). This night activity provides a useful device for investigating the energy requirements of migration in these species (*3*).

In Europe several species of birds have been demonstrated to show a seasonal and predictable orientation with respect to migratory restlessness. These birds apparently navigate by the sun if they are day migrants and by the stars if they are night migrants (*4*).

White-crowned sparrows (*Zonotrichia leucophrys gambelii and Z. l. pugetensis*) captured on their winter range in the vicinity of San Jose, Calif., were kept captive in an outdoor aviary on the roof of the Natural Sciences Building on the campus of San Jose State College in mid-town San Jose. Conditions in the aviary allowed the birds to maintain reasonably natural patterns of seasonal weight change, molt, and gonadal development. Birds captured in early 1958 were first tested in August 1958. Most birds tested in early 1959 had been captured in late 1958 and early 1959.

Our activity-orientation cage is a modification of that used by Kramer (*5*) and is designed for continuous automatic recording of activity. Orientation of restlessness was obtained by placing an individual bird in a circular cage 36 in. in diameter and 10 in. high (Fig. 1) under an open sky. All later tests were made with a 24-in. masonite screen around each cage to block out most surrounding "landmarks." Each cage has a central circular perch surrounding its food and water cafeteria, and has around its periphery eight separate activity-sensitive perches. Each perch occupies just under 45° of the 360° circle. Perches are monitored electrically on remotely located Esterline-Angus (20-pen) recorders. One activity-orientation cage was used in late 1958 and early 1959, and four were in operation as of April 1959.

In April and May (1959), birds which had completed their prenuptial molt and which had gained weight to more than 30 gm (normal weight at other periods is 25 to 27 gm) were active at night. This night restlessness was strongly oriented to the north and northwest (see Table 1). Daytime activity tended to be random or to show a slight southerly orientation. The increasing trend toward a northerly orientation during the first two or three nights in which significant activity occurred suggests that restlessness (i) may develop before a sense of

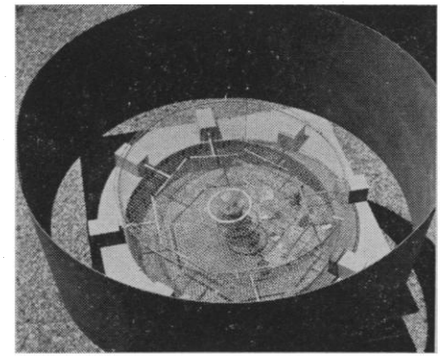

Fig. 1. Activity-orientation cage showing proportions of parts and the position of the 24-in. masonite screen.