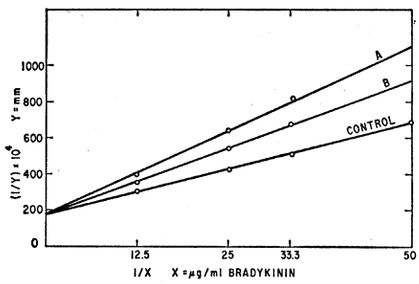

Fig. 2. Drug dose (x)–response (y) plot characterizing suppression of contractile response of smooth muscle (guinea pig ileum) by BHA. Control samples contain no antioxidant. Points on line A were obtained with 7.2×10^{-9} g of BHA per milliliter in test solution, and points on line B were obtained with 3.6×10^{-9} g of BHA per milliliter. All points represent data corrected for loss in gut sensitivity to bradykinin due to exposure to BHA.

being directly proportional to the amount of smooth muscle contraction elicited by bradykinin. Reduction in peak height by added antioxidant was taken as a measure of its inhibitory action.

With this procedure, it was found that the threshold concentration at which BHA began to suppress the ileum's response to 0.04 μg of bradykinin per milliliter in the reaction bath was $8 \times 10^{-9}M$. Increasing concentrations of antioxidant depressed gut response (Fig. 1).

Supposedly the mechanism of this inhibition can be ascertained from a standard study of the relation of drug to dose response as described by Roche e Silva (*2*). Here the reciprocals of effects ($1/Y$), where Y is peak height in millimeters, as measured on the kymograph chart are plotted against the reciprocals of the doses ($1/X$), where X is equal to the concentration of bradykinin in the reaction chamber. Our data in this form are presented in Fig. 2. From the relatively straight line plots and the common intercept shown in Fig. 2 it can be inferred (*2*) that BHA acts as a competitive inhibitor of bradykinin. Likewise, from the second plot, the relative inhibitor strength of BHA can be derived (*2*) as its inhibitory potency (pK_1). The value ascertained by this procedure, $pK_1 = 7.9$ g/ml, indicates that BHA is a relatively strong inhibitor.

The validity of this relation may be questioned since the inhibitory action of BHA is not completely reversible. After four 30-second exposures to solutions containing $1 \times 10^{-6}M$ BHA stock solution, only 92 percent of normal bradykinin response could be recovered by flushing with buffer for 15 minutes. The reason for this loss in sensitivity has not been established. It probably involves very strong or irreversible binding of the antioxidant on some bradykinin receptor sites since the smooth muscle contracts normally in response to 0.40 μg of histamine per milliliter in the presence of $10^{-5}M$ concentrations of BHA in the reaction bath. Regardless, our data demonstrate that relatively low concentrations of BHA can inhibit the contractile action of bradykinin on smooth muscle. The higher BHA concentrations used in these experiments approach the concentrations of BHA permitted in foods under present Food and Drug Administration regulations. The significance of this finding in regard to the widespread use of this antioxidant in food manufacture remains to be established.

The possibility that an impurity in the commercial BHA sample used in our study could have given rise to the described results has been considered. Standard gas-liquid chromatographic analysis of the antioxidant used showed it to be essentially 7 percent 2-BHA and 93 percent 3-BHA. Only two volatile contaminants in trace quantities could be found on the chromatogram. A sample of BHA further purified by sublimation also has the same inhibitory power as the commercial sample. Furthermore, we have some data that indicate other phenolic antioxidants similarly inhibit the response of smooth muscle to bradykinin.

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Catch Property in Single Mammalian Motor Units

Abstract. *The tension output of single motor units in the cat triceps surae muscle was studied during patterned stimulation of the innervating motoneurons. With repetitive stimulation within a rather narrow frequency range, the tension output of slow-twitch (type S) motor units may be quite sensitive to the pattern of stimulus intervals in the train. The presence of only one stimulus interval that is much shorter than the others in the train can cause marked, long-lasting tension enhancement, provided that the stimulus repetition rate in the basic train is within certain limits.*

A motor unit in skeletal muscle consists of a neural component, a motoneuron with cell body in the spinal cord and axon in a particular muscle nerve, and a muscle component, the bundle of muscle fibers innervated by that motoneuron (*1*). Motor units may be regarded as the indivisible functional elements of skeletal muscle action. Much work on the production and maintenance of muscle tension has involved whole muscle, with all motor units activated simultaneously (*2*). However, such simultaneous activation is rare in normal movement; usually, muscle tension is produced by motor units acting asynchronously and independently (*3*). Moreover, motor units in a given muscle may differ from one another in contractile properties (*4*). For these reasons, we have recently begun a study of the production and maintenance of tension by single motor units in the cat triceps surae muscle. In the course of this work we have observed a phenomenon similar to what has been termed the "catch property" of certain invertebrate muscles (*5*).

When a single motoneuron can be stimulated in isolation, the mechanical response produced in a muscle by one active motor unit can be studied (*4*). In these experiments adult cats, anesthetized with pentobarbital, were maintained with normal blood pressure and body temperature. The tendons of the three heads of the triceps surae muscle, medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL), were separated from one another and arranged for independent connection to a strain gauge myograph through a stiff link. The hind limb was rigidly fixed to a frame with drills through the femur and ankle. The strain gauge, mounted on a rack and pinion that permitted

adjustment of muscle length (and thus passive tension), was aligned with the muscle under study. All tension recordings were made with the test muscle held at constant length (isometric conditions). Triceps surae motoneurons were impaled with glass micropipettes filled with 3*M* KCl solution and were stimulated by injecting short depolarizing current pulses through the electrode. Motor unit contractions resulting from this stimulation produced tension changes, which were recorded with the appropriate muscle stretched to give some 50- to 100-g initial passive tension (*4*).

Activation of a single motor unit by two closely spaced stimuli (interpulse interval of 3 to 10 msec) produces a tension response much larger and longer lasting than the twitch response to a single stimulus (Fig. 1). The apparent tension added by the second stimulus (as in Fig. 1, dotted curve) is usually larger in peak tension and always longer in duration than the single twitch response. At short interpulse intervals tension enhancement is at maximum; it falls off progressively as the interval is lengthened. A similar effect has been observed with whole muscle (*6*). The electrical action potential (EMG response) produced by the active muscle fibers during the second response may be the same or somewhat smaller (upper trace) than the EMG during the first (or during the single) twitch. This suggests that the apparent tension enhancement in the second response is not due to additional muscle fibers that had remained inactive during the first response; thus the effect does not appear to be due to properties of the motor axon or neuromuscular junction (*7*). The motor unit in Fig. 1 was classed as a slow-twitch or type S unit on the basis of speed of twitch contraction [contraction time >35 msec (see *4*, *8*)]; such units are present in the nominally slow SOL and also in the nominally fast MG and LG muscles. Similar tension enhancement has been found in fast-twitch type F motor units [contraction time <35 msec (see *4*, *8*)], which are found in the MG and LG muscles but not in SOL. The effect in type F and type S units differs only in time scale.

Two stimuli with a short interpulse interval superimposed on continuous low-frequency stimulation of a type S motor unit may cause long-lasting tension enhancement, far longer in duration than the double-twitch response itself (Fig. 2). In Fig. 2 (left column) tension produced by a type S unit in the MG muscle, activated at various low rates, is shown compared with the tension output when the first stimulus in the train was followed by a single extra impulse (arrows) at an interval of 10 msec. The duration of tension addition following the single extra stimulus depended rather strikingly on the frequency of the underlying basic stimulus train. A convenient measure of the added tension was the area between the tension traces (with and without the extra stimulus) for different stimulus rates, with traces taken over equal, relatively long times. The graph illustrates the rather small range of "optimal" frequencies for long-lasting tension enhancement and shows that a stimulus rate of 9.8 pulses per second (pps) resulted in the most prolonged response to the extra stimulus. With this rate, tension enhancement lasted over 5 seconds after the single extra stimulus was added to the basic pulse train (Fig. 2, record below graph).

Prolonged enhancement of muscle tension output, consequent to interpolation of a single stimulus or a short high-frequency train into an ongoing low-frequency stimulus train, is present to a remarkable degree in some crustacean muscles and has been called the "catch" property (*5*). The phenomenon observed in our experiments with mammalian motor units is qualitatively similar; we will use the same term to describe it, although we recognize that the mechanism responsible may not be the same in invertebrate and mammalian muscle. The catch property as demonstrated here is particularly prominent in type S motor units. A similar behavior is exhibited by type F units, but the resulting tension enhancement is always short-lasting; the records at best resemble those in Fig. 2 for 7.2 pps, although of course the time scale is different (*9*). Our results indicate that the catch phenomenon is markedly dependent on the stimulus repetition rate in the basic train. In type S motor units, the optimal frequency has been between 6 and 11 pps, and units with longer contraction

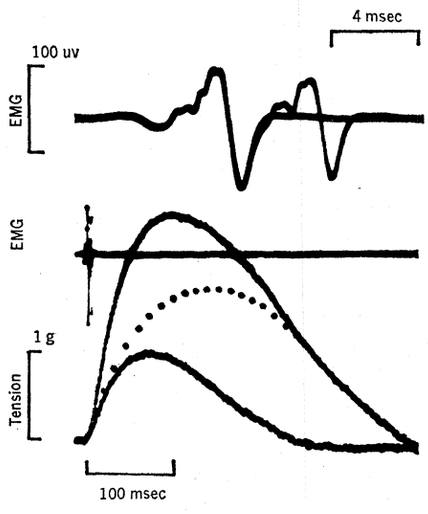

Fig. 1. Mechanical and electrical responses of a single motor unit in SOL muscle. Bottom traces (tension): superimposed records of mechanical responses (solid lines) to single pulse stimulation of the unit motoneuron (smaller response) and to two closely spaced pulses (larger response; pulse interval 4 msec). The dotted outline denotes the algebraic difference between the two responses; it shows the additional tension resulting from the second stimulus. Middle trace (EMG): electrical potential (EMG) generated by the activated muscle fibers and recorded with fine bipolar wire electrodes placed on the muscle surface directly over the contracting unit. Time calibration for both traces is 100 msec. Upper trace (EMG): the same EMG responses recorded on a fast time base (note 4-msec calibration). Muscle temperature was 35°C.

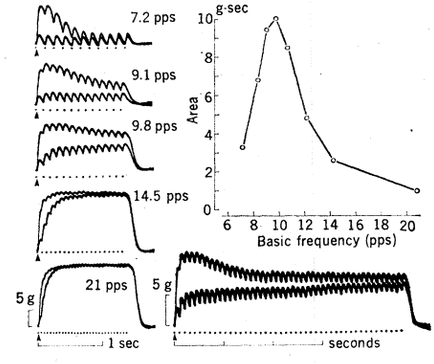

Fig. 2. Tension output of a MG slow-twitch (type S) motor unit activated at different frequencies. In each set of traces in the left column the tension produced by the unit at the indicated stimulus frequencies is shown as the lower trace. Then a single extra stimulus was introduced into each stimulus train at an interval of 10 msec (arrows) after the first pulse in the train; the resulting tension is shown in the upper trace in each set. Note that the duration of tension enhancement with the extra stimulus depended on the basic stimulus frequency. The graph shows the area (ordinate) between the tension curves for each stimulus frequency, measured over equal times (in this case, 1.5 seconds) and plotted against the basic stimulus frequency in pulses per second. Below the graph is a record of the tension output of the unit activated at 9.8 pps, with and without a single extra stimulus, taken on a slower time base to show the long duration of the effect of the extra stimulus. Muscle temperature was 36°C.

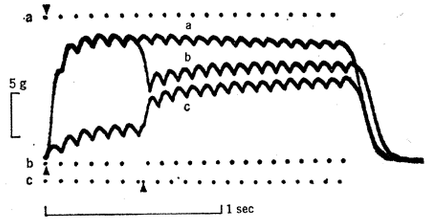

Fig. 3. Tension responses of a MG type S motor unit to three trains of 22 stimuli each, at a basic rate of 12.2 pps (interpulse interval, 82 msec). In each train, one or two stimulus intervals were altered. The tension traces are labeled (*a*, *b*, *c*), and the corresponding pulse sequences are similarly 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