similar to the one shown in Fig. 1C, while the f2a had no demonstrable effect on the staining of the chromosomes. The results suggest that the removal of the f1 histone is necessary prior to staining with Giemsa. To substantiate this interpretation, we treated the slides with trypsin (induction of G banding), added f1 and f2a histones (blocking of G banding), treated the cells with trypsin again, and stained with Giemsa. After the second trypsin treatment the chromosomes exposed to the f1 were again banded. However, exposure to the f2a and subsequent trypsin treatment induced no bands, even when the duration of the trypsin treatment was increased.

Another approach was to induce bands on the chromosomes with trypsin, block with either the f1 or f2a fraction, fix again in Carnoy's mixture or 0.2*N* HCl, and stain. This procedure was used to study the effect of fixation on removal of the histones. G bands reappeared. Therefore our data indicated that fixation in Carnoy's mixture or 0.2*N* HCl removed the histone fractions f1 and f2a from the chromosomes, thus allowing for the reappearance of the bands.

It has been reported that fixation in a mixture of ethanol and acetic acid (3 : 1) removed 7 to 8 percent of the total histone from calf thymus chromatin (*5*) and fixation in Carnoy's mixture removed 8 to 20 percent of the histones from nuclei (*2*). These results have been used to support the hypothesis that the histones are not involved in the mechanism of banding. Our data suggest that the histones play an important role in the mechanism of banding. The removal of the f1 and f2a fractions most likely occurs during fixation and appears to be a prerequisite for G banding. Since the Giemsa stain does not seem to interact with the f1 histone fraction, it may be necessary to remove the f1 fraction for staining chromosomes with Giemsa.

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Goldfish Abducens Motoneurons: Physiological and Anatomical Specialization

Abstract. *During natural movements, the motoneurons innervating a single muscle have different patterns of activity that are correlated with differences in synaptic input. The caudal abducens motoneurons fire phasically in synchronous bursts before rapid posterior eye movements; the rostral abducens motoneurons fire only tonically when the eye is fixed or moving slowly. This physiological difference is not related to motoneuron size. In this respect the abducens motoneurons violate the "size principle" that has been advanced for spinal motoneurons. The difference is probably related to the present finding that the caudal but not the rostral cells receive numerous electrical synapses that are known to have a role in synchronizing phasic activity.*

A vertebrate muscle is a collection of motor units, each consisting of a group of muscle fibers innervated by a single motoneuron. Fibers within a particular motor unit are quite homogeneous, but the motor units within a single muscle often exhibit marked physiological, biochemical, and morphological specialization. A rough classification of fibers into "fast" (or "phasic") and "slow" (or "tonic") types is generally recognized (*1*). It has been suggested that the nervous system may have mechanisms for activating different types of motor units in various combinations and patterns. For example, ballistic movements might be initiated by phasic motor units while other types of movements might begin with mobilization of slow motor units capable of long-lasting, tonic activity (*2*).

In support of this idea, Burke and his colleagues (*3*) have demonstrated qualitative physiological differences in the synaptic input to the motoneurons that supply fast and slow motor units in a single muscle. However, it has not yet been demonstrated that the different types of motoneurons supplying a single muscle actually show different activation patterns in naturally occurring movements, nor has it been shown anatomically that different types of motoneurons supplying a single muscle have qualitatively different synaptic inputs. This has been particularly difficult, since in most cases the neurons supplying the different types of motor units are intermingled and are not anatomically distinguishable. We describe here a part of the goldfish oculomotor system in which there is an extremely good correlation between naturally occurring fast and slow movements, anatomical specialization of fibers within a single muscle, and the physiological specialization of its motoneurons. An additional unique feature of the system is that the two physiological classes of motoneurons are grouped in distinct subnuclei. This has permitted us to demonstrate that the two groups of motoneurons differ in their synaptic inputs.

The posterior rectus muscle moves the fish's eye from anterior to posterior in the horizontal plane. Under different conditions, movement can occur as a rapid "saccade" at velocities up to 400° per second or as a slow drift. The muscle also acts in fixing the eye in various positions in the anterior-posterior axis (*4*). The goldfish posterior rectus is about 17 mm long and is composed of about 1100 fibers of two distinct types that are sharply segregated within the muscle (*5*) (Fig. 1B). The large fibers (Fig. 1A) are 8 to 32 μm in diameter, contain few mitochondria, and have no capillary supply. The small fibers (Fig. 1C) are 5 to 14 μm in diameter and, in contrast to the large fibers, are richly supplied with mitochondria and capillaries. Although no physiological or histochemical studies have been performed on this muscle, there are good reasons to believe from studies of other muscles that the large fibers are anaerobic and fast contracting and fatiguing, while the small fibers are aerobic and slowly contracting and fatiguing (*1*).

The motoneurons innervating the posterior rectus lie in two distinct cell groups in the medulla that are separated by about 200 μm in the rostro-caudal axis (Fig. 1F). Each group contains about 50 cells that, when measured in light microscope sections 50 μm thick, range from 11 to 30 μm in diameter. The distribution of cell sizes is the same for the two groups ($P > .89$). Axons from large and small neurons (arrows, Fig. 1F) in each group can be traced directly into rootlets that fuse to form the abducens nerve. The nerve, which in fish innervates only the posterior rectus (*6*), contains about 100 axons that range from 1 to 20 μm in diameter. The good correspondence between the number of axons in the nerve and the number of cell bodies suggests that most of the cells in each group are motoneurons. Furthermore, they must be alpha and not gamma motoneurons since fish do not have muscle spindles (*7*). All these considerations lead to the conclusion that the functional differences we found between the two cell groups cannot be attributed to differences in cell size.

We examined the functions of the two

cell groups by simultaneously recording eye movements and the activity of single neurons. A fish was anesthetized with tricaine methanesulfonate (MS-222) and clamped between two sponge rubber pads with its eyes under water. To record horizontal eye movements, a 1-cm stalk of polyethylene tubing bearing a small flag was fixed by suction to the cornea without obscuring the pupil (*4*). As the eye moved, the flag interrupted a beam of infrared light. The beam was detected by a photocell whose output was amplified and displayed on an oscilloscope. Further details of the system and its calibration will be presented elsewhere (*8*). The fish was respired by flowing aerated water over its gills through a tube tapered to fit its

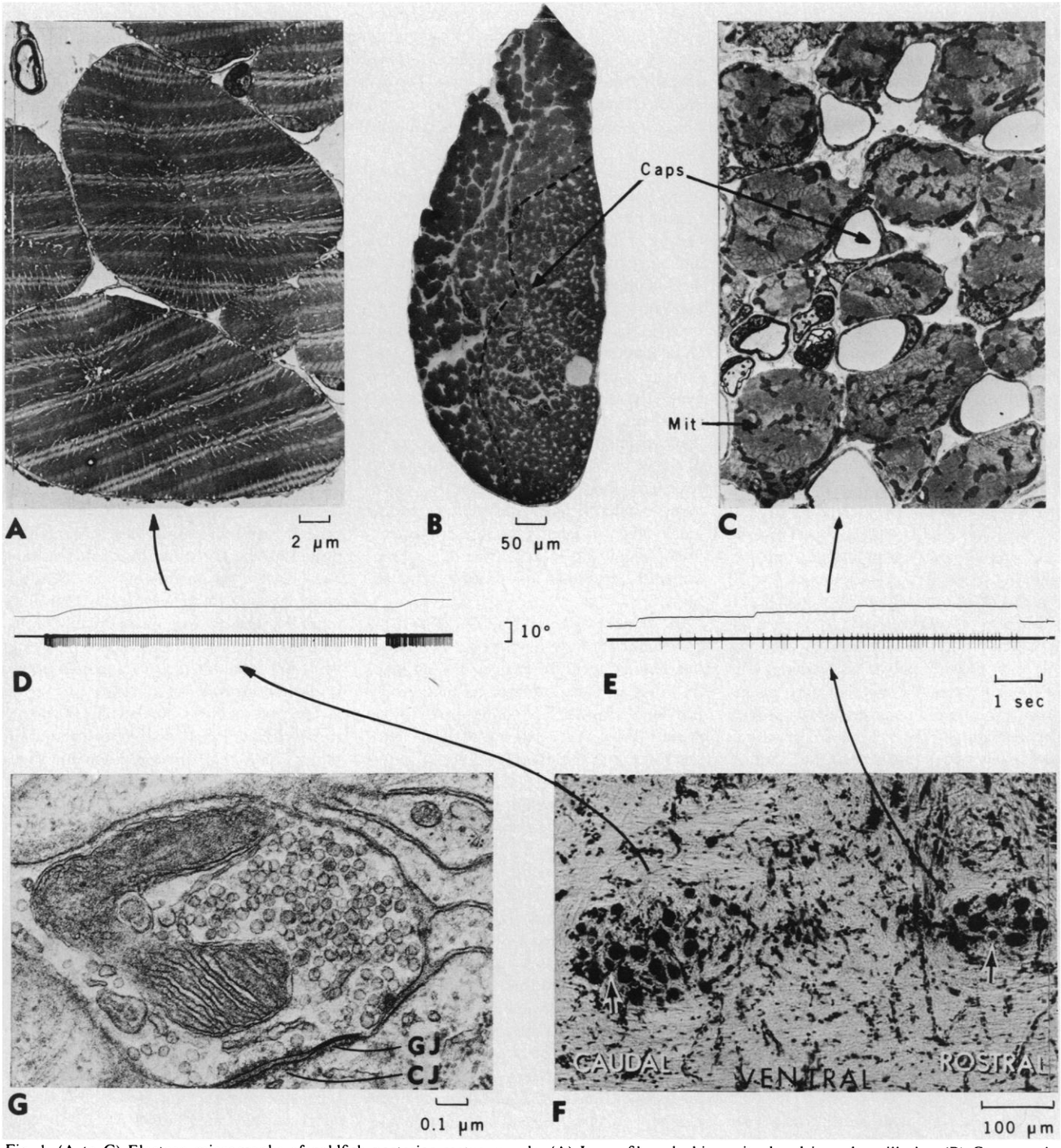

Fig. 1. (A to C) Electron micrographs of goldfish posterior rectus muscle. (A) Large fibers lacking mitochondria and capillaries. (B) Cross section through whole muscle. Dotted line marks major morphological division—large fibers on left, small fibers on right. (C) Small fibers rich in mitochondria (*Mit*) and capillaries (*Caps*). Magnifications in (A) and (C) are the same. (D and E) Records of abducens motoneuron action potentials (bottom) and eye movements (top). (D) Phasic-tonic neuron from caudal cell group fires burst before each posterior saccade and fires tonically in proportion to degree of posterior deviation. (E) Tonic neuron from rostral cell group does not burst with saccades but increases firing as eye moves posteriorly. Note "position threshold" for both cells and the lower maximum firing rate in (E) than in (D). (F) Light micrograph of sagittal section through the two abducens cell groups (Nissl stain). Axons from large and small neurons (short arrows) in both cell groups have been traced into the motor rootlets. Although it is not evident in this section, each group contains about the same number of cells. (G) Electron micrograph of axosomatic terminal in caudal cell group containing round vesicles and making both a chemical (*CJ*) and an electrical (*GJ*) synapse. Gap junction synapses were rare in the rostral cell group.

mouth. Within 15 to 30 minutes after discontinuing anesthesia the fish resumed its spontaneous scanning of the visual field with small horizontal saccades (*4*). Unitary extracellular action potentials were recorded with metal microelectrodes and conventional amplification and display. The abducens cell groups were systematically explored in a series of penetrations, and the coordinates of each unit isolated were recorded with reference to landmarks on the surface of the brain. The coordinates were later transferred to an anatomical map of the area constructed from histological sections. In each experiment, one or more recording points were marked with small electrolytic lesions and later examined histologically.

Roughly 250 units were studied in the general region of the abducens nucleus in 28 experiments. Several classes of cells were found that could not have been motoneurons because their firing did not have the proper relation to the eye movement (*8*). The present report concerns two major classes of neurons consisting of about 50 each, which by their firing patterns and localization within the abducens cell groups were almost certainly motoneurons.

Most of the cells in the caudal cell group fired a burst of impulses immediately before each posteriorly directed saccade and also fired tonically in the period between saccades (Fig. 1D). Impulse frequencies during a burst were as high as 485 per second; maximum tonic rates were as high as 185 per second but were more commonly 40 to 50 per second. The intensity of the presaccadic burst, judged by either the number of impulses in a burst or their frequency, increased monotonically with the size and velocity of the saccade (*8*). For most cells there was no threshold for the bursts; that is, they preceded every saccade, no matter what its size or velocity, and no matter what the position of the eye. The bursts in different cells were well synchronized with each other; the average time by which the beginning of the burst preceded the saccade was 23.7 $\pm$ 5.2 msec (*9*). This period shortened for all cells as the size of the saccade increased, so the degree of synchrony was always about the same (*8*).

The tonic firing during the periods between saccades showed a distinct "position threshold" that differed from cell to cell. Most neurons were silent with the eye in the extreme anterior position and began to fire slowly when the eye reached a particular degree of posterior deviation. Thereafter, the firing rate was proportional to the degree of posterior deviation and, if the eye was moving slowly, also to the velocity.

Neurons in the rostral abducens cell group resembled those in the caudal cell group in that their tonic firing rates increased as the eye moved posteriorly. Like the caudal units, the rostral units also had position thresholds and were sensitive to the velocity components of slow movements. The maximum tonic firing rates of these neurons were commonly 10 to 20 per second, much lower than the rates for the caudal cells. The rostral neurons differed most strikingly from the caudal neurons in that they never fired a burst preceding a saccade. The pattern of change in their firing rates during a saccade varied but was usually characteristic for each cell. Some increased their firing rates before, others during, and still others after a posterior saccade. Some cells made the transition to the new firing rate smoothly, others abruptly, while others paused before assuming the new rate. These observations eliminate the possibility that the rostral neurons could have been sensory elements.

Kriebel *et al.* (*10*) have shown that oculomotor neurons in fish are electrotonically coupled via "gap junction" synapses on their cell bodies. The coupling, thought to be mediated via afferent fibers with electronic junctions on several motoneurons, serves to synchronize the firing of impulses that arise at the cell bodies (*11*). This is useful in promoting rapid synchronous muscle contraction during the fast phase of nystagmus and in the rapid spontaneous movements described here. One would expect that if gap junctions serve to promote the synchrony of the presaccadic burst in motoneurons, their distribution in the abducens nucleus should be restricted to the caudal cell group where the phasic-tonic neurons are found. We examined both cell groups of the abducens nuclei in four fish with the electron microscope at magnifications of 60,000 to 100,000 $\times$. Micrographs were taken of all contacts that could possibly be considered gap junctions. After the micrographs had been scored blindly, the pictures were assigned to their proper cell groups. In almost every section synapses forming gap junctions (Fig. 1G) were found in the caudal cell group, mostly on the cell bodies, but also on proximal dendrites and axon hillock. A few gap junction synapses were found in the rostral cell group, but they were rare, about one-tenth as frequent as in the caudal group.

It appears that during spontaneous movements of the eye, the motoneurons innervating the fish posterior rectus muscle do not behave as a qualitatively homogeneous "pool" (*12*). Neurons in the caudal abducens cell group which probably innervate the large fast fibers of the posterior rectus fire synchronous bursts before rapid eye movements. Neurons in the rostral cell group which probably innervate the small slow muscle fibers fire only tonically when the eye is fixed or moving slowly. The functional differences between the two cell groups cannot depend on differences in cell size (*12*) since the distributions of cell and axon sizes (*8*) in the two groups are virtually identical. Very likely, the functional differences are related to specific differences in synaptic input. For example, the synchronized presaccadic bursts of the caudal neurons may well be synchronized by the gap junction synapses on their cell bodies. Other physiological differences between the two types of cells, such as their different tonic firing rates, may be related to other differences in synaptic input (*8*). It should be possible to identify such differences since the two types of cells are anatomically segregated.

Recordings from abducens and oculomotor neurons in mammals, particularly monkeys (*13*), have revealed mostly phasic-tonic neurons resembling those found in the caudal cell group of the fish, despite the fact that the fiber types in mammalian extraocular muscle are quite diverse (*14*). The present results suggest that the less frequent reports of additional motoneuron types in the mammalian oculomotor system (*15*) should be thoroughly explored.

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