Post-Tetanic Repetitive Activity in the Cat Soleus Nerve
Its origin, course, and mechanism of generation

FRANK G. STANDAERT
From the Department of Pharmacology, Cornell University Medical College, New York

ABSTRACT Subsequent to conditioning by a high frequency stimulus axons of the cat soleus nerve respond to single stimuli with brief trains of repetitive action potentials. This phenomenon, post-tetanic repetitive activity (PTR), was studied in individual axons and single motor units of an in situ cat soleus nerve-muscle preparation. The occurrence, intensity, and duration of PTR are principally dependent on the frequency and duration of the conditioning tetanus. PTR occurs synchronously in the axon and muscles of single motor units. An analysis of the temporal relationships of the repetitive nerve and muscle potentials showed that PTR is generated in the motor nerve terminal. It is postulated that PTR is produced by a generator potential which is developed in the post-tetanic period between the unmyelinated nerve terminal and the last node of Ranvier.

The after-effects of high frequency stimulation have interested many workers (for a review, see Hughes 1958). In the neuromuscular system, attention has focused chiefly on the post-tetanic augmentation of contractile response, frequently termed post-tetanic potentiation (PTP). Mechanisms underlying PTP production were sought for almost a century before Rosenblueth and Morison (1937), and Rosenblueth and Cannon (1940) discovered that repetitive muscle action potentials accompany the PTP. Feng, Li, and Ting (1939) confirmed these observations and added others. Of greatest importance was their discovery that the post-tetanic repetitive potentials arise in the terminal portions of the motor nerve. They also noted that in the cat post-tetanic repetitive activity occurs less frequently in the gastrocnemius than
in the soleus. This difference explained the failure of several previous attempts to confirm the observations of Rosenblueth et al.

These early investigators were hampered by the limitations of available recording techniques. Particularly troublesome was the reliance on gross recordings which were incapable of resolving the temporally dispersed repetitive potentials. As a consequence many workers failed to detect the repetitive potentials or tended to interpret them as experimental artifacts (Brown and von Euler, 1938). Others confused them with ephaptic potentials which can be produced in the same preparations (Lloyd, 1941; Lloyd, 1942; Brown and Matthews, 1960), under different conditions (cf. Werner, 1961 a).

Recently, work in this laboratory (Werner, 1960) has affirmed the earlier report of Feng et al. (1939) that indirect tetanic conditioning of a cat nerve-muscle preparation in vivo can provoke post-tetanic repetition (PTR) in both the nerve and the muscle. In the present study, an attempt has been made to investigate the origin, mechanism of generation, and course of this PTR as it occurs in the cat soleus nerve. Particular effort was exerted to determine the site of origin of PTR. For this purpose recordings were taken from single ventral root axons and single motor units of an in vivo soleus nerve-muscle preparation.

METHODS

All experiments were performed on cats prepared essentially as described by Riker, Roberts, Standaert, and Fujimori (1957) and Riker, Werner, Roberts, and Kuperman (1959 a); this is shown schematically in Fig. 1. In brief, dorsal laminectomies (L4 to
S1) were performed on cats anesthetized with 80 mg/kg of α-chloralose intravenously. The dura mater of the exposed spinal cord was opened and the ventral root of the seventh lumbar nerve sectioned close to the spinal cord. The homolateral popliteal fossa was also dissected. The popliteal artery was located and all branches except the posterior tibial artery were ligated. The soleus nerve was dissected free from the lateral head of the gastrocnemius muscle and all other branches of the sciatic nerve severed. The animal was mounted in a rigid frame and mineral oil pools were formed in the back and in the leg. These were continuously bubbled with 95 per cent O₂ and 5 per cent CO₂ and kept at 37°C by means of thermoregulated heating lamps.

A bipolar platinum electrode in the lumbar region (Rn in Fig. 1) was used first as a stimulating electrode and with this, filaments from the ventral root were tested until one innervating a restricted region of the soleus muscle was located. The Rn electrode was then connected to the recording system and the soleus nerve stimulated by means of a peripheral electrode on the soleus nerve (Sn in Fig. 1). Further subdivision of the ventral root filament was made until a strand containing a single active axon was obtained. At intervals during the experiment, the functional connec-

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**Figure 2.** Post-tetanic repetitive activity in an axon of the cat soleus nerve. The first tracing was obtained prior to a 10 sec. 400 cps stimulus. The other tracings were obtained at the indicated times after the stimulus. In each case a single stimulus applied to the soleus nerve elicited the neural activity shown.
tion between the filament and the soleus muscle was verified by stimulating the filament and observing the soleus muscle for activity.

In some experiments, simultaneous recordings were made from a ventral root axon and from the motor unit of that axon. The animals were prepared as above. After a ventral root filament containing a single active axon had been located, it was placed across the two bipolar platinum electrodes, the one for stimulation ($S_v$ in Fig. 1) and the other for recording ($R_m$ in Fig. 1). The motor unit of this fiber was located visually and a glass-insulated platinum microelectrode ($R_m$) (Wolbarsht, MacNichol, and Wagner, 1960) placed in the region of the motor end-plates. The criterion for correct placement was the recording of a diphasic muscle action potential with an initial fast rising negative phase and with minimum latency (Eccles and O'Connor, 1939). The accuracy of its positioning could also be checked by the recording of characteristic end-plate potentials during the Wedensky block associated with high frequency nerve stimulation. The nerve and muscle potentials were displayed simultaneously on a dual trace oscilloscope and were recorded on magnetic tape for subsequent photography and analysis.

In all experiments stimulation was accomplished with rectangular pulses of 0.01 msec. duration and an intensity slightly above the threshold of the fiber on the recording electrodes. For tetanic stimulation these pulses were applied for a period of 10 sec. at the frequency specified, usually 400 cps. Between tetani the nerve was stimulated continuously at a rate of 0.4 cps. When only nerve potentials were to be recorded the stimulus was applied to the soleus nerve. When both nerve and muscle activity were to be recorded, the stimulus was applied to the ventral root filament.

**RESULTS**

PTR was readily seen in these soleus nerve-muscle preparations. As illustrated in Fig. 2, a single stimulus applied in the post-tetanic period elicited
one or more repetitive potentials. The intensity of this repetitive activity was proportional to the frequency of the conditioning stimulus. After a 10 sec., 400 cps stimulus, the maximum used in this study, trains containing as many as ten repetitive potentials and lasting as long as 25 msec. were seen. In these the maximum frequency attained by the PTR was about 600 cps.

The occurrence and duration of the repetitive activity were also dependent on the frequency of the conditioning stimulus. Figs. 3 and 4 show the results of a study of 116 axons in 83 cats. In Fig. 3, where the percentage of these axons which showed PTR is plotted on the ordinate and the frequency of the 10 sec. conditioning tetanus on the abscissa, it can be seen that the occurrence of PTR is a linear function of the logarithm of the frequency. The duration of the PTR is simply a linear function of the frequency as shown in Fig. 4 where the duration, expressed as a percentage of the PTR duration following a 400 cps stimulus, is plotted against frequency on a linear scale. The average duration of PTR after the 400 cps tetanus was 48 sec. The values of the 104 individual fibers which responded to this frequency were distributed approximately log normally (Fig. 5).

The intensity and duration of PTR are additionally dependent on other stimulus parameters. Principal among these are the duration of the tetanic stimulation, the interval between successive periods of tetanization, and the frequency with which the single testing stimuli are applied after the tetanus. The effect of a change in tetanus duration was complex and depended in part on the frequency of the stimulation. It was not explored beyond noting

**Figure 4.** PTR duration as a function of stimulus frequency. Ordinate, duration expressed as per cent of the PTR duration following a 400 cps stimulus.
that for a given frequency a longer period of application generally led to more intense and longer lasting PTR. The influence of the interval between successive periods of tetanization was more definitive. If 10 sec. periods of stimulation were applied more often than once every 2 min., cumulation of effect occurred at a rate and to an extent which were inversely proportional to the interval. Finally, it was noted that when the single stimuli were applied more frequently than once per second after the tetanus, PTR either did not appear or was considerably reduced in intensity and duration. When these factors were controlled by fixing the stimulation parameters, the repetitive activity generated in a given filament was remarkably reproducible; the results of replicate trials usually agreed within 10 per cent of each other over periods of 3 to 6 hours.

The intervals between the stimulus-evoked and first repetitive potential usually were shortest at the time of peak intensity. Since part of this interval represented conduction time from the stimulating electrode to the peripheral end of the nerve and return, it therefore varied with the placement of the stimulating electrode. However, even in preparations in which the electrode was placed on the nerve within a few millimeters of the origin of its intra-muscular branches, a repetitive potential was never observed to occur less than 2.0 msec. after the evoked potential. As repetitive activity waned, the interval between the evoked and first repetitive spike tended to increase usually to 4 or 5 msec., but occasionally to as much as 15 msec.

Simultaneous recording from a single axon and from muscle fibers innervated by it showed that PTR also occurs in the muscle and that the muscle and nerve activities are synchronous (Fig. 6). In this figure, the repetitive muscle potentials precede the repetitive nerve potentials, but an apprecia-
tion of the true relationship between them can only be gained by considering the relative conduction times of the two potentials. Since the muscle electrode is at the end-plate region, the conduction time of the muscle potential is negligible. The nerve-recording electrode, on the other hand, is in the lumbar region, remote from the origin of the repetitive activity and therefore conduction time to it is appreciable. For example, in the experiment of Fig. 6, antidromic conduction in the extramuscular portion of the nerve $(S_n \rightarrow R_a$, Fig. 1) required 2.55 msec. Since this is considerably greater than the 1.45 msec. difference between the repetitive muscle and nerve potentials, it is apparent that the repetitive activity is generated presynaptically and then transmitted orthodromically to the muscle and antidromically to the axon.

A precise localization of the point of origin of PTR was made utilizing the method diagrammed in Fig. 7. The synchrony between the nerve and muscle
PTR indicates that they are generated simultaneously to a point in the presynaptic structures, designated arbitrarily as point 0 in the figure. From this point, the repetitive activity is propagated in both directions and results in the recording of a nerve action potential at the ventral root electrode and a muscle action potential at the muscle electrode. The antidromic and orthodromic conduction times from point 0 to the recording electrodes are indicated as $t_a$ and $t_0$ respectively. Any difference between these two times will cause the two potentials to be recorded at different times as shown in the lower half of the figure. Since the location of point 0 is initially unknown, neither $t_a$ nor $t_0$ can be measured directly, but it is possible to establish two relationships between them. Their sum is equal to the total conduction and transmission time of the system, i.e. the interval between the application of a stimulus to $S_n$ and the recording of the muscle potential at $R_m$, represented as $T$ in the figure and their difference is equal to the interval between the recording of the two repetitive potentials, represented as $t_i$ in the figure. These relationships can be expressed as:

$$T = t_a + t_0$$

$$t_i = t_a - t_0$$

Solving for $t_a$

$$t_a = \frac{T + t_i}{2}$$
The total time $T$ and the interval $t_4$ are readily determined by direct measurement of photographs of oscilloscopic tracings. Therefore $t_4$ and $t_0$ can be calculated and point 0 located. In this manner an approximate $t_0$ of 0.9 msec. was established indicating that the repetitive activity originates in the nerve terminal 0.9 msec. before the beginning of the repetitive muscle potential.

Two difficulties were encountered in employing this procedure. First although every repetitive muscle potential was associated with a synchronous nerve potential, there occasionally appeared a repetitive nerve potential not associated with a muscle potential (Fig. 6, trace 3). Moreover, while most of the repetitive muscle potentials were identical with the stimulus-evoked muscle potential, occasional distorted muscle potentials occurred (Fig. 6, trace 4). From examination of the records it became apparent that the occurrence or non-occurrence of a normal muscle action potential was dependent upon the interval between successive nerve potentials, and that absent or distorted muscle potentials were occasioned by a high frequency burst of neural repetitive activity. This conclusion was verified by applying paired stimuli to the ventral root filament at appropriate intervals. When the two stimulating pulses were 2.5 msec. or less apart, no second muscle potential developed. With intervals between 2.5 and 4.0 msec., distorted second potentials were produced. Only when the interval was greater than 4.0 msec. did two normal muscle potentials appear (cf. Fig. 6, trace 5). These effects produced by nerve stimuli at selected intervals agreed exactly with those that occurred from comparable spontaneous neural discharges during a repetitive train. In addition, these experiments revealed that the distorted muscle potentials were generated after a greater than normal latency. In both appearance and latency the distorted repetitive muscle potentials closely resembled the “newborn” or “abortive” muscle potentials described by Eccles and O’Connor (1939) and Kuffler (1942). Consequently $t_0$ values were tabulated only for those pairs which consisted of a nerve potential associated with a normal muscle potential.

The second difficulty in the calculation of $t_4$ arose from the fact that the total time $T$ increased considerably after a period of high frequency stimulation. The amount of increase varied somewhat but averaged 0.46 msec. or about 13 per cent of the control $T$, at a time 2.5 sec. after the 10 sec., 400 cps stimulation. The rate of recovery varied greatly from animal to animal but was in each case an exponential function. Both the intramuscular and extramuscular portions of the axon contributed to the increased conduction time. The extramuscular component, which was measured between the stimulating electrode on the soleus nerve ($S_N$) and the recording electrode on the ventral root filament ($R_N$), showed an average increase of 0.24 msec. (about 10 per cent) at $2\frac{3}{2}$ sec. after the 400 cps stimulus and had a one-half
recovery time of 15 sec. The intramuscular component, determined as the difference between \( T \) and the extramuscular conduction time, had an average increase of 0.23 msec. (23 per cent) and a 50 per cent recovery time of 40 sec.

In tabulating \( t_a \) the post-tetanic increase in \( T \) was taken into account as follows: \( t_a \) was calculated as described above using the \( T \) actually observed for each stimulus in the post-tetanic period. This resulted in values of \( t_a \) which progressively diminished and approached a limiting value. The values of \( t_a \) declined exponentially and in each instance this rate proved identical with that for the return of the extramuscular axonal conduction time to normal. Therefore, each individual value of \( t_a \) was normalized by subtracting

\[
t_a = \text{the post-tetanic increase in } T \text{ taken into account}
\]

from the initially calculated value an amount equal to the increase in extramuscular axonal conduction time which existed in the preparation at the time that the particular repetitive potential was observed. The corrected values of \( t_a \) showed very little variation during the course of an experiment. In no case did the standard deviation of the mean \( t_a \) exceed 0.05 msec., the estimated error of the method. The normalized values of \( t_a \), \( t_b \), and related data are presented in Table I.

The site of PTR origin was determined in another way, the results of which verified the presynaptic locus, as found through calculation of \( t_a \). This method was based on the fact that the time between the application of a post-tetanic stimulus to the ventral root and the recording of a repetitive nerve potential at that site is composed of three parts: the conduction time of the potential evoked at the ventral root electrode to the site of origin of the repetitive activity, the latency of generation of the repetitive activity, and the conduction time of the retrograde repetitive potential to the ventral root electrode. Since the interval between stimulus application and the first repetitive retrograde response is readily measured, it remains only to determine and to sub-

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>( T )</th>
<th>( t_a )</th>
<th>( t_b )</th>
<th>( S_{y} \rightarrow R_{n} )</th>
<th>( R_{m} \rightarrow R_{n} )</th>
<th>( T - R_{m} - R_{n} )</th>
</tr>
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<tr>
<td>1</td>
<td>3.85</td>
<td>3.35 ± 0.03</td>
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<td>3.25 ± 0.10</td>
<td>2.84</td>
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<td>2</td>
<td>3.24</td>
<td>2.63 ± 0.05</td>
<td>0.61</td>
<td>2.53 ± 0.06</td>
<td>2.44</td>
<td>2.96</td>
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<tr>
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<td>3.35</td>
<td>2.33 ± 0.03</td>
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<td>—</td>
<td>2.18</td>
<td>2.50</td>
</tr>
<tr>
<td>4</td>
<td>3.40</td>
<td>2.40 ± 0.04</td>
<td>1.00</td>
<td>—</td>
<td>2.19</td>
<td>2.50</td>
</tr>
<tr>
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<tr>
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<td>2.13 ± 0.05</td>
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<td>—</td>
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</tr>
<tr>
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<td>2.78 ± 0.04</td>
<td>1.17</td>
<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>9</td>
<td>3.50</td>
<td>2.45 ± 0.04</td>
<td>1.05</td>
<td>—</td>
<td>2.35</td>
<td>—</td>
</tr>
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</table>
tract the latency of repetitive generation to complete a calculation of \( t_a \), i.e. subtraction of latency from the total specified time will leave a remainder which is twice the conduction time from the point of origin of the repetitive activity to the ventral root electrode \( (2t_a) \).

The latency of generation of repetitive activity was determined indirectly from the following consideration: wherever the point of origin of the repetitive activity, both the stimulus-evoked and the repetitive nerve potentials must traverse the same orthodromic pathway. Consequently, the latency will be evident from the interval between the time that the stimulus-evoked potential passes through the site of origin of the repetitive activity and the time when the repetitive potential is generated. Therefore in two experiments photographs were made of every repetitive nerve and muscle potential which occurred after a 10 sec. period of 400 cps stimulation. In the same preparation, stimulus pairs of varying intervals were applied to the ventral root filament and the muscle potentials produced by these stimuli were photographed. Comparison of these photographs with those of the spontaneous repetition made possible selection of stimulus pairs which exactly reproduced the pattern of muscle repetitive activity. In these corresponding photographs measurement of the interval between the artifacts of the applied stimulus pair gave the latency of the generation of the repetitive activity. The results obtained with this method are indicated as \( t_a \) (pairs) in Table I.

This procedure for locating the point of origin of the repetitive activity was more tedious and less accurate than the more direct one employed earlier, but it offered two advantages. First, it afforded a means of checking the results obtained with the first method. Second, it could be employed on all records including those exhibiting distorted or absent muscle potentials. By this means, therefore, it was possible to show that in each preparation the site of origin of the repetitive activity was unvarying regardless of the time of its occurrence and regardless of the capacity of the muscle to respond to the repetitive impulses.

The data in Table I clearly show PTR to be presynaptic in origin but by themselves are not sufficient to indicate which of the presynaptic structures is responsible for its generation. Two additional procedures were employed to locate anatomically the origin of the repetitive activity. In each experiment stimulating electrodes \( (S_N) \) were placed on the soleus nerve as close as possible to its entrance into the muscle and conduction times, antidromic and orthodromic, were measured. The orthodromic conduction time measurements were unsatisfactory, partly because stimulation could not be restricted to the single motor unit under study and partly because the results were found to be dependent upon stimulus strength. The measurements of antidromic conduction times were not restricted by either of these factors and the results are reported in Table I \( (S_N \rightarrow R_a) \). It can be noted that they
are in each case less than the antidromic conduction time for the repetitive activity.

Second, it was possible to stimulate the intramuscular portion of the nerve with the needle electrode used to record the muscle action potentials ($R_m$). In every case this could be done without changing the position of the electrode. In fact, the slightest movement of the electrode destroyed the ability to stimulate the nerve in this manner; an observation which substantiated the belief that the nerve was stimulated directly by the electrode and not by retrograde transmission from the muscle. The improbability of the latter was ascertained by noting that stimulation of the nerve-free tendon end of the muscle fibers led to the recording of a muscle action potential at the needle electrode but not of a nerve action potential at the ventral root electrode. In every preparation the antidromic conduction time from the needle electrode ($R_m \rightarrow R_n$, Table I) was less than the total time $T$ of the preparation but greater than the antidromic conduction time $t_a$ of the repetitive activity. As a result of these two procedures it was concluded that the repetitive activity was generated in intramuscular nerve branches, but at a point proximal to the termination of the nerve.

**DISCUSSION**

Within the presynaptic structure, the most probable site of origin of PTR is at the junction between the myelinated and the unmyelinated portions of the motor axon. Only at this site are there present the structural and functional discontinuities requisite for the production of PTR. From the data of Table I, it is clear that the antidromic conduction time of a PTR spike exceeds that of a spike generated by the stimulating electrode on the soleus nerve at its entrance into the muscle. To obtain an approximation of the length of axon represented by this time difference a constant conduction velocity of 65 m/sec was assumed (Eccles, Eccles, and Lundberg, 1958) and the equivalent conduction distance calculated. In each preparation this distance agreed within 2 mm with the distance between the electrode on the soleus nerve and the electrode placed at the motor end-plate region. Such calculations provide only crude approximations, but they serve to indicate that the repetitive activity arises very close to the anatomic end of the nerve. The measurements made with the microelectrode located at a junctional region confirm this. The appearance of end-plate potentials during the Wedensky block associated with high frequency stimulation indicates that in each case the electrode was less than 1 mm from the end-plate (Eccles and O'Connor, 1939). Despite this proximity, the conduction time of an antidromic potential evoked by nerve stimulation through this electrode, when compared with $t_a$, revealed that conduction from the end-plate locus to the site of origin of the repetitive activity requires several tenths of a millisecond. This time
most likely represents a conduction distance in the order of tens of microns, since the fibers of the unmyelinated terminal have a diameter of less than 0.5 μ (Coers and Woolf, 1959) and therefore probably have a conduction velocity of less than 1 m/sec. (Grundfest and Gasser, 1938; Gasser, 1950; Iggo, 1960). This order of distance is commensurate with the 30 to 100 μ unmyelinated terminal length determined anatomically (Coers and Woolf, 1959; Couteaux, 1960).

In neural structures, repetitive activity may occur in response to the application of a constant depolarizing current (Katz, 1937; Barron and Matthews, 1938; Hodgkin, 1948; Katz, 1950; Eyzaguirre and Kuffler, 1955; Adelman, 1956; Shanes, 1958; Wall, 1959; Knadel and Spencer, 1961). Often the source of the depolarizing current is external to the structure under study; i.e., it is furnished by a cathodal stimulating electrode or by the depolarizing influence of an adjacent structure. However, it is also possible for a depolarizing current to be provided by functional discontinuities in a single fiber. A depolarizing potential gradient between two parts of an axon has been termed a generator potential and has been demonstrated to be responsible for the firing of muscle spindle neurons (Katz, 1950) and mechanoreceptors (Loewenstein, 1959). It has also been postulated as the mechanism by which PTR is produced in dorsal root fibers (Wall and Johnson, 1958; Wall, 1959) and by which drug-induced repetitive activity is produced in the motor nerve terminal (Werner, 1960, 1961 b).

In motor nerves, the juxtaposition of the large diameter axon to the small diameter unmyelinated terminal (Tiegs, 1953) provides a setting for PTR generation. Particularly pertinent are the differences in the afterpotentials of the two structures. In large medullated fibers the negative afterpotential is relatively small and lasts only a few milliseconds (Gasser and Grundfest, 1936). In small unmyelinated fibers the negative afterpotential is much larger and may continue for several hundred milliseconds. The positive afterpotentials are in similar proportion (Grundfest and Gasser, 1938; Rushton, 1951; Ritchie and Straub, 1957; Greengard and Straub, 1958; Shanes, 1958; Eccles and Krnjevic, 1959; Straub, 1961).

Following high frequency stimulation in axons of all sizes the afterpotentials are exaggerated in magnitude and duration. Of primary concern is the positive afterpotential which, because of its prominence and persistence is referred to as a post-tetanic hyperpolarization (Lloyd, 1950; Douglas and Ritchie, 1962). The magnitude of this change is also greatest in small diameter fibers and accordingly, with reference to the motor nerve, it is reasonable to expect that the hyperpolarization of the unmyelinated terminal will be greater than that of the myelinated axon. Although the terminal of the tetanically stimulated motor nerve will thus become positive relative to the axon, the current elicited by this potential difference is insufficient to initiate
an action potential. However, if this hyperpolarized system is now disturbed by the passage of a single action potential, the subsequent development of negative afterpotentials will cause the potential gradient to reverse. The negative afterpotential of the large myelinated axon will be brief relative to that of the unmyelinated terminal. Consequently, the membrane potential of the axon will return to its prestimulus value while the prolonged negative afterpotential of the terminal persists. Hence a potential difference between the terminal and the axon will be created, with the former negative relative to the latter. It appears that repetitive potentials are generated when the current produced by this potential difference exceeds the rheobase of the last node of Ranvier.

Data from the present experiments are compatible with this proposed mechanism of PTR generation. The increased conduction time noted during the post-tetanic period in all probability indicates hyperpolarization of the axon (Lloyd, 1950). Moreover the relatively greater increase in the intramuscular conduction time implies an even greater hyperpolarization of the unmyelinated terminal than of the myelinated axon (Hubbard and Willis, 1962). The observed changes in the latency and the frequency of the repetitive potentials are also what one would expect for this mechanism. The minimum latency was always observed early in the post-tetanic course and then increased with time after the tetanus. Also, the frequency of the repetitive discharge was greatest early in the post-tetanic period. These findings suggest that the depolarizing influence of the terminal's negative afterpotential causes a current which initially is considerably above the rheobase of the last node of Ranvier and that this structure responds with a short utilization time and a high frequency of repetition (Tasaki and Mizuguchi, 1948). Furthermore, as the hyperpolarization and the magnitude of the negative afterpotential wane, the strength of the depolarizing current would be expected to decline and accordingly an increase in latency and a decrease in frequency were observed. Thus, cessation of the repetitive activity signals the decline of the current flow between terminal and axon to below the rheobase. In all instances PTR was observed to cease at the time the conduction velocity of the extramuscular portion of the motor axon returned to control, although the conduction time of the intramuscular portion of the system was still 5 to 10 per cent above control.

These experiments were intended as an examination of the properties of the motor nerve terminal and were not directly concerned with the mechanism of neuromuscular transmission. The results, however, are pertinent to the latter problem in that they lead to an estimate of synaptic delay which is less than that usually cited (Kuffler, 1949). In each experiment the sum of neural conduction time and synaptic delay was determined and is tabulated as $T$ in Table I. In seven experiments, the neural component of $T$ was esti-
mated by stimulating the nerve via the needle electrode \((R_m\text{ in Fig. 1})\) and noting the time for antidromic conduction to the ventral root. These data are presented in Table I as \(R_m \rightarrow R_n\). The difference between \(T\) and \(R_m \rightarrow R_n\), the maximum possible value of the synaptic delay, is shown in the last column of the table. It can be seen that in four experiments the difference is less than 0.5 msec. and in two less than 0.3 msec. A similar upper limit can be obtained from the recent report of Hubbard and Schmidt (1962). Measurements of their photographs indicate a nerve-muscle latency of approximately 0.35 msec. in their in vitro rat phrenic nerve-diaphragm preparation. Notwithstan- ding these small values, it seems clear that in neither the present experiments, nor in those of Hubbard and Schmidt were the electrodes in contact with the final branches of the motor nerve. In the former, the 5 to 10 micron diameter of the needle tip probably precluded exclusive contact with the smallest fibers, while in the latter, the shape of the nerve action potentials indicates that the electrode was at least 50 microns from the source of the electrical activity (Furshpan and Furukawa, 1962). Since the electrodes were not in contact with the smallest branches of the nerve terminal, it follows that the true value of the synaptic delay must be less than the figures given above. Furthermore, considering the fact that the smaller the fiber diameter, the slower the conduction, it is possible that virtually all of this remaining time could be accounted for by conduction in the terminal branches in which case synaptic delay would become vanishingly small.

It is noteworthy that PTR in soleus nerve can be produced by stimulus frequencies in the range of normal physiologic function. Fig. 3 shows that PTR occurred in 11 per cent of axons subjected to a 25 cps stimulus for 10 sec. With longer stimulus durations, the percentage of responding fibers was greater and the minimal effective frequency lower; i.e., about 5 cps. In addition, axons stimulated for several minutes at rates of 5 to 20 cps occasionally discharged repetitively during the course of the stimulus. This took the form of one, or more rarely, two, repetitive potentials in a fixed relationship 3 to 5 msec. after the stimulus-evoked potential. When these occurred they bore a striking resemblance to the “double potentials” seen in cat and human slow muscle electromyograms (Hoff and Grant, 1944; Denslow, 1948), indicating that the latter are a form of PTR. These reports and the present findings indicate that PTR generation may be a normal event for motor nerve terminals of slow muscles. Locally generated repetitive activity seems an efficient means of maintaining tension in these postural muscles. The significance of PTR for muscle function will be discussed more fully in a forthcoming report on the relationship between PTR and PTP (Standaert, 1963).

The technique described herein for the generation of PTR in soleus motor nerves provides a new tool for the study of the pharmacology of the neuro-
muscular synapse. Since this PTR originates entirely in motor nerve, drugs which increase or decrease the repetitive activity must do so by an effect on the nerve. Therefore it is possible to study in an in vivo situation the presynaptic action of a drug uncomplicated by the presence of another drug. This has now been accomplished for d-tubocurarine (Standaert, 1961, 1962), succinylcholine (Standaert, 1962; Adams, Dietrick, and Gordon, 1963), diphenylhydantoin (Parisi and Raines, 1963), diethyl ether and cyclopropane (Van Poznak, 1963). The present results as well as these latter findings reemphasize the conclusions of Riker et al. (1957, 1959 a, 1960) that the motor nerve terminal is a functional entity distinct from the motor axon and that this presynaptic structure is an important element in the control of neuromuscular transmission.

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