SYNAPTIC STIMULATION OF MOTONEURONS
AS A LOCAL PROCESS

RAFAEL LORENTE DE Nó
From the Laboratories of The Rockefeller Institute for
Medical Research, New York*

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Three lines of evidence justify the assumption that the initiation of an impulse by synaptic stimulation of a motoneuron is attributable to a process reaching threshold within a discrete zone of the soma, underneath a group of active synaptic knobs. (1) The soma of the motoneuron is electrically excitable and has a number of properties in common with peripheral nerve fibers. It must conduct propagated disturbances according to the all-or-nothing law, while subliminal disturbances must remain localized (1935d), i.e., their spread away from the stimulated zone must be decremental, as is known to be the case in nerve (Hodgkin, 1937; Lorente de Nó, 1938a; cf. Eccles, 1936, 1937). (2) The period of effective summation of two volleys of impulses arriving in succession at different synapses is brief and summation does not essentially alter the time course of stimulation by the second volley (Lorente de Nó 1935b, c, 1938b, c; cf. Eccles, 1937). (3) Activation of a large number of synapses belonging to a certain set of pathways remains ineffective in the absence of bombardment of the motoneurons by impulses that have originated in internuncial pools of neurons (Lorente de Nó 1935c, p. 507; 1938c, Fig. 7, 13 to 20).

The last line of evidence is doubtless the more direct one, and further experiments conducted on this basis have yielded results that seem to be unequivocal. These findings are presented here, together with anatomical data on the distribution of synaptic junctions on the soma of the motoneurons, which are necessary for an understanding of the facts observed.

TECHNIQUE

The experiments have been carried out on the oculomotor preparation of the rabbit, as previously described (1935a, 1938c). The anatomical studies have been conducted on the brainstem and spinal cord of young cats, and the specimens were stained with silver chromate after Golgi.

RESULTS

Experimental. During mild ether narcosis, not deep enough to modify the corneal reflex in an appreciable manner, but still sufficient to reduce or abolish the tonic labyrinthine innervation of the eye muscles, single F shocks are ineffective in setting up a synaptic response of ocular motoneurons, even when they are so strong that they must stimulate every fiber of the posterior

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longitudinal bundle and adjacent pathways. Under these conditions it cannot be doubted that a large number of synapses on the motoneurons are activated. In point of fact, in the absence of ether, large numbers of motoneurons, and eventually the total population of the motor pool, do respond. The absence of response during narcosis cannot be attributed, at least not wholly, to a direct effect of ether on the motoneurons, for the following reasons: (1) When two F shocks are delivered in succession at the proper interval, the second sets up responses that include large numbers of motoneurons (Figs. 1 and 2). (2) During ether narcosis shocks delivered to the nuclei of the reticular substance in front of the oculomotor nuclei set up synaptic responses of the motoneurons (1938d, Fig. 2). (3) Single F shocks are rendered ineffective if the vestibular nuclei and the reticular formation in the medulla are destroyed. (1935c.) (4) Single F shocks also remain ineffective if previous stimulation has temporarily suppressed the normally existent background of subliminal bombardment of the motor nucleus by internuncial impulses (1936, 1938c, Fig. 7).

In conditions such as these the conclusion is unavoidable that the activation of any number of synapses in isolation is insufficient to stimulate the motoneurons to discharge impulses into their axons. A typical experiment is illustrated in Fig. 1. Two shocks in succession were delivered: F2 had twice the strength of F1; F1, in the absence of ether narcosis, was almost maximal for the F response to single shocks (Fig. 1, 11 and 15). During ether narcosis both F1 and F2 delivered in isolation were ineffective (Fig. 1, 1), but when F2 was preceded by F1, at intervals of 0.6 to 5 msec., it was followed by strong responses, which at certain intervals (Fig. 1, records 3 and 4) had almost half the size of the maximal motor twitch (record 10). A few minutes after discontinuing the narcosis, both F1 and F2 were individually able to set up strong responses (records 13 and 15), and when delivered in succession at intervals of 0.6 to 5 msec., the F2 response also was facilitated.

The facts described below are significant. At intervals of 0.41 msec. (record 1) or 0.48 msec. (record 11) the F2 shock was ineffective, either in setting up
any response at all, or in adding anything to the response produced by F1 (cf. records 11 and 15). However, at 0.6 msec. (record 3) or 0.65 msec. (record 12), the F2 response was large, indicating that between the intervals of 0.41-0.48 to 0.6-0.65 msec. a change in the excitability of the motoneurons had taken place. Since in both records 3 and 12 the F2 response had the minimal synaptic delay, there is no doubt that in both cases the F2 response had been produced by f fibers not reached by the F1 shock, for the absolutely refractory period of f fibers is 0.52 msec. (1935b) and the impulses set up in refractory fibers by the F2 shock arrive at the motor nucleus after some delay and set up responses after lengthened latencies (1935b; cf. Fig. 2, 11). The increase in excitability was so great that in the case of record 3 a limited number of f fibers set up a large response after the minimal synaptic delay (cf. 1938b, Fig. 1), while in record 1, a more powerful volley of f impulses was unable to fire any motoneuron. On the other hand, as the response in record 3 was caused by fibers not stimulated by F1, the change in excitability cannot be ascribed to repetitive stimulation of synapses.* Under conditions such as these the only plausible assumption is that internuncial neurons responded to the impulses set up by the F1 shock (cf. 1938b, Fig. 1), and that these internuncial impulses when added to the f2 impulses were capable of stimulating the motoneurons to discharge. Therefore, it must be concluded that the f1 impulses, although they were unable to fire motoneurons, could fire internuncials; and that the internuncial impulses alone were incapable of firing motoneurons.

The same facts are presented in a more striking manner by the records shown in Fig. 2, which belong to another experiment. For the series of records 12 to 17, since F2 had twice the strength of F1, the same conditions were created as were present in the case of Fig. 1, 1 to 9, and identical results were obtained. It will be noted that the response at the start of the period of facilitation (record 13) had the minimal synaptic delay, and consequently was caused by impulses conducted by the fibers not reached by F1.

* In the absence of ether narcosis, when the excitability of internuncial neurons is high, facilitation has been observed to begin 0.43-0.48 msec. after delivery of F1, i.e., at a moment when all the fibers stimulated by F1 were still absolutely refractory (1935c, 1938d).
In the case of records 1 to 11, the F2 shock preceded the smaller F1, so that repetitive stimulation of f fibers was necessary to produce the response. It will be seen that at the 1.4 msec. interval, when it was expected that a large number of f fibers had recovered the response (record 7) was practically identical with that shown in record 17. But at shorter intervals the responses in series 11 to 8 were consistently smaller than those at the same intervals in series 13 to 16. They were also delayed, which indicates that the f impulses had been conducted to the motor nucleus with subnormal speed. As a matter of fact, the series of records 11 to 7 is like that regularly obtained with the nerve-muscle preparation after delivery of two shocks at similar intervals. This result can be graphically expressed in a simple manner. The inter-nuncial discharge started by the conditioning shock created a subliminal stimulation of the motoneurons, so that the motoneurons responded to every f impulse that they received, with the result that the number of responding motoneurons became proportional to the number of recovered f fibers. Thus we have the following facts: (1) in record 1 a maximal f volley was unable to set up a response, while in records 13 and 11 small f volleys did produce responses; (2) in records 11 to 8 the numbers of responding motoneurons were roughly proportional to the numbers of the f impulses. The conclusion lies at hand that the strength of stimulation of motoneurons depends not only on the number of simultaneously activated synapses, but also on other conditions, one of which must be the topographical distribution of the synapses over the soma of the motoneuron.

Anatomical. It is at present generally recognized that the synapses on the motoneurons are formed by small thickenings of nerve fibers, located on the body and dendrites.*

The synaptic thickenings have been designated in the literature by various terms, which as a rule include the adjective "terminal" and often the noun "feet," for instance, the end or terminal feet of Held. Recently the designation,boutons terminaux, used in the French translation of Cajal's book (1909) has been widely employed. Given the extreme variety of forms of the thickenings of the axon (cf. Cajal, 1935), any term already used to designate another object is obviously improper, but this impropriety is unavoidable in the present case. Here the term "knob," suggested by Auerbach, is used. The adjective "terminal" also is improper, and even more, it is misleading; here use is made of the non-committal designation "synaptic."

The distribution of the synaptic knobs on the motoneurons and other multipolar neurons has in the past been studied in stains made with reduced silver according to the methods of Cajal, Bielschowsky, or their modifications which, however, yield only incomplete pictures and as a rule give the impression that each knob forms the ending of a fibril. It is exceptional to obtain pictures which demonstrate the fact that several knobs belong to a single fibril. But even in the best anatomical picture ever published (Cajal, 1909, Fig. 436), the distribution of all the knobs belonging to each of the fibrils cannot be observed. Ordinarily, the silver picture is incomplete on another count; it fails to show many of the knobs present on a cell, and even all the knobs on by far the largest number of cells in the gray matter. For example, many of the knobs reproduced in Figs. 3 and 4,

* In some cells, such as the Purkinje cells of the cerebellum, the synapses extend to the initial segment of the process called axon. The same seems to be true for some types of interneurals in the spinal cord. As the axon often starts from dendrites at considerable distances from the body of the cell, it is a matter of definition whether to place the origin of the axon at the cell body and to consider that its initial part may carry synapses, or to hold that the axon starts at the level of ending of the synaptic scale of the cell.
in point of fact all those establishing synaptic junctions on dendritic branches, are not stained or cannot be identified with the use of the ordinary silver stains.

The silver staining methods are, of course, capable of improvement, and the yield obtained is in relation to the experience and knowledge of the worker using them. No theoretical reason exists why it should be impossible eventually to stain any synaptic junction with reduced silver. But in the experience of the present author, the silver chromate method of Golgi is the only one known to be capable of staining simultaneously and completely the synaptic knobs and the fibers to which they belong. Satisfactory stains can be obtained of sections from the spinal cord of cats even 20 days old (older animals have not been used), and sections of the hypoglossus, facial and oculomotor nuclei of 30 day old cats have been so stained. As a rule, the synaptic knobs in the cord can be studied in the 10–15 day old cat. It is a rather general belief that the knobs develop late, but the fact underlying this belief is that reduced silver methods fail to yield satisfactory stains at those ages. However, when synaptic junctions from animals at ages at which completed myelinization begins to prevent obtaining a satisfactory Golgi stain are compared with synaptic junctions from young but fully developed or even adult animals, stained with the Golgi-Cox method, no essential difference is observed.

Cells A to E in Fig. 3 are motoneurons and cell I presumably is large internuncial. All of these cells are from the anterior horn of the lumbar cord of a 15–16 day old cat. In the original preparation they are stained a light orange color, while the fibers and knobs are stained black or a deep red. The number of stained fibers is small, and hence there is opportunity to follow each fiber and its branches throughout their entire trajectory within the 100μ thick section.

From the majority of fibers near the group of motoneurons A to E only relatively short segments are included in the section; but there is a fiber 6 which has a relatively large segment and is seen to establish synaptic junctions with two motoneurons, B and C. Fiber 6 which also establishes synaptic junctions with dendrites is a branch of a slightly thicker fiber, which in turn is a collateral of another fiber of the anterolateral tract. The parent fiber, therefore, establishes connections with many motoneurons and internuncials—a well-known fact which does not require emphasis.

It will be noted that during its trajectory along cell C, fiber 6 gives off a series of branches, each one of which ends on cell C by means of one, two, or even a cluster of knobs. The total number of knobs is large, but it is remarkable that these knobs are not located in the immediate proximity of one another, being separated by spaces which are no doubt occupied by knobs belonging to other fibers. Fiber 6 then divides into two branches, 6 a and 6 b. Fiber 6 a has five knobs on the body of cell B and finally ends in an intercellular space having several small synaptic thickenings (d) obviously in contact with dendrites. Fiber 6 b establishes contacts presumably only with dendrites.

The connections of fiber 2 with cell C also are clearly seen in the preparation. Branch 2 a obviously establishes contacts only with dendrites, for no cell body can be located in that narrow intercellular space; but branch 2 b, after having formed some knobs d in contact with dendrites, has a trajectory parallel to the border of cell body C, in the course of which it gives off tiny branches terminated by knobs on cell body C. It will be observed that the second fibril before reaching C has two tiny thickenings which must necessarily be in contact with dendrites.
Another type of fiber having contacts with cell C is fiber 5, which ends with a cluster of knobs at the base of a large dendrite. In the case of cell C the orientation of the section is such that fibers 2 and 6 happen to be in the equatorial plane of the cell body, so that the true relations of cell, fibers, and knobs can be observed. This relationship can still be seen, although but partially, in the case of fiber 7 and cell D. The knobs are in contact with the cell body,
but the fibrils connecting the knobs are not in contact with the cell. In the
spaces left by the connecting fibrils and the cell, other fibers and tiny dendritic
branches are located. In the case of cells A, B, and E, the stained synapses
are located on the upper surface of the cell and for this reason the distances
between the knobs formed by each fiber are better observed than in the
other cells.

All these pictures are, of course, incomplete because only a few fibers
were stained. The body of the cell is in fact always entirely covered with
synaptic knobs belonging to many fibers, which form a continuous syn-
aptic scale. It is obvious from the drawings of Fig. 3 that the synaptic
scale is a mosaic of interlacing clusters of knobs, so that the activation
of any discrete zone of the synaptic scale demands the conduction of im-
pulses by several fibers. On the other hand, one fiber may help activate
several different zones of the synaptic scale. An attempt to illustrate
these facts in graphic form is made in the drawing of cell I taken from
another section, and seven (10 to 16) of the stained fibers having
knobs on the upper surface of the cell. It will be noted that while fibers
10, 11, 13 and 14 have but a small number of knobs, fibers 15 and espe-
cially 12 and 16 have numerous
knobs distributed over a wide area of the cell body. The motoneurons and
large internuncials receive knobs from so many fibers that a complete picture
is obtained only when the preparation is so heavily stained that the course of
the individual fibers cannot be investigated in detail; but the small inter-
nuncials, especially in Cajal’s intermediate nucleus, receive knobs from a rela-
tively small number of fibrils, and therefore not infrequently one observes
pictures such as that shown in Fig. 4, in which an important part of the syn-
aptic scale is stained, while the cell itself has remained unstained.

Ten fibers (f1 to f10) having synaptic junctions with the upper surface of
the cell body or dendrite d, or both, have been reproduced in the drawing.
It can be seen that fibers f7 to f10 join dendrite d at some distance from the
cell body, presumably at its point of bifurcation; the fibers follow the den-
drite, having small thickenings in contact with it, and upon reaching the body form synaptic knobs in a manner similar to the fibers represented in Fig. 3. Fibers $f_3$, $f_5$, and $f_4$ reach the cell at the origin of dendrite $b$, and fibers $f_5$ and $f_6$ at the origin of dendrite $c$; a collateral of fiber $f_1$ reaches the cell body at a point which has no dendrite. It is obvious that if only a few more fibers had been stained, the synaptic scale would have been complete. Presumably it is almost complete at the level of dendrite $d$. While neighboring synaptic knobs are near each other, the distances being measured in fractions of $\mu$, they are still not in actual contact. Between them there seems to exist an amorphous substance, which by Golgi's method is at times stained a brownish red color. The nature of this substance is unknown; at present it cannot even be said whether or not it differs from the intercellular fluid. Neither can it be determined definitely whether it corresponds to the pericellular network described by Cajal, Golgi and others (cf. Cajal, 1935, Fig. 47). We can only state with certainty that between the synaptic knobs there is something which takes the Golgi stain in a manner different from the knobs themselves.

The drawing of Fig. 4 also illustrates another important fact. The continuous synaptic scale formed by interlacing clusters of knobs extends over the dendrites, but for only a short distance, usually up to the point where the dendrites branch out, and sometimes even for a shorter distance. This, with but few exceptions, is the general rule for all the types of neurons studied by the present author in the spinal cord, medulla, midbrain, thalamus, and cerebral cortex. The differences observed refer chiefly to the extension of the synaptic scale beyond the limits of the cell body, the size of the knobs, the homogeneity of the fibers contributing to the scale, etc.

For the synaptic junctions of dendrites the description must be made in general terms, for the reason that a detailed analysis has not yet been completed. On the other hand, not all dendrites are alike, and there are important differences between dendrites of cells of various types, and even between the dendrites of one cell.

A general description may be attempted in the following terms. In the intercellular spaces, i.e., in the spaces between cell bodies, there are besides glia cells, blood vessels, and myelinated fibers, two plexuses—one formed by dendritic branches, and the other by unmyelinated fibers which carry numerous synaptic thickenings. Fibers 17 and 18 of Fig. 3 are typical fibers of the plexus in the anterior horn of the cord, and so are the thin collateral branches of the other fibers in the same figure. Accurate representations of the dendritic plexuses have been given in other publications (cf. 1938c, Fig. 1; 1933, Fig. 12). Both dendritic and fibrillar plexuses have numerous synaptic junctions. It can be said that a dendrite while crossing the fibrillar plexus establishes junctions with fibrils of most varied origin, and that a fibril while crossing the dendritic plexus makes junctions with numerous dendrites of various types of cells. An attempt to illustrate graphically the relation between the two plexuses is made in the diagram of Fig. 5.

In parts of the nervous system where the arrangement of the dendritic
and fibrillar plexuses is systematic, as for example in the cerebral cortex and the tuberculum acusticum, it is possible to establish the existence of an orderly regional arrangement of synapses on the various dendrites (1933, 1934); but in the anterior horn of the spinal cord and the motor nuclei of the cranial nerves, an analysis of the dendritic connections is made difficult by the apparent lack of regularity.

There are motoneurons with dendrites distributed exclusively within the limits of the motor pool, and consequently the dendrites have connections with fibers also contributing to the synaptic scale on the cell bodies. But other motoneurons have dendrites extending far beyond the limits of the motor pool, and these have connections with the fibrillar plexus of internuncial pools. There are internuncial cells located in the immediate proximity of the motor pool, with no dendrite penetrating the latter; but there are also internuncial cells, sometimes with the bodies at considerable distances from the motor pool, which send dendrites into the pool, where they establish contacts with the fibrillar plexus of the latter. It is obvious that for the dendrites the same general rule holds as for the cell bodies, namely that activation of all the synapses in any discrete length of a dendrite demands the simultaneous conduction of impulses by several fibers of the plexus.

**DISCUSSION**

Threshold stimulation of a motoneuron does not require the activation of all the synaptic knobs, for the stimulation may have all gradations of intensity, from subliminal to supramaximal, *i.e.*, capable of firing a refractory neuron. On the other hand, the stimulation may be produced by the activation of different sets of knobs. This conclusion may be reached from a study of the experiments shown in Figs. 1 and 2, but more direct proof has been obtained in experiments in which it was shown that the same motoneurons may respond either to an *f* volley or to one of internuncial impulses (1938c, Figs. 3, 4 and 5). Increase in the number of knobs active constitutes an increase in the strength of stimulation; but the number of active knobs is not the only determining factor, because it has been shown that a small volley of *f* impulses may set up a response, while a large *f* volley may fail to do so. In view of the anatomical data, the most reasonable explanation seems to be that stimulation of the motoneuron demands the activation of all the synapses
on a discrete zone of the motoneuron, a condition that in the present experiments cannot be fulfilled by the activation of f or i fibers alone, undoubtedly because the synapses of each kind are scattered over the neuron. For example, let it be assumed that in Fig. 3, I, fibers 10, 11, 12, 13, 15 and 16 are f fibres. Stimulation of any number of these fibers will fail to activate all the synapses or any discrete zone of the soma; but if to the f volley an impulse through fiber 14 be added, all the synapses on the zone marked with s will be activated and the neuron will fire. Obviously, if fiber 14 conducts an impulse, there is no need for all the other fibers to be activated; threshold stimulation will be obtained if fibers 12 and 16 only are active. Obviously, other sets of fibers will cause the impulse to start at another zone of the soma.

This explanation, which is supported also by the first two lines of evidence mentioned in the introduction, applies as well in the case where the effective knobs are located on the cell body as in that of knobs located on dendritic branches. The latter, for example, is true of the olfactory bulb, because the olfactory fibers establish synapses with the dendritic bushels of the mitral cells. However, much work must still be done before the role of the various types of dendrites is fully understood.

The fact that liminal stimulation is obtained by the activation of a discrete zone of the soma does not indicate that the subliminal disturbance produced underneath any knob does not spread for a certain distance. The fact that summation takes place demonstrates conclusively that it does spread, at least to the next knob (Eccles and Sherrington, 1931). But the available evidence does not provide us with sufficient data to estimate the amount of the spread. The only permissible assumption is that effective summation demands the activation of knobs located at a distance from each other which is shorter than in the case of knobs belonging to one fiber.

An interesting conclusion to be drawn from this discussion is that the f and i synapses, which during ether narcosis are activated by shocks delivered to the posterior longitudinal bundle, are scattered over the soma of the motoneuron, so that an f or an i volley in isolation cannot result in the activation of all the synapses on any discrete zone of the motoneurons, but the f synapses must be so concentrated on some i cells that an f volley fires them. This conclusion is in agreement with known facts.

From the anatomy of the oculomotor preparation (cf. 1938c, Figs. 1 and 2) it follows that the F shock activates the posterior longitudinal bundle and adjacent pathways, which after giving collaterals in the oculomotor nuclei, end in the internuncial nuclei containing chiefly small neurons. The synaptic scales on these neurons are of the type illustrated in Fig. 4 and may be activated, at least in large zones, by the volley of f impulses. Thus it could be assumed beforehand that these neurons would respond to any f volley which is sufficiently large. Similarly, it could have been predicted that a volley of impulses conducted by the axons of these cells would in isolation be insufficient to fire motoneurons, for as these axons are thin, they can have but few contacts with any neuron. Neither is there difficulty in explaining why under
ether narcosis facilitation is of short duration. The large neurons of the internuncial nuclei having synaptic scales of heterogeneous origin are not activated by the $f$ impulses. Therefore, long closed chains of neurons do not come into play, and facilitation ceases as soon as the impulses have crossed all the synapses in the open delay paths, which in the case of the experiments recorded in Figs. 1 and 2 means four or five internuncial synapses. But what was rather astonishing was to find the $f$ volley in isolation incapable of firing motoneurons, for the powerful branches of the posterior longitudinal bundle form extensive arborizations in the motor nucleus. Attempts to analyze the distribution of the $f$ synapses in the oculomotor nucleus have as yet failed, for the anatomical conditions present are unfavorable for detailed study. But in the hypoglossus nucleus it has in fact been found that each one of the long bundles sending collaterals into this nucleus has a certain type of terminal fibers in the nucleus, and that the axons of the small internuncials located in the neighborhood of the motor pool again have their specific distributions. It is, therefore, likely that the $f$ synapses on the ocular motoneurons are systematically arranged on strategic zones of the neurons, so that only the summation of $f$ impulses with impulses carried by other pathways is possible. It will be remembered that if a shock is delivered to the internuncial nuclei in front of the oculomotor nucleus, so that $f$ and $i$ fibers are stimulated simultaneously, ether narcosis does not prevent some motoneurons from responding; and that if a background of internuncial activity is present, the $f$ volley may fire the entire population of the motor pool.

**Summary**

Although the threshold stimulation of motoneurons requires that several impulses be delivered simultaneously at their synapses, not all the fibers contributing to the synaptic scale need be active. Experimental results demonstrate that while the activation of a large number of synapses may remain ineffective, activation of a limited number of those synapses, effected simultaneously with the stimulation of other fibers, results in the setting up of a response. Furthermore, it is shown that effective stimulation of motoneurons may be produced by the activation of different groups of fibers.

Anatomical studies have revealed that the synaptic scale of the motoneurons consists of interlacing clusters of knobs belonging to a large number of fibers and that the synaptic terminals of each fiber are located at some distance from one another. The activation of all the synaptic knobs at any discrete zone of the soma demands that several fibers be conducting impulses. It is obvious that different groups of fibers will cause stimulation at different zones of the soma.

It is concluded, therefore, that the setting up of a new impulse by the motoneuron results from a localized process taking place underneath a dense group of active synaptic knobs. Sublimal responses must be propagated decrementally but the information available is insufficient to estimate the amount and rate of the spread.
REFERENCES


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