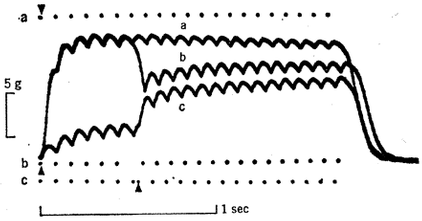

Fig. 3. Tension responses of a MG type S motor unit to three trains of 22 stimuli each, at a basic rate of 12.2 pps (interpulse interval, 82 msec). In each train, one or two stimulus intervals were altered. The tension traces are labeled (*a*, *b*, *c*), and the corresponding pulse sequences are similarly designated. The arrows at the first pulses in *a* and *b* indicate double stimulation with interpulse interval of 10 msec as in Fig. 2. The arrow in *c* denotes a single pulse following the previous pulse with an interval shorter than that in the basic train but longer (about 26 msec) than the double stimuli in *a* and *b*. In trace *b*, note the drop in tension to a new level when one interval in the train was lengthened to about 117 msec. Muscle temperature was 36°C.

times tended to have lower optimal frequencies. The optimal frequencies for type F units were a good deal higher than those for type S, but we have insufficient data at the moment to specify a range for them.

In essence, it appears that the catch phenomenon results when at least one stimulus interval within a train of relatively low repetition rate is considerably shorter than the rest. Tension output, particularly of type S motor units, may thus be quite sensitive to the pattern of stimulus intervals within a train when the basic repetition rate is in the optimal range. For example, the MG type S unit in Fig. 3 produced markedly different tensions when activated by three stimulus trains with the same basic frequency (12.2 pps), which differed from one another in only one or two stimulus intervals. The mean frequencies of the three trains hardly differed (mean frequencies of *a*, *b*, and *c* were 12.8, 12.5, and 12.6 pps, respectively). A stimulus interval shorter than intervals in the basic train caused tension enhancement, which was maintained (*a*, *c*) unless reset to a lower level by interpolation of an interval longer than the basic one (*b*). Permutation of the many possible stimulus interval sequences appeared to set the tension output at quite different levels, which were then maintained for some seconds as long as there was no subsequent change in the basic train.

It is not at present clear whether or not motor units participating in normal motor behavior fire in patterns that utilize the catch property. It has, however, been observed that type S motor units responding to stretch of their own muscle tend to fire more rapidly at the onset of stretch than during maintained stretch (*8*; see also *3*, *10*). Furthermore, the optimal frequencies found for the catch phenomenon in type S units are in the same low range as the firing frequencies observed for motor units in slow-twitch muscles activated by maintained stretch (*3*, *10*). It seems reasonable to suppose that, at least with regard to the slow-twitch type S motor units, the catch property may operate to extend the range of output tensions that can be produced by a given unit without a large change in mean firing frequency of the motoneuron.

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9. The situation in type F motor units is complicated by the presence of a mechanism that seems to cause reduction in tension output per stimulus after the first two to four stimuli in a low-frequency train. This mechanism is apparent as a "sag" in tension output of type F units in low-frequency tetani, and the effect can also be seen in whole, nominally fast muscle (see *6*). This effect, not observed in type S units, also appears to be involved in the limitation of tension enhancement duration with interpolation of an extra impulse (as in Fig. 2). The reason for this difference between types F and S motor units and the mechanism for the catch phenomenon itself are currently under study.
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A Short-Latency Labyrinthine Input to the Vestibular Nuclei in the Pigeon

Abstract. *Electrical stimulation of the pigeon labyrinth evokes responses in many second-order vestibular neurons with a latency shorter than the monosynaptic delay. These early responses are probably due to electrically mediated synaptic transmission, or perhaps to antidromic invasion of cells supplying efferent fibers to the labyrinth. In either case the results demonstrate a difference between cat and pigeon with respect to connections between labyrinth and vestibular nuclei.*

The labyrinth plays a more important role in the motor activities of birds than it appears to play in the motor activities of terrestrial vertebrates. Ewald demonstrated that following bilateral labyrinthectomy pigeons will not fly, while the long-term effects of such a procedure in mammals are relatively mild (see *1*). This in turn suggests the possibility that there are qualitative or quantitative differences between these two groups of animals with respect to the interconnections between labyrinth and vestibular nuclei. We have therefore investigated the vestibular input to second-order neurons in the pigeon, and compared it to the well-studied analogous input in the cat.

Pigeons were anesthetized by intramuscular injection of Equithesin (Jensen-Salsbery Laboratories), paralyzed with Flaxedil (American Cyanamid Co.), and artificially ventilated with a mixture of O_2 and CO_2 (95 : 5). An active (cathode) platinum ball electrode, insulated except at the tip, was inserted between the bony and membranous labyrinths, and an indifferent electrode was inserted nearby. The head was clamped in a holder similar to that described by Karten and Hodos (*2*). The head and upper cervical region were held ventral side up during the experiments; in order to prevent circulatory failure the rest of the body was rotated and maintained

dorsal side up. If the body was kept ventral side up, failure occurred frequently. The ventral surface of the brain stem was exposed for subsequent insertion of microelectrodes. The upper cervical cord was also exposed for antidromic stimulation of vestibulospinal fibers by means of a pair of ball electrodes. Single unit activity was recorded with microelectrodes filled with 2*M* NaCl saturated with Fast Green FCF for marking of electrode location (*3*). Conventional recording circuits were employed.

We have recorded from cells located in the rostral part of the descending nucleus, in Deiters' nucleus, in the tangential nucleus, and in the area medial to the tangential nucleus and ventral to Deiters' nucleus occupied by vestibular nerve fibers (see *2*). There are numerous cells intermingled with afferent fibers in the latter location, which, following Bartels (*4*), we shall call ventral Deiters'. Although stimulation of the labyrinth with our method is likely to stimulate cochlear as well as vestibular fibers, we have assumed the responses we have recorded result from activity in vestibular fibers: it seems unlikely that cochlear fibers would make monosynaptic contact with cells in these regions of the vestibular nuclei (*5*).

Electrical stimulation of the labyrinth produces in the vestibular nuclei focal potentials that are particularly prominent in the region occupied by vestibular nerve fibers, and have a time course similar to that illustrated in Fig. 1. The first positive peak, representing the arrival of afferent volleys and comparable to the P wave in the cat (*6*), is at 0.25 to 0.35 msec. The positivity reverses at 0.3 to 0.5 msec to a negativity that often consists of two or more components. The positivity and first negativity are shown in Fig. 1A, in which the second negativity is not marked. Unit discharges are superimposed on these fields (Fig. 1). The unit spikes are usually negative, sometimes negative-positive or positive-negative, and are generally typical of recordings obtained from cell bodies rather than fibers. Latency of the unit potentials varies and depends on stimulus strength (Fig. 1). With the weakest shocks the responses of most cells occur at least 1.2 msec after the stimulus. Some of these may be late monosynaptic spikes, but most are probably polysynaptic (see below). As the stimulus strength is increased, latency may decrease to within 0.7 to 1.2 msec: this is monosynaptic firing, similar to that observed in cat second-order vestibular neurons with extracellular recording (*7*). With stronger, but still moderate, shocks some cells fire right behind or on the first negative peak (see Fig. 1A2 and 1A3). Some cells fire even earlier, near the positive peak, with latencies as short as 0.4 msec, as is the case with the exceptional giant extracellular spike illustrated in Fig. 1B. The decrease in latency with increasing stimulus strength in such cells often was not gradual, but abrupt. Activation occurring earlier than firing that is obviously monosynaptic has not been seen in the cat. We have called it early firing, and it is the subject of the remainder of this report.

Polarity of the extracellular potentials suggests that we are not recording from fibers, and any possibility that we are recording from vestibular afferent fibers is further diminished by the fact that some units that fire early could be fired anti- or orthodromically by stimulation of the spinal cord. In addition, it is unlikely that an afferent fiber would yield a response at such a variable latency, or that the change in latency with stimulus strength would be as pronounced as was observed for many extracellularly recorded units. We have performed some intracellular recording to obtain further information on the nature of the early response. The cells in that area of the vestibular nuclei (tangential and ventral Deiters' nuclei) where we have seen most early responses are small, and because of the difficulties encountered in recording from these cells intracellular spike potentials have not been large. Some action potentials were as large as 40 to 50 mv, but most were between 20 to 30 mv (Figs. 2 and 3). Early responses have been seen in many cells, usually occurring with little or no delay after the juxtacellularly recorded positive peak of the afferent volley, even earlier than is usually the case with extracellular recording. It was possible to record monosynaptic excitatory postsynaptic potentials (EPSP's) in some early firing cells. In those cells the EPSP's came later than the early responses (Figs. 2 and 3). The average latency of monosynaptic EPSP's recorded in these and other nearby neurons was 0.3 msec after the positive peak (range 0.1 to 0.8, mode 0.3) and 0.6 msec after the stimulus artifact (range 0.4 to 1.0, mode 0.5). In contrast the earliest onset of intracellularly recorded early responses had an average latency 0.3 msec after the stimulus artifact (range 0.2 to 0.4, mode 0.3). The range of latencies of the monosynaptic EPSP's resembles the range of latencies of those extracellular spikes described above that we considered monosynaptic. In some cells a later EPSP could be seen behind the peak of the monosynaptic potential (Fig. 2A). The latency of this second EPSP was similar to that of the late firing recorded extracellularly and considered polysynaptic. In the case of intracellular recording the threshold of early firing is very low, and early spikes are often observed with shocks weaker than those required to produce a significant EPSP. This contrasts with the results obtained with extracellular recording (described above), where weak shocks usually evoke late firing and early spikes are evoked only with stronger stimuli (Fig. 1). This difference suggests that the safety factor for early firing is low, and that depolarization incident to penetration of the cell lowers the threshold of the cell to early firing. Perhaps the events leading to early firing are usually too small to fire a resting, unpenetrated cell. Under such conditions a prerequisite for early firing

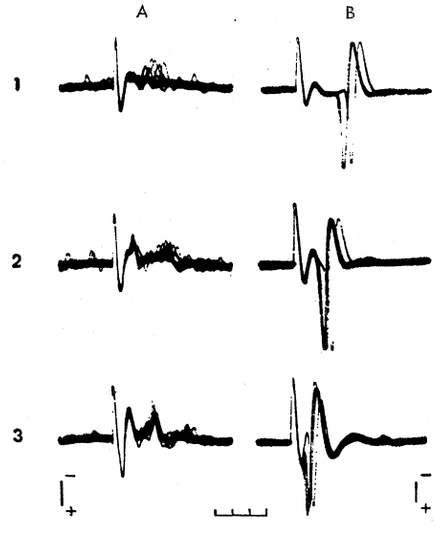

Fig. 1. Extracellular records from two cells (from two experiments) in the vestibular nuclei of the pigeon. (A) This cell was located somewhat dorsal to vestibular nerve fibers, lateral to the lateral nucleus of Deiters'. Labyrinth was stimulated at a frequency of five shocks per second with shocks 1.7P (1.7 times P wave threshold) in A1, 1.8P in A2, and 1.95P in A3. The P wave threshold was 0.9 volt. Voltage calibration, 500 μv; each unit on time scale, 0.5 msec. (B) This cell was located in the midst of vestibular nerve fibers, either in ventral Deiters' or in the descending nucleus. Labyrinth was stimulated at one shock per second with shocks 1.2P in B1, 1.4P in B2, and 1.8P in B3. The P wave threshold was 1.75 volts. This unit grew in size throughout the recording period. Voltage calibration, 2 mv; each unit on time scale, 0.5 msec. In this and succeeding figures all traces consist of several superimposed sweeps.

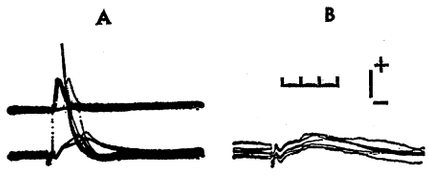

Fig. 2. Intracellular records from a cell in the descending nucleus. Low-gain records in upper trace; high-gain capacitatively coupled recording in lower trace. Input capacitance not neutralized. In (A) labyrinth was stimulated at five shocks per second with shocks 2.8P (P wave threshold was 0.8 volt). In (B) stimulus strength was the same, but spike was blocked by passage through the recording microelectrode of hyperpolarizing current just large enough to produce block. Voltage calibration, 20 mv for upper trace, 1 mv for lower trace. Each unit on time scale, 1 msec.

may be facilitation of the cell by extracellular negativity, as observed by Nelson (*8*) for spinal motoneurons. Such facilitation would be expected to become more pronounced as the extracellular negativity increases with increasing shock strength. Alternatively, summation of several short-latency inputs may be required to produce firing.

At threshold the early spike appears in an all-or-none fashion with no indication of a prepotential. To try to reveal a prepotential we have blocked firing with hyperpolarizing current passed through the recording microelectrode. This procedure has always blocked the early spike without revealing any other potentials (Fig. 2). Small potentials, that could be prepotentials, have occasionally been revealed by high-frequency stimulation. Early spikes usually follow frequencies of stimulation of several hundred shocks per second. At such frequencies there is usually enhancement of A/B separation (upper trace in Fig. 3C), and when failure of A and B spikes occurs a still smaller potential is sometimes seen (arrow labeled *S* in middle trace of Fig. 3C).

Early firing might originate from: (i) direct electrical excitation of the cell from stimulus spread; (ii) antidromic invasion of cells projecting to the labyrinth; (iii) electrically mediated synaptic transmission. Because the early responses are evoked in penetrated cells at low threshold (1 to 2 times P wave threshold), and because they are evoked by weak shocks in some cells but not in other nearby cells even by very strong shocks, it is unlikely that they are generated by stimulus spread. In addition, early responses are clearly separated from the stimulus artifact (Figs. 2 and 3) and have never been observed before the positive peak of the afferent volley, indicating that they are fired by the arrival of this volley rather than directly by the electrical stimulus itself. Some of our results do not seem consistent with the idea of antidromic invasion. First is the difference in threshold of early spikes that results from different recording conditions (intra- and extracellular); we have seen no evidence that antidromic invasion of vestibular neurons from the spinal cord has a low safety factor, although we have not been able to compare the behavior of such an antidromic input with that of a short-latency labyrinthine input in the same cell. Second is the latency shift with increasing stimulus strength seen with extracellular recording (A2, A3, B2, and B3 in Fig. 1). We feel that the possibility that early spikes are due to stimulation of efferent fibers and subsequent antidromic invasion of cell bodies is not likely, but it is not ruled out. Our findings are consistent with the presence of electrical coupling between vestibular fibers and second-order neurons. This possibility is suggested by Cajal's description of the contacts between vestibular afferent fibers and cells in the tangential nucleus in birds (*9*) and is reinforced by the recent findings of junctions with closely apposed membranes in the tangential nucleus of chicks described as gap junctions (*10*). Against this interpretation is the lack of any visible coupling potential when the early spike is blocked by hyperpolarization. It is possible, however, that hyperpolarization also blocks the presynaptic terminals (see *11*).

If the early spikes are antidromic, this suggests that the efferent innervation of the pigeon labyrinth is rather ample, because we have seen numerous cells that show early firing. In the cat, on the other hand, the efferent projection seems rather scanty on anatomical grounds (*12*). If the early spikes are due to electrical coupling, this means that there is present in the pigeon vestibular nuclei a type of synaptic interaction not seen in the cat vestibular nuclei, where there has been no electrophysiological evidence for the presence of electrical synapses. Therefore, whether early firing is due to antidromic invasion of cells giving rise to efferent fibers, or to electrical synapses, it demonstrates a difference between cat and pigeon. While we believe that our findings are explained more readily by the presence of electrical synapses than by numerous efferents, this problem may be more readily settled unequivocally by anatomical studies.

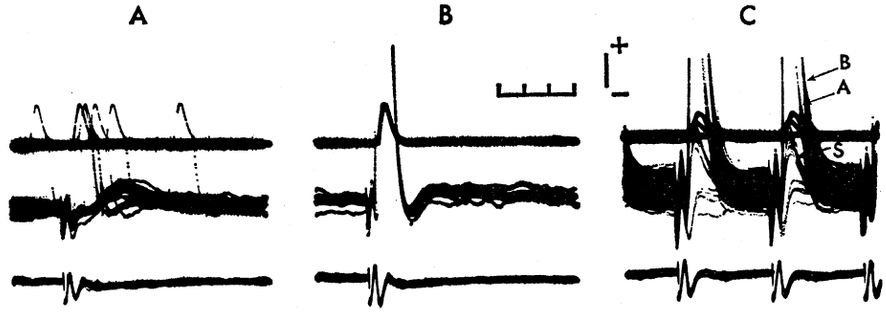

Fig. 3. Records from a cell in the descending nucleus. Low-gain intracellular records are shown in the upper traces. Middle and lower traces show high-gain capacitatively coupled records obtained, respectively, intracellularly and after withdrawal of the electrode from the cell. Input capacitance not neutralized. Labyrinth was stimulated at five shocks per second with shocks 1.9P in (A) and 2.3P in (B). The P wave threshold was 0.8 volt. In (C) frequency of stimulation was 250 shocks per second and intensity was 3.4P. The upper beam in (C) shows that the cell sometimes appeared to fail completely. The falling phase of the *A* and *B* spikes can be seen in the middle beam (arrows marked *A*, *B*), which also shows that when the *A* and *B* spikes failed, a smaller potential was present in some cases (arrow marked *S*). Voltage calibration, 20 mv for upper trace, 1 mv for middle and lower traces. Each unit on time scale, 1 msec.

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Z Disc Ultrastructure in Scutal Depressor Fibers of the Barnacle

Abstract. *The ultrastructure of Z discs in nonglycerinated, striated muscle fibers of the barnacle* Balanus nubilus *Darwin was examined in contracted, resting, and stretched preparations. At all sarcomere lengths, the Z discs are perforated sheets comprised of "Z rims" and "Z perforations," extending continuously across the myofibril; the dimensions of the rims and perforations change with changes in sarcomere length. Accordingly, with shortening of the sarcomere, there was an accompanying decrease in the transverse width of the Z rims and an increase in the diameter of the Z perforations.*

Striated muscle fibrils are composed of a serially repeating structure, the sarcomere. Bounded by transversely situated Z material, each sarcomere contains an ordered array of thick and thin protein filaments. Thick filaments occupy the central region of the sarcomere while thin filaments originate in the Z material and extend into the center of the sarcomere where both sets of filaments interdigitate. Contraction is characterized by the sliding of thick and thin filaments relative to each other.

Physiologically many types of striated muscle do not contract to the extent that the ends of the thick filaments make contact with the Z bands, but other muscles "supercontract" to less than 50 percent of rest length, and the fate of the thick filaments is not fully known. The questions remain whether the thick filaments crumple against the Z material when the sarcomeres shorten, whether they pass through unobstructed, or whether supercontraction occurs in another manner.

Hoyle and his group (*1*, *2*), in their studies of supercontracting fibers of the barnacle *Balanus nubilus* Darwin, believe that the Z discs in resting muscle are comprised of individual dense bodies held together closely by elastic bridges, so that the discs appear as continuous, nonperforated structures. Hoyle *et al.* postulate that, when muscle is activated, the dense bodies become rearranged and discontinuous; perforations now appear in the discs so that the thick filaments from adjacent sarcomeres may pass through unobstructed.

Our work was designed to study the ultrastructure of the Z discs of barnacle fibers, in resting, stretched, and contracted muscle. Our conclusions differ somewhat from those of Hoyle *et al.* in that we believe that, under all conditions, the Z discs of the myofibrils are continuous but perforated sheets; and that during supercontraction the Z perforations enlarge and permit unrestricted passage of the sarcomere filaments.

Relaxed nonglycerinated preparations of scutal depressor fibers were obtained by injecting 5 ml of *d*-tubocurarine chloride (aqueous, 1.5 mg/ml) through the connective tissue layer located between the shell and the operculum (hard platelike valves protecting the soft parts of the barnacle). After 5 minutes, three "windows" were drilled in the shell and an additional 8 ml of the tubocurarine was added through the windows. The depressor muscles were then fixed by 4.0 percent formaldehyde and 0.5 percent glutaraldehyde in 0.1*M* phosphate buffer, *p*H 7.3. The relaxed fibers were fixed in situ.

To obtain "physiologically" contracted preparations, the barnacle operculum was lightly tapped, so that the depressor muscles contracted. The fixative then was injected through the shell windows. To obtain stretched preparations, the operculum of curarized barnacles was forcibly extended from the shell before the fixative was added.

Longitudinal and transverse sections (thickness, 350 to 600 Å and 500 to 900 Å) were examined with an electron microscope (RCA EMU-3).

The longitudinal sections reveal that the Z discs manifest different structural appearances under different physiological conditions. Most frequently in rest-

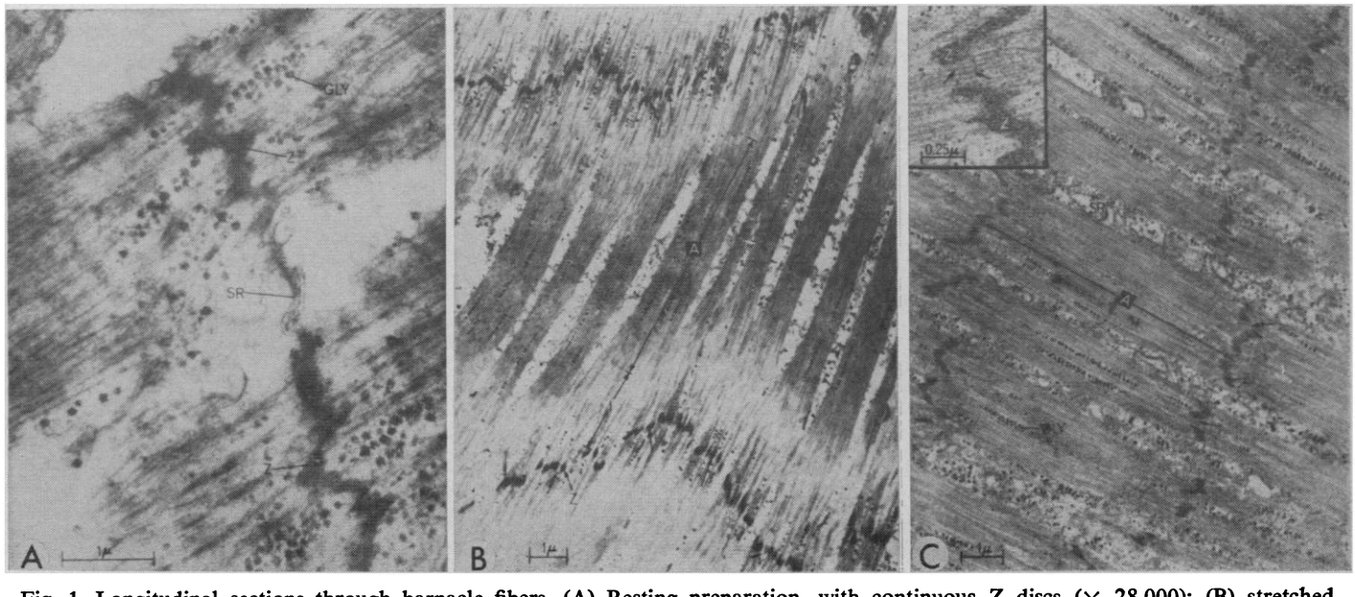

Fig. 1. Longitudinal sections through barnacle fibers. (A) Resting preparation, with continuous Z discs (× 28,000); (B) stretched preparation, with Z discs appearing as separate Z bodies (× 10,000); (C) supercontracted preparation, with absence of I bands. Inset shows intersarcomeric passage of filaments (× 48,000); *Z*, Z discs; *SR*, sarcoreticular tubules; and *GLY*, glycogen.