MONOSYNAPTIC REFLEX RESPONSE OF SPINAL MOTONEURONS TO GRADED AFFERENT STIMULATION

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Among the factors required for synaptic transmission in motoneurons is that many presynaptic elements be active in near synchrony. The earliest evidence for such a requirement stemmed from the experiments of Eccles and Sherrington (3) on spinal reflexes mediated through chains of synapses. It was not until the pioneer investigations of Lorente de N6 (23, 24), however, that a direct approach to the problem was made and spatial summation was studied in a reflex response mediated through a single synapse. He analyzed the response of oculomotor neurons to graded presynaptic volleys and concluded that discharge of a motoneuron depends upon activation of a dense zone of the synaptic scale rather than of a critical number of synaptic knobs.

The subsequent discovery of the monosynaptic reflex response of spinal motoneurons (2, 29) provided an experimental situation in which the relation between graded presynaptic activity and motoneuron discharge could be further analyzed. Certain primary afferent fibers from muscle establish direct synaptic connections with spinal motoneurons, the anatomical basis for the myotatic reflex (13, 15). The afferent fibers concerned are of large diameter and, in part, comprise group I of the afferent fiber diameter spectrum of muscle nerves (14). Activity in graded numbers of such fibers may be initiated by appropriate stimulation and the size of afferent volleys as well as of motoneuron response may be measured.

Monosynaptic reflex response to graded afferent volleys was studied in the segmental spinal reflex by Lloyd (13), who found that as size of dorsal root volleys, recorded in the stimulated root was increased from zero to maximum, reflex discharge was elicited when the volley size reached 5 to 15 per cent of maximum. Discharge zone grew until the volley size reached about 60 per cent of maximum; further increase in size of dorsal root volleys then failed to increase monosynaptic reflex discharge. These findings demonstrated the need for spatial summation in monosynaptic reflex discharge of spinal motoneurons.

Size of volleys recorded in a stimulated dorsal root does not provide a direct measure of the number of active monosynaptic excitatory fibers, for contribution to volley size is made by a variety of other fibers chiefly of higher threshold than those concerned in the monosynaptic reflex path. The number of mono-

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synaptic excitatory fibers activated by graded afferent stimulation may be more directly estimated by initiation of volleys in muscle nerves and measurement of volley size as they enter or near the cord, a device used by Rall (26). Significance of afferent volley size so measured depends upon the afferent fiber composition of muscle nerves for the latter contain several functional groups.

Myelinated afferent fibers in muscle nerves display diameter distributions with three distinct modes (19, 30) permitting classification into three groups: I (12 to 20 μ), II (4 to 12 μ), and III (1 to 4 μ). On the basis of recent studies fibers of groups I and II may be further classified as to receptor function (8). Within group I are two functional categories: group IA, containing fibers from muscle spindles, and group IB, comprising fibers from tendon organs. Group II is a nearly homogeneous collection of smaller afferent fibers from muscle spindles. The afferent fibers may also be separated on the basis of their reflex actions: the large diameter fibers from muscle spindles are afferent for the monosynaptic reflex response (6); fibers from tendon organs are concerned in the inverse myotatic reflex, mediated disynaptically (6, 12); and the smaller muscle spindle afferent fibers are concerned with a flexor reflex of proprioceptive origin (5, 7, 12, 14).

Now the distance between nerves to hind limb muscles and spinal cord of cat is such that volleys initiated in muscle nerves suffer temporal dispersion before they reach the spinal cord and to such a degree that impulses in group I fibers alone contribute to amplitude of volleys as measured at dorsal root. Rall (26) has studied the relation between size of afferent volleys, produced by graded stimulation of muscle nerves and recorded from intact dorsal root, and monosynaptic reflex response. He found that reflex discharge was initiated by volleys about 20 per cent of maximum and was complete when volleys reached about 70 per cent of maximum, suggesting that monosynaptic excitatory fibers are confined to the larger diameter fraction of group I.

Subsequent investigation has revealed the bimodal diameter distribution of muscle spindle afferent fibers. The group of larger fibers (IA) extends throughout the group I range with a mode at about 16 to 18 μ, while the smaller spindle afferent fibers occupy group II and are distributed about a maximum at 5 to 6 μ. The two distributions are not completely separate but merge to some extent. The major portions of the distributions are sufficiently separate to suggest that the bulk of the fibers in both groups are functionally distinct. Thus, most of the spindle afferent fibers in the group I band might be expected to comprise a functionally homogeneous group. From these considerations it might be expected that monosynaptic excitatory fibers, being large afferent fibers from muscle spindles, would contribute to a group I volley throughout nearly its full extent.

The present investigation of the relation between size of graded muscle

afferent volleys and monosynaptic reflex response has two purposes: to determine the diameter distribution of afferent fibers producing monosynaptic excitatory actions and to evaluate the requirements for spatial summation in spinal motoneurons by analysis of the input-response relation.

Method

Two methods have been used to record afferent volleys and the resultant monosynaptic reflex response of motoneuron pools. The first has been to stimulate intact dorsal roots while recording monophasically from a selected muscle nerve. A volley initiated in dorsal root courses antidromically to be recorded in the muscle nerve, and orthodromically into the spinal cord where it initiates reflex action. Since monosynaptic reflex response is reflected into the same muscle nerve that contains the exciting afferent fibers (14), the monophasic record displays the afferent volley and, after delay incurred by added conduction and synaptic transmission, a nearly synchronous monosynaptic reflex response. This method permits accurate measurement of afferent volleys and of reflex response and allows the delivery of relatively synchronous volleys to a motor nucleus. The second method has been to stimulate selected muscle nerves, recording afferent volleys by a volume lead at the root-cord junction and reflex response in distally severed ventral root. Afferent volleys are then measured by amplitude of triphasic response from onset of negativity to peak negativity. Accuracy of measurement of afferent volley size is limited by amplifier noise at the gain required and by the considerable fluctuation in potential of the "resting" cord.

Response of individual medial gastrocnemius motoneurons has been recorded in filaments of ventral root following graded stimulation of the muscle nerve, afferent volleys being recorded at the root-cord junction. As a measure of response, firing index has been used, defined as the per cent of monosynaptic reflex responses elicited by 50 trials of a test volley. A similar index has been employed in a study of individual motoneuron response to maximal afferent volleys (21).

In all experiments acutely decapitate cats have been used, maintained by artificial respiration. Stimuli have been delivered at the rate of 30 per minute. Although there is slight depression of response at this interval (11) the large number of responses required for analysis has forced the selection of such a repetition rate. Long term stability is an additional requirement when many responses must be measured: this can be achieved by maintaining constancy of body temperature.

Response of Motoneuron Populations

Considerations Pertaining to Measurement of Afferent Volleys and of Reflex Response.—Monosynaptic reflex responses of triceps surae and of deep peroneal motoneurons to graded afferent volleys have been investigated. For the particular muscles studied afferent volleys are conducted for a distance of about 15 cm. from the point of initiation to the point of recording. Temporal dispersion at such a conduction distance is appreciable and determines which among the fibers of the afferent diameter spectrum contribute to the measured volley. In the case of monophasic recording one may determine the diameter

band of fibers that will add to spike potential amplitude by reconstruction of the latter after the method of Gasser and Grundfest (4). Using the histological diameter spectrum of afferent fibers in triceps surae nerve from data of Lloyd and Chang (19) and a factor of 6 for conversion of diameter to conduction velocity (10), it is found that fibers in the range 14 to 21 μ contribute to recorded spike potential amplitude after conduction over a distance of 15 cm. From these considerations it may be concluded that size of measured input volleys provides a measure of the number of group I afferent fibers above 14 μ which have been stimulated. Since the afferent fiber diameter spectrum appears to be fairly uniform among different cats (8) the measure of input volleys, expressed in per cent of maximum, may be compared from one preparation to another.

Monosynaptic reflex discharge produces a nearly synchronous volley in the fairly homogeneous group of large motor fibers. Although large motor fibers differ in diameter (ranging from 9 to 15 μ) there is no reason to suppose any relation between diameter of efferent fiber and motoneuron behavior in reflex response. Hence it may be assumed that monosynaptic reflex response amplitude, recorded monophasically in distally severed ventral root or muscle nerve, is proportional to the number of motoneurons that discharge. Amplitude of reflex response, unless total pool response is determined, provides only a relative measure of the number of motoneurons responding and cannot be compared in absolute terms from one experiment to another. Thus reflex responses, expressed as per cent of mean amplitude of response to maximal afferent volleys, may represent activity in different numbers of motoneurons in different preparations.

Relation between Input and Response Amplitude.—The characteristic relation between afferent volley size and amplitude of monosynaptic reflex response is illustrated in Fig. 1, volleys being initiated in triceps surae nerve and recorded at the root-cord junction. As input was varied from zero to maximum, no reflex discharge occurred until the input level reached about 20 per cent of maximum, after which incrementing input caused discharge to grow at increasing rate, then at nearly linear rate and finally at decreasing rate until input reached maximum. Further increase in stimulus strength caused no increase in discharge.

Each response amplitude in Fig. 1 represents the mean of 25 successive determinations, the use of mean response amplitude being necessary because of the considerable temporal variation in response. Certain features of the input-response curve are a consequence of its use: In the preceding communication (9) it was demonstrated that initial growth of mean response amplitude depends upon the characteristics of excitability fluctuation such that, if synaptic drive were linearly increased from zero, growth of discharge as measured by mean response amplitude would exhibit initial curvature. Only after af-

erent volleys have reached a size sufficient to complete the zone of variation can mean response amplitude be considered a linear measure of synaptic excitation.

Growth of discharge zone is related to the degree of transmitter potentiality developed by afferent volleys in members of the pool. Initial growth of mean pool transmitter potentiality with incrementing input cannot be stated with certainty for at least part of the initial curvature in the input-response curve is a consequence of excitability fluctuation. Following this initial curvature the
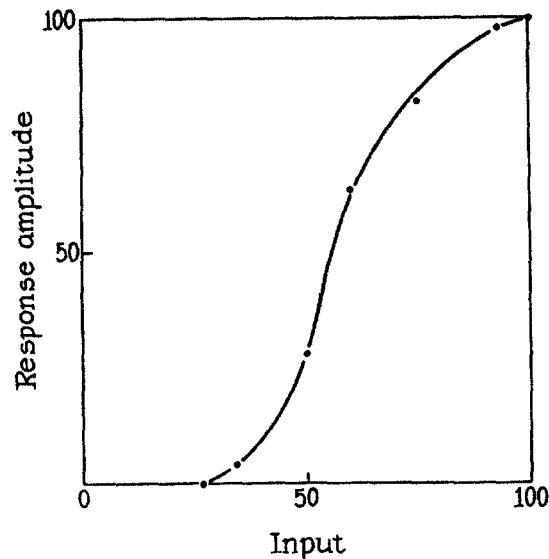

FIG. 1. Input-response relation in a population of triceps surae motoneurons. Afferent volleys initiated in muscle nerve and recorded at root-cord junction; reflex response recorded in distally severed ventral root. Ordinate, per cent of mean response to maximal input. Abscissa, per cent of measured input.

growth of mean pool transmitter potentiality is nearly linearly related to input until, for the final 40 per cent of input, the rate of increase in transmitter potentiality diminishes.

It will be noted that the input-response curve obtained by dorsal root stimulation (Fig. 2A) has less final decrease in rate of growth of discharge as input nears maximum than the response curve found with muscle nerve stimulation (Fig. 1). Since dorsal root volleys suffer less temporal dispersion than volleys initiated in the muscle nerve, the former must arrive more synchronously at the motor nucleus than the latter. Hence the greater decrease in rate of growth of discharge zone with muscle nerve volleys may be attributed to the greater delay in arrival of impulses in the higher threshold fibers. Even

with dorsal root volleys some asynchronism must occur in onset of activity in the synaptic knobs concerned; purely spatial summation cannot be studied. It will be shown that decrease in rate of growth of discharge zone as input volleys near maximum, is, in large part, due to the rapid temporal decay of transmitter potentiality.
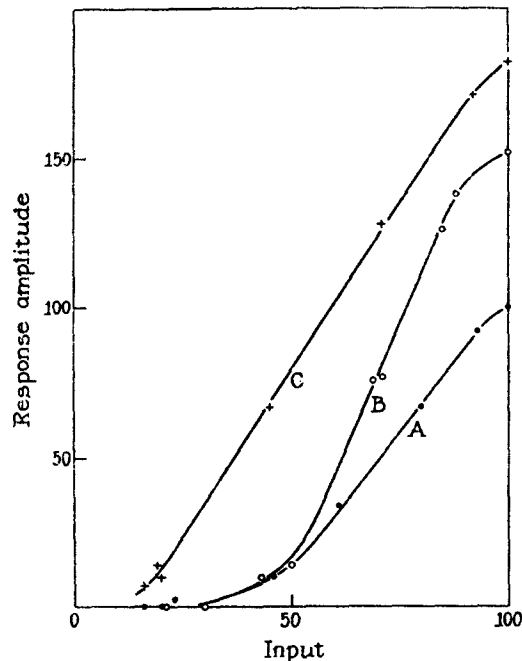

FIG. 2. Input-response relation in a population of deep peroneal motoneurons. Afferent volleys initiated in dorsal roots; afferent and reflex volleys recorded in muscle nerve. A, control, temperature, 36.5°C. B, after cooling to 35°. C, facilitated curve, test volleys delivered after standard sural nerve volleys, temperature, 36.5°C. Volley interval 4.0 msec.

Alteration of Reflex Response to Graded Afferent Volleys.—The input-response relation has been studied in circumstances which increase or decrease response amplitude to maximal afferent volleys. The curves obtained thereby may be compared with the experimental input-response relations found by Rall (27) and with the theoretical input-response relations predicted by his mathematical formulation of the requirements for spatial summation (28).

One convenient means of changing the number of motoneurons that discharge to maximal afferent volleys is by alteration of body temperature. Reduction in body temperature by a few degrees Centigrade causes a large increase in monosynaptic reflex response although it may be assumed that no

change occurs in the number of presynaptic fibers active in an input volley a given per cent of maximum. The effect of change in body temperature on a monosynaptic reflex response of deep peroneal motoneurons is illustrated in Fig. 2. At 36.5°C. the input-response relation is described by curve A, while curve B indicates the relation found after lowering body temperature to 35°C. Although response amplitude to maximal input volleys was considerably greater at the lower temperature, discharge was initiated at nearly the same level of input, about 30 per cent of maximum, at both temperatures. In fact, if curves A and B are plotted so that their maxima coincide, they are nearly identical. Reduction in body temperature, therefore, increases the number of motoneurons that respond to maximal afferent volleys but fails to reduce the number of active monosynaptic excitatory afferent fibers required for initiation of discharge in the most powerfully driven motoneurons.

The effect of change in body temperature may be contrasted with the modification in the input-response relation of the same reflex brought about by addition of standard convergent facilitatory volleys. Fig. 2C indicates the response to graded afferent volleys delivered following a facilitatory volley of fixed size in sural nerve and should be compared with the control curve A taken at the same temperature. Addition of standard facilitatory volleys increased the discharge to maximal afferent volleys and also shifted the response curve to the left (from A to C) permitting input volleys previously ineffective to secure discharge. A comparable effect on the input-response relation is produced by the facilitatory action of monosynaptic excitatory fibers (Fig. 5). Facilitation shifts the input-response curve to the left permitting activity in fewer than the otherwise minimal number of monosynaptic excitatory afferent fibers to secure discharge. This effect, produced by addition of active synaptic knobs from another source, is clearly not equivalent to the effect produced by a reduction in body temperature.

The input-response relation has been studied during inhibitory action in which circumstance the number of active monosynaptic excitatory afferent fibers for an input volley, a given per cent of maximum, will remain unaltered. Inhibition has been direct, resulting from standard volleys in the antagonist nerve, or indirect, due to the action of sural nerve volleys on the monosynaptic reflex response of extensor motoneurons. In either case the effects are similar. Fig. 3 depicts a representative experiment in which input-response relations in triceps surae motoneurons were determined before (A) and during (B) inhibition produced by standard volleys of fixed size in sural nerve. It will be noted that inhibition decreased the response to maximal afferent volleys but had little effect on the input level which initiated discharge in the most powerfully driven motoneurons.

Taken in conjunction the effects of alteration in body temperature and of inhibition reveal that considerable change can occur in the size of mono-

synaptic reflex response to maximal input without significant change in the minimal input level required to initiate discharge in the most powerfully driven motoneurons. On the other hand, addition of standard facilitatory volleys, which may be considered to add active excitatory knobs from another source, causes a marked reduction in the minimal input level required for the initiation of discharge. The above findings may be compared with the results of Rall (27) who found that change of excitability of the system, by varying the level of anesthesia or by post-tetanic potentiation, caused a parallel trans-
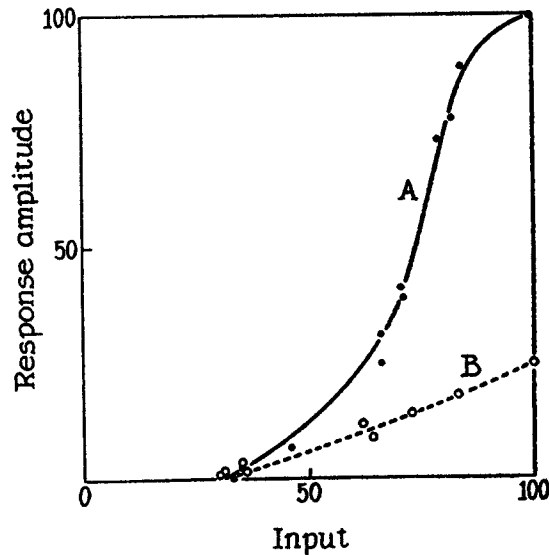

FIG. 3. Input-response relation in a population of triceps surae motoneurons. Afferent volleys initiated in dorsal roots; afferent and reflex volleys recorded in muscle nerve. Temperature, 36.5°C. A, control. B, test volleys delivered after standard sural nerve volleys. Volley interval 4.0 msec.

lation of the input-response curves after he had applied a correction factor to his measured input. The present series of input-response curves determined at different levels of responsivity tend to pivot about a minimal input value. The latter is not compatible with Rall's theory in its simplest application (26) but could be accommodated by different adjustment of theoretical parameters (see 28); the validity of the assumptions now required may be questioned.

It seems likely from the present results that activity in excitatory synaptic knobs must fulfill some minimal anatomical requirement in order to secure motoneuron discharge. This could explain the facts that a minimal number of monosynaptic excitatory afferent fibers must be activated to initiate discharge in a motoneuron pool and that the minimal effective input may show little

change at different levels of responsivity. The lack of shift in the minimal effective input level during inhibition indicates that inhibition does not result from block of impulse transmission in monosynaptic excitatory afferent fibers before reaching the motoneurons (25) for such a hypothesis would demand that the minimal input level which secured discharge in the most powerfully driven motoneurons be increased during inhibition.

Facilitation Produced by Graded Afferent Volleys.—The growth of discharge to graded input volleys depends upon the development of transmitter potentiality by monosynaptic excitatory fibers for motoneurons of a given pool. Transmitter potentiality is developed to the greatest extent by fibers homonymous for a particular group of motoneurons. In contrast, monosynaptic excitatory fibers from the heteronymous fraction of a muscle possess considerable potentiality for facilitation but comparatively little transmitter potentiality. It has been suggested that transmitter and facilitator potentialities are not necessarily covariant (20).

The relation between afferent volley size and degree of facilitation has been measured by recording the effect on a test monosynaptic reflex response in medial gastrocnemius motoneurons of graded synchronous volleys in the nerve to lateral gastrocnemius-soleus. In measuring this relation between degree of facilitation and size of facilitatory volleys it is preferable to record reflex response in a pool containing no motoneurons homonymous for the lateral gastrocnemius-soleus volleys for, to the extent that such motoneurons are present in the pool, the curve of facilitation will be influenced by their input-response relation. In practice reasonable separation of a portion of the medial gastrocnemius pool may be effected. In selected preparations ventral root S1 or the caudal part thereof contains an appreciable number of medial gastrocnemius motoneurons and but few pertaining to lateral gastrocnemius-soleus. Four such preparations have been studied by delivering maximal test volleys in the medial gastrocnemius nerve and graded volleys in the heteronymous nerve. The results are plotted in Fig. 4 (curve marked 100 per cent). Facilitation was produced by the lowest threshold afferent fibers and grew in approximately linear relation to input volley size until the latter reached about 85 per cent of maximum, beyond which input level facilitation grew at decreasing rate.

The nearly linear increase in degree of facilitation as heteronymous input is increased from zero to 85 per cent of maximum suggests that the number of monosynaptic excitatory fibers increases approximately in proportion to input volley size over this range. This finding also suggests that there is no large correlation between fiber diameter and facilitator potentiality.

The decrease in rate of growth of facilitation over the final 15 per cent of heteronymous input suggests that there is a decrease in the proportion of monosynaptic excitatory fibers among the highest threshold group I fibers. It ap-

pears likely that the diameter distribution of monosynaptic excitatory fibers must overlap with that of the functionally different group of smaller spindle afferent fibers. However, monosynaptic excitatory fibers do occur throughout the 14 to 21 μ band, and over most of its range the measured input may be considered, in first approximation, a nearly linear measure of the number of monosynaptic fibers stimulated.

Degree of facilitation bears a nearly linear relation to size of afferent volleys over the range zero to 85 per cent of maximum whereas rate of growth of mean

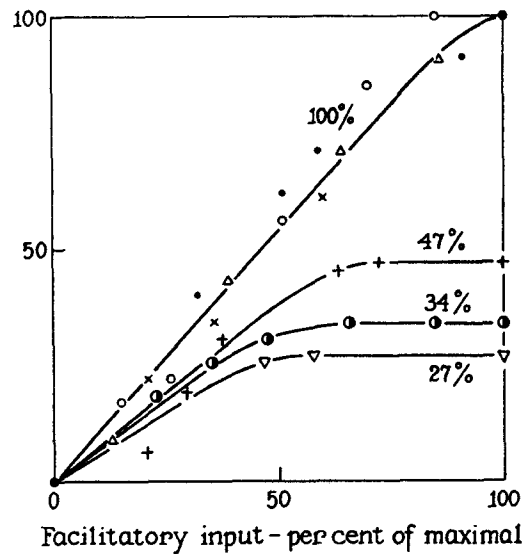Facilitatory input - per cent of maximal

FIG. 4. Relation between degree of facilitation and size of heteronymous volleys. Facilitation of response of medial gastrocnemius motoneurons to homonymous test volleys by graded volleys in the nerve to lateral gastrocnemius-soleus. Test volleys fixed at 100, 27, 34, and 47 per cent of maximum. Ordinate, growth of facilitation. Abscissa, measured heteronymous input.

transmitter potentiality decreases after incrementing input reaches about 60 per cent of maximum. It is suggested that the latter results from temporal dispersion of afferent volleys initiated in the muscle nerve and from the rapid temporal decay of transmitter potentiality. One may enquire as to why facilitation produced by heteronymous volleys, which suffer similar temporal dispersion, should not be influenced in the same manner by rapid decay of transmitter potentiality. Impulses in heteronymous fibers, dispersed in their arrival at the motor nucleus, will arrive in near synchrony with some fraction of the similarly dispersed maximal homonymous volleys. Therefore, volleys in higher threshold heteronymous fibers can effectively increase transmitter potentiality

even though the latter has a rapid temporal decay. This question may be put to a test by determining the relation between heteronymous input and degree of facilitation, using homonymous volleys considerably less than maximum. In this case the higher threshold, slower conducting component of group I homonymous volleys is not activated; if transmitter potentiality has a rapid temporal decrement the high threshold fraction of heteronymous input should be considerably less effective, by virtue of its late arrival, than when maximal homonymous volleys are used. In Fig. 4 are shown curves relating growth of facilitation to heteronymous input determined when test volleys were restricted in their dispersion by stimulation of only their lower threshold, faster conducting components. It will be noted that with submaximal test volleys (27, 34, and 47 per cent) the higher threshold fraction of incrementing heteronymous input failed to facilitate response whereas that fraction was effective with maximal test volleys (100 per cent). Thus, with test volleys 27 per cent of maximum, facilitation failed to increase when heteronymous input was raised from 58 to 100 per cent of its maximum. The failure of the higher threshold, slower conducting fraction of heteronymous input to increase response when homonymous test volleys are restricted in their dispersion demonstrates that transmitter potentiality does indeed have the postulated rapid temporal decay. The rate of decay cannot be directly determined, but, since temporal dispersion of the input volleys concerned would lead to a difference in conduction time of only 0.5 msec. between the fastest and slowest components, transmitter potentiality must decay considerably within 0.2 to 0.3 msec.

The Effect of Interacting Homonymous and Heteronymous Volleys.—Two measurable effects result from volleys in monosynaptic excitatory fibers: initiation of response in a "resting" pool and facilitation of response in a pool already caused to discharge by volleys from another source. In both cases increase in discharge depends upon the development of an increment in transmitter potentiality by volleys in monosynaptic excitatory fibers. Heteronymous volleys can produce powerful facilitation of response initiated by homonymous volleys, yet in isolation volleys in the heteronymous nerve have feeble transmitter potentiality (20). The effects of interacting graded homonymous and heteronymous volleys have been studied in order to compare the development of transmitter and facilitator potentialities.

The response of a population of medial gastrocnemius motoneurons to synchronously initiated homonymous (G.M.) and heteronymous (G.L.S.) volleys is shown in the isometric diagram of Fig. 5. In practice homonymous volleys were fixed at a succession of given sizes and response observed as heteronymous input was varied from zero to maximum. In order to avoid contribution to response amplitude by discharge in lateral gastrocnemius motoneurons, a preparation was selected in which a portion of the medial gastrocnemius outflow in ventral root S1 was anatomically separate. Response

at the peak of post-tetanic potentiation indicated that the root used for recording contained very few motoneurons pertaining to lateral gastrocnemius-soleus.

It will be noted that the response curve to incrementing homonymous input in isolation (G.M. = 0 to 100, G.L.S. = 0) is sigmoid in form and similar to the curve already shown in Fig. 1. With heteronymous volleys of maximal
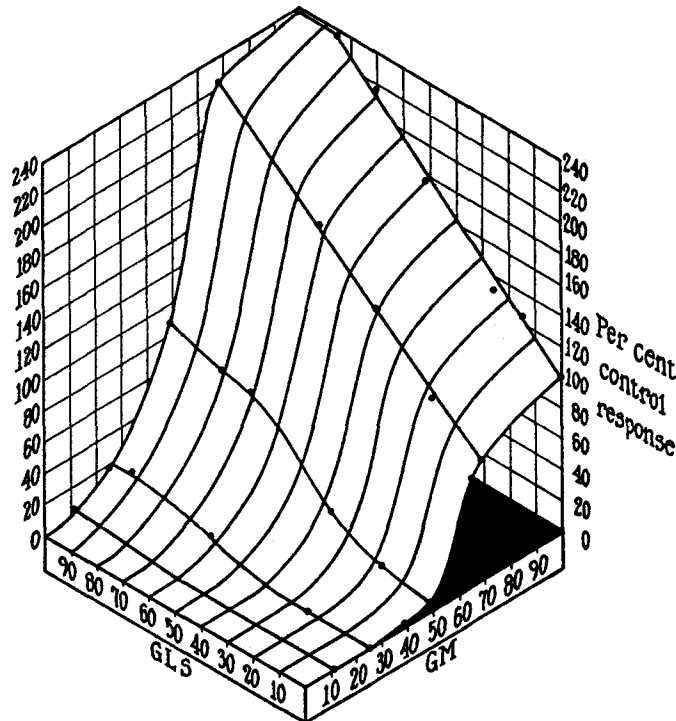

Fig. 5. Isometric diagram of the response of a population of medial gastrocnemius motoneurons to interacting graded volleys initiated synchronously in the homonymous (G.M.) and heteronymous (G.L.S.) nerves. Each point is the mean of 50 successive responses.

size the curve of response to homonymous input (G.M. = 0 to 100, G.L.S. = 100) is again sigmoid in form but shifted to the left and increased in slope by the facilitatory action, features already considered in connection with Fig. 2. Similarly, at fixed levels of heteronymous input the interpolated curves relating response to homonymous volley size are sigmoid.

The relation between degree of facilitation and size of heteronymous volleys at several fixed levels of homonymous input may be seen in Fig. 5. When homonymous input was fixed at 68 or 100 per cent of maximum, degree of

facilitation had an approximately linear relation to size of heteronymous volleys (G.M. = 68 or 100, G.L.S. = 0 to 100). However, when homonymous input was fixed at 48 per cent of maximum the increase in response to incrementing heteronymous input followed a sigmoid curve (G.M. = 48, G.L.S. = 0 to 100). The initial curvature in this relation may be attributed, in part at least, to the fact that the zone of variation was not complete at this level of homonymous input, hence growth of response was initially influenced by the effect of excitability fluctuation on mean response amplitude (9). The final decrease in rate of growth of facilitation is considered to result from the rapid temporal decay of transmitter potentiality. Homonymous volleys 48 per cent of maximum do not include impulses in the higher threshold, slower conducting fibers; impulses in higher threshold heteronymous fibers produced less effect because they arrived later than the slowest impulses in homonymous volleys of the size used. With homonymous volleys fixed at the 26 per cent of maximum level, heteronymous input was weakly effective in increasing discharge. Further reduction in homonymous input to 12 per cent of maximum caused heteronymous volleys to be even less effective. In general, it may be seen that the increment in response produced by heteronymous volleys is highly dependent upon the level of homonymous input. Although maximal heteronymous volleys in isolation were ineffective, when combined with maximal homonymous volleys the former increased response amplitude nearly two and one-half times.

Direct Inhibitory Action by Graded Afferent Volleys.—Monosynaptic afferent fibers exert excitatory effects on motoneurons of a synergic unit and, in addition, inhibitory effects on motoneurons of the antagonist group (17). The relation between degree of direct inhibition and size of afferent volleys has been measured by subjecting a test monosynaptic reflex response in triceps surae motoneurons to the action of graded volleys in the antagonist nerve. The test response was recorded in ventral root following maximal group I volleys in triceps surae nerve which reached the cord 0.4 to 0.5 msec. after graded volleys in deep peroneal nerve. Direct inhibition increases in degree from zero to maximum as the volley interval is increased from zero to 0.5 msec. (16). Unfortunately it has been necessary to study the effects of graded inhibitory volleys during this incremental phase in order to avoid disynaptic reflex actions which begin at volley intervals of 0.5 to 0.6 msec. (12).

The relation between size of inhibitory volleys and degree of inhibition was determined in 6 experiments, the results of which are illustrated in Fig. 6. The relation is nearly linear, the deviation from linearity being in the direction of proportionately less inhibitory action with smaller volleys. With shocks sufficiently weak to stimulate only the largest fibers delay in initiation of impulses can cause the volley interval to be reduced, which reduction might well explain the weaker inhibitory action of small volleys.

Comparison of the relations between degree of facilitation and size of heteron-

ymous volleys and between degree of inhibition and size of antagonist volleys reveals their general similarity. It should be noted that the measures of input volley size are not strictly comparable since the diameter spectra of afferent fibers in triceps surae nerve and in the nerves to deep peroneal muscles differ considerably (19). Nevertheless the general similarity of input-facilitation and input-inhibition curves indicates that both these monosynaptic actions are mediated by the same fiber group. This is in accord with the observation that the relations of facilitation and inhibition to size of dorsal root volleys are similar (13).
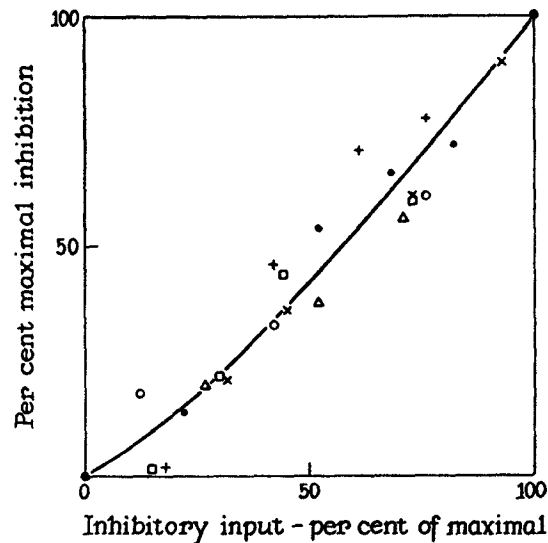

FIG. 6. Relation between degree of inhibition and size of volleys in the antagonist nerve. Response of triceps surae motoneurons subject to direct inhibitory action of graded volleys in deep peroneal nerve. Volley interval 0.4 to 0.5 msec.

The essentially linear increase in degree of inhibition with incrementing antagonist volleys suggests that fibers producing such action are distributed throughout the fiber range contributing to measured input and further that inhibitory potentiality does not vary widely as a function of fiber diameter.

Responses of Individual Motoneurons

In the preceding section the input-response relations of motoneuron populations were considered. Responses of individual motoneurons have been examined in similar circumstances and the information obtained provides a basis for interpretation of population responses and also indicates differences relating to functional organization of motoneurons that could not be inferred from observation of the population.

A total of 60 medial gastrocnemius motoneurons have been examined for their response to graded volleys in the homonymous muscle nerve. The sample was selected to represent all significant varieties of response in motoneurons liminally excited by afferent volleys. Of the 60 units examined at maximal homonymous input, 9 had a firing index of zero, 42 had intermediate firing indices (between zero and 100), and 9 had a firing index of 100. This sample represents only a fraction of the total pool (20), but the composite response of the 60 motoneurons to graded input was found to reproduce the essential features of natural pool responses in similar circumstances.
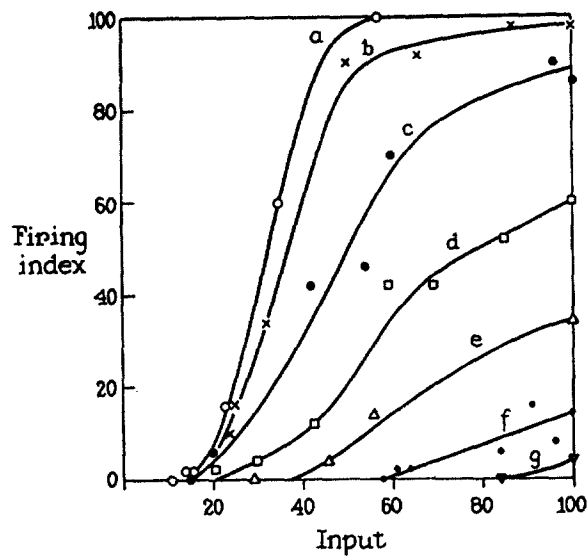

FIG. 7. Relation between homonymous input and firing index in a representative series of individual medial gastrocnemius motoneurons.

The Relation between Homonymous Volley Size and Firing Index.—Fig. 7 illustrates the manner in which firing indices increase with increase in size of homonymous volleys (input) from zero to maximum. Each of the curves plotted therein describes the relation between input and firing index for a particular motoneuron. The motoneurons shown were selected to include individuals that displayed, at maximal input, firing indices varying throughout the range of values greater than zero. Collectively these motoneurons illustrate the complete range of input–firing index relations and, but for minor variations, the plotted curves have a form characteristic of all units examined in which maximal input volleys secured discharge.

As input volleys were increased from zero to maximum the most powerfully driven motoneurons (a) discharged with low firing index in response to volleys 15 to 30 per cent of maximum, with further increase in input firing index rose

rapidly to reach 100 with the use of volleys 50 to 60 per cent of maximum. Less powerfully driven motoneurons (b to g) showed a more gradual rise in firing index as the input volley was similarly increased. In the most powerfully driven units the rapid rise in firing index always occurred within a characteristically limited range of input, that between 20 and 50 per cent of maximum.

Fig. 7 illustrates another characteristic feature of the development and growth of motoneuron response to incrementing afferent input, namely the level of input at which individual motoneurons pass from the subliminal zone to the zone of intermediate firing indices. Although it may be seen that individuals do this over a considerable range of input levels greater than some minimal value, those individuals destined to reach firing indices in excess of 50 when input is maximum (*i.e.*, a, b, c, d), all enter the intermediate firing zone at input levels very close to the minimal value. By way of contrast relatively large successive increments of input are necessary for the recruitment into the intermediate firing zone of those motoneurons that fall progressively short of firing index 50 at maximal input (*i.e.*, e, f, and g).

Relation of Transmitter Potentiality to Input Volley Size.—For consideration of the factors responsible for effective spatial summation it would be preferable to express the input-response relation for an individual motoneuron in terms of transmitter potentiality rather than firing index. Analysis of the input-response relation in populations of motoneurons suggests the manner in which mean pool transmitter potentiality grows with incrementing input but provides no evidence as to the development of transmitter potentiality in individual motoneurons.

There is no direct measure of transmitter potentiality in an individual motoneuron; to any given volley the measurable motoneuron response is discharge or no discharge. If a motoneuron were subject to no influence other than a series of test volleys of incrementing size one would expect the firing index to rise abruptly from zero to 100 once transmitter potentiality had reached a critical value. However, motoneurons are subject to continuous temporal fluctuation in excitability such that for any given degree of transmitter potentiality the firing index is determined by the frequency with which excitability levels are reached permitting this value of transmitter potentiality to satisfy the requirements for discharge. An increment in transmitter potentiality causes the firing index to rise since the lower excitability level now required for discharge is attained more frequently. If the frequency distribution of fluctuating excitability levels were known for a particular motoneuron it would be possible to predict the manner in which firing index would rise with linear increase in transmitter potentiality.

Actually the relation between firing index and transmitter potentiality may be derived in first approximation by assuming: (1) that variation in amplitude of a pool monosynaptic reflex response is directly proportional to the fluctua-

tions in excitability level in individual motoneurons and (2) that all motoneurons are subject to identical fluctuations in excitability. Lloyd and McIntyre (21) have used a similar derivation to assign values of transmitter potentiality among motoneurons in the intermediate firing zone of a synthetic pool at fixed levels of drive. Fig. 8 shows the relation between transmitter potentiality and firing index used in the present study, based on a frequency distribution of response amplitude variation in a motoneuron pool (9). Using this relation input–transmitter potentiality curves have been derived from input–firing index curves of a number of individual motoneurons.
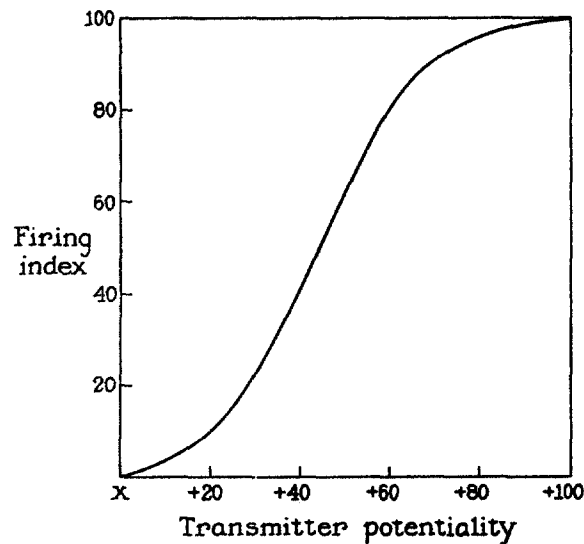

FIG. 8. Relation between firing index and transmitter potentiality.

In Fig. 9 curves of the input–transmitter potentiality relation have been constructed for a number of individual motoneurons which collectively form a synthetic pool. The units are so arranged with regard to differences in input–transmitter potentiality relations as to satisfy several requirements:

1. The series of curves of input–transmitter potentiality relation represent curves derived from the full range of experimental input–firing index relations.

2. The number of units in the zone of intermediate firing remains the same once this zone is complete. This requirement is posed by the fact that variation in population response amplitude remains essentially constant as drive is increased above that level at which the zone of variation is complete (9). Also, within a synthetic pool the number of motoneurons in the intermediate firing zone remains substantially the same at several fixed levels of synaptic drive (21).

3. Mean pool transmitter potentiality grows at decreasing rate as the input size nears its maximum. This follows from the fact that input-population response curve decreases in slope as the input size approaches maximal.

In Fig. 9 the limits of the intermediate firing zone are represented by two finite values of transmitter potentiality (x and $x + 100$). Individual motoneurons subject to transmitter potentiality $x + 100$ or greater will display a firing index of 100; those with transmitter potentiality of x or less have a firing index of zero. In the range between x and $x + 100$ the relation between firing index
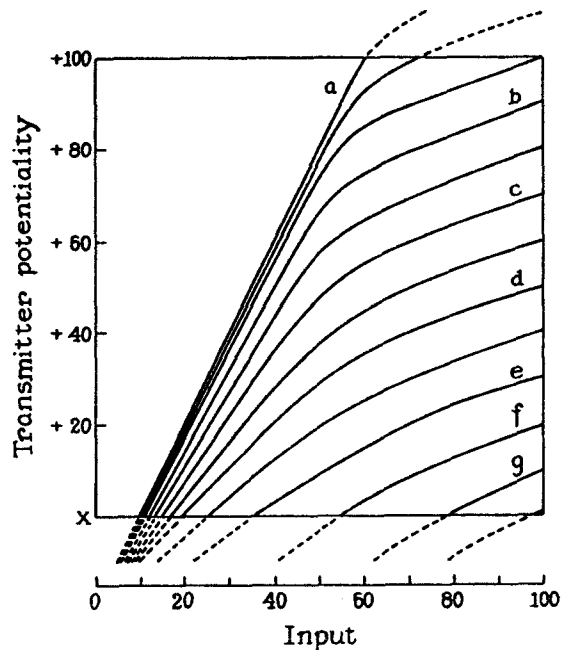

Fig. 9. Derived input–transmitter potentiality relations of individual motoneurons comprising a synthetic pool. See text.

and transmitter potentiality is obtained from Fig. 8. The series of input–transmitter potentiality curves when converted to input–firing index curves are representative of the behavior of individual motoneurons. Thus curves a to g of Fig. 7 correspond closely to input–firing index curves derived from similarly labeled input–transmitter potentiality curves of Fig. 9. In fact, a series of input–firing index curves, derived from the input–transmitter potentiality curves of Fig. 9, reproduce the full range of input–firing index relations observed experimentally.

The series of input–transmitter potentiality curves (Fig. 9) display several characteristic features. In the most powerfully driven motoneuron (a) trans-

mitter potentiality, over the range x to $x + 100$, increases linearly with incrementing input. Motoneuron b, which acquires transmitter potentiality to the degree $x + 90$ at maximal input, has a nearly linear increase in transmitter potentiality as input is increased to 50 per cent of maximum but with further increase in input the rate of growth of transmitter potentiality decreases. Successively less powerfully driven motoneurons (b to d) exhibit a progressive decrease in the initial slope of the input–transmitter potentiality relation but the differences in initial slope are small as compared to the differences in final degree of transmitter potentiality attained. Only when the transmitter potentiality is below the level $x + 40$ at maximal input is the initial slope markedly less (d to g).

In considering input–transmitter potentiality curves of individual motoneurons it should be recalled that mean *pool* transmitter potentiality grows at decreasing rate to incrementing muscle nerve volleys over the final 40 per cent of input. This decrease in rate is largely due to temporal dispersion of the input volleys and the rapid temporal decay of transmitter action. Temporal dispersion of afferent volleys will also affect the input–transmitter potentiality curves of individual motoneurons. Consider, for example, the curve of motoneuron b in Fig. 9. It is likely that, in the absence of temporal dispersion, the rapid rise in transmitter potentiality which occurred in the 10 to 50 per cent of maximal input range would have continued as input was further increased. However, by the time impulses in higher threshold fibers arrive, transmitter potentiality produced by the lower threshold fibers will have decayed to some extent and in this way slope of the input–transmitter potentiality curve may decrease. Similar considerations apply to other motoneurons of the series. Although transmitter potentiality produced by the lowest threshold fibers may undergo considerable decay by the time impulses arrive in the higher threshold fibers impulses in the former may, by the residual facilitation they produce, reinforce the action of the latter.

The present observations bear on the problem of the requirements for spatial summation. Two opposing views have previously been suggested: that discharge depends upon activation of all or nearly all the knobs in a restricted zone of the synaptic scale (23, 24) and that discharge depends upon activation of a critical number of excitatory synaptic knobs irrespective of location (1). If discharge depended upon total activation of a discrete zone one would expect input–transmitter potentiality curves to rise rapidly from x to $x + 100$ at widely different levels of input in different motoneurons dependent upon the input level at which all synaptic knobs in a sufficient zone became active. The input–transmitter potentiality curves derived from experimental observations indicate that rapid rise in transmitter potentiality occurs over a characteristic and limited range of input. The fine gradation of transmitter potentiality with incrementing input suggests that the process leading to discharge is dependent

to a considerable extent on number of active knobs. On the other hand, if number of active excitatory synaptic knobs irrespective of location determined transmitter potentiality, one might expect the intercepts of input–transmitter potentiality curves at ordinate x (Fig. 9) to shift in proportion to the degree of transmitter potentiality attained at maximal input. Observation reveals that the intercepts tend to be clustered together until the level of transmitter potentiality at maximal input is below a certain value. This discrepancy from prediction does not necessarily disprove the hypothesis that number of active excitatory knobs alone determines transmitter potentiality. If it be conceded that transmitter potentiality has a rapid temporal decay, there is no feature of the series of input–transmitter potentiality curves that forces acceptance of the view that variables other than number of active excitatory knobs determine growth of transmitter potentiality with incrementing input. It will be shown below that degree of aggregation of active knobs must be a significant variable. The observed input–transmitter potentiality relations are compatible with the latter hypothesis.

Effect of Temperature Change on the Input–Firing Index Relation.—Lowering body temperature has been shown to increase amptitude of pool monosynaptic reflex response without causing significant reduction in the minimal input level necessary to initiate discharge in the most powerfully driven motoneurons. The effect of change in body temperature on the input-response relation in individual motoneurons has been studied as well. By determining input–firing index curves for a number of motoneurons at several temperatures and in the same preparation, the effect of a given temperature change has been compared from one unit to another. The four units of Fig. 10 were all studied in one preparation at three different temperatures. At 38.5°C. these units differed considerably in their response, although all in the intermediate range of firing indices at maximal input. However, after temperature had been reduced to 32° all four units approached a similar input–firing index relation. Certain details of the response curves deserve mention:

Unit A responded with high intermediate firing index, 94, to maximal input at 38.5°C., discharge beginning at the 40 per cent input level. Cooling to 35°, shifted the input–firing index curve so that entry into the intermediate zone occurred at about 30 per cent of maximal input and a firing index of 100 was reached at 70 per cent of maximal input. Further reduction of body temperature to 32° caused additional change in the input–firing index curve, principally increasing its rate of rise.

Unit B was nearly in the subliminal fringe at 38.5°, responding to maximal input with a firing index of 4. Successive reductions in body temperature to 35° and to 31.8° caused two distinct shifts in the input–firing index curve so that at the lowest temperature level entry into the intermediate zone occurred at the 30 per cent input level and the neuron acquired a curve characteristic of individuals powerfully driven in ordinary circumstances.

Unit C also displayed two distinct shifts in the input–firing index relation as temperature was reduced from 38.5 to 35° and then to 32°. This motoneuron, having a high intermediate firing index to maximal input at normal temperature, exhibited little change in the minimal input level required for entry into the intermediate firing zone.
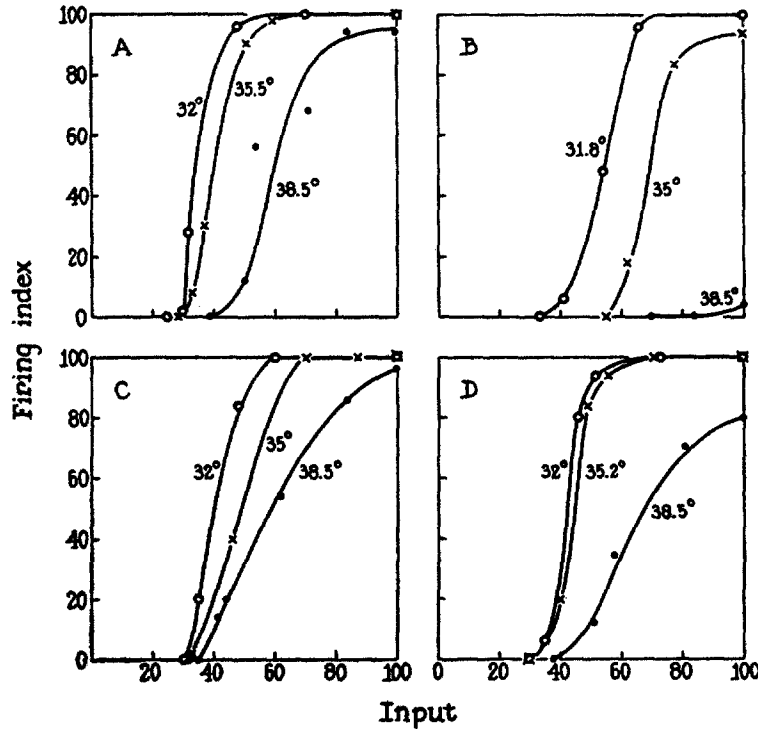

FIG. 10. Homonymous input–firing index curves of four medial gastrocnemius motoneurons determined at three levels of body temperature in the same preparation.

Unit D is of especial interest in that lowering body temperature from 38.5 to 35.2° caused a marked shift in the input–firing index curve but a further reduction in temperature to 32° resulted in no additional significant change.

Considered together these observations indicate that motoneurons differing widely in their input–firing index curves at normal temperature tend to approach a similar, and limiting, relation when body temperature is reduced. Cooling may cause previously ineffective input volleys to secure discharge but the limiting input–firing index relation indicates a minimal anatomical requirement for discharge. The fact that the motoneurons examined tended to approach the same limiting relation suggests that they received the minimal anatomical requirement at nearly the same level of input.

The input–firing index curve at the limiting relation may be seen to be similar to the curve already described for the most powerfully driven motoneuron of Fig. 7 (a). The limiting input–firing index relation corresponds to the derived relation between transmitter potentiality and input of the most powerfully driven neuron in Fig. 9 (a). From the evidence already considered it may be concluded that temperature reduction alters the input–transmitter potentiality relation so that a given motoneuron acquires a curve characteristic of a more powerfully driven individual.

Effect of Facilitation and Inhibition on the Input–Firing Index Relation.— Each medial gastrocnemius motoneuron has been examined for its response, not only to homonymous volleys in isolation but, in addition, for its response during monosynaptic facilitation produced by standard maximal group I volleys in the heteronymous nerve and during direct inhibition produced by standard maximal group I volleys in the antagonist nerve. Facilitatory volleys were initiated in the nerve to lateral gastrocnemius-soleus in synchrony with homonymous volleys in medial gastrocnemius nerve. Inhibitory volleys in deep peroneal nerve were timed to reach the cord 0.4 to 0.5 msec. before homonymous volleys.

Fig. 11 shows the input–firing index relations of 9 motoneurons examined in the three circumstances: homonymous input alone (h), with standard facilitatory volleys (f), or with standard inhibitory volleys (i). The examples in Fig. 11 have been selected to illustrate representative behavior of motoneurons in three categories of response to maximal homonymous volleys: units with a firing index of 100 (A, B, and C), units with intermediate firing indices (D, E, and F), and units with zero firing index (G, H, and I).

The input–firing index curves (h) of units A, B, and C are comparable to the curve of the most powerfully driven motoneuron of Fig. 7 (a) and to the limiting input–firing index relation obtained by lowering body temperature (Fig. 10). However, addition of standard facilitatory volleys to the graded input volleys in units A, B, and C shifted the response curves to the left (f) and permitted discharge to occur with input volleys smaller than the previously minimal effective values.

Units D, E, and F, intermediate in firing index to maximal homonymous input, also displayed distinct changes in the input–firing index relation on addition of standard facilitatory volleys. Facilitation merely shifted the response curve to the left in unit F (h to f) but in units D and E the slope of the response curve was increased as well. In fact, although unit E was much less powerfully driven by homonymous volleys in isolation than were units A, B, and C, on addition of standard facilitatory volleys this motoneuron acquired a response curve (f) similar to the facilitated curves of the most powerfully driven units.

Units G, H, and I were subliminally excited by homonymous input in isola-

tion but when delivered in conjunction with standard facilitatory volleys, homonymous volleys secured discharge. Thus, in the facilitated state maximal homonymous input elicited a low intermediate firing index in G, a somewhat
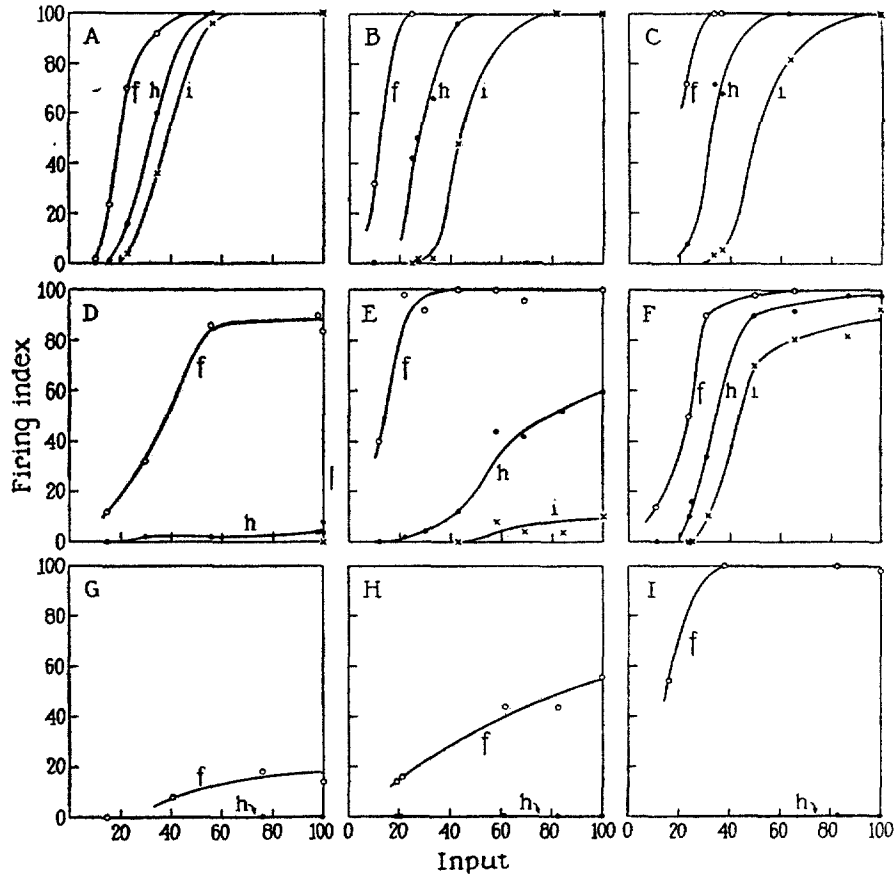

FIG. 11. Homonymous input–firing index relations of medial gastrocnemius motoneurons. Effects of facilitation and of inhibition. h, control curves. f, with maximal heteronymous volleys, synchronously initiated. i, with maximal antagonist volleys initiated in deep peroneal nerve, volley interval 0.4 to 0.5 msec.

higher value in H, and a firing index of 100 in I. It is noteworthy that the facilitated input–firing index curve of unit I is similar to that exhibited during facilitation by the powerfully driven motoneurons A, B, and C, although homonymous volleys in isolation did not evoke discharge.

All the 60 medial gastrocnemius motoneurons examined have shown evidence of monosynaptic facilitatory action by volleys in the heteronymous nerve.

Similarly, Lloyd and McIntyre (22) have demonstrated heteronymous facilitatory effects in all the 110 tricipital motoneurons they examined. Taken together the evidence indicates that each gastrocnemius motoneuron receives monosynaptic excitatory connections from afferent fibers which have their source in the heteronymous fraction of the muscle.

It is apparent that facilitatory volleys of fixed size can shift the input–firing index curve to the left even in units which are at the limiting value of this relation. This indicates that addition of active synaptic knobs from the heteronymous source reduces the minimal number of active knobs from the homonymous source required for discharge. In less powerfully driven motoneurons standard facilitatory volleys may increase the slope as well as shift the input–firing index curve to the left. An increase in slope of the input–firing index relation signifies that the slope of the input–transmitter potentiality relation has also increased (Figs. 7 and 9). If a fixed increment in transmitter potentiality were added by standard facilitatory volleys, one would expect the degree of transmitter potentiality at any level of homonymous input to be increased by a fixed amount and therefore the slope of the input–transmitter potentiality relation would not change. The observed change in slope indicates that number of synaptic knobs, irrespective of location, does not determine both transmitter and facilitator potentialities, for such a situation would require that standard facilitatory volleys add fixed increments of transmitter potentiality.

The effect of standard facilitatory volleys on the input–firing index relation of individual motoneurons (Fig. 11) reveals the manner in which similar fixed facilitatory volleys alter the input-response curve of a population (Figs. 2 and 5). If, in the population, some motoneurons have the limiting input–firing index relation they will discharge during facilitation to input volleys smaller than the minimal effective value (A, B, and C). The input–firing index curves of motoneurons less powerfully driven will increase in slope as well as shift to the left during facilitation (D, E, and F). Finally, motoneurons in the subliminal fringe will enter the discharge zone during facilitation (G, H, and I). Thus the population input-discharge curve will be shifted to the left, increased in slope, and attain a higher maximal value during facilitation.

Units A to F of Fig. 11 illustrate the effect of standard maximal inhibitory volleys on the input–firing index relation. Units A, B, and C exhibited no reduction in firing index when standard inhibitory volleys preceded maximal homonymous volleys but as input volleys were progressively reduced inhibition became evident. The over-all effect of inhibitory volleys in A, B, and C was to shift the input–firing index curves slightly to the right (h to i). The action of inhibitory volleys may not only shift the input–firing index curve to the right but may also decrease its slope (E). In terms of transmitter potentiality this means that inhibition may cause a motoneuron with a given input–transmitter potentiality relation to acquire a relation similar to that of a less powerfully driven unit.

The effect of fixed inhibitory volleys on the input–firing index relation (Fig. 11) throws light on the change produced in the population input-response curve by standard inhibition (Fig. 3). If some motoneurons in the population have the limiting input–firing index relation, inhibitory volleys will cause little increase in the input level required for entry of these neurons into the zone of intermediate firing (A, B, and C). In less powerfully driven motoneurons inhibition will cause a decrease in slope and a shift to the right of the input–firing index relation (D, E, and F). Thus the population input-response curve will exhibit little change in the minimal input level required for discharge but its slope and maximal amplitude will be reduced. However, if in a particular pool response no motoneurons are powerfully driven, standard inhibitory volleys can cause a significant increase in the minimal input level that initiates discharge. Such a reflex would have to be so small that no significant number of motoneurons are at the limiting input–firing index relation. It is noteworthy that in all the individual motoneurons examined, maximal volleys in the antagonist nerve have caused inhibition of response to homonymous volleys of appropriate size.

The input–firing index relations shown in Fig. 11 also indicate that the degree of convergence of homonymous monosynaptic excitatory fibers on an individual motoneuron is considerable. In the three circumstances examined (f, h, and i), many of the units show evidence of monosynaptic excitatory effects from almost the entire range of homonymous input. While the number of homonymous monosynaptic excitatory fibers which converge on a particular neuron cannot be stated, that number must be large.

The Interaction of Graded Homonymous and Heteronymous Volleys.—It is clear from the foregoing considerations that heteronymous volleys delivered in conjunction with graded homonymous volleys can cause large increases in the degree of transmitter potentiality developed. Yet heteronymous volleys in isolation produce very little transmitter potentiality as is evidenced by the fact that such volleys normally fail to secure discharge. However, heteronymous volleys can initiate discharge in some circumstances, as during post-tetanic potentiation, suggesting that homonymous and heteronymous volleys have a qualitatively similar action (20). The question remains as to whether potentialities for transmission and for facilitation are dependent on the same factors. The following observations have been made on the effects of interacting homonymous and heteronymous volleys in an attempt to define the factors responsible for transmitter and facilitator potentialities.

A number of individual medial gastrocnemius motoneurons have been examined for response to graded volleys initiated synchronously in the homonymous (G.M.) and heteronymous (G.L.S.) nerves. Isometric diagrams have been constructed to display the relations between firing index and the two independent variables. Several examples will be presented, these being representative of the types of behavior that have been observed.

Fig. 12 illustrates the response of a medial gastrocnemius motoneuron with low intermediate firing index, 28, to maximal homonymous volleys in isolation (G.M. = 100, G.L.S. = 0). Maximal heteronymous volleys alone elicited no discharge (G.M. = 0, G.L.S. = 100). In this unit small homonymous volleys in synchrony with small heteronymous volleys were highly effective in producing discharge. Thus with homonymous input fixed at 30 per cent of maximum,
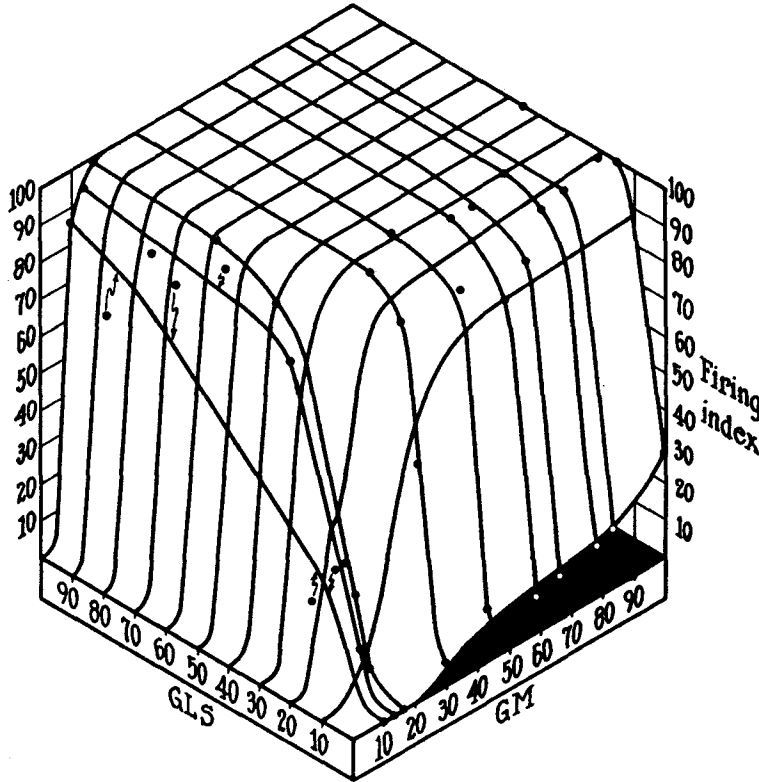

FIG. 12. Isometric diagram of response of a medial gastrocnemius motoneuron to graded volleys in the homonymous (G.M.) and heteronymous (G.L.S.) nerves.

incrementing heteronymous volleys caused a rapid rise in firing index so that synchronous volleys 30 per cent of maximal in both nerves caused a firing index of 100 (G.M. = 30, G.L.S. = 30).

Examination of the contour of the isometric diagram permits comparison of homonymous and heteronymous volleys with respect to their ability to increase firing index. In actual experiment, homonymous input was fixed at selected levels and at each level the firing index measured as heteronymous volleys were varied in size. The experimental curves express the relation between size

of heteronymous volleys and firing index at selected levels of homonymous input. An additional series of curves has been constructed to intersect the experimental curves at selected levels of heteronymous input; these indicate the homonymous input–firing index relation that would be expected with heteronymous input fixed at successive levels by increments 10 per cent of maximum. The more rapid the rise of the curve at a fixed level of heteronymous input the greater is the increment in transmitter potentiality per unit of homonymous input.

Returning now to Fig. 12 one may note that the maximal rise of firing index to incrementing heteronymous volleys at some fixed level of homonymous input (G.M. = 82 for example) is nearly as great as the maximal rate of rise observed for incrementing homonymous volleys at some fixed level of heteronymous input. This indicates that in some circumstances heteronymous fibers are as effective as homonymous fibers in increasing discharge. If transmitter and facilitator potentialities were due to identical factors such an effect would not be expected since heteronymous volleys in isolation have extremely low potentiality for producing discharge. It appears then that the increase in discharge produced by heteronymous volleys has an unique dependence on activity in homonymous fibers.

Examination of Fig. 12 indicates in addition that the degree of convergence of homonymous and heteronymous fibers on this motoneuron must have been considerable. It will be noted that at appropriate levels of heteronymous input the full range of homonymous input was effective in raising the firing index. Thus incrementing homonymous volleys in the range 20 to 100 per cent of maximum (G.M. = 20 to 100) increased discharge in the absence of heteronymous input (G.L.S. = 0) while effectiveness of homonymous input in the range zero to 20 per cent of maximum (G.M. = 0 to 20) was evidenced with heteronymous input fixed at levels 10 or 100 per cent of maximum (G.L.S. = 10 or 100). The effectiveness of heteronymous input throughout the range zero to maximum (G.L.S. = 0 to 100) may be seen with homonymous input fixed at 10 per cent of maximum (G.M. = 10). It is apparent that many afferent fibers in both homonymous and heteronymous nerves must have innervated this motoneuron.

Fig. 13 illustrates the response of a motoneuron which had a high intermediate firing index, 82, to maximal homonymous volleys in isolation (G.M. = 100, G.L.S. = 0). As in the previous unit, heteronymous volleys had strong facilitator action. With homonymous volleys fixed at 29 per cent of maximum, addition of heteronymous volleys 8 per cent of maximum (G.M. = 29, G.L.S. = 8) raised the firing index from 8 to 90 which is a higher level than that produced by addition of the remaining 71 per cent of homonymous input alone (G.M. = 100, G.L.S. = 0). The maximal rate of rise of firing index is nearly as great for increments in heteronymous volleys as in homonymous volleys. This

again indicates that heteronymous fibers in appropriate circumstances were equally as effective as homonymous fibers in increasing discharge although maximal heteronymous volleys alone evoked no discharge (G.M. = 0, G.L.S. = 100). The diagram of Fig. 13 indicates that the number of both homonymous and heteronymous fibers that made monosynaptic excitatory connections with
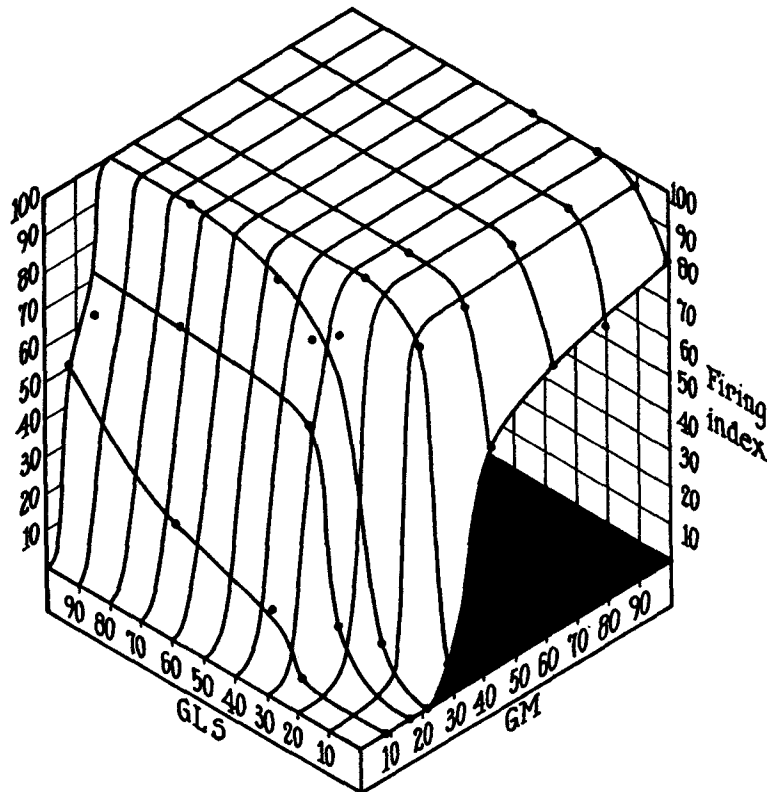

Fig. 13. Isometric diagram of response of a medial gastrocnemius motoneuron to graded volleys in the homonymous (G.M.) and heteronymous (G.L.S.) nerves.

this unit must have been large for, in appropriate combinations, increments in volleys from both sources produced increments in discharge throughout the range of volley size.

It has been shown that the sigmoid relation between firing index and transmitter potentiality results from the nature of excitability fluctuation. In consequence the curve relating homonymous input and firing index is sigmoid (Fig. 7). Reference to Figs. 12 and 13 reveals the fact that the curve relating heteronymous input to firing index is likewise sigmoid when this curve extends

throughout the full range of the intermediate firing zone. By way of contrast it will be recalled that the relation between degree of facilitation and size of heteronymous volleys in a motoneuron pool subject to maximal test volleys (Fig. 4) is nearly linear; the influence of excitability fluctuation in this case is eliminated by the use of mean response amplitude (9).
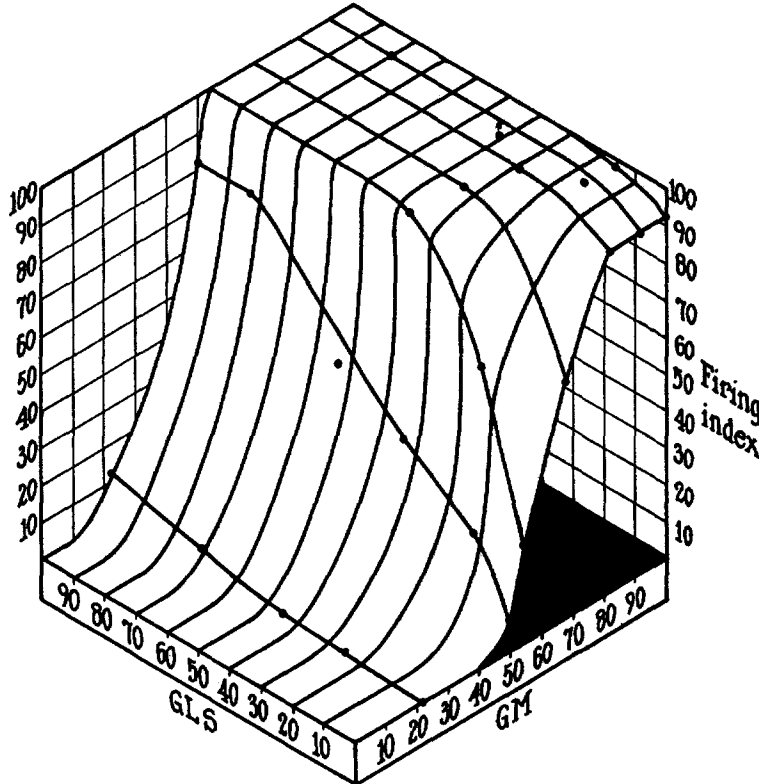

Fig. 14. Isometric diagram of response of a medial gastrocnemius motoneuron to graded volleys in the homonymous (G.M.) and heteronymous (G.L.S.) nerves.

The motoneurons of Figs. 14 and 15 had high intermediate firing indices to maximal homonymous volleys in isolation but were less powerfully facilitated by heteronymous volleys than the previous two units. The rise of firing index with increments in heteronymous input, at any fixed size of homonymous input, is considerably less than that exhibited in similar circumstances by the units of Figs. 12 and 13. However, the potency of facilitatory connections was considerable as evidenced, in the unit of Fig. 15, by the increase in firing index obtained by adding graded heteronymous volleys to homonymous volleys fixed

at the 38 per cent of maximum level (G.M. = 38, G.L.S. = 0 to 100). In this circumstance addition of 40 per cent of maximal heteronymous volleys (G.M. = 38, G.L.S. = 40) raised the firing index to approximately the same degree as increase in homonymous volleys alone to maximum (G.M. = 100, G.L.S. = 0). As in the units previously described, the considerable degree of convergence
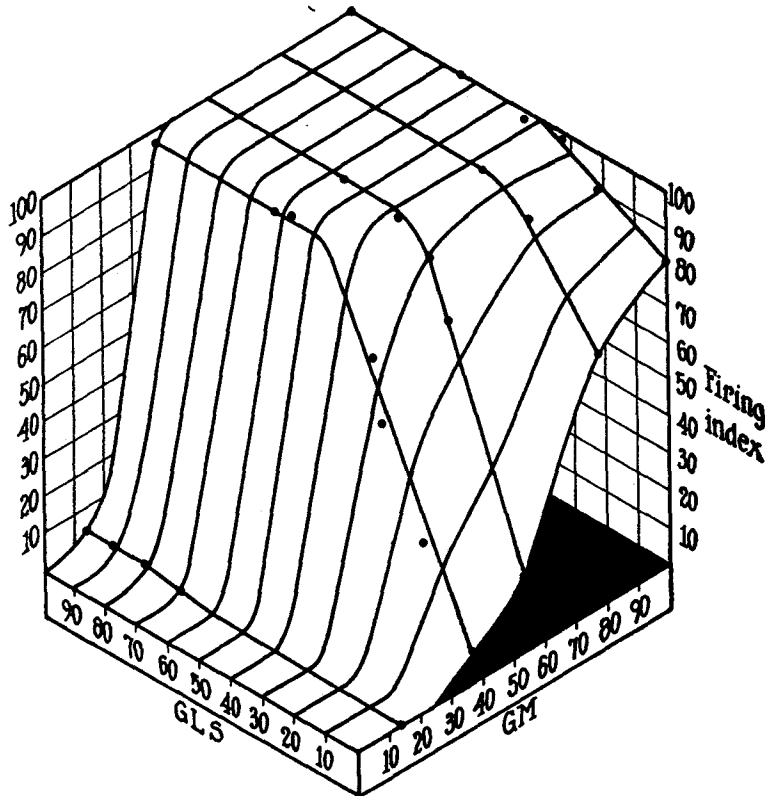

FIG. 15. Isometric diagram of response of a medial gastrocnemius motoneuron to graded volleys in the homonymous (G.M.) and heteronymous (G.L.S.) nerves.

from homonymous and heteronymous sources may be seen by inspection of the isometric diagrams.

The response of a unit in the firing index 100 category to maximal homonymous input is displayed in Fig. 16. This motoneuron was less powerfully facilitated by heteronymous volleys than any of the preceding four units, as evidenced by the fact that the rate of rise of firing index to incrementing heteronymous volleys at any fixed level of homonymous input was least in this unit. The graded rise in firing index to heteronymous volleys of increasing size

is especially well seen in the diagram of Fig. 16 at homonymous input levels 28, 43, and 58 per cent of maximum.

The unit of Fig. 17 was a motoneuron only subliminally excited by homonymous volleys in isolation. With synchronously initiated maximal volleys in both homonymous and heteronymous nerves the firing index reached 60.
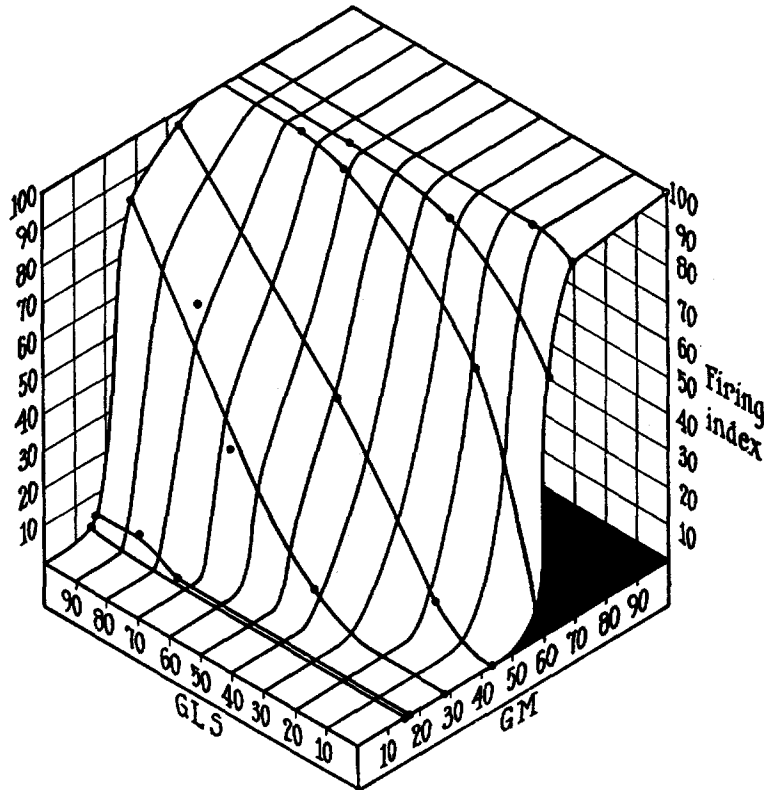

FIG. 16. Isometric diagram of response of a medial gastrocnemius motoneuron to graded volleys in the homonymous (G.M.) and heteronymous (G.L.S.) nerves.

Although homonymous volleys alone failed to secure discharge, this unit must have received excitatory connections from a large number of homonymous fibers for increase in size of volleys from this source over a considerable range caused a graded increase in firing index when delivered with heteronymous volleys of appropriate size. In a similar manner, graded heteronymous volleys may be seen to have increased discharge when delivered with homonymous volleys of adequate size.

Consideration of the homonymous input–firing index curves in the preceding

diagrams indicates that their slope is changed by addition of fixed heteronymous input. Since the rate of rise of firing index to incrementing homonymous input reflects the development of transmitter potentiality it is apparent that the effect of heteronymous volleys is not simply to add a fixed increment of transmitter potentiality for, were this so, the homonymous input–firing index curve should
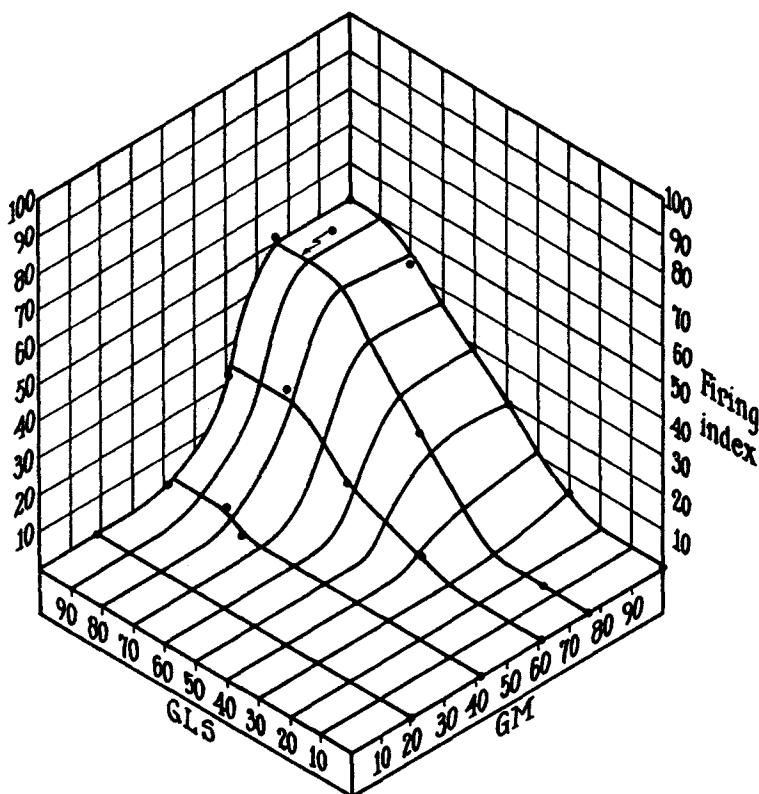

FIG. 17. Isometric diagram of response of a medial gastrocnemius motoneuron to graded volleys in the homonymous (G.M.) and heteronymous (G.L.S.) nerves.

be merely shifted in its position and not changed in slope. The observed change in slope would not be expected if transmitter potentiality depended on number of active excitatory synaptic knobs regardless of location since, in that case heteronymous volleys of fixed size would add a certain number of active knobs resulting in a constant increment of transmitter potentiality, upon which that developed by homonymous input would be superimposed.

Medial gastrocnemius motoneurons do not discharge to volleys in the heteronymous nerve in unusual circumstances. However, during cooling and in the period of post-tetanic potentiation heteronymous volleys may secure discharge

in some motoneurons (22). Most of the motoneurons examined for response to interacting homonymous and heteronymous volleys were also tested for their response to maximal volleys in the heteronymous nerve following tetanization of the latter at a frequency of 500/sec. for 10 seconds. While the number of units examined has been small, there appears to be a definite correlation between occurrence of post-tetanic cross-firing and degree of facilitation produced by heteronymous volleys in the resting state. Thus, one of the most powerfully facilitated units, that of Fig. 12, discharged 14 times to heteronymous volleys alone repeated every 2 seconds after the period of tetanization. On the other hand, the less powerfully facilitated units of Figs. 14, 15, and 16 failed to discharge to heteronymous volleys in the post-tetanic period. It is of interest that the unit of Fig. 17, although subliminally excited by homonymous volleys, discharged 4 times to heteronymous volleys post-tetanically, an indication of its powerful heteronymous connections.

Lloyd and McIntyre (22), in an analysis of response of a synthetic pool, found that most of the motoneurons showing post-tetanic discharge to heteronymous volleys were those with intermediate firing indices to maximal homonymous volleys. Moreover, within the intermediate firing zone some motoneurons exhibited such cross-firing while others did not. There was no direct correlation between firing index to homonymous volleys and ability to respond to post-tetanically potentiated heteronymous volleys indicating that motoneuron responsivity is not the principal determinant of both types of response. The present limited series of observations on interacting graded homonymous and heteronymous volleys are in harmony with their results for the most powerfully facilitated motoneurons were in the intermediate firing index category of homonymous response (Figs. 12 and 13). Also, units with the same firing index to maximal homonymous volleys were found to differ greatly in the potency of their heteronymous connections (compare Fig. 13 with Figs. 14 and 15).

In some motoneurons heteronymous volleys, added to activity in homonymous fibers, are found to be as effective in increasing discharge as are homonymous volleys. This means that a fraction of heteronymous input may produce an increment in net transmitter potentiality as great as the increment produced by a like fraction of homonymous input. Yet heteronymous volleys in isolation have extremely low transmitter potentiality as compared with homonymous volleys (20, 22). The capacity to develop transmitter potentiality thus differs from the capacity to increase transmitter potentiality.

The number of active excitatory synaptic knobs from homonymous sources must be greater than the number from heteronymous sources but difference in number alone is not a sufficient explanation for the difference between homonymous and heteronymous effects. There is no reason to suppose a qualitative difference in action of synaptic knobs derived from the two sources (20). The other possible variant concerns the spatial orientation of synaptic knobs from

the two sources. It is suggested that active synaptic knobs from homonymous fibers develop transmitter potentiality dependent on number and degree of aggregation, and that active excitatory synaptic knobs from heteronymous fibers are so dispersed as to develop very little transmitter potentiality in isolation but in the presence of activity in significant assemblages of homonymous synaptic knobs can cause large increments in transmitter potentiality: The greater the number of active homonymous knobs the greater would be the chances for dispersed heteronymous knobs to increase transmitter potentiality. The less aggregated state of synaptic knobs from the heteronymous source would enable activity in such endings to be highly effective in increasing discharge for such a distribution implies that heteronymous knobs would be interspersed among aggregations of knobs from homonymous fibers. Facilitator potentiality would then be chiefly dependent on number of active knobs.

DISCUSSION

The earlier investigations of spatial summation by Lorente de Nó (23, 24) and by Lloyd (13) have been mentioned. More recently Rall (27) has analyzed the relation between graded muscle nerve volleys and monosynaptic reflex response of spinal motoneurons and has proposed a statistical theory (28, see also 31) which predicts input-response curves in agreement with his results. This formulation was found to provide an equally sufficient explanation, whether number of active excitatory knobs irrespective of location or number of knobs in a defined zone were assumed to be the significant determinant of discharge. Rall preferred the former alternative on the basis of its simplicity but suggested that additional evidence would be required to distinguish between the two possibilities. The present study represents a subsequent attempt to define the requirements for spatial summation.

Volleys in monosynaptic excitatory afferent fibers evoke discharge in a motoneuron pool dependent on the degree of transmitter potentiality developed among its members. The relation between input and pool response indicates the manner in which mean pool transmitter potentiality grows with incrementing input. Alteration of this relation by change in motoneuron responsivity poses certain requirements that must be met by any theoretical formulation of the input-response relation but the population response fails to define the manner in which transmitter potentiality grows with incrementing input in the individual motoneuron. The latter, which provides more critical information as to the requirements for spatial summation, must be determined from the input-response relations in individual motoneurons. The input–firing index curves and, in turn, the derived input–transmitter potentiality curves of a representative group of liminally excited motoneurons form a characteristic series. The growth of transmitter potentiality, in the measurable range, is finely graded by incrementing input indicating that transmitter potentiality

has considerable dependence on number of excitatory synaptic knobs. This finding would not be expected if transmitter potentiality depended upon total or nearly total activation of a discrete zone of the synaptic scale. If the latter were the case one would expect transmitter potentiality to rise abruptly with incrementing input when such a zone was fully activated; rapid rise in transmitter potentiality would not be confined to a characteristic range of input as has been observed but might occur at other levels of input when zonal requirements were satisfied. The change in individual input–firing index curves brought about by altering responsivity is limited by a minimal input–firing index relation which signifies that there is a minimal anatomical requirement for discharge.

If number of active excitatory synaptic knobs, irrespective of location, determined discharge the action of each knob would be independent. This being the case increases in transmitter potentiality in the individual motoneuron, produced by different increments of input, would be expected to add in a linear manner. It has been noted that the rate of rise of firing index per unit of homonymous input may be increased by addition of standard volleys in the heteronymous nerve. Further, heteronymous volleys which, in isolation, have feeble transmitter potentiality may, in conjunction with suitable homonymous activity, produce increments in transmitter potentiality per unit input as great as that exhibited by homonymous volleys. This means that synaptic knobs activated by heteronymous input have little capacity to develop transmitter potentiality but considerable capacity to increase transmitter potentiality. These effects could not result if transmitter potentiality resulted from a simple summation of independent knob actions. There must be an interaction between excitatory synaptic knobs. This suggests that spatial orientation of active knobs is an important variable, degree of mutual interaction being a function of the distance between active knobs.

It has been demonstrated that transmitter potentiality has a rapid temporal decay, declining appreciably in a few tenths of a millisecond. No component of the post-synaptic potential, recorded intracellularly or in ventral root, exhibits a time course similar to that of transmitter potentiality. From this fact it may be concluded that transmitter potentiality does not result from a generalized reaction. Indeed, the rapid decay of transmitter potentiality serves to exclude the post-synaptic potential as the essential step in the normal production of discharge. Transmitter potentiality appears to have rapid temporal decay and significant spatial decrement in contrast to residual facilitation which decays to $1/e$ in 4 msec (16) and which must be generalized over the motoneuron soma.

The present results suggests that the following factors are concerned with the development of transmitter potentiality to incrementing input:

1. Degree of transmitter potentiality developed for a particular motoneuron

is a function of the number of active excitatory knobs, the intensity of action per knob, and the degree to which active knobs are aggregated. The requirement for discharge will be met at a given level of responsivity when an assemblage of knobs is active, sufficient in number, in degree of aggregation, and in intensity of action.

2. The limiting input–firing index relation indicates that there is a minimal anatomical requirement for discharge. It appears that a considerable number of monosynaptic excitatory knobs must be active in order that a significant assemblage may develop.

3. Transmitter potentiality has a rapid temporal decay and appreciable spatial decrement. Effectiveness of an assemblage of aggregated excitatory synaptic knobs will be greatest when activated synchronously. Activity in such an assemblage will also lead to an action which is generalized over the cell body, has a comparatively slow decay, and is associated with residual facilitation. If a given assemblage of excitatory synaptic knobs is active, addition of synchronous activity in other excitatory knobs will produce an increment in transmitter potentiality by addition of numbers and by increase in degree of aggregation. In this manner addition of activity in knobs from the heteronymous source to activity in knobs from homonymous fibers may cause a large increase in transmitter potentiality although the heteronymous knobs, fewer in number and more dispersed, have feeble transmitter potentiality in isolation. The effect of change in intensity of action per knob is evident during posttetanic potentiation when the feeble and dispersed heteronymous knobs may secure discharge in some motoneurons (22). The fact that heteronymous volleys can produce discharge suggests that total activation of a discrete zone of the synaptic scale is not needed for initiation of discharge since it appears unlikely that heteronymous knobs are distributed in a dense zone. The degree of transmitter potentiality produced by a given assemblage of active excitatory knobs may also be changed by alteration of body temperature. Cooling reduces, in a manner not yet known, the degree of aggregation of a given number of active knobs required for discharge.

The above statement of the requirements for spatial summation may be contrasted with other views. Lorente de Nó's (23, 24) conclusions, based on a study of oculomotor neurons, have generaly been taken to indicate that total or near total activation of a discrete zone of the synaptic scale is required for discharge. He considered the action produced by each synaptic knob to have a very sharp spatial decrement and rapid temporal decay. On the other hand a distinctly different view has been suggested, namely that transmitter action is essentially without spatial decrement and that motoneuron discharge is dependent on number of active excitatory knobs irrespective of location. Thus, Brock, Coombs, and Eccles (1) have recently concluded that each active excitatory knob independently exerts a depolarizing action on the motoneuron soma,

the individual effects summing to give a generalized post-synaptic potential which, in turn, initiates discharge. Rall (28) considered both the above possibilities. As distinguished from the above views, the present formulation suggests that transmitter action has an appreciable but gradual spatial decrement such that the effective interaction between two active excitatory knobs is finely graded with interknob distance. This is in contrast to the very sharp spatial decrement proposed by Lorente de Nó (23, 24). On the other hand the present view does consider that activation of a localized portion of the motoneuron soma is probably essential to the normal initiation of discharge, although total activation of a discrete zone is not necessary. The generalized post-synaptic potential is considered to play a supportive role in initiation of discharge. The significant spatial decrement and rapid temporal decay of transmitter potentiality, indicated by the present observations, are not compatible with the view that the post-synaptic potential causes motoneuron discharge in normal circumstances. The post-synaptic potential may be considered to result from a *net* depolarization of the motoneuron soma. It seems likely that a significant assemblage of active excitatory knobs can initiate an impulse without the necessary antecedent production of such a post-synaptic potential. Indeed, observations indicate that during a certain stage of anoxia monosynaptic reflex discharge of motoneurons can occur without the appearance of a post-synaptic potential of measurable size (18).

Among members of a motoneuron pool there is considerable difference in response to volleys in monosynaptic afferent excitatory fibers. The wide differences in transmitter potentiality developed by monosynaptic input for motoneurons of a pool might depend on several factors:

1. Individual motoneurons might differ in mean responsivity. There is, unfortunately, no direct means of measuring this variable. If motonueron responsivity were the major factor determining difference in motoneuron discharge, one would have to assume a very wide variation and one would expect those motoneurons most powerfully driven by homonymous input to exhibit the greatest response to heteronymous volleys. Observations reported herein as well as those of Lloyd and McIntyre (22) indicate that response to homonymous and to heteronymous input is not covariant. Difference in mean motoneuron responsivity may contribute to difference in response to individual motoneurons but does not appear to be a major determinant.

2. Motoneurons might vary widely in the number of excitatory synaptic knobs they receive from primary afferent fibers. The fact that motoneurons liminally excited but differing widely in response to graded homonymous input approach the same limiting input–firing index relation suggests that they acquire the minimal anatomical requirement at nearly the same level of input. The fact that most of the motoneurons of the pool will discharge to maximal afferent volleys in the nerve to the entire synergic unit during post-tetanic

potentiation indicates that the minimal anatomical requirements must be met in nearly all the members of the pool at this level of input. These considerations suggest that variation in number of monosynaptic excitatory knobs distributed from primary afferent fibers to individual motoneurons does not have very wide limits. Difference in number of monosynaptic excitatory knobs may be an important but not necessarily the most significant variable determining monosynaptic reflex response.

3. Motoneurons might differ in their response to input volleys because of difference in degree of aggregation of their monosynaptic excitatory knobs. The present results suggest that this is a highly significant variable. The most powerfully driven motoneurons are considered to be those which receive monosynaptic excitatory synaptic knobs in the most highly aggregated state. This interpretation is in accord with the finding that motoneurons weakly driven by homonymous input in isolation may be powerfully driven when convergent activity is added to homonymous volleys.

The present study affords an explanation for the powerful facilitatory effects of heteronymous volleys which in isolation have weak potentiality for initiation of discharge. It is suggested that monosynaptic excitatory knobs from heteronymous sources are more highly dispersed, as well as fewer in number, than those from homonymous sources. Activity in such dispersed heteronymous knobs can produce only feeble transmitter potentiality in isolation but in conjunction with activity in homonymous knobs can cause large increments in transmitter potentiality.

The author wishes to express his appreciation for an opportunity to read the doctoral thesis of W. Rall on "Spatial summation and monosynaptic input-output relations in the mammalian spinal cord" (University of New Zealand, 1953) before this material has become generally available (27, 28).

SUMMARY

Monosynaptic reflex response of spinal motoneurons to graded afferent volleys has been studied in natural populations and in a representative sample of individual motoneurons. By analysis of input-response relations certain of the requirements for initiation of reflex discharge have been defined.

Initation of motoneuron discharge by monosynaptic afferent excitatory volleys results from the development of transmitter potentiality among members of a pool. Transmitter potentiality is considered to have the following characteristics:

1. It is a function of the number of active excitatory synaptic knobs, the degree to which such knobs are aggregated on the motoneuron soma, and the intensity of action per knob.

2. It has an appreciable spatial decrement and rapid temporal decay.

3. While transmitter potentiality has considerable dependence on number of

active excitatory knobs, proximity of such knobs is an important variable. Total activation of a discrete zone does not appear to be necessary for initiation of discharge.

In addition to initiation of discharge, volleys in monosynaptic afferent excitatory fibers facilitate response otherwise engendered. Such facilitation depends upon the production of an increment in transmitter potentiality. Facilitator potentiality has the following characteristics:

1. It depends principally on number of active excitatory synaptic knobs and intensity of action per knob.

2. Facilitatory action may result from synchronous activity in knobs interspersed among aggregations of knobs otherwise activated, thus fulfilling spatial requirements for transmitter potentiality. Alternatively a residual facilitation may result from a generalized action.

3. Residual facilitation has a slow temporal decay in comparison with transmitter potentiality.

BIBLIOGRAPHY

1. Brock, L. G., Coombs, J. S., and Eccles, J. C., The nature of the excitatory and inhibitory processes in the spinal cord, *Proc. Roy. Soc. London, Series B*, 1952, **140**, 169.

2. Eccles, J. C., and Pritchard, J. J., The action potential of motoneurons, *J. Physiol.*, 1937, **89**, 43p.

3. Eccles, J. C., and Sherrington, C. S., Reflex summation in the ipsilateral spinal flexion reflex, *J. Physiol.*, 1930, **69**, 1.

4. Gasser, H. S., and Grundfest, H., Axon diameters in relation to the spike dimensions and the conduction velocity in mammalian A fibers, *Am. J. Physiol.*, 1939, **127**, 393.

5. Henneman, E., and Hertz, H., Alterations in the monosynaptic reflexes of flexor muscles with static stretch, *Fed. Proc.*, 1953, **12**, 65.

6. Hunt, C. C., The effect of stretch receptors from muscle on the discharge of motoneurons, *J. Physiol.*, 1952, **117**, 359.

7. Hunt, C. C., Diameter and function of afferent fibers from muscle (abstract), *XIX Internat. Physiol. Congr.*, Montreal, 1953, 485.

8. Hunt, C. C., Relation of function to diameter in afferent fibers of muscle nerves, *J. Gen. Physiol.*, 1954, **38**, 117.

9. Hunt, C. C., Temporal fluctuation in excitability of spinal motoneurons and its influence on monosynaptic reflex response, *J. Gen. Physiol.*, 1955, **38**, 801.

10. Hursh, J. B., Conduction velocity and diameter of nerve fibers, *Am. J. Physiol.*, 1939, **127**, 131.

11. Jefferson, A. A., and Schlapp, W., Some effects of repetitive stimulation of afferents on reflex conduction, *in* The Spinal Cord. A Ciba Foundation Symposium, (G. E. W. Wolstenholme, editor), Boston, Little, Brown and Company, 1953, 99.

12. Laporte, Y., and Lloyd, D. P. C., Nature and significance of the reflex connections

established by large afferent fibers of muscular origin, *Am. J. Physiol.*, 1952, **169**, 609.

13. Lloyd, D. P. C., Reflex action in relation to pattern and peripheral source of afferent stimulation, *J. Neurophysiol.*, 1943, **6**, 111.

14. Lloyd, D. P. C., Neuron patterns controlling transmission of ipsilateral hind limb reflexes in cat, *J. Neurophysiol.*, 1943, **6**, 293.

15. Lloyd, D. P. C., Conduction and synaptic transmission of reflex response to stretch in spinal cats, *J. Neurophysiol.*, 1943, **6**, 317.

16. Lloyd, D. P. C., Facilitation and inhibition of spinal motoneurons, *J. Neurophysiol.*, 1946, **9**, 421.

17. Lloyd, D. P. C., Integrative pattern of excitation and inhibition in two neuron reflex arcs, *J. Neurophysiol.*, 1946, **9**, 439.

18. Lloyd, D. P. C., data to be published.

19. Lloyd, D. P. C., and Chang, H.-T., Afferent fibers in muscle nerves, *J. Neurophysiol.*, 1948, **11**, 199.

20. Lloyd, D. P. C., Hunt, C. C., and McIntyre, A. K., Transmission in fractionated monosynaptic spinal reflex systems, *J. Gen. Physiol.*, 1955, **38**, 307.

21. Lloyd, D. P. C., and McIntyre, A. K., Monosynaptic reflex response of individual motoneurons, *J. Gen. Physiol.*, 1955, **38**, 771.

22. Lloyd, D. P. C., and McIntyre, A. K., Transmitter potentiality of homonymous and heteronymous monosynaptic reflex connections of individual motoneurons, *J. Gen. Physiol.*, 1955, **38**, 789.

23. Lorente de Nó, R., Synaptic stimulation of motoneurons as a local process, *J. Neurophysiol.*, 1938, **1**, 195.

24. Lorente de Nó, R., Transmission of impulses through cranial motor nuclei, *J. Neurophysiol.*, 1939, **2**, 402.

25. McCulloch, W. S., Lettvin, J. Y., Pitts, W. H., and Dell, P. C., An electrical hypothesis of central inhibition and facilitation, *Proc. Assn. Research Nerv. and Ment. Dis.*, 1952, **30**, 87.

26. Rall, W., Input-output relation of a monosynaptic reflex, *Proc. Univ. Otago Med. School*, 1951, **29**, 17.

27. Rall, W., Experimental monosynaptic input-output relation in the mammalian spinal cord, *J. Cell. and Comp. Physiol.*, in press.

28. Rall, W., A statistical theory of input-output relations, *J. Cell. and Comp. Physiol.*, in press.

29. Renshaw, B., Activity in the simplest spinal reflex pathways, *J. Neurophysiol.*, 1940, **3**, 373.

30. Rexed, B., and Therman, P. O., Calibre spectra of motor and sensory fibers to flexor and extensor muscles, *J. Neurophysiol.*, 1948, **11**, 133.

31. Rosenblueth, A., Wiener, N., Pitts, W., and Garcia Ramos, J., A statistical analysis of synaptic excitation, *J. Cell. and Comp. Physiol.*, 1949, **34**, 173.