Catchlike property of rat diaphragm: subsequent train frequency effects in variable-train stimulation

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Van Lunteren, Erik, and Christopher B. Sankey. Catchlike property of rat diaphragm: subsequent train frequency effects in variable-train stimulation. J. Appl. Physiol. 88: 586–598, 2000.—A high-frequency burst of pulses at the onset of a subtetanic train of stimulation allows skeletal muscle to hold force at a higher level than expected from the extra pulses alone because of the catchlike property of muscle. The present study tested the hypothesis that the presence and degree of force increase induced by a high-frequency burst are strongly modulated by the subsequent train frequency. Rat diaphragm muscle strips (studied in vitro at 37°C) underwent two-, three-, or four-pulse bursts [interpulse interval (IPI) of 5 or 10 ms] at the onset of 10- to 50-Hz subtetanic trains. Force was quantified during the train with respect to its peak value (Fpeak), mean value (Fmean), and force-time integral (Farea), and it was compared with that produced during subtetanic trains of an equal number of pulses without preceding pulse bursts (Diff-Fpeak, Diff-Fmean, Diff-Farea). Fpeak and Fmean increased with two-, three-, and four-pulse bursts, and Diff-Fpeak and Diff-Fmean increased progressively with decreasing frequency of the subtetanic train. Farea, the best reflection of catchlike force augmentation, was increased mainly by the four-pulse bursts with an IPI of 10 ms, and Diff-Farea was maximal at subsequent train frequencies of 15–25 Hz. The use of incorrect patterns of burst stimulation could also precipitate Farea decreases, which were observed with the four-pulse, 5-ms IPI paradigm. The time required to reach 80% of maximal force (T80%) became shorter for each of the pulse burst stimulation patterns, with maximal reduction of Diff-T80% occurring at a subsequent train frequency of 20 Hz in all cases. These data indicate that extra-pulse burst stimulation paradigms need to incorporate the optimal combinations of extra-pulse number, IPI, and the frequency of the subsequent subtetanic train to take greatest advantage of the catchlike property of muscle.

The contraction of skeletal muscle is a dynamic process, the force of which is regulated by modifying the frequency of activation of recruited motor units. Conventional investigations into the relationship between the stimulation frequency of skeletal muscle and the amount of resultant force have monitored the response of nonfatigued muscle to constant-frequency trains (CFTs) of regularly spaced electrical pulses (13). However, it has since been demonstrated that the force-frequency relationship of muscle depends on the activation history of the muscle (3, 4, 8, 12, 23) and that motoneurons often stimulate muscle with nonrepetitive firing patterns (1, 14, 20, 21, 24). These factors prevent accurate forecasts of force output from a given pattern of stimulation and have precipitated more recent endeavors into the area of variable-frequency-train (VFT) stimulation (a stimulation pattern in which the frequency changes during the course of the stimulus train). Most (but not all) variable-frequency studies have demonstrated an impact on the force output of muscle (15, 16, 22, 27, 28, 30, 35). These investigations asserted that the largest amount of force generated per pulse occurred when a small number of pulses were delivered at a high frequency (short interpulse interval) at the onset of a lower frequency, subtetanic train. This methodology of irregular stimulation has also been used to exploit the catchlike property of muscle, another factor that can influence muscle output force (2–4, 9, 10). The catchlike property has been well documented as the nonlinear augmentation in muscle force resulting from a high-frequency burst of pulses at the onset of a subtetanic train. The force output of muscle stimulated by a train of variable frequency is greater than would be expected from the presence of the additional pulses alone; this finding gives rise to the notion that muscle is able to “catch” or “hold” tension and sustain it for the duration of a train. Previous studies in variable-train stimulation, most specifically that by Binder-Macleod and Barrish (3), have elucidated the ways in which the numbers of extra pulses and the size of the interpulse interval (IPI) of the initial pulse burst affect muscle output force. The role of the frequency of the subsequent subtetanic train that succeeds the burst of extra pulses has been examined to a more limited extent. The purpose of the studies by Burke et al. (9, 10) was to examine the functional impact of the “doublet” on muscle contraction rather than to define optimal stimulation parameters to take advantage of the catchlike property of muscle. Experiments were restricted to a doublet with a single IPI. Furthermore, the number of pulses in the subsequent train was not kept constant, so that trains with a preceding doublet had more pulses than trains without the doublet. More recently, Binder-Macleod et al. (7) examined effects of varying the frequency of the subsequent train, but the emphasis was on how the salu-
tory effects of catchlike-inducing trains are modulated by muscle fatigue. They also restricted their studies to a doublet with a single IPI. Furthermore, their stimulation paradigm increased peak force but did not result in an augmentation of the force-time integral in nonfatigued muscle at any frequency. In fact, the force-time integral was decreased slightly by VFT relative to CFT stimulation in nonfatigued muscle.

The restoration of muscle contraction by functional electrical stimulation may allow subjects with paraplegia and quadriplegia to regain partial use of their arms and legs and permit them to engage in some normal activities of daily living (26). Functional electrical stimulation has also been used for the restoration of diaphragm contraction and hence ventilation in subjects with high cervical quadriplegia (18,19). Considerable attention has been paid to defining patterns of neuromuscular stimulation to optimize the magnitude and temporal pattern of force output and to minimize the rate at which fatigue occurs (2,3,5–8,16,28), with data from animal models allowing more focused studies to be performed in humans. Many of these studies have been performed in limb muscles composed of relatively homogeneous fiber subtype populations. Responses in these muscles may not predict the behavior of a muscle such as the diaphragm, which contains substantial populations of most fiber subtypes, as the ensemble response of a heterogeneous population of fibers will represent the summation of the varied responses of all of the motor units activated simultaneously.

The purpose of our present investigation was therefore to further describe the effects of a high-frequency burst of pulses at the beginning of a subtetanic train by delineating the extent to which muscle is influenced by the frequency of the remainder of the subtetanic train. We investigated the hypothesis that the stimulation frequency of the second portion of a VFT strongly modulates the presence and the magnitude of the force increase induced by the extra burst of pulses. Because the catchlike property of muscle involves the persistence of a level of muscle force throughout the entirety of a train, we believed that the postburst portion of a VFT would play an integral part in total force held or "caught." We also hypothesized that, with certain stimulation parameters of the subsequent train, the increase in force engendered by the extra burst of pulses could be mitigated or entirely prevented. Finally, we wished to examine the responses of a muscle composed of a mixture of fiber types, because information about the catchlike property of such a muscle would be important for applications in which functional electrical stimulation is used to restore ventilatory function.

**METHODS**

Surgical procedure and experimental protocol. Studies were performed in vitro on diaphragm muscle of 250- to 425-g male Sprague-Dawley rats. The animals were anesthetized with urethan (Sigma Chemical, St. Louis, MO) (1 g/kg ip), and the diaphragm was surgically excised. Small muscle strips of 1- to 1.5-mm in diameter were prepared, ensuring that rib origin and central tendinous insertion remained intact. The strips were mounted vertically in a double-jacketed bath (37°C) containing oxygenated (95% O2-5% CO2) physiological solution consisting of (in mM) 135 NaCl, 5 KCl, 2.5 CaCl2, 1 MgSO4, 1 NaH2PO4, 15 NaHCO3, and 11 glucose, with pH adjusted to 7.3–7.4. The muscles were field stimulated electrically at supramaximal voltage and optimal length with platinum electrodes (Radnotti Glass, Monrovia, CA). Muscle force was measured with a high-sensitivity isometric transducer (Kent Scientific/Radnotti Glass Technology).

Diaphragm muscle strips underwent isometric twitch stimulation with subtetanic CFTs and with VFTs containing a high-frequency burst of pulses at the beginning of a subtetanic train of lower frequency pulses. Figure 1 depicts CFT and VFT pulse patterns (A2, B2), along with characteris-
tic amounts of force generated by each (A1, B1). Two-, three-, or four-pulse bursts at the onset of a VFT were fixed at an IPI of either 5 or 10 ms. Muscle strips were each assigned to one of six possible stimulation patterns defined by the number of extra pulses and IPI, and a sample size of six strips was investigated for each pattern. All strips were subjected to the following stimulation paradigm. A series of trains consisting of a VFT preceded and followed by a corresponding CFT (separated by ~15 s) was given sequentially for each frequency tested. Frequencies were varied at 5-Hz intervals from 10 to 50 Hz and from 50 to 10 Hz, yielding a total of two stimulations at each frequency. The muscle was allowed 1 min of recovery before being stimulated at the next frequency in the sequence. The number of pulses was kept uniform for all trials (8 pulses) so that VFTs and CFTs could be reasonably compared. Muscle force records were digitized, collected on-line (Axotape, Axon Instruments, Foster City, CA), and stored on the hard drive of a computer for future analysis.

Data management and analysis. Ordering effects, which may have occurred as a result of sequential stimulation, were controlled by averaging the two measurements obtained from each frequency tested: one in the progressive ascent from 10 to 50 Hz and the other in the descent from 50 to 10 Hz. Furthermore, averaging CFT measurements before and after corresponding VFTs compensated for any potential bias or temporary effect created by the extra-pulse stimulation. Four main parameters were quantified to examine the effects of VFTs: peak force (Fpeak), mean force (Fmean), area of force (Farea), and time required for the VFT to reach 80% of peak force generated by the muscle at any time during the train. Fmean is the average amount of force produced by the muscle during the time of contraction. Farea is the area under the force-time curve and was calculated by multiplying Fmean by the duration of time over which muscle force persisted. T80% may have some clinical importance in establishing how quickly a desired level of force can be reached when muscles are externally stimulated with VFTs. All measurements and calculations were made off-line with manually controlled cursors using Axoscope software (Axon Instruments). Isometric force was measured in grams. These data were normalized in a fashion identical to that performed by previous investigators (3), by dividing a given VFT measurement (Fpeak, Fmean, Farea) by the average value of the CFTs that surrounded it. To ascertain the differential (Diff) force produced between VFT and CFT figures, one normalized force unit was subtracted from the normalized measurements. For example, the differential value for Fmean was obtained in the following manner

\[
\text{Diff-Fmean} = \left( \frac{\text{VFT-Fmean}}{\text{CFT-Fmean}} \right) - 1
\]

We viewed Diff-Farea, akin to the measure used by Binder-Macleod and Barrish (3), Parmiggiani and Stein (27), Stein and Parmiggiani (30), and Zajac and Young (35), as the most critical of the preceding measurements because it represents the total force generated by the muscle for the entirety of a train. Diff-Farea is therefore the best means of quantifying the catchlike property of skeletal muscle. Because the number of pulses was the same for each train (8 pulses), Farea also yields a per-pulse approximation of the relative amount of force produced. Consequently, augmentation of Farea indicates an increase in the total force output of the muscle, the amount of force caught by the muscle, and the quantity of force affected per pulse by each train. In contrast, Fpeak and T80% are affected predominantly by the effects of the pulse burst itself, rather than the effects of the pulse burst on the remainder of the train, and hence are poor indexes of the catchlike property of muscle.

Peakburst, potentiationburst, and catchburst measures, originally characterized by Binder-Macleod and Barrish (3), were calculated for the four-extra-pulse, 10-ms IPI data (Fig. 1B1).

Fig. 2. Values for peak force (Fpeak; A), mean force (Fmean; B), force-time integral (Farea; C), and time required to reach 80% of maximal force (T80%; D) during CFT and VFT stimulation, in which 4-pulse, 10-ms IPI paradigm was used for VFT stimulation. *Significant differences between CFT and VFT stimulation (P < 0.05).
We focused on a single combination of number of pulses and IPI in performing these additional analyses principally to ensure that the varied parameter was the frequency of the subsequent train, our chief interest in this study. Given the fact that we restricted ourselves to a single stimulation pattern, the particular combination of number of extra pulses and IPI was chosen on the basis of increases in \( F_{\text{area}} \). For reasons just described, \( F_{\text{area}} \) is the best approximation of the catchlike property of skeletal muscle, and we therefore determined it appropriate to perform the “burst” measurements on the parameter that took greatest advantage of this property. The four-pulse, 10-ms IPI pattern was chosen because it resulted in the largest increase in \( F_{\text{area}} \) among the six stimulation patterns examined (See Fig. 6). Fig. 1B illustrates an example of the burst measurements applied to data from the four-pulse, 10-ms IPI pattern of stimulation.

The values of \( \text{peakburst} \), \( \text{potentiationburst} \), and \( \text{catchburst} \) address force measurements specific to the burst of extra pulses; they were proposed by Binder-Macleod and Barrish (3) to correlate with an increase in muscle force. We were interested to discern whether this correlation persisted when the frequency of the subtetanic train rather than the IPI of the extra-pulse burst was manipulated. \( \text{peakburst} \) is the maximum muscle force that is achieved by the initial burst of extra pulses, but it is not necessarily the maximum force produced over the entire train. \( \text{catchburst} \) is the level of force to which the muscle drops at the time of stimulation by the first pulse following the high-frequency burst. This parameter pertains directly to the catchlike property of muscle because it reflects the amount of force the muscle is attempting to hold or augment for the rest of the train. The increase in muscle force elicited by the pulse that follows the initial IPI is the \( \text{potentiationburst} \), and it represents the force augmentation facilitated by the preceding, high-frequency burst of pulses (potentiation\( _{\text{burst}} \) shown in Fig. 1B is actually negative). Potentiation\( _{\text{burst}} \) is calculated by subtracting the peak\( _{\text{burst}} \) values from the level of force attained by the first pulse that follows the high-frequency burst.

The additional burst measures were normalized to CFT values by the differential method previously described (burst values for the CFTs were calculated based on the burst consisting of a single pulse, rather than the two to four pulses that constituted the burst for the VFTs) (3). To determine whether \( \text{peakburst} \), \( \text{catchburst} \), and \( \text{potentiationburst} \) were associated with an increase in muscle force, they were plotted vs. the \( \text{Diff-Farea} \), \( \text{Diff-Fpeak} \), and \( \text{Diff-Fmean} \) values obtained for the same frequencies and correlated with a linear regression model. \( R \) and \( P \) values were calculated for each plot, with a threshold of significance of \( P < 0.05 \). Analyses of statistical differences for \( \text{Diff-Fpeak} \), \( \text{Diff-Fmean} \), \( \text{Diff-Farea} \), and \( \text{Diff-T80%} \) between VFTs and CFTs were performed by using two-way ANOVA followed by the Newman-Keuls test to compare differential force values to zero (a differential value of zero occurs when the VFT and CFT values are identical). One-way ANOVA and the Newman-Keuls test were used to establish statistical differences in a given measurement (\( \text{Diff-Fpeak} \), \( \text{Diff-Fmean} \), \( \text{Diff-Farea} \), \( \text{Diff-T80%} \)) as a function of the frequency of the subsequent subtetanic train (10–50 Hz). Comparison of

![Fig. 3. Effects of VFT stimulation on Fpeak. Differential Fpeak (\( \text{Diff-Fpeak} \)) is illustrated for 2-pulse (A), 3-pulse (B), and 4-pulse (C) bursts at 10-ms IPI, as well as 2-pulse (D), 3-pulse (E), and 4-pulse (F) extra-pulse bursts at 5-ms IPI. Fpeak for VFTs at each frequency was normalized to values for flanking CFTs, and it represents increase in peak force afforded by extra burst of pulses. Values are means ± SE. *Statistically significant differences between \( \text{Diff-Fpeak} \) and 0, corresponding CFT values (\( P < 0.05 \)). Significant differences in \( \text{Diff-Fpeak} \) as a function of stimulation frequency are indicated in Table 1.](http://jap.physiology.org/ by 10.2233.5 on November 4, 2016)
VFTs as a function of stimulation frequency across the six paradigms was performed with two-way ANOVA, followed in the event of a significant difference by the Newman-Kuels test. Statistical significance in all cases was indicated by $P < 0.05$.

RESULTS

Examples of force records elicited by CFT and VFT stimulation are depicted in Fig. 1. During VFT stimulation, force increased relative to that of the CFT stimulation in response not only to the burst but also thereafter for several (Fig. 1B) or all (Fig. 1A) of the postburst pulses. The persistent elevation of force during the latter part of the stimulus train is what is attributed to the catchlike property of muscle. The actual (nonnormalized) forces elicited by CFT and VFT stimulation during the four-pulse, 10-ms IPI paradigm are depicted in Fig. 2, A–C. Compared with CFT stimulation, the four-pulse, 10-ms IFTVFT trains produced large increases in $F_{\text{peak}}$, more modest increases in $F_{\text{mean}}$, and small increases in $F_{\text{area}}$. The increase in $F_{\text{peak}}$ was statistically significant at all stimulation frequencies, that of $F_{\text{mean}}$ was statistically significant at all but the highest stimulation frequency, and that of $F_{\text{area}}$ was statistically significant at stimulation frequencies of 10–35 Hz.

Large force increases were evident in $F_{\text{peak}}$, which resulted in positive Diff-$F_{\text{peak}}$ values with two-, three-, and four-pulse bursts at both 5- and 10-ms IPIs (Fig. 3). In most instances, the increases in $F_{\text{peak}}$ were attributable to the effects on force of the pulse burst itself, rather than to the effects of the pulse burst on force during the remainder of the stimulation train, especially when the pulse burst consisted of three or four pulses (thus $F_{\text{peak}}$ is not a good index of the catchlike property of muscle). Diff-$F_{\text{peak}}$ for all six combinations of IPI and number of extra pulses progressively increased with decreasing frequency of the subsequent train from 10 to 50 Hz. This trend in Diff-$F_{\text{peak}}$ was most evident for the four-pulse, 10-ms IPI stimulation, which had a maximal Diff-$F_{\text{peak}}$ of $1.77 \pm 0.08$ at 10 Hz and a minimal value of $0.22 \pm 0.03$ at 50 Hz (Fig. 3C). Force increases attributed to extra-pulse stimulation were statistically significant in all cases at low-to-intermediate subsequent train frequencies (10–30 Hz) but not consistently so at stimulation patterns between 30 and 40 Hz. The four-pulse, 10-ms IPI paradigm was the only stimulation that retained significant differences between VFTs and CFTs for all frequencies from 10 to 50 Hz. Significant differences in Diff-$F_{\text{peak}}$ as a function of the frequency of the subsequent train were present for all patterns of stimulation (Table 1). The pronounced decreases observed in $F_{\text{peak}}$ resulted in significance between a majority of the subsequent train frequencies from 10 to 50 Hz.

VFT stimulation also elicited augmentations in $F_{\text{mean}}$, which produced positive Diff-$F_{\text{mean}}$ values with two-, three- and four-extra-pulse bursts at 5- and 10-ms IPIs (Fig. 4). The most marked increases were again observed with the four-pulse, 10-ms IPI stimulation, the maximum Diff-$F_{\text{mean}}$ value of which at 10 Hz was $0.73 \pm 0.03$ and which declined to $0.12 \pm 0.03$ at 50 Hz (Fig. 4C). Diff-$F_{\text{mean}}$ increased moderately with decreasing subsequent train frequency, and statistically significant increases in Diff-$F_{\text{mean}}$ persisted for nearly all frequencies from 10 to 50 Hz in four of the six patterns of stimulation. Statistically significant differences in Diff-$F_{\text{mean}}$ between subsequent train frequencies were

Table 1. Significant differences in $F_{\text{peak}}$, $F_{\text{mean}}$, and $F_{\text{area}}$ as a function of subsequent train stimulation frequency

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Significance between frequencies for a particular combination of extra-pulse number and interpulse interval (IPI) is listed across a row, and frequency ranges listed are significantly different from the frequency at the top of the column to which they correspond ($P < 0.05$). Peak force ($F_{\text{peak}}$), mean force ($F_{\text{mean}}$), and force-time integral ($F_{\text{area}}$) are for data depicted in Figs. 3, 4, and 5, respectively.
present for all but the two-pulse, 10-ms IPI stimulation (Table 1). Significance between frequencies was again dispersed throughout the entire range from 10 to 50 Hz. 

\( \text{Diff-F}_{\text{area}} \) and \( \text{Diff-F}_{\text{mean}} \) are akin to the measures used in previous studies (3, 9, 10, 27, 30, 35) for quantifying the catchlike property of muscle. \( \text{F}_{\text{area}} \) was minimally affected by two- or three-pulse bursts and either increased (10-ms IPI) or decreased (5-ms IPI) with four-pulse bursts (Fig. 5). \( \text{Diff-F}_{\text{area}} \) was relatively stable with increasing frequency of the subsequent subtetanic train for most stimulation patterns, except for the four-pulse, 10-ms IPI burst. \( \text{Diff-F}_{\text{area}} \) during four-pulse, 10-ms IPI bursts was maximal at subtetanic train frequencies of 15–25 Hz (Diff-F\text{area} values were 0.19 ± 0.03, 0.17 ± 0.04, and 0.19 ± 0.04, respectively), and declined to a Diff-F\text{area} of 0.02 ± 0.02 at 50 Hz (Fig. 5C). Positive values for Diff-F\text{area} were present only for the two-pulse, 10-ms IPI and the four-pulse, 10-ms IPI stimulations at frequencies of 10–35 Hz. Negative values for Diff-F\text{area} were observed for the three-pulse, 5-ms IPI and the four-pulse, 5-ms IPI patterns. Statistically significant alterations in Diff-F\text{area} as a function of subsequent train frequency were present in all stimulations except for the two-pulse, 5-ms IPI stimulation pattern (Table 1). Significance in this case was most prevalent between the lowest (10–20 Hz) and highest (40–50 Hz) ends of the frequency range.

\( \text{Diff-F}_{\text{area}} \), \( \text{Diff-F}_{\text{mean}} \), and \( \text{Diff-F}_{\text{peak}} \) were correlated with the \( \text{Diff-catch}_{\text{burst}} \), \( \text{Diff-peak}_{\text{burst}} \), and \( \text{Diff-potentia}_{\text{burst}} \) measurements by using linear regression models (3). The burst measurements were performed for a single stimulation parameter to isolate the frequency of the subsequent train as the manipulated variable. The four-pulse, 10-ms IPI pattern of stimulation was chosen because it had the greatest values for \( \text{Diff-F}_{\text{peak}} \), \( \text{Diff-F}_{\text{mean}} \), and \( \text{Diff-F}_{\text{area}} \) among the stimulation paradigms (Fig. 6). Diff-F\text{area} and Diff-F\text{mean} correlated with Diff-catch\text{burst} (Figs. 7A and 8A), whereas Diff-F\text{peak} did not (Fig. 9A). However, only 20% and 13% of the variability in Diff-F\text{area} and Diff-F\text{mean}, respectively, could be attributed to variability in Diff-catch\text{burst}. None of the differential force values correlated with Diff-peak\text{burst} (Figs. 7B, 8B, and 9B). Diff-F\text{mean} and Diff-F\text{peak} correlated with Diff-potentia\text{burst} (Figs. 8C and 9C), whereas Diff-F\text{area} did not (Fig. 7C). Only 28 and 41% of the variability in Diff-F\text{mean} and Diff-F\text{peak}, respectively, could be attributed to variability in Diff-potentia\text{burst}.

\( T_{80\%} \) became shorter during VFT compared with CFT stimulation for all six combinations of extra-pulse number and IPI (Fig. 2D and Fig. 10). In most instances, the increases in \( T_{80\%} \) were attributable to the effects on force of the pulse burst itself, rather than to the effects of the pulse burst on force during the remainder of the stimulation train, especially when the...
pulse burst consisted of three or four pulses (thus T_{80\%} is not a good index of the catchlike property of muscle). As the frequency of the subsequent subtetanic train was increased from 10 to 50 Hz, Diff-T_{80\%} in all cases decreased to a minimum at 20 Hz and then progressively ascended from 20 to 50 Hz. Minimum Diff-T_{80\%} values were very similar across the various stimulation patterns, each of which was between 0.80 and 0.75.

The differences in T_{80\%} between VFT and CFT stimulations were significant for all frequencies from 10 to 50 Hz in all stimulation patterns tested. Statistical analyses of the differences between frequencies within each of the stimulation patterns are depicted in Table 2. The decrease of Diff-T_{80\%} to a minimum value at 20 Hz and the ensuing increase from 20 to 50 Hz affected significance in such a way that intermediate frequencies (15–35 Hz) were most commonly significantly different compared with the highest (45 and 50 Hz) and lowest (10 Hz) frequencies.

DISCUSSION

The principal finding of this study was that the frequency of the subsequent subtetanic train following a high-frequency burst of pulses strongly modulates the force output of muscle and the time required to reach a desired level of force. In general, F_{peak}, F_{mean}, and F_{area} were significantly affected by the manipulation of the subsequent train frequency within a particular stimulation paradigm. More specifically, the augmentations in these force measurements generated by variable-train stimulation, as quantified by Diff-F_{peak}, Diff-F_{mean}, and Diff-F_{area}, declined substantially with increasing frequency of the subsequent train from 10 to 50 Hz. T_{80\%} was shortened for all subsequent train frequencies, and Diff-T_{80\%} reached a minimum value at 20 Hz in each of the stimulations. Of the six patterns of stimulation tested, the four-pulse, 10-ms IPI pattern engendered the greatest increases in force. However, the differential force values generally correlated weakly or not at all with Diff-catchburst, Diff-peakburst, and Diff-potentiationburst measurements.

Burke et al. (9) originally characterized the catchlike property of skeletal motor units after observing in cats a property similar to what Wilson and Larimer (34) had documented as the “catch property.” Wilson and Larimer discovered a means by which arthropod muscle tension could be sustained by a stimulation frequency lower than that necessary to create it. Additionally, they found that a relatively high frequency is initially necessary to produce a force output large enough to be caught by the lower level of excitation. Burke et al. (9, 10) concluded that this phenomenon was heavily influ-
enced by the frequency of the subsequent train of pulses, on the basis of their studies of area increases elicited by a single extra-pulse pattern (two-pulse, 10-ms IPI) and each of the other paradigms are indicated; number of asterisks indicates number of other stimulation paradigms that resulted in values that differed from the 4-pulse, 10-ms IPI stimulation paradigm (e.g., 5 asterisks indicates that values for the 4-pulse, 10-ms IPI paradigm differ from all other values). Statistical differences among other five paradigms were present in a few instances, but to maintain clarity they are not indicated.

property, in that the extra force initially generated by the extra-pulse burst gradually diminished to a plateau at a lower level of force. For this reason, Burke et al. (10) observed the catchlike property for much shorter durations in F than in S units.

The results of the present study concerning the effects of the subsequent train frequency corroborate those of Burke and colleagues (9, 10) and extend their work in several ways. First, we examined an array of variable-train stimulation patterns consisting of six different combinations of extra-pulse number and interpulse interval. In contrast, Burke et al. (9, 10) investigated only a single extra-pulse pattern, a two-pulse stimulation paradigm with a 10-ms IPI, as the purpose of their study was to examine the functional impact of the doublet. The exploration of extra-pulse bursts with higher pulse numbers was necessitated because Binder-Macleod and Barrish (3) found greater force augmentation with three-pulse bursts than with two-pulse bursts. Our data corroborate those of Binder-Macleod and Barrish in that extra-pulse bursts comprising more than two pulses elicited greater force augmentations than two-pulse bursts. Second, we tested force differences in a wider range of tested frequencies (10–50 Hz) than did Burke et al. (9, 10) in their animal studies. They examined a relatively limited range of stimulation frequencies of the subsequent subtetanic train (listed above), which is lower than that at which motor units fire during high-intensity motor acts (e.g., leg movements during running, breathing during exercise) or the most commonly utilized frequencies in functional electrical stimulation applications. Third, Burke et al. (9, 10) used a stimulation paradigm in which the VFT contained a greater number of total pulses than the CFT stimulation, thereby magnifying the increases in F_area afforded by the VFT. Furthermore, because they analyzed trains over a fixed interval of time, increasing the subsequent train frequency also meant increasing the number of pulses per train at higher frequencies of stimulation. Our investigation was therefore better able to isolate the force increases attributable solely to the catchlike property at a particular subsequent train frequency by examining VFTs and CFTs with the same total number of pulses. By progressively shortening the train duration as the subsequent train frequency was increased, we were also able to maintain a constant number of pulses per train at each subsequent train frequency and thus isolate the subsequent train frequency as the only manipulated variable.

Binder-Macleod et al. (7) recently examined the effects of fatigue on the catchlike properties of human quadriceps muscle. As part of this study, they examined a wide range of stimulation frequencies of the subsequent stimulation train (~8–100 Hz) on force output, although they restricted their studies to a doublet with a single IPI (5 ms). They found in nonfatigued muscle that significant increases in F_peak were found at very low frequencies (8–10 Hz) but not higher frequencies (11–100 Hz) of the subsequent train. The present study concurs that doublet-induced increases in F_peak are
most prominent at a low frequency of the subsequent train. However, we found increases in $F_{\text{peak}}$ over a greater range of frequencies of the subsequent train (10–35 Hz). Furthermore, we found with triplets and quadruplets that the frequency of the subsequent train had an even greater effect on $F_{\text{peak}}$ than was noted with doublets. Thus the present study extends the observations by Binder-Macleod et al. (7) by delineating the interactive effects, which have not been reported previously, of varying the high-frequency burst and varying the subsequent tetanic train. Furthermore, Binder-Macleod et al. (7) did not find an augmentation of $F_{\text{area}}$ in nonfatigued muscle at any frequency subsequent to the doublet. In fact, $F_{\text{area}}$ was decreased slightly by VFT stimulation in nonfatigued muscle. Thus that study did not use a paradigm that took optimal advantage of the catchlike property of muscle. In contrast, the present study used at least one VFT protocol (four-pulse, 10-ms IPI) that did take good advantage of the catchlike property, in that not only was $F_{\text{peak}}$ increased but $F_{\text{area}}$ was also increased. Of interest is that Binder-Macleod and colleagues (7) found that force improvements with the antecedant doublet were considerably greater in fatigued than in nonfatigued muscle. In addition, there was a considerably greater dependence of force augmentation on the frequency of the subsequent train in fatigued than in nonfatigued muscle, and, in fatigued muscle, force augmentations were generally most prominent at low frequencies of the subsequent train. On the basis of these findings, we suspect that the force increases noted in the present study would have been even greater had we also tested fatigued muscle.
Muscle twitch kinetics (rates of isometric contraction and relaxation) and twitch-to-tetanic-force ratios exert a large influence on the catchlike property, the generation of muscle force, and the optimal pattern of stimulation. Burke et al. (9, 10) examined both F and S motor units, individually, and found different optimal stimulation parameters and duration of effect for each. Other studies have tended to focus on either fast- or slow-

![Diagram of relationships between Diff-F_peak and Diff-catch_burst (A), Diff-peak_burst (B), and Diff-potentiation_burst (C).](image-url)

Fig. 9. Relationships between Diff-F_peak and Diff-catch_burst (A), Diff-peak_burst (B), and Diff-potentiation_burst (C). Each symbol represents data from a single muscle strip, and each plot was correlated with a linear regression model. Burst measurements were performed only on 4-pulse, 10-ms IPI stimulation data. See text for details.

![Diagram of effects of VFT on T_80% at different stimulation frequencies.](image-url)

Fig. 10. Effects of VFT on T_80%. Diff-T_80% is illustrated for 2-pulse (A), 3-pulse (B), and 4-pulse (C) bursts at 10-ms IPI, as well as 2-pulse (D), 3-pulse (E), and 4-pulse (F) extra-pulse bursts at 5-ms IPI. T_80% for VFTs at each frequency was normalized to values for flanking CFTs, and it represents amount of time necessary for VFT to reach 80% of the force generated by the CFT. Values are means ± SE. *Statistically significant differences between Diff-T_80% and 0, the corresponding CFT values (P < 0.05). Significant differences in Diff-T_80% as a function of stimulation frequency are indicated in Table 2.
twitch muscle (3, 15, 30), and most of the investigations of both twitch types looked at each separately (4, 21, 22, 27). Recent human studies (5, 6) examined effects of variable-frequency stimulation on the quadriceps femoris muscle, which is composed of both slow and fast fibers. However, the stimulator amplitude was set at that which elicited a force equal to ~20% of maximal contraction, so that a relatively homogeneous population of fiber sizes and therefore types might have been activated. The behavior of a muscle with a mixed population of fibers is not necessarily predictable from data of each fiber type on slow fibers and data from fast fibers. A stimulation paradigm that optimally elicits the catchlike property in one fiber type may result in a decrease in force of another fiber type with different contractile properties. Thus, in a mixed muscle, the net effect may be no change in force output because of the offsetting effects. The diaphragm muscle used in our study has faster isometric contraction and half-relaxation times (24–25 ms) (32) than the S units described by Burke et al. (9, 10) (~60–70 ms) but is slower than F units (~20 ms). The rat diaphragm is composed of a mixed population of fiber types, including 40% slow and 60% fast (33). These and other factors, such as differences in species, temperature, and type of experimental preparation, may affect the optimal stimulus parameters for eliciting catchlike force increases in response to variable-frequency stimulation, so that these parameters cannot be extrapolated directly to humans.

The present study found that, with an IPI of 10 ms, the two-pulse and four-pulse bursts augmented $F_{area}$, whereas the three-pulse bursts did not alter $F_{area}$. This finding may be attributable to the diaphragm being composed of a heterogeneous population of fiber types. Burke et al. (9) studied both S and F motor units. They did not examine the effects of altering the parameters of the initial burst, but they did examine the effects of altering the frequency of the remainder of the train. They found that “the optimal frequencies for type F units were a good deal higher than those for type S.” On the basis of their findings, it is possible that F and S units may also differ from each other in the optimal parameters of the initial pulse burst with respect to eliciting the catchlike property. The present findings could therefore be explained if for one subpopulation of fibers the two-pulse but not the three- and four-pulse bursts increased $F_{area}$, and for another subpopulation of fibers the four-pulse but not the two- and three-pulse bursts increased $F_{area}$. The net effect therefore would be increases in $F_{area}$ in response to two- and four-pulse bursts but not in response to three-pulse bursts.

The burst measurements were devised to describe the contractile response of the muscle to the high-frequency burst of pulses and possibly to allow forecast of force increases evident over the entire train of stimulation (3). The correlations made by Binder-Macleod and Barrish (3) showed that catchburst was the most reliable predictor of force increase when the frequency or pulse number of the initial train is varied. Overall, they found catchburst and peakburst, but not potentiationburst, to positively correlate with $Diff-F_{mean}$ for two- and three-pulse stimulations. Increases in $Diff-F_{mean}$ could therefore be estimated or predicted on the basis of these two burst measurements in their study, with >90% of the variability in $Diff-F_{mean}$ being attributed to variability in the burst values. The present results assert that the differential burst values are not very predictive of differential force when the frequency of the subsequent tetanic train is varied. Of the nine relationships examined, statistically significant correlations were noted in only four, and among these four variability in differential burst measurements accounted for only 13–41% of the variability in differential force measurements.

$T_{80\%}$ is a measurement specifically quantified by Binder-Macleod and Barrish (3), but the concept of the rate of rise of force was addressed in other investigations of initial tension enhancement resulting from extra-pulse stimulation at various interpulse intervals (9, 10, 15, 21, 22, 27, 30, 35). These investigators found that optimal extra-pulse stimulation engenders an increase in initial tension magnitude and hence a shorter amount of time required to reach a given level of force. Binder-Macleod and Barrish (3) reported ~50%, ~75%, and ~80% shortening of $T_{80\%}$ as the result of two-, three-, and four-pulse, 10-ms IPI stimulations, respectively. Our figures for shortenings in $T_{80\%}$ were in the vicinity of 75–80% at optimal subsequent train frequencies, regardless of the pattern of stimulation used. As noted previously, $T_{80\%}$ has a unique dependence on the subsequent train frequency, becoming shortest at 20 Hz. This further the findings of Binder-Macleod and Barrish (3) in that $T_{80\%}$ depends both on the frequency of the subsequent train and on the different patterns of stimulation. Viewed collectively,
the T_{90%} and force measurement results are in accord-
ance with the theory postulated by Binder-Macleod and Barker (2) that the augmentations in force elicited by variable-train stimulation can be attributed to two main factors: a more rapid rate of force development and the catchlike property. The rate of force development within a contraction may be more important for some functional electrical stimulation applications than others. A rapid rate increases the maximum speed at which ambulation can occur and thereby may also increase its efficiency (11, 25). In contrast, respiratory motor output during breathing increases gradually during the course of inspiration, so that the rate of rise of force at the onset of contraction is an important factor mainly at high respiratory rates. However, during coughing, expiratory muscles are activated intensely with a fast rate of force development (31), so that rate of force development may be highly relevant to functional electrical stimulation applications that are being developed for coughing.

Variable-train stimulation may increase muscle force and/or maximize the rate of force achievement in functional electrical stimulation applications. These applications include partial restoration of arm and leg movements, diaphragm muscle activation to provide ventilatory support in subjects with spinal cord injury (18, 19, 26), and cardiomyoplasty in subjects with congestive heart failure (17). However, our results indicate that some care must be taken in the practice of VFT stimulation, for the use of incorrect patterns can actually precipitate decreases in force production. Although this study and many of its predecessors have demonstrated the dependence of force increase on a few unique combinations of IPI and number of extra pulses, such paradigm specificity is not required if the goal of the VFT stimulation is the rapid achievement of a certain targeted level of force, which occurs regardless of the particular pattern used.

VFT stimulation is also of great interest because it has been documented in biological systems and thus cannot be considered merely an external phenomenon. High-frequency, two-pulse (doublet) motor unit firing at the initial portion of a lower frequency train has been identified in a number of instances, among which are human voluntary muscle contraction (1, 14, 20), controlled cat locomotion (29), and unrestricted muscle movement in rats (21, 22). Doublet stimulation in rats was found only in fast-twitch muscle (type F), a finding that gives rise to the question of why only certain kinds of muscle are observed to take advantage of a catch property that is potentially available to all muscle types. One conceivable explanation in this case is that the rate of rise of force is the most important benefit of doublet stimulation, and that is why the doublets were seen only in type F muscle. Evidence that sudden or “ballistic” movements in humans seem to elicit doublet stimulation seems to support such a theory; however, repetitive doublets have been recorded in high-as well as low-threshold motor units. Further investigation is therefore necessary in the area of variable frequency stimulation as a natural phenomenon.

During natural contractions of the diaphragm and limb muscles, force recruitment is rarely maximal. In a mixed muscle such as the diaphragm, slow motor units are activated to produce submaximal contractions, and faster motor units are recruited to further augment force. In the present study, supramaximal stimulation was used to elicit maximal contractions. Although many functional electrical stimulation paradigms utilize submaximal stimulation, phrenic nerve pacing as described by Glenn and colleagues (18, 19) uses stimulation voltages that produce maximal contractions, with force output being modulated by the frequency of stimulation. Thus the use of supramaximal voltages to elicit maximal contraction in the present study may be directly relevant to clinical phrenic nerve pacing.

In clinical applications of phrenic nerve pacing, the diaphragm undergoes a gradual transition in its fiber subtype composition, changing from a mixed muscle to one consisting predominantly of slow fibers (see Ref. 18). This transition is believed to underlie the ability to pace the diaphragm for extended periods of time without substantial development of muscle fatigue. Whether these structural and functional changes alter the optimal stimulation parameters for eliciting the catchlike property of muscle cannot be determined from the present data, but this question is worthy of further study.

In conclusion, the results of the present study indicate that the subsequent train frequency strongly influences the force increases engendered by an antecedent high-frequency burst of pulses. Our results support the conclusions made by Burke et al. (9, 10) that the basic train frequency plays an important role in the amount of force caught and maintained by the muscle. Our data indicate that, among the stimulation patterns examined, the greatest augmentations in force occurred with the four-pulse, 10-ms IPI pattern in rat diaphragm. It is likely, based on the observations of others, that each muscle will have its unique optimal stimulation paradigm based on the contractile properties of its constituent fibers. VFT stimulation is presently being investigated for efficacy in various clinical applications, and its prevalence in biological systems is being assessed as well. The present data indicate that these paradigms need to incorporate the optimal combinations of extra-pulse number, interpulse interval, and the frequency of the subsequent subtetanic train to allow muscle to take greatest advantage of the catchlike property.

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