

TEMPORAL FLUCTUATION IN EXCITABILITY OF SPINAL
MOTONEURONS AND ITS INFLUENCE ON MONO-
SYNAPTIC REFLEX RESPONSE

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In the acutely decapitate, unanesthetized cat repeated testing of a monosynaptic reflex response to standard afferent volleys reveals considerable variation in response amplitude, indicating that motoneurons are subject to fluctuation in excitability. This temporal variation in response amplitude has led to the use of the mean of successively determined response amplitudes in analysis of pool monosynaptic reflex responses. Successive application of test volleys has been employed to measure the response of individual motoneurons as well. Thus, Lloyd and McIntyre (8) have used firing index as a measure of individual motoneuron response, this index being determined by the number of times a motoneuron responds with monosynaptic latency to a given number of repeated test volleys. They have studied the distribution of firing indices, at fixed levels of synaptic drive, in individual motoneurons comprising a synthetic pool and have suggested that the distribution of firing indices of units in the intermediate firing range (between zero and 100) results from the distribution of fluctuating excitability levels.

Although it is clear from the foregoing considerations that the usual measures of motoneuron response, both of individuals and of pools, will be influenced by temporal fluctuation in excitability, no systematic investigation has previously been made of the nature and sources of excitability fluctuation. The present study has this aim and the results obtained provide information essential to the interpretation of observations, presented in the following paper (4), on the responses of motoneurons to graded afferent volleys.

Monosynaptic reflex response to afferent test volleys repeated once per 2 seconds has been recorded, in the acutely decapitate cat, after establishment of steady state conditions. Response has been recorded in distally severed ventral roots following stimulation of selected muscle nerves or, alternatively, in muscle nerves following stimulation of distally cut dorsal roots. The latter method has the advantage that spike potential amplitude of a volley in all the large motor fibers may be recorded in the muscle nerve following stimulation of the ventral root outflow. With this datum available, amplitude of reflex response may be expressed relative to amplitude of response that would be

given by discharge of the entire motoneuron pool. Since the total number of motoneurons in selected pools is known in approximation it follows that an estimate can be made of the number of motoneurons involved in any given manifestation of reflex response. Satisfactory measurement of variation in pool response has required the recording of several hundred to one thousand successive reflex responses in each experimental situation. Since amplitude of monosynaptic reflex response is greatly influenced by body temperature, the latter must be maintained constant within a few tenths of a degree Centigrade during such determinations.

Variation in Monosynaptic Reflex Response to Maximal Afferent Volleys.—For each experimental series of monosynaptic reflex responses a frequency distribution of response amplitude has been constructed. By this means temporal variation in response has been compared, in different experimental circumstances, both as to range of variation and as to form of frequency distribution. In addition, the standard deviation (s.d.) has been used as a convenient over-all measure of variation.

A typical frequency distribution, derived from 1000 responses of a medial gastrocnemius pool, is illustrated in Fig. 1. Successive response amplitudes from a part of the same series are shown in the plot of Fig. 2. Reflex response amplitude has been expressed relative to total pool discharge. It will be noted that the frequency distribution has a nearly normal form with slight negative skewness. The mean of the series amounted to 22 per cent of the total pool response (s.d. 1.9). Variation in response amplitude, as indicated by the width of the distribution, amounted to an appreciable fraction of the total pool response; a considerable number of motoneurons must have been influenced in their discharge by excitability fluctuation.

There are a large number of motoneurons, approximately 300, in a medial gastrocnemius pool;¹ the number of motoneurons contributing to mean response amplitude may be estimated. Similarly, the number of neurons discharging in the maximal and minimal responses of a series may be determined. Clearly, that number of neurons represented by the difference between maximal and minimal responses must be influenced by excitability fluctuation. The number of motoneurons responding to every application of the test volley, *i.e.* in the firing index 100 category, must be equal to or less than the number concerned in the minimal response. Likewise the number of motoneurons in the never responding, firing index zero category must be equal to or less than the number in the fraction not discharging in the maximal response.

If all the motoneurons in the pool were subject to completely correlated

¹ Eccles and Sherrington (1) found an average of 410 myelinated efferent fibers in the nerve to medial gastrocnemius. Since about 30 per cent of these are small motor fibers, efferent to muscle spindles (7), a reasonable figure for the number of motor neurons in this pool is about 300.

excitability fluctuation the minimal response of a pool would be equal to the number of motoneurons in the firing index 100 category and the size of the intermediate firing zone would be given by the difference between the maximal and minimal responses. However, if fluctuation were completely uncorrelated,

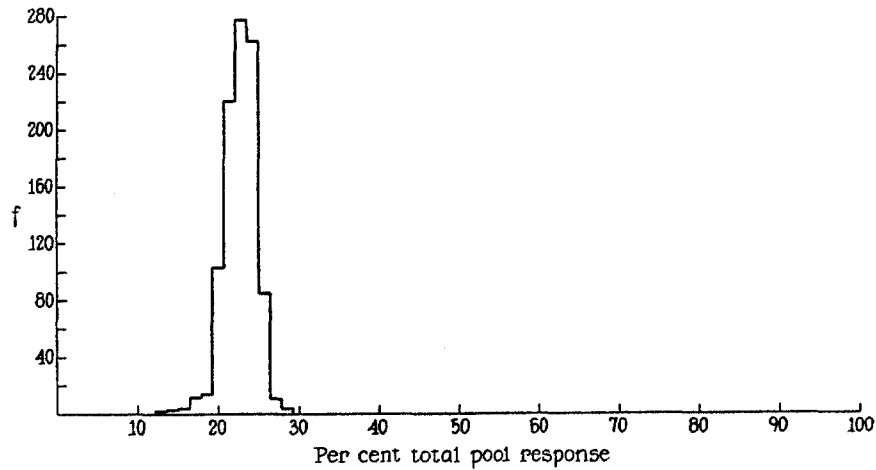


FIG. 1. Frequency distribution of 1000 successive response amplitudes of a monosynaptic reflex response recorded in medial gastrocnemius nerve following a maximal stimulus to dorsal roots L7 and S1. Ordinate, frequency of occurrence. Abscissa, measured amplitude of response.

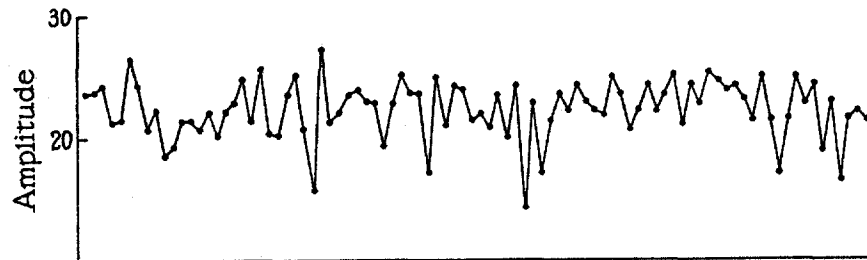


FIG. 2. Plot of amplitude of successive responses from a portion of the record used for the frequency distribution of Fig. 1. Points represent measured amplitudes of response determined at successive 2 second intervals.

coincidence of discharge or no discharge among motoneurons in the intermediate zone would be on a purely chance basis. Were this the case the difference between the minimal and maximal responses would fall short of the difference expected with completely correlated fluctuation. Thus, if fluctuation were completely random the statistical chance of all the motoneurons in the intermediate zone coinciding in their excitability swings would be very small.

A comparison of observed population variation with the predicted behavior of a synthetic pool subject to completely correlated excitability fluctuation may be made. The data of Lloyd and McIntyre (8) provide the number and firing indices of motoneurons comprising a representative synthetic pool. As a measure for comparison, the difference between observed minimal and maximal pool responses has been used, this difference being designated zone of variation. The limits of the zone of variation have been set to include 98 per cent of observed response amplitudes of each series for the reason that 50 trials of a test volley are usually employed to measure firing index of individual motoneurons. If comparison is to be made between number of motoneurons in the zone of variation and the number of motoneurons in the intermediate firing range (indices between zero and 100), an equal number of trials must be considered, for both values will increase their absolute limits as the number of trials is extended.

TABLE I

Experiment No.	Temperature	Mean amplitude	Range of variation	Zone of variation	s. d.
	°C.				
1	36	22.4	15.0-27.1	12.1	1.9
2	36	23.4	16.0-30.0	14.0	3.4
3	36	16.8	7.6-22.7	15.1	3.0
4	35.7	14.5	9.8-19.7	9.9	2.2
5	35	8.7	3.0-14.5	11.5	2.6

Variation in response amplitude of a motoneuron pool may be compared from one preparation to another if reflex response is, in each case, measured relative to total pool response by the method already described. This has been done for medial gastrocnemius motoneuron pools in 5 preparations. Table I presents the essential results. It is noteworthy that in each preparation all monosynaptic afferent fibers engaging the pool, homonymous and heteronymous, were excited by the dorsal root stimuli. Mean response amplitude varied in the different preparations from 8.7 to 23.4 per cent of total pool response while the standard deviation varied from 1.9 to 3.4. Less difference in the zone of variation was found, the largest range observed being 15.1 and the smallest 9.9 per cent of total pool response. The average value of the zone of variation was 12.5 per cent of total pool response.

Now the number of tricipital motoneurons in the intermediate firing zone found by Lloyd and McIntyre (8), when their synthetic pool is raised to the number, 300, of motoneurons in an actual medial gastrocnemius pool, would amount to 55 to 82 neurons. The intermediate firing zone thus corresponds to about 18 to 27 per cent of the pool. Since zone of variation of a natural pool

response is appreciably smaller, on the average 12.5 per cent of total pool response, it follows from the considerations already discussed that excitability fluctuation cannot be completely correlated. On the other hand, zone of variation of natural pool response corresponds to an appreciable fraction of the intermediate firing zone of the synthetic pool, suggesting that excitability fluctuation is correlated to some, as yet undefined, degree. If one makes the likely assumption that the degree of correlation in a given experiment remains essentially constant, it should be possible to utilize zone of variation of pool response as an indirect measure of the size of the intermediate firing zone. This has been done in the following section which considers variation of pool response with graded afferent stimulation.

Variation in Monosynaptic Reflex Response to Graded Afferent Volleys.—The foregoing observations indicate that temporal variation in response amplitude to maximal afferent volleys is a conspicuous feature of monosynaptic reflex response. It is evident that mean amplitude of reflex response in any series will be determined by the minimal response plus the mean of the zone of variation. If variation changed appreciably at different levels of drive, it would have important consequences for the significance of mean response amplitude. For this reason a study has been made of temporal variation in response to afferent volleys of graded size. Fig. 3, taken from a representative experiment, displays frequency distributions of response amplitudes of a monosynaptic reflex at four levels of afferent stimulation. The reflex in question pertained to triceps surae motoneurons, afferent volleys being initiated in the muscle nerve and reflex responses being recorded in distally severed ventral root. Frequency distribution of response amplitude to maximal afferent volleys is plotted in A; the ensuing distributions were derived from series recorded at progressively reduced strengths of afferent stimulation. Reduction in afferent volley size, and in consequence reduction in mean response amplitude, had no appreciable effect on the range or distribution of variation (A to D). Only when drive was reduced to a level which failed to elicit a minimal response on every trial was variation reduced. In the last mentioned circumstance response variation no longer serves as an index of excitability fluctuation. However, at levels of synaptic drive sufficient to secure some response on every trial it may be seen that variation was essentially constant both as to range and as to form of frequency distribution. This is evidenced by the essential constancy of the standard deviation (s.d.) in the series A to D of Fig. 3.

In some experiments the zone of variation may be considerably reduced at a level of drive sufficient to elicit a very small response on every trial. Incrementing afferent volley size then causes an increase in variation as mean response amplitude increases but as soon as the zone of variation attains its full size it remains constant with further increase in drive. The reduced zone of variation sometimes noted with very small mean amplitude may be at-

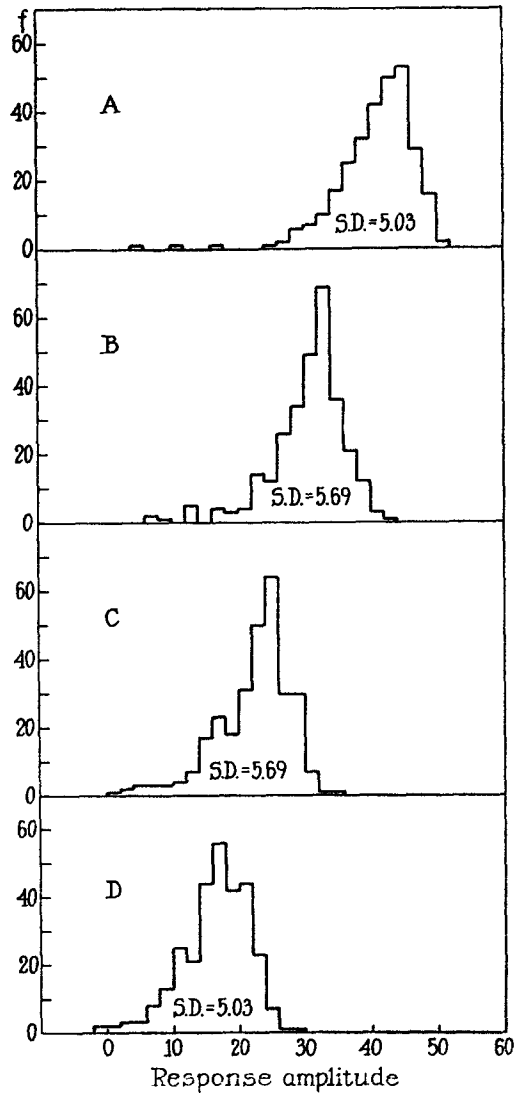


FIG. 3. Frequency distributions of successive response amplitudes at four levels of drive. Monosynaptic reflex response recorded in ventral root following stimulation of triceps surae nerve. In A stimulation was maximal for group I fibers. Stimulus strength reduced in steps from A to B, B to C, and C to D. Note negative skewness. Response amplitude is in relative units and cannot be compared with total pool response.

tributed to the fact that at this level of drive the intermediate firing zone is less than complete, but, because excitability fluctuation is only partially cor-

related, each trial elicits discharge in some of the motoneurons in the incomplete intermediate zone. Only when the intermediate zone is complete does variation appear to be constant.

It is common experience in observation of monosynaptic reflex response that a response of large amplitude appears subject to less variation than one of small amplitude (5). This impression is gained from the fact that as mean reflex amplitude is increased variation does diminish relative to mean amplitude whereas in absolute terms it does not (Fig. 3).

The range of pool variation and frequency distribution of response amplitudes have been considered indirect measures of the number of motoneurons in the intermediate firing range and of their distribution of firing indices. Since response variation remains substantially the same, both as to range and standard deviation, when drive is increased above that level at which the intermediate zone is complete, one may conclude that the size of the intermediate zone and the distribution of firing indices therein will also remain constant. This conclusion rests on the assumption that the proportions of correlated and uncorrelated excitability fluctuation, also determinants of pool variation, remain the same. To assume otherwise would require that these proportions vary systematically with synaptic drive, an improbable condition.

The constancy of the intermediate zone at different levels of drive is also suggested by Lloyd and McIntyre's (8) analysis of a synthetic pool in which they found that the number of motoneurons in the intermediate zone was nearly the same at three fixed levels of drive. The present results indicate an even greater constancy of intermediate zone than could be concluded from the behavior of the synthetic pool.

The Effect of Response Variation on the Measurement of Pool Monosynaptic Reflex Response.—Variation in a monosynaptic reflex response remains essentially constant above the level at which the zone of variation reaches its full size. Thus, over a considerable range the influence of variation on response amplitude remains constant and may be eliminated as a factor by using the mean of a series of responses. When reflex response to graded afferent excitation is studied however, initial growth of response to incrementing afferent volleys will be influenced by response variation. Fig. 4 is a schematic representation of the relation between mean response amplitude and synaptic drive in a motoneuron pool. This hypothetical pool is considered subject to synaptic drive which increases linearly until sufficient to discharge all members of the pool. In the absence of excitability fluctuation the relation between discharge zone and degree of drive may be assumed to follow the dashed line: Once the level of drive is sufficient to secure discharge in the most powerfully driven motoneuron, further increments of drive will cause a linear increase in discharge zone until all the members of the pool have discharged. Introduction of excitability fluctuation would alter the growth of mean response amplitude to the relation given by the solid line: At levels of drive previously ineffective

(10 to 30) fluctuation in the direction of increased responsivity would cause discharge on some trials and therefore mean response amplitude would have some finite value. Initial growth of mean response amplitude would depend on the extent and distribution of excitability fluctuation. Since the latter has a nearly normal distribution, one would expect initial curvature of the sort shown in the relation between synaptic drive and mean response amplitude. Only after synaptic drive was sufficient to complete the zone of variation (> 50) would mean response amplitude provide a linear measure of degree of synaptic drive. Further increase in drive would then be directly related to mean response amplitude until limitation in size of the pool became a factor. When the latter occurs the zone of variation will undergo progressive diminu-

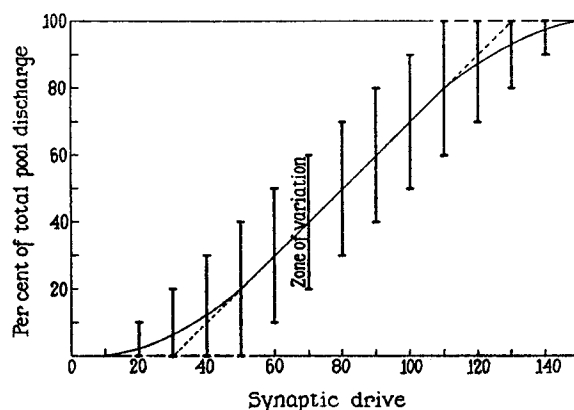


FIG. 4. Schematic representation of the effect of temporal variation on the measure of mean response amplitude of a hypothetical pool. For details see text.

tion as drive is increased (> 110) until all motoneurons of the pool discharge to every test volley regardless of excitability fluctuation.

The considerations relating to modification of discharge by limitation of pool size have little practical importance for only a small fraction of the pool discharges in usual tests of monosynaptic reflex response (Table I). However, the influence of excitability fluctuation on the initial growth of mean response amplitude has considerable significance for analysis of monosynaptic excitatory action. It will be noted that after development of the zone of variation is complete, mean response amplitude may be considered to provide a direct measure of the degree of monosynaptic excitation over a considerable range of pool response. For such a relation to exist there must be differences in transmitter potentiality among motoneurons of the pool such that equal increments of drive recruit equal numbers of motoneurons. Evidence for a distribution of transmitter potentialities such as would satisfy this requirement has been

presented in the preceding section (*cf.* reference 8). Before the zone of variation is complete additional factors determine mean response amplitude; namely, the range and frequency distribution of fluctuating excitability levels. These considerations are of particular importance in analysis of response of motoneuron populations to graded afferent volleys, a subject to be considered in the following paper (4).

Sources of Excitability Fluctuation.—Excitability fluctuation in spinal motoneurons could arise from several sources. Fluctuation might depend upon factors intrinsic to the motoneuron and its synaptic connections. Included in this category would be fluctuation in background excitation by synaptic "noise" of the sort described at the neuromuscular junction (2) and instability in responsiveness of the motoneuron itself. Among factors extrinsic to the motoneuron which might produce excitability change are (1) variation in background activity in interneurons producing excitability change by specific synaptic effects or by non-specific current flows, and (2) changes in responsiveness due to systemic factors. Intrinsic fluctuation in excitability may be assumed to be uncorrelated among the motoneurons comprising a pool whereas extrinsic factors might clearly affect motoneurons with some degree of correlation.

One of the possible sources of excitability fluctuation may be modified, namely that due to variation in background activity of interneurons. In recent years several agents have been described, chief among these myanesin, that block reflex transmission through polysynaptic paths in doses which do not reduce monosynaptic reflex responses (6, 9). A suggestion may be found in the experiments of Henneman, Kaplan, and Unna (3) that myanesin might reduce excitability fluctuation of spinal motoneurons for they noted that variation in successive tests of knee jerk reflex response was greatly reduced by this drug.

Fig. 5 illustrates the effect of myanesin on amplitude variation in a monosynaptic reflex response of deep peroneal motoneurons. In the upper portion of the figure are shown plots of successive response amplitudes taken before (A) and immediately after (B) the intravenous administration of 120 mg. of myanesin. Below these plots are frequency distributions of response amplitude for the corresponding periods. Myanesin caused a striking reduction in response variation as may be seen by comparing the two series of successive response amplitudes and the frequency distributions. It will be noted that the range of variation was significantly reduced. Accompanying the reduction in variation of monosynaptic reflex response there occurred a marked decrease in polysynaptic reflexes; such has been the uniform effect of myanesin. One may reasonably conclude that the reduction in response variation caused by myanesin is due to block of internuncial activity, thereby revealing the latter as a significant source of excitability fluctuation in spinal motoneurons. With the passage of time after a single dose of myanesin (about 30 to 40 mg/kg.) varia-

tion gradually increases and reaches its former size after some 20 to 30 minutes.

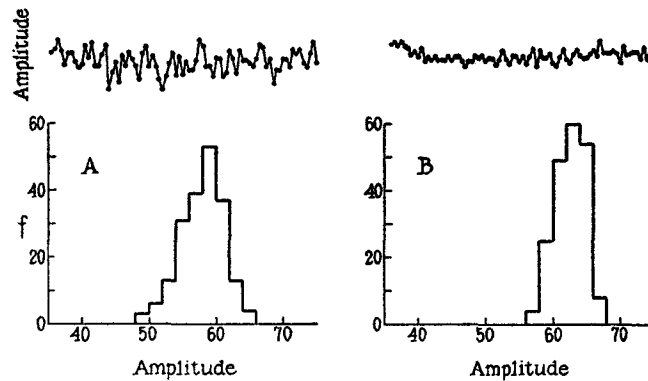


FIG. 5. Upper, plot of successive response amplitudes of a monosynaptic reflex response in deep peroneal motoneurons. A, control. B, after administration of 120 mg. of myanesin. Lower, corresponding frequency distributions of response amplitudes. Reflex response recorded in L6 ventral root following stimulation of deep peroneal nerve. Note reduction in response variation after myanesin. Response amplitude is in arbitrary units.

On the Functional Role of Excitability Fluctuation.—The present study indicates that motoneurons are subject to fluctuation in excitability such that the responsiveness of any particular motoneuron is subject to continuous change. It is likely that excitability fluctuation of the sort described exists in the normal animal and serves a useful purpose in providing for finer gradation of response. In the absence of excitability change the responsiveness of a motoneuron would be fixed and discharge would result whenever transmitter potentiality exceeded a critical value in the otherwise resting state. However, in the presence of fluctuation in excitability the statistical chance that a motoneuron will discharge can be finely graded as transmitter potentiality is varied over a considerable range.

SUMMARY

Observations on temporal variation in monosynaptic reflex response in the acutely decapitate cat indicate the following:

1. Frequency distribution of response amplitude has a nearly normal form often with some degree of negative skewness. Response variation differs only moderately in form and magnitude from one preparation to another.
2. Temporal variation remains essentially constant at different levels of drive above that level required to complete the zone of variation.
3. The role of response variation in the determination of mean response amplitude is considered.

4. One of the major sources of excitability fluctuation in the "resting" cord is variation in background activity of interneurons.

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