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Synaptic Activity in Motoneurons during Natural Stimulation of Muscle Spindles

Abstract. *Synaptic activity evoked in cat motoneurons during stretch stimulation of muscle spindles in the homonymous muscle has been studied by intracellular recording. A class of miniature excitatory postsynaptic potentials evoked by such physiologic stimulation results from activity in single group Ia spindle afferent fibers, and the criterion for identification is a predictable pattern of rhythmic occurrence of the synaptic potentials. Statistical examination of the amplitude distributions of this class of miniature synaptic potentials suggests that some group Ia fibers liberate a relatively large number of quantal excitatory postsynaptic potential units per fiber impulse.*

Intracellularly recorded synaptic activity, evoked in a motoneuron by stretch stimulation of muscle spindle receptors in the muscle innervated by that motoneuron, consists of a complex series of membrane potential variations or wavelets, the aggregate of which is usually impossible to analyze visually into discrete components (*1*). However, in favorable circumstances when individual wavelets can be examined in isolation, they correspond in wave form to the familiar excitatory postsynaptic potential (EPSP) which results from simultaneous electrical activation of a large population of group Ia spindle afferent fibers in a muscle nerve (*2*, *3*). It is reasonable to suspect that stretch-evoked wavelets may indeed be miniature EPSP's that result from activity in single group Ia fibers and thus represent unitary components of the volley-evoked EPSP. Kuno has, in fact, demonstrated that an electrically evoked EPSP can be fractionated into such all-or-none unitary components by limiting the stimulus to one or a few group Ia afferents (*4*).

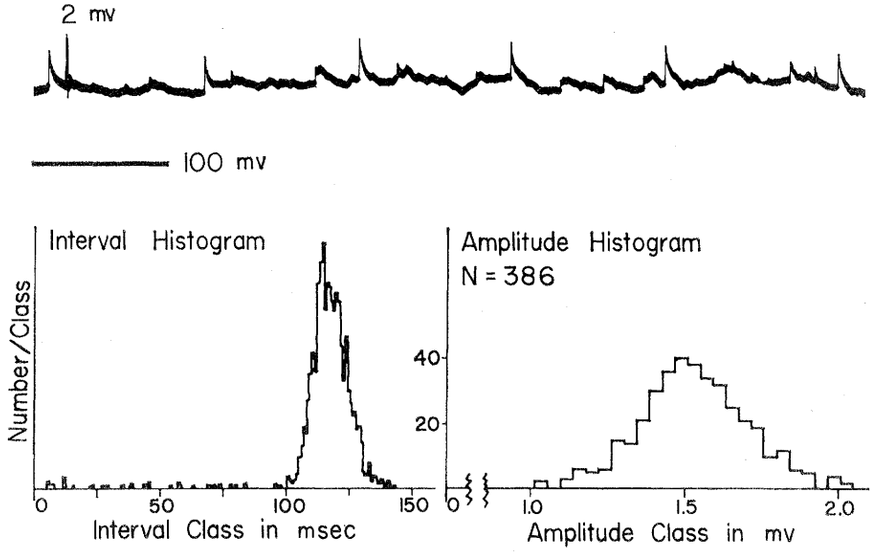


Fig. 1. (Top) High-gain intracellular potential recording from a plantaris motoneuron with the muscle slack. Note regularity of occurrence of large EPSP of typical waveform. (Lower left) Histogram of the time interval in milliseconds between successive large rhythmic EPSP's, developed over a 5-minute period on a CAT computer (*11*). The narrow peak indicates a high degree of periodicity which is quite stable. (Lower right) Histogram of the amplitude distribution of 386 successive EPSP's from the rhythmic pattern, measured from film records. Mean amplitude is 1.57 mv ± 0.16 S.D. Note the break in the abscissa. There were no failures of occurrence (breaks in the rhythmic pattern), and no constituent synaptic potential was less than 1.0 mv in amplitude.

Afferent fibers of muscle spindles discharge quite rhythmically during muscle stretch and often even without stretch; the regularity of firing is particularly evident when central nervous system control of spindle sensitivity through the gamma loop has been interrupted by section of the ventral roots (*5*). Therefore, rhythmic occurrence of stretch-evoked miniature EPSP's should be a useful criterion for identifying synaptic potentials which result from impulses in single group Ia afferent fibers. Our present results demonstrate this.

The experiments were performed on cats that had been anesthetized with pentobarbital and firmly fixed in a steel frame. The right leg was denervated completely, and only muscle nerves to the medial and lateral gastrocnemius heads and to the plantaris muscle were left intact. Tendons of these muscles were separated and arranged for independent stretch with weights. After laminectomy, ventral roots of lumbar segments L6 and L7 and sacral segment S1 were cut, and the L7 segment was explored with micropipettes (diameter of tip, 2 μ) filled with potassium citrate. Potentials from gastrocnemius and plantaris motoneurons, recorded intracellularly, were identified by the pattern of postsynaptic potentials evoked from appropriate muscle nerves (*6*). After identification, the homonymous muscle was stretched tonically with small weights, and the resulting synaptic activity was recorded photographically and on an FM magnetic-tape recorder for later analysis.

In about 90 percent of the extensor cells that we examined, muscle stretch evoked the apparently asynchronous and unpatterned barrage of miniature potentials already described (see *1*). However, in the other 10 percent (numbering 11 cells to date), careful grading of the stretch from zero to moderate amounts (about 100 g) evoked miniature EPSP's of typical wave form which were clearly unitary events and which occurred in a strikingly regular rhythmic pattern. This rhythmic pattern was recognizable primarily because most of the successive constituent

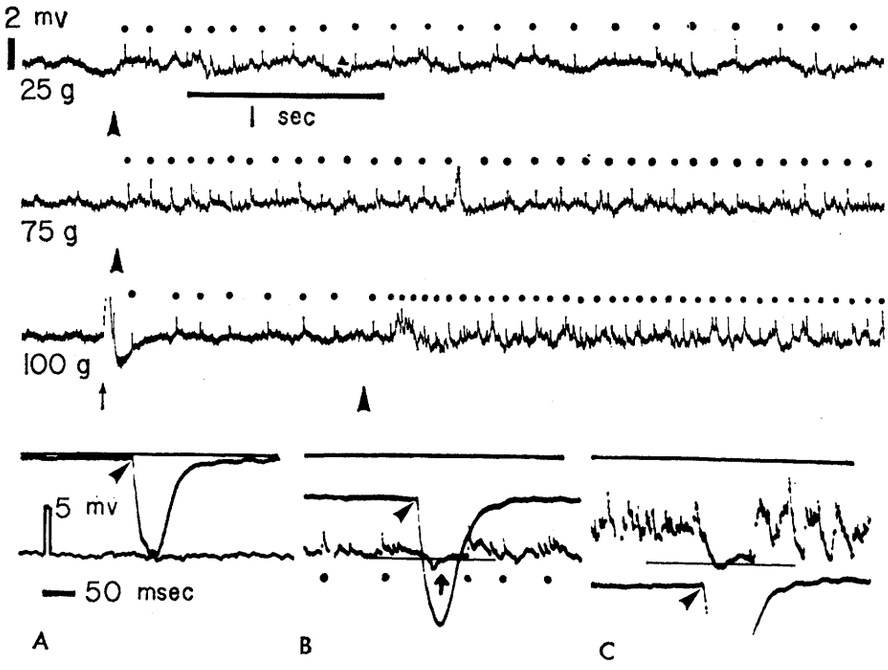
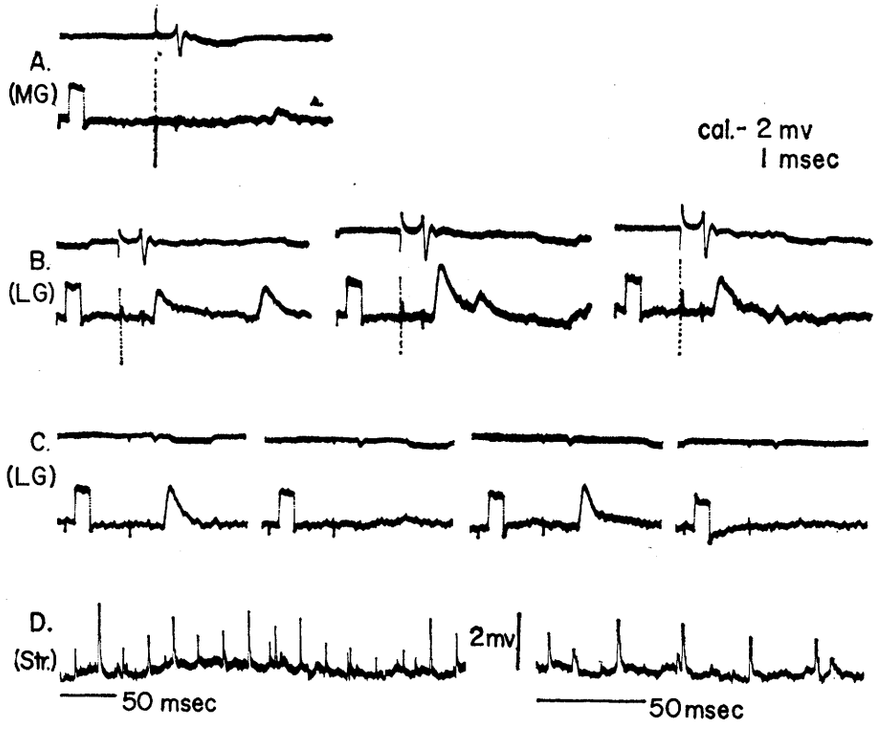


Fig. 2. (Top section) Gastrocnemius motoneuron. High-gain intracellular records showing a rhythmic EPSP (marked with dots) evoked by gastrocnemius muscle stretch of three successively increasing magnitudes (25, 75, and 100 g). Onset of stretch signaled by the arrowhead. (Initial mechanical artifact in the 100-g record at the small arrow; stretch fully on at the arrowhead.) Note the tendency for the discharge rate to slow during maintained stretch, a point clearly visible on longer segments of the original records. Note also the addition of smaller, apparently unpatterned, EPSP's to the response as stretch is increased to 100 g. (Bottom section) Same cell. In the lower beam, d-c–coupled intracellular trace with 5 mv calibration mark. Isometric myograph trace (center beam) records gastrocnemius muscle tension by downward displacement from the base line (initial tension in *A* is zero; that in *C* is about 500 g). Twitch of the muscle from stimulation of cut ventral root of L7 appears in the middle of each sweep (diagonal arrow). Note apparently complete cessation of the EPSP discharge during the twitch, with transient return of the intracellular potential to resting level (indicated by the line). Rhythmic EPSP is visible in *B*, marked with dots; the third expected occurrence (lower arrow) is aborted by the twitch, but the unit fires again during muscle lengthening.



synaptic potentials were of relatively large amplitude and could be identified positively over long segments of the intracellular potential records. The miniature EPSP's we are considering (termed "rhythmic EPSP's") are grouped together because they fit into a predictable occurrence pattern.

A clear example of the phenomenon is shown in Fig. 1, top line. This is an intracellular recording made at high gain on moving film from a plantaris motoneuron, in this case without muscle stretch. Wave form of the recurrent miniature potential is typical of an EPSP, and occurrence rate is quite constant over a 5-minute period, as shown by the narrow peak of the successive interval distribution histogram shown in Fig. 1, lower left. When the plantaris muscle was stretched slightly, the rate of occurrence increased and rhythmic EPSP became obscured by other, almost equally large, postsynaptic potentials elicited by the stretch. The constant rate of occurrence demonstrated by this unit is characteristic of primary afferent fibers, and the response to stretch suggests that muscle spindles are the source.

In order to prove that muscle spindles are indeed the source of the rhythmic EPSP's, it must be shown that (i) the occurrence rate of synaptic potentials increases with increasing stretch, and (ii) the EPSP's cease during an active twitch of the appropriate muscle (*5*). Both of these parallels between spindle afferent behavior and that of the rhythmic EPSP's are illustrated

Fig. 3 (left). Interneuron in L7 segment, intracellular recording. No response to antidromic shock to the L7 ventral root. (Line A) Shock to medial gastrocnemius nerve, twice maximal for group Ia fibers. Incoming volley appears in the dorsal root entry zone potential in the upper beam. Intracellular record (below) shows no evoked EPSP at monosynaptic latency. The small potential late in the sweep is unrelated. (Line B) Same supramaximal shock, now to the lateral gastrocnemius nerve, producing a typical monosynaptic EPSP at each trial. Again, the later EPSP's are spontaneously occurring and appear unrelated to the stimulation. (Line C) Lateral gastrocnemius shock decreased to a threshold point at which the EPSP occurs in an all-or-none manner, without intermediate stages of development. Over 50 trials, the firing index was 0.4. (Line D) Stretch of the gastrocnemius muscle with a 300-g weight elicits rhythmic EPSP, shown at different sweep speeds. Note that the discharge to stretch is relatively uncomplicated by other synaptic potentials (see Fig. 2, 100-g trace).

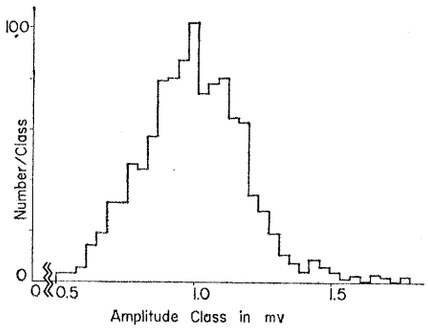


Fig. 4. Histogram of the amplitude distribution of 1000 successive rhythmically occurring EPSP's recorded from the gastrocnemius cell shown in Fig. 2, measured from film records on an OSCAR analog-digital film reader (*12*). Note break in the abscissa.

in Fig. 2 (data obtained from a gastrocnemius motoneuron). In contrast to Fig. 1, the rhythmic synaptic potential was not present during control periods but appeared only during muscle stretch, the rate of occurrence increasing as the stretch was increased (Fig. 2, top three records). There is also a definite slowing of the occurrence rate of rhythmic potential during maintained stretch, which reflects adaptation to tonic stretch that is typical of muscle spindle Ia discharge (*5*). The lower set of records (Fig. 2, *A*, *B*, and *C*) displays the cessation of the stretch-evoked synaptic potential discharge, which includes the rhythmic EPSP, during an active twitch of the gastrocnemius muscle. Another important point, which is not illustrated, is that in the several instances in which rhythmic EPSP's were seen without stretch, these ceased for several seconds after release of a muscle stretch and then resumed the original rate. This cessation of discharge after stimulation is again typical of group Ia spindle afferents (*5*).

Close correspondence between behavior of the rhythmic EPSP's and that of group Ia afferent fibers strongly suggests that rhythmic synaptic potentials result from impulse activity in single afferent fibers ending directly (monosynaptically) on the motoneuron being studied. It has been assumed (*7*) that the group Ia fibers are the only primary afferents with monosynaptic connection to mammalian motoneurons, and it seems likely, therefore, that the spindle afferents with which we are dealing are those of group Ia (see *8*).

This hypothesis is strongly supported by the data shown in Fig. 3 which is from the only interneuron included in the present results (*9*). The rhythmic EPSP discharge elicited in this cell by gastrocnemius stretch (line D) differs from that in other cells of the series in that rhythmic potential appears to be accompanied by few, if any, other postsynaptic potentials related to the stimulus, despite the relatively large amount of stretch used (300 g). This suggests that there are few connections between the gastrocnemius spindles that were stimulated and the cell under study. In fact, the all-or-none behavior of the EPSP which is electrically evoked from the lateral gastrocnemius nerve (line C) indicates that there is only one fiber in this nerve which produces both volley-evoked and stretch-evoked EPSP's. This is confirmed by the fact that amplitude distributions and wave forms of both volley-evoked and stretch-evoked EPSP's were exactly the same. The conduction velocity of this fiber was about 120 m/sec, which proved its identity as a group Ia fiber.

Thus far we have eight sets of data for rhythmic EPSP, out of a total of 11 examples, which include sufficiently large numbers of measurements on successive responses to generate meaningful amplitude distributions. Mean amplitudes of these distributions range between 0.68 mv $\pm$ 0.13 S.D. (standard deviation) and 1.57 mv $\pm$ 0.16 S.D., and typical examples are shown in Fig. 1 (lower right) and Fig. 4. All eight distributions have more or less Gaussian form with no failures of occurrence (no breaks in the rhythmic pattern) in 400 to 1000 successive responses. These distributions are quite different from those obtained by Kuno (*4*), who used small dissected bundles of group Ia fibers stimulated electrically. In those instances in which he stimulated only one fiber, the amplitude of the resulting EPSP's fitted a Poisson distribution with a relatively large number of failures of occurrence in about 200 trials. His data suggest that monosynaptic EPSP's that result from impulses in single group Ia fibers are made up of unitary quantal steps with an average of one such unit responding per fiber impulse and that the average unit size is about 0.12 to 0.25 mv. The present results, on the contrary, suggest that some group Ia fibers produce much larger "least" EPSP's, and statistical treatment of the amplitude distributions of potentials produced by these fibers indicates a rather high average number (m) of quantal units responding to each fiber impulse. This appears analogous to results obtained in some frog motoneurons by Katz and Miledi (*2*) and at the unblocked myoneural junction (see *10*); the probability of the existence of such fibers was discussed by Kuno (*4*). We cannot calculate the exact m number of unitary components implied by our amplitude distributions since we have no failures of occurrence in large samples and there is therefore no way to measure the size of the quantal steps that make up the rhythmic EPSP amplitudes (see *2*, *4*). Reasonable assumptions, however, with the use of either Poisson or binomial distribution functions, suggest that the m number is at least 5 or 6, and quite possibly as high as 10 or 15, quantal units released per fiber impulse. Kuno reported a number of instances in which the calculated m number was in excess of 3, but attributed these to stimulation of more than one afferent fiber. The large m numbers indicated by our data cannot be explained on this basis, since it appears highly probable that the rhythmic EPSP's that we studied result from activity in single fibers.

It would appear that the difference between the present results and those of Kuno is one of selection of different classes of group Ia fibers by the experimental design. Kuno worked with fibers that evoked EPSP's in the cells that he studied without selecting them on the basis of amplitude of response, while those fibers that we studied were highly selected on the basis of amplitude, since the criteria for identification of the rhythmic pattern definitely favored large amplitude synaptic potentials. We interpret our data as demonstrating afferent fibers at one extreme of a range of group Ia fibers, that is, fibers which produce unitary miniature EPSP's by releasing relatively large numbers of synaptic potential quantal units per fiber impulse, with the other extreme represented by the fibers studied by Kuno, which appear to release on the average only one quantal unit per fiber impulse. It is reasonable to suppose that there are examples intermediate between these two extremes. Our working hypothesis is that fibers that release large numbers of EPSP quanta per fiber impulse have a larger number of synaptic terminals on the motoneuron than do those fibers that release smaller numbers.

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9. Since this paper was completed, results exactly like those illustrated in Fig. 3 have been obtained from a cell quite definitely identified as a plantaris motoneuron. This cell appeared to be connected monosynaptically with only one spindle in the plantaris muscle, and conduction velocity of the afferent fiber was in the group Ia range.
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5 November 1965

Lactate Dehydrogenase of Trout: Hybridization in vivo and in vitro

Abstract. *Speckled trout and lake trout contain five forms of lactate dehydrogenase, but a different electrophoretic distribution of isozymes characterizes each species. The hybrid splake, which is produced artificially by fertilizing lake trout eggs with speckled trout sperm, contains nine isozymes. This complement of isozymes in vivo could be produced in vitro by recombination of subunits from tissues of the parent species. In the splake trout, this complement is the result of heterozygosity at the gene locus responsible for synthesis of LDH-5. Extracts of trout eyes contain at least two additional forms of LDH which could not be demonstrated in other tissues.*

In describing the lactate dehydrogenase isozyme composition of various tissues from the speckled trout, *Salvelinus fontinalis*, I proposed that three active nonallelic genetic loci controlled the synthesis of LDH subunits (*1*). Evidence that requires modification of this hypothesis and provides a firm genetic basis to describe the multiplicity of lactate dehydrogenases in these trout is now presented.

As many as five forms of electrophoretically distinguishable lactate dehydrogenases may be produced in most vertebrates by the random association of two classes of polypeptide subunits (*2*). These subunits are coded by two nonallelic genes (*3*). Polyacrylamide-gel electrophoretograms exhibited nine regularly spaced bands of LDH activity from various tissues of *S. fontinalis* (*1*). In order to fit these data within the framework of the subunit hypothesis it was reasonable to propose that there was active in this species a third cistron coding a third LDH polypeptide subunit. This then would result in the occurrence of three homopolymers of subunit composition, AAAA, BBBB, and CCCC, and of 12 heteropolymers produced by the random combinations of the three polypeptides.

I have now determined that nine LDH isozymes occur in the hybrid (heterozygous) trout, while in the parent (homozygous) species there are the usual five forms of the enzyme. The original data on trout LDH were obtained with fish from the Gaspé Hatchery of Quebec, Canada, and those reported now were obtained with *S. fontinalis*, *S. namaycush* (lake trout), and the hybrid splake from various hatcheries in the United States and Canada.

The lactate dehydrogenase isozymes were separated electrophoretically on polyacrylamide gels. Enzyme activity was localized on the gels as sites of nitro blue tetrazolium formazan deposition. The reaction mixture at *p*H 8.3 contained, in final concentration, 0.1*M* tris (hydroxmethyl) aminomethane, nicotinamide adenine dinucleotide (NAD) (1.3 mg/ml), nitro blue tetrazolium (0.45 mg/ml), 0.05*M* L(+)-lactic acid sodium, and phenazine methosulfate (0.14 mg/ml). The mixture was incubated at 37°C, usually from 5 to 30 minutes, depending upon the activity of the preparation. Isozymes were better separated on a 5-percent polyacrylamide gel rather than on the standard 7½-percent gel.

Organs were removed from freshly killed fish, washed in ice-cold 0.05*M* sodium phosphate buffer at *p*H 7 to remove excess blood, blotted with filter paper, weighed, and then homogenized in an amount of buffer sufficient to make a 10-percent homogenate. Testes were homogenized and then disrupted with high-frequency sound. The extracts were centrifuged at 4°C for

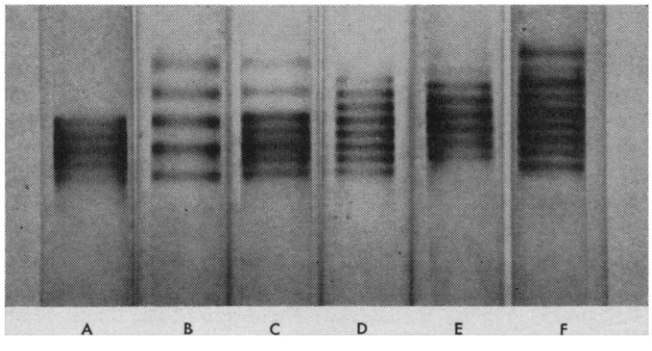

Fig. 1. LDH electrophoretograms from trout. The sample was applied to the top of the gel, and the proteins migrated toward the anode. LDH-1 is the isozyme closest to the anode. Patterns: A, lake trout; B, speckled trout; C, mixture of A and B, demonstrating coincidence of LDH bands; D, splake; E, recombinant isozyme pattern formed by combining A and B and freezing; F, mixture of B and E, demonstrating coincidence of LDH bands.

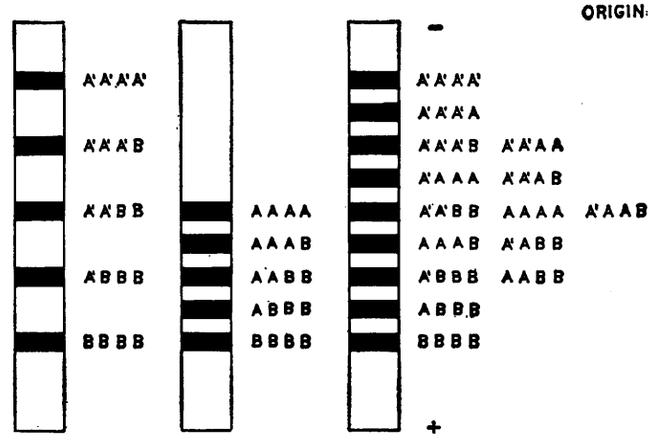

Fig. 2. Diagrammatic representation of LDH isozymes showing proposed subunit composition of (left to right) speckled trout, lake trout, and splake.