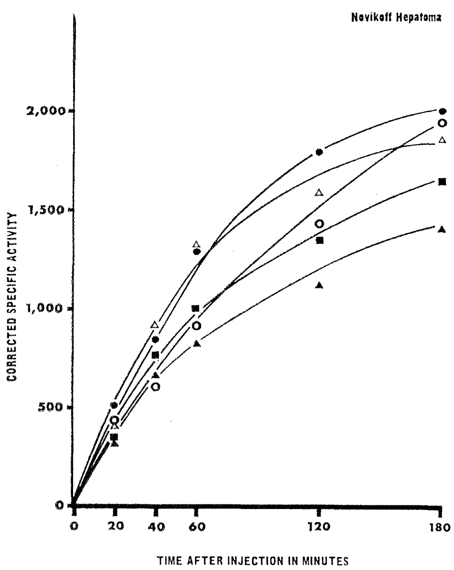

Fig. 3. The incorporation of uniformly labeled L-lysine-C^{14} into histones and nuclear acidic proteins in Novikoff hepatoma cells. The symbols are as indicated in Fig. 1.

In normal rat liver each of the four histone fractions is different in its incorporation of the L-lysine-C^{14}. The arginine-rich fraction 3 and the very lysine-rich fraction 1 incorporated the label most rapidly. The rapid increase in the activity of the fraction 3 histones followed by a decline within approximately 60 minutes after administration of the isotope suggests a high turnover of this fraction. The acidic nuclear proteins are more highly labeled than any of the histone fractions. The incorporation kinetics suggests that histones are biosynthesized more rapidly than the acidic nuclear proteins in liver.

In the regenerating-liver fractions, the incorporation of the isotope was more rapid with an approximately five-fold increase in the specific activity of the histone over that in rat liver. Fraction 3 and fraction 1 are again more active than the fractions 2*a* and 2*b*; however, the differences are much smaller. As in normal rat liver, a rise of activity of fraction 3 is followed by a slow decline, beginning about 1 hour after the administration of L-lysine-C^{14}. In regenerating rat liver as in normal liver, the label appeared faster in histones than in the acidic nuclear proteins. However, the initial differences were much smaller.

In Novikoff hepatoma cells, the incorporation of isotope in the histone fractions was very high (Fig. 3), about 12-fold higher than the values obtained for rat liver, and there is much less difference between fractions in the incorporation of the isotope. In contrast to normal and regenerating liver, there is little difference in the specific activities of the acidic nuclear proteins and of the histones in Novikoff hepatoma.

These data confirm reports that the biosynthesis of basic nuclear proteins increases with increasing rate of cell division (*9*). The different rates of incorporation of L-lysine-C^{14} into various histone fractions of rat liver indicate that the fractions are biosynthesized at different speeds. Dedifferentiation, such as in the first 48 hours of liver regeneration or in hepatoma, substantially lessened the differences in the incorporation of labeled lysine into the four main histone fractions. Rapidly dividing cells seem to have an increased demand for all the histone fractions. The balance between the biosynthesis of the acidic nuclear proteins and the histones becomes upset in favor of the histones. In Novikoff hepatoma, the histones were synthesized at about the same rate as the acidic nuclear proteins. The difference in the biosynthesis of the four main histone fractions, especially in rat liver, support the proposal that different quantities of histones and their structural variations may both be essential to the regulation of genetic activity.

The high rate of biosynthesis of the acidic nuclear proteins in well-differentiated tissues, such as rat liver, suggest that these proteins are closely related to the cellular functions and may be more important in the regulation of nuclear processes than the histones. According to Caspersson (*10*) the most marked change which occurs during prophase is a decrease of the amount of protein in the cell nucleus. The protein which remains is rich in arginine (*11*) and represents most of the basic proteins of the cell (*12*). Our data are compatible with such observations and indicate more specifically that these changes involve mainly the acidic nuclear proteins and all the histone fractions.

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23 September 1965

Parkinsonism: Electromyographic Studies of Monosynaptic Reflex

Abstract. *Electromyographic studies of the monosynaptic reflex in 70 Parkinsonian patients and 12 normal subjects show four types of abnormal facilitation and recovery curves in the Parkinsonian group, types that correspond to variations in the clinical syndrome. Cryosurgical lesions in the ventro-lateral and ventro-postero-lateral nuclei of the thalamus restore essentially normal curves.*

In searching for explanations of the basic neurophysiological events that lead to development of the various signs and symptoms in different neurological disorders, many investigators have concentrated on the alterations observed in the functioning of the most distal elements in the nervous system: namely the motor neuron and the muscle spindle (*1–3*) .

Apart from intrinsic disorders that may affect its function, the motor neuron is constantly exposed to influences from the higher centers that act upon its excitability, either increasing or decreasing its responsiveness or giving special character to the rapidity of its recovery following stimulation (*3, 4*). This superior control can be better studied during two critical periods of physiological activity: one, the brief period

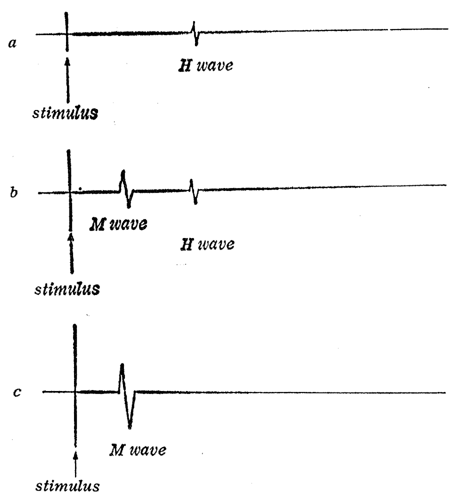

Fig. 1. Relation between intensity of the stimulus and the appearance of H and M waves.

immediately following neuronal stimulation; the other, the phase of recovery of excitability after discharge of the motor neuron.

It is well known that peripheral nerves (mixed) have both afferent and efferent fibers of different diameters (*5*). Fibers of large diameter have a lower threshold of stimulation than those of small diameter; thus, when the posterior tibial nerve is stimulated by current of low intensity, only fibers of large diameter (*I*a) are stimulated. This afferent fiber arising from the muscle spindle travels to the spinal cord to connect with motor neurons that upon discharging originate a response in the gastrocnemius muscle that can be recorded electromyographically; this is the H-wave response (Figs. 1 and 2).

When the stimulus used is of medium intensity, efferent fibers, in addition to afferent fibers, are stimulated and produce a direct muscle response called the M wave. The H wave is also obtained and follows the M wave in the electromyogram (Fig. 1*b*). If the stimulus is too strong, antidromic impulses in the efferent fibers interfere with production of the H wave and only an M wave is recorded (Fig. 1*c*) (*6*).

Eccles used intracellular electrodes to study membrane potentials in cats and showed that six different changes follow stimulation. Shortly after a stimulus there is a spike potential of less than 2-msec duration, followed by an after-negative potential for another 8 msec. After 10 msec there is a phase of hyperpolarization lasting almost 100 msec. Concurrently with these events other changes occur: (i) during the phase of spike potential an excitatory postsynaptic potential appears and lasts for 15 msec; (ii) 2 msec after the stimulation the excitability of the lower motor neuron is inhibited by impulses arising from the *I*b fibers, showing an inhibitory postsynaptic potential 15 msec after the stimulus and lasting some 60 msec (Fig. 2, *A* and *B*) (*7*). The algebraic summation of all these potentials is shown in Fig. 2*B* (after Lloyd).

The recovery curve of H responses, first described by Magladery and McDougal in 1950, indicates the excitability of the lower motor neuron by a second stimulation, given at various intervals after a first stimulation, of the afferent fibers of the posterior tibial nerve in the popliteal fossa. The potentiality of response is closely connected with the changes in membrane potentials, which are controlled by axon terminals of many descending tracts and fibers coming from the muscle receptors (*8*); these tracts include the pyramidal and extrapyramidal systems carrying impulses originated or elaborated in the thalamus, globus pallidus, nucleus, caudatus, putamen, cerebellum, labyrinth, cortex, and such.

When, instead of one single stimulus of adequate intensity to obtain H waves, two successive stimuli are given, there are variations in the H wave corresponding to the second stimulus (H_2) that depend upon the time interval between the stimuli. This relation between the two H waves is recorded on a percentage basis, taking as a unit index the amplitude of H_1 ($H_2/H_1 = x/100$). Two different moments in the physiological process of excitability and recovery were chosen: one was the 10-msec period following a first stimula-

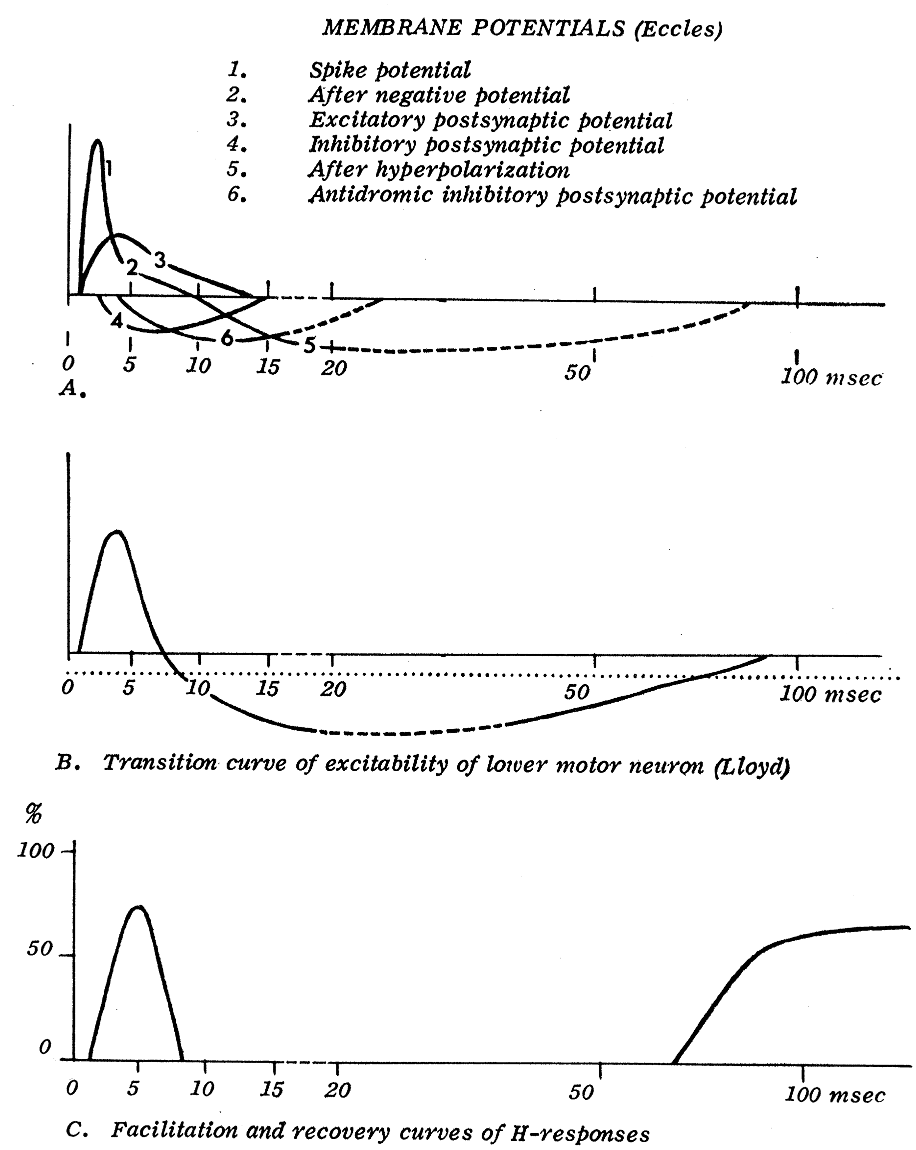

Fig. 2. Comparative graphs of membrane potentials, excitability of the lower motor neuron, and facilitation and recovery curves of H responses.

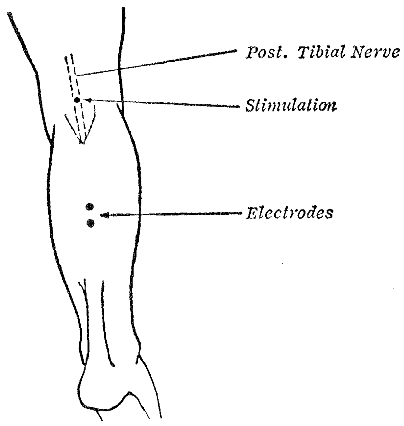

Fig. 3. Location of stimulating and recording electrodes in the lower extremity.

tion (yielding a "facilitation curve"); the second recording determined the shortest time necessary for recovery of excitability after a conditioning shock (yielding a "recovery curve"). The former had been studied by Ioku (*2*). For convenience and simplicity the two curves were plotted on the same graph (Fig. 2*C*).

We shall now attempt to correlate the work described with some of the clinical aspects of Parkinsonism and to provide an objective measurement of certain symptoms that are otherwise difficult to quantitate. This study is divided into two sections: The first deals with the results of comparative electromyographic examination of monosynaptic reflexes in Parkinsonian patients and in normal persons, and with classification of the Parkinsonian patients into four groups. The second section compares the preoperative electromyographic studies of the facilitation and recovery curves with the postoperative recordings from Parkinsonian patients who underwent cryogenic thalamic surgery; this technique enables classification of certain motor abnormalities of Parkinsonism and provides objective data after the placement of thalamic lesions in such patients.

Electromyographic studies were carried out on 70 Parkinsonian patients and 12 normal controls. Muscle action potentials were recorded by means of a pair of silver-disc electrodes, 0.5 cm in diameter, placed over the skin surface of the calf muscle. The tibial nerve was stimulated percutaneously in the popliteal fossa. The indifferent electrode was placed over the skin in the lower part of the thigh. Rectangular pulses lasting 0.5 to 0.6 msec were given to the posterior tibial nerve (Fig. 3):

1) Ten double stimulations were given separately. The interval between the conditioning shock and the second shock ranged from 1 to 10 msec in progressive steps of 1 msec. H_1 and H_2 waves were recorded.

2) Twenty-four double stimulations were given about 2 seconds apart, with the interval between the conditioning shock and the second shock increasing steadily from 20 to 270 msec. H_1 and H_2 waves were recorded.

3) The ratio H_2: H_1 was determined for each double stimulation and the results were plotted in a curve of percentage against time-interval between the conditioning shock and the second shock.

In the 12 normal subjects the maximum height of the facilitation curve of *H* responses was between 50 and 80 percent. The shortest interval in the recovery curve in which the H_2: H_1 ratio was greater than 10 percent was of 80 msec (Fig. 4). The Parkinsonian patients, however, yielded two types of response for each curve, combination of which was used to classify the patients into four groups. Those in group 1 showed a high facilitation curve and a fast recovery curve. Those in group 2 showed a low facilitation curve and a fast recovery curve. Patients in group 3 showed a high facilitation curve and slow recovery curve, while low facilitation and slow recovery curves were recorded for group 4 (Fig. 4).

From the clinical standpoint we made the following observations. In group 1 the outstanding symptoms were tremor or rigidity, or both—mild to severe. Walking difficulties when present directly resulted from the muscle rigidity. There was no clinical evidence of muscle wasting; in some patients exaggerated stretch reflexes suggested pyramidal components in their neurological disorders.

In group 2 also the main symptoms were tremor or rigidity, or both. Clinical evidence of muscle wasting could be detected as well as moderate degrees of muscle weakness. Walking difficulties, when present, were related to the degree of rigidity.

The patients in group 3 generally did not show severe rigidity, although most showed tremor—sometimes very pronounced. No clinical evidence of muscle wasting was noticed. However, walking difficulty was considerably out of proportion to the rigidity, with severe loss of balance and many instances of propulsion (also of retropulsion and lateropulsion). In addition to tremor and rigidity, group-4 patients showed clinical evidence of muscle wasting, balance disturbances, and a number of vegetative (autonomic) symptoms.

The second section of this study consisted in comparing facilitation and recovery curves in 70 Parkinsonian patients before and 4 to 10 days after cryothalamectomy—freezing lesions in

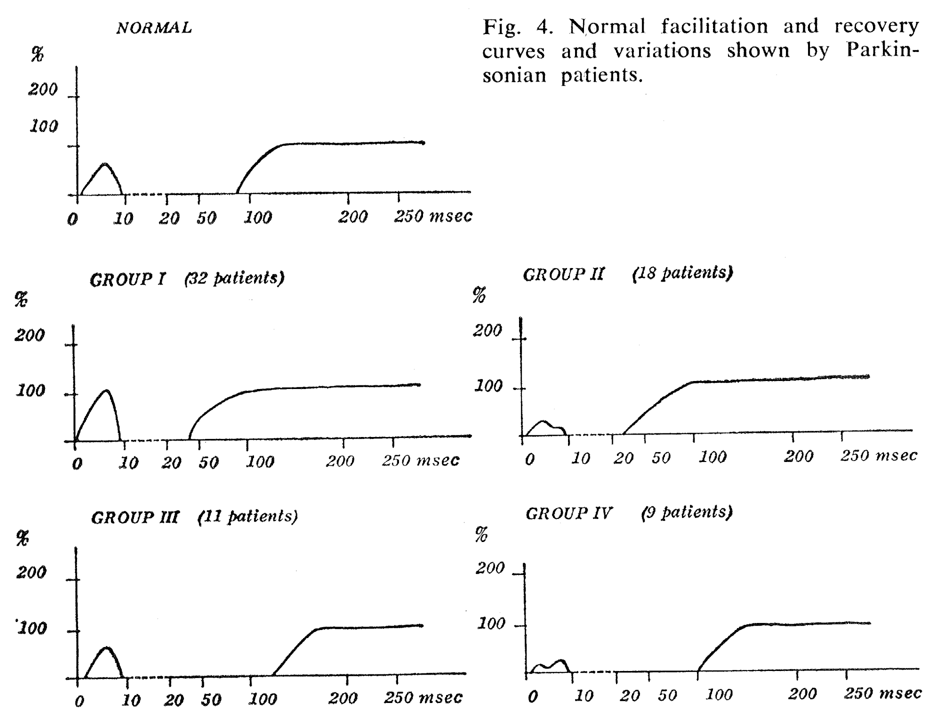

Fig. 4. Normal facilitation and recovery curves and variations shown by Parkinsonian patients.

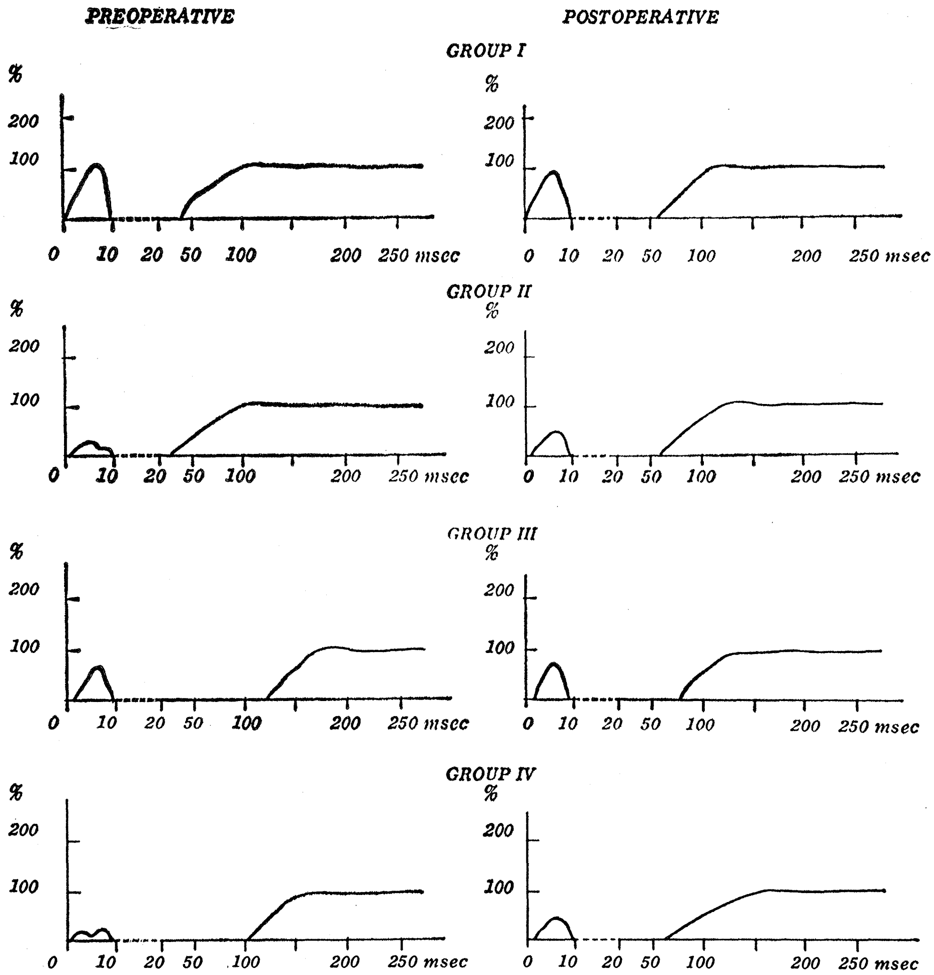

Fig. 5. Comparison of facilitation and recovery curves before and after cryothalamectomy.

the ventrolateral and ventro-posterolateral nuclei of the thalamus (Fig. 5). (Recordings of these curves during surgery constitute a separate study.) This comparison demonstrated that such lesions restored to normal, or near normal, both the facilitation and recovery curves of these patients. The degree of restoration of the monosynaptic reflex to normal correlates with the amelioration of the clinical motor signs of Parkinsonism.

When trying to correlate the electromyographic findings regarding the monosynaptic reflex with the clinical picture of a random group of Parkinsonian patients, we observed that severe rigidity usually accompanied fast recovery curves that suggested hyperexcitability of the lower motor neuron. Slow recovery curves were recorded in patients who showed severe balance problems.

The facilitation curve was low in patients with clinical evidence of muscle wasting (thenar eminence and interossei muscles of hands) and with autonomic disturbances. Facilitation curves were high in patients with severe tremor, some of whom demonstrated exaggerated tendon jerks and hyperactive stretch reflexes.

In summary, electromyographic studies of the monosynaptic reflex in 70 Parkinsonian patients and in 12 normal subjects indicated that the former are characterized by four types of abnormal facilitation and recovery curves that correspond with variations in the clinical syndrome. Cryosurgical lesions in the ventro-lateral and ventro-posterolateral nuclei of the thalamus restored essentially normal facilitation and recovery curves; this indicates that lesions of these thalamic nuclei, which relieve tremor and rigidity of Parkinsonism, also tend to restore normalcy of the monosynaptic reflex in such patients.

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9 September 1965

Pogonophora: Living Species Found off the Coast of Florida

Abstract. *A population of pogonophorans, consisting of species attributable to at least four genera and living at a depth of about 200 meters in an accessible locality off south Florida, has been discovered.*

Members of the phylum Pogonophora have been reported from the Western Atlantic only by Bayer (*1*), Wigley (*2*), and Southward (*3*). The last two reports dealt with material from depths of 366 and 567 meters on the continental slope south of Martha's Vineyard, Massachusetts; a deep, cold-water habitat characteristic of the animals. Bayer, however, reported specimens from relatively shallow water off the coast of Florida, a rather unusual site. Unfortunately, that material was scanty and consisted only of tubes, the worms themselves having disintegrated. Dredgings made off Miami Beach, Florida, during April 1965 revealed abundant living material of several species in muddy bottom at a depth of about 200 meters, with temperature about 8°C in the mud. Thus far, four genera have been recognized in collections made on several occasions since the initial discovery: *Oligobrachia, Nereilinum, Siboglinum*, and a new genus of the family Lamellisabellidae. Preliminary examination of the living specimens indicated that the species concerned are new.

The discovery of several species of pogonophorans, one of them quite large, in a readily accessible location a short distance from a marine laboratory provides a source of living material that is indeed unique. Initial attempts to main-