Evoked potentials in cat extraocular muscle

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Cat extraocular muscle consisted of a peripheral portion with red histochemical properties and a central part with white muscle findings. Recordings were obtained from the two regions by extracellular electrodes. Third nerve stimulation produced action potentials which were prolonged monophasic units (slow units) along the edge, and short-duration, biphasic spikes (fast units) in the center of the muscle. Third nucleus stimulation induced an immediate fast eye movement and an electromyographic pattern similar to III nerve stimulation. Vestibular complex stimulation brought about a slow movement that did not begin until several seconds after excitation. The motor unit activity consisted of slow units throughout the muscle which began at the start of stimulation. Intravenous succinylcholine produced fast and intermediate spike activity from all layers but very few slow units. Evoked fast eye movement appeared to be accompanied by fast and slow action potentials while slow vestibular movement was associated with only slow units. The motor unit pattern appeared to correlate with the histochemical organization and the type of movement.

Physiologic studies in extraocular muscle of the cat have shown the presence of different types of muscle fibers. Two investigators, believed that they had identified slow fibers by intracellular recording. This finding was not confirmed by another group who were only able to demonstrate twitch fibers in the superior rectus and inferior oblique of the cat. The latter divided the cells into “slow multi-innervated twitch fibers” located near the outer and inner surface, and fast fibers in the central portion of the muscle.

Histologic studies have also indicated that extraocular muscles were not homogeneous throughout but contained a layer of small cells around the border and larger cells in the center. Muscle cells in the periphery of guinea pig eye muscle had the morphologic characteristics of slow fibers. In the monkey cells with the histochemical properties of red fibers were located principally around the outer portion of the muscle and those having the features of white fibers were predominantly in the central part. Electron micrographs indicated that the marginal cells did not contain an M line and in this respect resembled the slow fibers of the frog. They also had a poorly developed sarcoplasmic reticulum in the A band region and by light microscopy would be classified as felderstruktur fibers.

Since past electromyographic studies have not taken into account the heterogeneity of extraocular muscle, an attempt was made to differentiate between the outer red

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and inner white portions of the muscle of the cat with the use of extracellular electrodes. Eye movements were produced by stimulating selected portions of the brain. Fast movements were obtained from III nucleus excitation and slow movement from vestibular complex or adjacent reticular formation. The effect of III nerve stimulation and succinylcholine was also evaluated.

**Method**

**Stimulation.** Twenty-four adult cats weighing 2 to 3 kilograms were anesthetized with intraperitoneal sodium pentobarbital (35 mg. per kilogram), supplemented by intravenous injection. The cats were placed in a Kopf stereotaxic apparatus and portions of the temporal bone, the roof, and the lateral wall of the orbit on one or both sides were removed. One or more of the eye muscles (MR, SR, IR, IO, LR) were held at resting length by grasping the tendon of insertion with an immobile muscle clamp.

In 13 cats the dura was opened and the temporal lobe elevated. The III nerve was identified and stimulated through a pair of platinum electrodes by a Grass S4 stimulator and a 4B stimulus isolation unit. Cathodal stimulus parameters varied from 0.5 to 10 v. with the use of frequencies as high as 600 per second. A pulse duration of 0.1 millisecond was utilized in the majority of experiments.

In the second group of 11 cats, insulated monopolar 22 gauge needles were introduced into the nucleus of the III nerve and the vestibular complex or adjacent reticular formation by stereotaxic coordinates. These areas were stimulated with single and repetitive pulses at frequencies of 1 to 300 per second and voltages of 0.5 to 8 v.

During vestibular complex stimulation, slow eye movements were observed by inspection and records were taken with the globe both fixed and mobile. Third nucleus stimulation produced fast movements of the globe. Displacements were of small amplitude and in the upward and nasal direction. Most of the recordings were obtained in an essentially isometric state.

**Recording.** Recordings were obtained with a 31 gauge needle containing two insulated stainless steel wires. The electrode was placed in the orbital aspect (outer fibers), in the global portion (inner fibers), and/or within the center of the muscle. In 18 cats simultaneous recordings from global and orbital aspects were obtained. In 2 cats the muscles were removed from the globe and the insertion elevated. The electrode was placed in the global or the orbital aspect and inserted perpendicular to the muscle at increments of 0.1 mm. Responses were obtained at every level of penetration until the needle had passed completely through the muscle.

The electrode was connected to a combination of a Tektronix FM-122 preamplifier with a time constant of 1 second and a Honeywell differential preamplifier providing a total gain of x2,000. One to three channels were used for recording. The muscle response and the stimulus were viewed on a 4 channel Tektronix RM-561A oscilloscope and simultaneously recorded on a Honeywell 8100 FM tape recorder. After the experiment, moving strip photographs were taken with a Grass C4 camera at paper speeds up to 1,000 mm. per second.

Succinylcholine (10 to 125 μg per kilogram) was injected by vein in 7 cats to produce muscle contraction. The larger quantities were injected at the end of the experiment.

In 5 cats an electrolytic lesion was made through the stimulating electrode in the III nucleus, the vestibular complex, or reticular formation. The lesions were obtained with a direct current of 30 Ma. for 20 seconds. The location of the lesions was verified in histologic sections stained with methylene blue.

![Fig. 1. Stimulation of III nerve adjacent to pons; ↓ stimulation. Calibration for time base and amplitude is the same for all figures. A, Stimulation: 5 v., frequency of 10 per second. Fast motor units of different latency recorded from inner portion of medial rectus. B, Stimulation: 4.5 v., frequency of 100 per second. Recording from inferior rectus. Upper trace: fast action potentials in global aspect. Lower trace: slower units in orbital (outer) aspect.](image-url)
Fig. 2. Stimulation of III nerve. Frequency of 10 per second. Recordings from one area adjacent to the globe. Lower stimulating voltage produced a single prolonged motor unit; higher voltage induced motor units of short duration. A, Stimulation: 4 v. Slower single motor unit. B and C, Stimulation: 4.5 and 5 v., respectively. Faster response recorded.

Fig. 3. A, Fast units from inner portion of superior rectus obtained by stimulation of III nucleus (4.5 v., 50 per second). B, Motor units of longer duration recorded from outer layer of medial rectus (3.5 v., 100 per second). This was characteristic of vestibular complex stimulation. Similar units were recorded from all layers of the muscle.

Results

Stimulation of III nerve. Recordings from the global and central or orbital parts of the muscle showed two different types of motor units (Fig. 1). The first type (Fig. 1, A) had a biphasic or polyphasic spike form (fast unit). Duration was 0.2 to 0.5 millisecond and amplitudes measured 50 µv to 1.5 mv. The faster motor units were found in the global portion and the center of the muscle and were recorded from the outer aspect in only two experiments. The second type of motor unit (Fig. 1, B, lower trace) was monophasic, longer in duration (0.5 to 3 milliseconds), and usually lower in amplitude (50 to 700 µv). The latter response was recorded mainly from the outer part of the muscle and was considered to represent slow units.

The latency of both types was 1 millisecond in response to the lowest stimulating voltage. Higher voltages produced many motor units (2 to 14) in the central and global portions of the muscle for each stimulus, with a delay of 1 millisecond for the first motor unit followed by a train of action potentials extending over 5 milliseconds. Thus there was a distribution of latencies indicating a gradation of nerve and muscle conduction velocities.

The most frequent response to III nerve stimulation was of the faster type in the global portion and the slower type in the orbital aspect (Fig. 1, B). Occasionally the slower type response was obtained in the global or central part with low stimulating voltage. An increase in stimulus strength produced a fast response which obscured the slower one (Fig. 2). In the orbital portion the slower motor units were observed with both low and higher voltage.

Stimulation of III nucleus. Stimulation of the III nucleus gave essentially the same response as III nerve stimulation, with slower motor units in the orbital aspect and faster units in the center and global portions (Fig. 3, A). Fast responses were sometimes recorded from the orbital layers.

Stimulation of vestibular complex. Stimulation in the region of the vestibular complex caused slow eye movements. Motor units of the slower type (Fig. 3, B) were recorded from the orbital and global aspects and the belly of the muscle. Stimulation was often accompanied by action potentials occurring at irregular intervals which were not directly related to stimulus frequency. Spontaneous slow units often appeared after stimulation was stopped. Slow eye movements did not occur until several
Fig. 4. Spontaneous activity. A, Fast units. B, Intermediate units. C, Slower action potentials.

Fig. 5. Comparison of spontaneous activity following administration of succinylcholine and vestibular stimulation. Upper trace: outer layer of muscle. Lower trace: inner layer of muscle. A, Spontaneous activity after 20 μg succinylcholine intravenously. B, 250 μg succinylcholine intravenously in same cat. C, Spontaneous activity following vestibular nucleus stimulation with 4 v., frequency of 300 per second. Same spontaneous activity followed different frequencies.

Fig. 6. Cross sections of cat superior rectus ×30, prepared to demonstrate αGPD and succinic dehydrogenase. The top of the figure is the outer portion of the muscle; the lower part is the center of the muscle. The central portion contains a mixture of cells varying in activity. A, Histochemical preparation for αGPD. The cells nearest the periphery had the least activity and the majority of the central fibers were darker indicating greater reaction. B, Succinic dehydrogenase. The outer portion was more reactive than the center.
seconds of stimulation had elapsed, while motor unit activity began with the first stimulus. This was in contrast to the fast eye movement which appeared almost immediately after stimulating the III nucleus.

Spontaneous activity. Spontaneous activity was recorded from the global and orbital aspects of the muscle. This activity appeared to be peripheral since it was still present when the III nerve was severed at the brain stem. The form of the spontaneous motor units was usually a biphasic spike wave extending over 0.2 to 0.5 millisecond. The amplitude for all types ranged from 50 \( \mu \text{V} \) to 1.2 mv., and the duration from 0.2 to 2.5 milliseconds (Fig. 4).

The intravenous administration of succinylcholine (10 to 125 \( \mu \text{g} \) per kilogram) was followed by activity occurring in both the inside and outside of the muscle with a duration of 0.2 to 1.5 milliseconds and an amplitude of 50 \( \mu \text{V} \) to 1.3 mv. (Fig. 5, A and B). Motor units were usually biphasic spikes and the activity lasted from 3 to 15 seconds.

Discussion

Muscle fibers that are considered slow in physiologic terms have been identified in lower vertebrates and invertebrates. These cells undergo local partial depolarization adjacent to the motor end plate and do not propagate an action potential. It is necessary to record the restricted membrane alterations by intracellular electrodes. However, a group of slow fibers capable of propagating an action potential have been identified in birds. The response was monophasic, prolonged, and graded when measured by extracellular electrodes. Intra-

cellular action potentials of similar configuration were obtained from rabbit and cat extraocular muscle following nerve stimulation and labyrinthine excitation. It was also proposed that the slow multi-innervated twitch fibers of eye muscle were similar to avian slow fibers.

In addition to the variation of action potentials among slow fibers, alterations in the form of twitch potentials were observed between red and white cat skeletal muscle. The rise time to peak was prolonged in red tonus fibers and the amplitude was less when compared with white phasic fibers. The resting potential was lower and the action potential more prolonged in red soleus muscle of the rat than in the white extensor digitorum longus. Red twitch fibers more closely resembled slow fibers in their response to acetylcholine applied to the surface membrane. Depolarization developed outside of the motor end-plate zone, provided higher concentrations of the cholinergic agent were used. In contrast, white fibers did not respond outside of the motor end-plate region.

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Since the present study utilized extracellular electrodes, only fibers that propagated an action potential were recorded. However, again the topographical arrangement of the action potentials obtained by the III nerve and III nucleus stimulation were similar to past results obtained by intracellular recording and were compatible with the histochemical studies. In addition, with vestibular complex or reticular formation stimulation, motor units of prolonged duration were consistently present about the periphery, but were also distributed throughout the muscle.

It can also be observed (Fig. 1 A, 2, C, 3, A) that stimulation of the III nucleus or nerve initiated a series of motor units with varying latencies. The first motor unit was observed 1 millisecond after stimulation while the last occurred 5 to 6 milliseconds later. This signifies that an evoked fast movement is produced by motor units with fast, intermediate, and slower conducting velocities, and presumably varying contractile properties. At the same time, the slower motor units were occurring in the periphery of the muscle. Thus fast movement appeared to be a mixture of slow, intermediate, and fast components while the slower vestibular-induced movement was accompanied solely by units low in amplitude and prolonged in duration. Vestibular movement also appeared to be graded in that it did not occur until several seconds of stimulation had elapsed.

The succinylcholine experiments indicated that the faster motor units, not the slow group, were activated by this agent since the contracture was accompanied by action potentials of short and intermediate duration. Furthermore, it was often possible to stimulate the vestibular or reticular formation while the induced contraction was still in effect and obtain the monophasic slower units.

Another indicator of a spectrum of motor units was spontaneous activity. Some motor units were of very short duration (Fig. 4, A), others intermediate (Fig. 4, B), and the long action potential noted with vestibular stimulation was also observed (Fig. 4, C).

In two III nerve experiments, fast responses were obtained from the orbital aspect and were occasionally observed after III nucleus stimulation. These exceptions may have been due to the limitations of the recording system. The diameter of the recording electrode was approximately the width of the fine cell layer. It was difficult to maintain the needle in or adjacent to this fine layer, and the contraction may have resulted in the passage of the needle into the more interior part of the muscle.

Recordings from the central aspect occasionally gave slower responses for low stimulating voltage and faster response for higher voltage (Fig. 2). Fibers having the same enzymatic reactions as the outer fibers were found throughout the muscle (Fig. 6). The recording electrode may have been near red fibers that were excited by a low level of stimulation. As the amplitude of stimulation was increased, the predominating white fiber responses would then override the initial response. A low threshold for response implies that the slow motor units are supplied by heavily myelinated nerves. This correlates with past histologic determinations of eye muscle showing that multiple motor end plates of en grappe configuration were often derived from thick medullated nerves.18,29

The present study does not indicate whether nonpropagating slow fibers are found in extraocular muscle. It does illustrate, however, that it is possible to produce action potentials of different configuration by stimulating regions of the brain that will induce slow or fast eye movement. It was also shown that the variations in action potentials coincides with the histologic characteristics of the muscle. Potentials of longer duration were more prevalent in the red portion while the white component was more complex since it contained a variety of motor units varying in duration and latency.

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