Clinica Europea

ATTUALITÀ DI MEDICINA

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ULTRASTRUCTURE AND FUNCTIONS OF SYNAPSES IN THE CENTRAL NERVOUS SYSTEM

EDIZIONI CLINICA EUROPEA
ROMA

Estratto dagli Atti dei Convegni Medici Europa dell'Istituto De Angeli:
« Il sistema nervoso centrale »: 29-30 Maggio 1970
Porto Cervo - Sardegna

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Ultrastructure and functions of synapses in the central nervous system *

The synapse is the single most important element of the nervous system. Its significance was first recognized by SHERRINGTON in 1897. He coined the term « synapse » and used the concept widely in his famous book on the « Integrative Functions of the Nervous System ». Decisive progress was made after world war II when more powerful tools became available for the direct electrophysiological study of single synapses (KATZ, 1965; ECCLES, 1964). Electronmicroscopists (PALAY, 1967; TAXI, 1965; DE ROBERTIS, 1964) soon discovered specializations at synaptic membranes and described the presence of a 200-300 Å gap between presynaptic and postsynaptic elements, thereby settling at long last the conflict between continuity and discontinuity theorists.

CHEMICAL VERSUS ELECTRICAL TRANSMISSION

One of the first major discoveries was that of chemical transmission. It came as a surprise to most physiologists who believed that excitation across the synaptic gap was mainly achieved by electrical currents. Fig. 1 shows the basic difference between the two types of synaptic transmission. It turned out that electrical coupling exists in specialized regions of the invertebrate nervous system (PAPPAS and BENNETT, 1966), but only rare instances of electrical transmission are known in higher forms. The chemical theory rests upon several solid lines of evidence. Furthermore, by systematic analysis of spontaneous and

* *Supported by Grant Nr. 3.133.69 of the Swiss National Foundation for Scientific Research.*
The collaboration of Prof. H. MOOR (Eidg. Technische Hochschule, Zürich), K. PFENNINGER, E. KAWANA, *and Miss.* C. SANDRI, *is gratefully acknowledged.*

stimulus-induced changes of the postsynaptic membrane potential it was shown by KATZ and his school that the chemical transmitter is released in a quantal fashion. About the same time, electronmicroscopists discovered the presence of small spheric vesicles of fairly regular size (500 Å diameter) in the presynaptic terminal and biochemical examination suggested that they represent packages for storage and release of the transmitter.

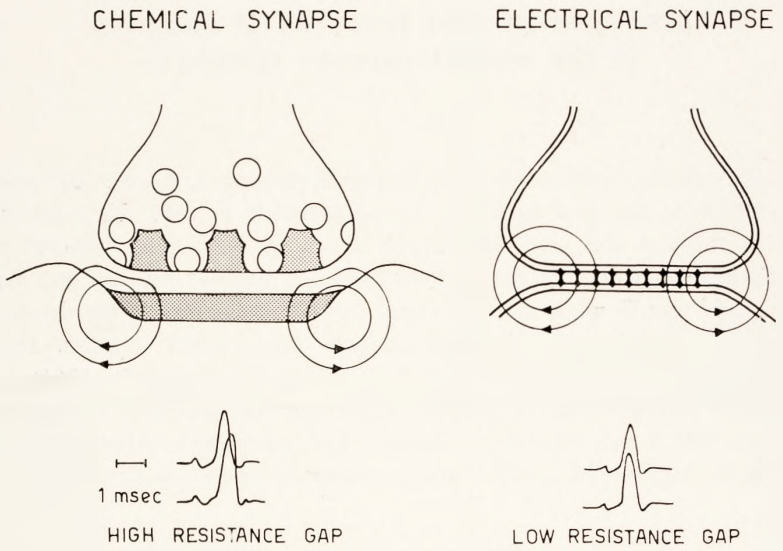


FIG. 1. - Comparison of chemical and electrical synapses. The upper diagrams are derived from electronmicroscopic research. The lower traces are nerve impulses recorded from the presynaptic fiber and the postsynaptic side. Note the delay at the chemical synapse between impulses. The intercellular space is considerably wider than in electrical junctions. Note also the strict symmetry of membrane structure and the absence of synaptic vesicles in the latter as compared with the asymmetry of the former

The problem of the chemical identification of the active substances is only partially solved today. However, neuropharmacological studies in vitro and in vivo (microiontophoretic application), in peripheral and central synapses, in vertebrates and in invertebrates have lead to the conclusion that biogenic amines and certain amino acids are suitable candidates because « they are there » and because their effect in situ corresponds to the effect of the physiological transmitters. What is still lacking is the evidence that effective amounts of these substances are liberated from nerve terminals under physiological conditions.

Morphological studies of synaptic vesicles have revealed that several types may be differentiated on the basis of size, shape and chemical affinity. These data are represented in Table 1. The correlation between vesicular morphology and biochemistry is still incomplete and may not be reviewed here. Unfortunately, cytochemically specific staining methods for the demonstration of transmitter substances in synaptic vesicles at the electronmicroscopic level are still painfully missing.

Table 1: Synaptic vesicles

Size (mean diameter	Form	Transmitter
500 Å	Spheric, clear ZIO*-positive	Acetylcholine, Glutamic acid (?)
400 Å	Spheric, ZIO*-positive (flattenable)	Inhibitory transmitter (?) Gamma-Aminobutyric acid (?), Glycine (?)
500 Å	Spheric, ZIO*-positive granulated	Catecholamines, Serotonin (?)
800-1500 Å	Spheric, dark core	? (Reserpine resistant)

* ZIO = Zinc iodide-Osmium tetroxide impregnation (Akert et al., 1968).

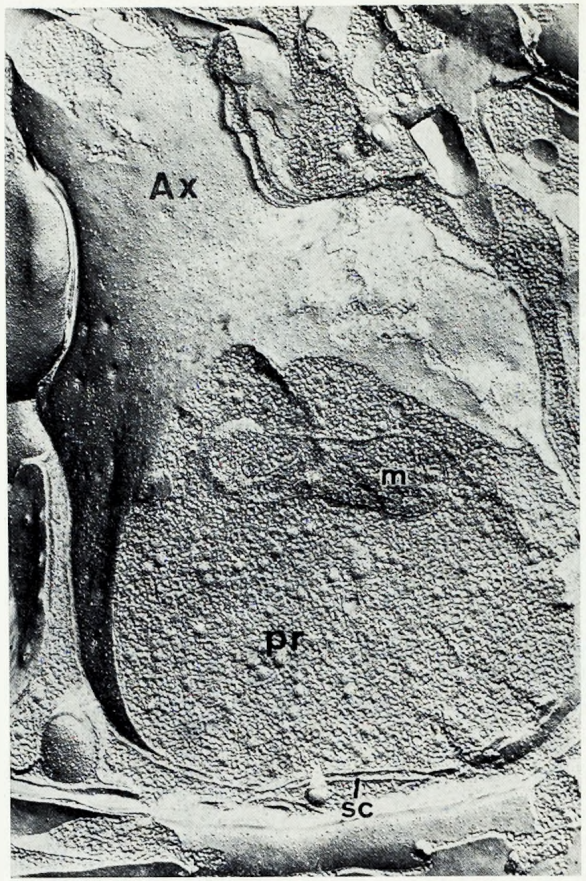
The main conclusions that can be drawn from the work on chemical transmission is a follows: 1. Chemical synapses are polarized junctions (uni-directional traffic). Consistent with this principle is the fine structural asymmetry of membrane complexes and the limitation of vesicles to the presynaptic side. In contrast, electrical junctions are bi-directional in function and symmetrical in structure (Fig. 1). 2. Chemical transmission offers more regulatory control of excitation at the expense of a measurable time lag which is minimal in electrical transmission (Fig. 1). 3. Although the origin of synaptic vesicles and their precise role in the mechanism of transmitter release is still incompletely understood, it seems clear that the neuron can no longer be considered exclusively as an electrical unit. It appears that the *secretory aspects* are of considerable significance and their role in so-called neural processes seems to be firmly established.

The consequences of the new concept for clinical medicine are manyfold. Slight irregularities in the chemical mediation of information at the synapse (e. g. caused by metabolic failure of transmitter synthesis) may cause a marked deterioration of mental or neurological functions. A good example is Parkinson's disease and its pathogenetic relations to dopamine deficiency of synapses in the extrapyramidal motor system (PLETSCHER et al., 1970; EHRINGER and HORNYKIEWICZ, 1960). On the other hand, the new insight has stimulated the use of drugs in the management of various brain disorders and further significant advances in neuropharmacology are expected in the near future.

INHIBITORY VERSUS EXCITATORY SYNAPSES

The skillful application of intracellular micropipettes for the recording of postsynaptic electrical events by ECCLES and his school lead to the discovery of the *inhibitory synaptic potential* and its relationship to an increased chloride permeability of the postsynaptic membrane. This method enables one to distinguish between excitatory and inhibitory actions of afferent impulses on the basis of postsynaptic membrane polarization changes, and thus represents a major step towards network analysis. With the thousands of excitatory and inhibitory inputs which are known to converge upon it, a single nerve cell may be visualized as a *cybernetic unit* with an astonishing range of integrative and logical functions.

The search for morphological differences between the two types of synapses has been less successful. Nevertheless, two criteria have been proposed: i) *site of contact*: excitatory synapses being preferentially located on dendrites, while inhibitory contacts seem to be more numerous on the soma. ii) *ultrastructure*: GRAY (1966) has found two types of synapses in the cerebral cortex which differed with respect to dimensions and shape of membrane complexes, as well as to the width of the synaptic cleft. UCHIZONO's (1968) finding that Gray's type 1 and type 2 synapses may be further characterized by differential size and shape of synaptic vesicles and that their location may correspond — within limits — to physiologically defined excitatory and inhibitory junctions respectively, opened up a lively discussion amongst the specialists (GRAY, 1969). Although the morphological dichotomy has been amply confirmed by many subsequent investigators as well as by recent data from our own laboratory, its correlation with functional properties remains to be further elucidated. Should Gray's classification be eventually accepted as a criterion for the iden-



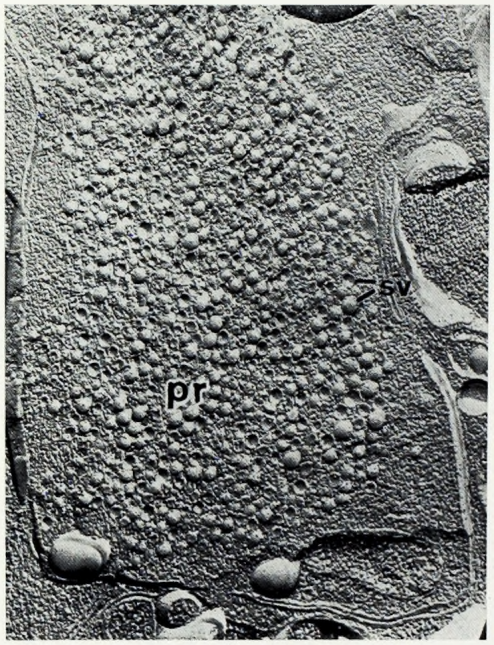


Fig. 2. - Synapse in the freeze-etched neuropil of the cat subfornical organ. Ax = axon, m = mitochondrion, pr = presynaptic terminal, sc = synaptic cleft, sv = synaptic vesicles. Magnification: 40'000 x.

Fig. 3. - Freeze-etched nerve terminal (pr) with large number of spheric profiles (convex and concave) of synaptic vesicles (sv). The synaptic site is not clearly seen. Magnification: 47'000 x.

tification of excitatory and inhibitory synapses, it would undoubtedly represent a powerful means of analyzing the structural and functional organization of the brain.

THE PRESYNAPTIC VESICULAR GRID

Fig. 2 and 3 provide an opportunity to review the classical inventory of structural components of chemical synapses. The presynaptic terminal is markedly enlarged and forms a classical bag or endknob. The freeze-etching method (MOOR and MUEHLETHALER, 1963) gives highly accurate replicas of cellular profiles and surfaces in quasi-threedimensional fashion. The tissue is in the unfixed frozen state, pretreated with 25 % glycerol to prevent the formation

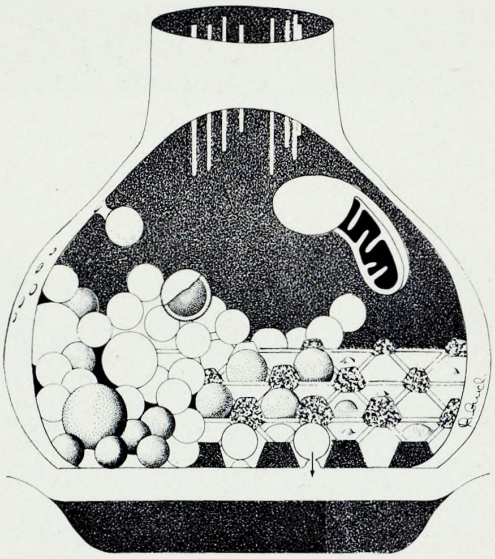


FIG. 4. - Schematic diagram of a synapse. Synaptic vesicles and their relation to the presynaptic vesicular grid. Note the attachment sites in the grid holes, where transmitter is released (arrow).

of ice crystals. Thus, the convex and concave spheric profiles of synaptic vesicles may be seen in the nearest-to-native state.

Fig. 4 shows the membrane specializations which can be demonstrated by means of heavy metal complexes such as bismuth iodide, uranyl, lead and osmium. A grid-like structure (AKERT et al., 1969; AKERT and PFENNINGER,

1969) may be identified at the presynaptic membrane. The nodal points of this filamentous network are arranged in a hexagonal pattern. The holes of the grid are large enough to accommodate single files of synaptic vesicles. The presynaptic grid seems ideally suited to guide the synaptic vesicles to their attachment sites at the presynaptic membrane (PFENNINGER et al., 1969). Details of the contact between vesicles and membrane have been revealed by means of surface views of synaptic membranes in freeze-etched preparations. A small invagination of the membrane seems to be involved and the coupling between membrane excitation and transmitter release seems to require the presence of calcium.

The question arises as to whether the grid is continuous and fully occupied by synaptic vesicles or whether the « occupation index » (number of vesicles/ number of holes) varies with function and thus represents a criterion of synaptic « viability ». This problem is presently under study.

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S.T.E. - Stabilimento Tipografico Editoriale - Città di Castello

American Journal of Physical Medicine
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Vol. 48, No. 1
Printed in U.S.A.

ELECTROMYOGRAPHIC ANALYSIS OF A RAPID VOLITIONAL MOVEMENT[1]

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The aim of this study was to analyze the sequence of events in two antagonistic muscles during a standardized, rapid, volitional movement. By comparing a simple and a complex motor reaction including a visual discrimination, we were able to measure the influence of a decision process on the motor reaction. The experiments were designed to study bradykinesia in parkinsonian patients. In an earlier report we have described the results obtained from a first group of controls and parkinsonian patients (Wiesendanger *et al.*, 1967); since that time we have modified the experimental situation slightly and therefore this report will mainly be concerned with the results of this second experimental series.

METHODS

The experimental situation has been described (Wiesendanger *et al.*, 1967). In brief, the subjects were required to respond as quickly as possible to one light signal (simple reaction) or differentially to two light signals in juxtaposition and of unequal intensity (complex reaction). The response consisted of a flexion movement of the forearm until the hand made contact with a key which operated a switch. In the complex reaction the subject had to reach the key below the brighter light which changed position randomly from right to left. The following properties of the response were analyzed; the electromyographic (EMG) innervation pattern of the biceps and triceps muscles, the reaction time measured from the "go" signal (light on) to the contact at the key (light off), the reaction time of the muscle activity, the duration of the biceps activity, and the errors in visual discrimination. In this second experimental series, we have also examined the onset of movement. Thus, four latencies were measured: the EMG latency of biceps and triceps muscles, movement latency, and contact latency. After preliminary training, 20 trials of a simple reaction and 20 trials of a complex reaction were evaluated. The means and standard deviations of the latencies were calculated for each subject. The brightness difference of the two lights was slightly increased in this second experimental series.

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Received for publication, April 25, 1968.

Presented at the International Meeting on Electromyography, Glasgow, Scotland, June 28, 1967. This work was supported by the Swiss National Foundation for Scientific Research (grant 2728).

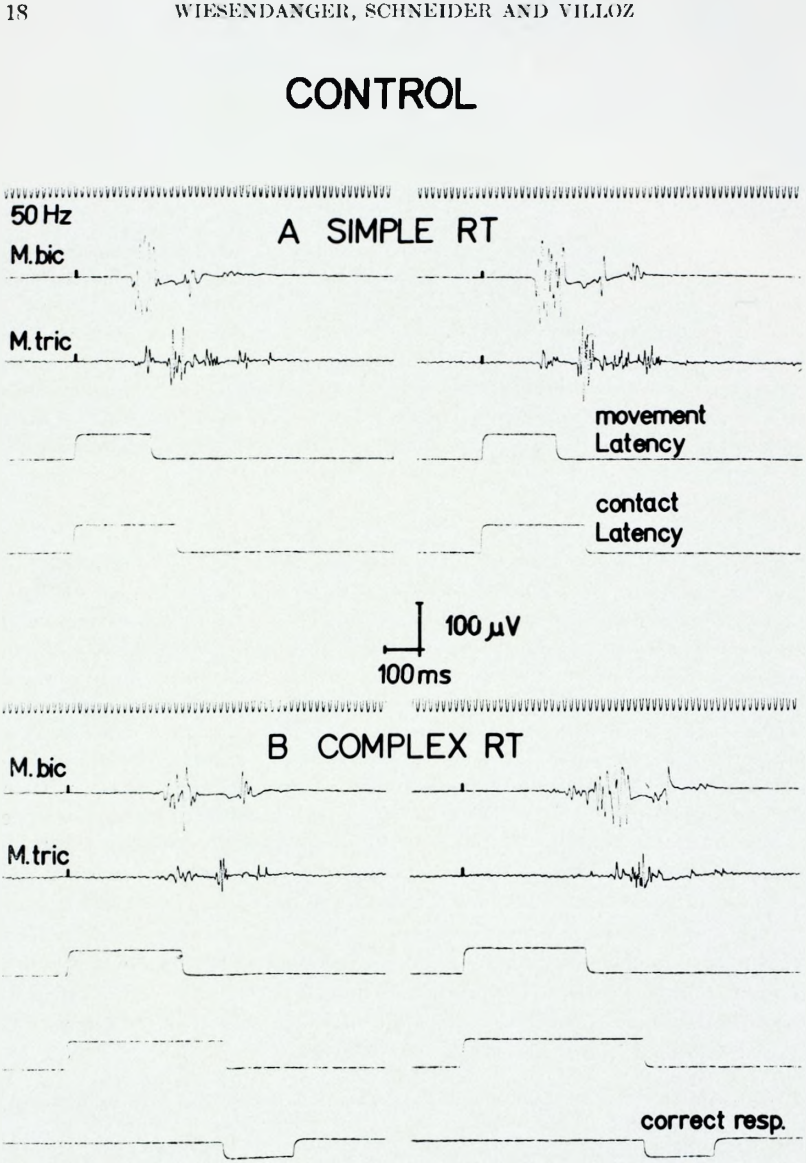

FIG. 1. Reaction time (*RT*) experiment in a normal subject. Age 54 years. For explanation, see text.

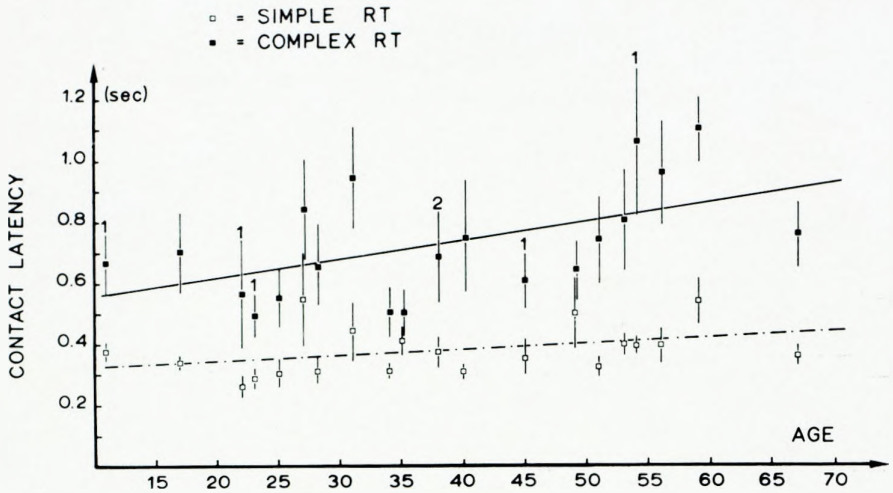

FIG. 2. Mean and standard deviations of simple and complex RTs as a function of age (contact latencies). The mean increase of the latencies is significant for the complex RT (fourfold tables, $P < 0.010$), but not for the simple RT. Number of errors marked on top of the bars.

RESULTS

Innervation pattern and latencies in normal subjects

Figure 1 illustrates the typical pattern recorded from a 54-year-old control subject. In *A* there are two consecutive trials of a simple reaction, in *B* two consecutive trials of a complex reaction. The time from the visual "go" signal to the moment the hand leaves the platform (movement latency) is given by the first rectangular pulse; the time required from the "go" signal to the moment the hand reaches the key is given by the second rectangular pulse. The EMG activity, recorded in the upper two traces, started before the hand left the platform. For all control subjects the mean time lag between hand movement and biceps activity was 90 msec. The biceps activity stopped well before the hand reached the key, and there was actually little or no activity during the movement. Typically, the silent period was followed by a small burst just before, or at the moment of, contact. The triceps activity started on the average 25 msec. after onset of the biceps response, the main bursts fell into the silent period of the biceps muscle. The principle of reciprocal innervation was not always clear cut. The triceps activity was sometimes very weak or absent and occasionally there was a co-contraction of the two muscles without clear inhibition in the middle phase of the biceps activity. A pronounced synchronization of the biceps activity during the movement was, however, a constant feature. Essentially the same pattern was observed for the complex reaction (fig. 1*B*), although the activity was weaker in most instances. As was to be expected, the latencies were longer. The mean time lag between the first electrical muscle activity and the onset of

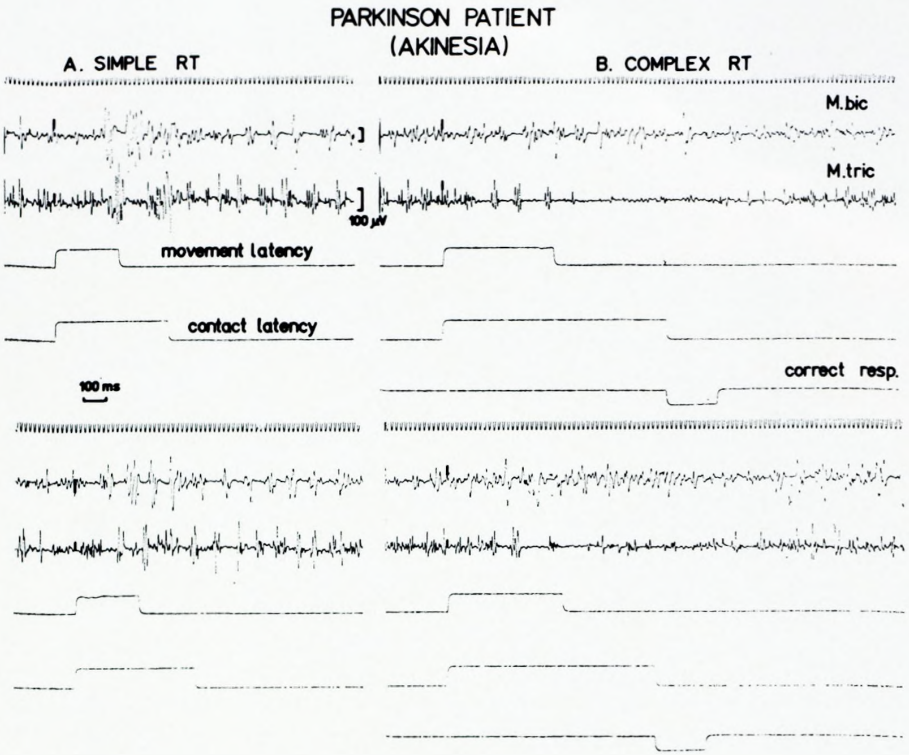

FIG. 3. RT experiment in a parkinsonian patient with pronounced akinesia. Age 64 years. For explanation, see text.

movement was longer for the complex reaction (150 msec.) than for the simple reaction (90 msec.). A deflection in the lowest signal in *B* indicates a correct discrimination, 10 per cent of the subjects made one or two errors.

In figure 2 the mean and standard deviation of the contact latencies are plotted for all subjects as a function of age. There was a clear tendency for contact latency of the complex reaction to increase with age, whereas the mean increase was not significant for the simple reaction. Variability is more pronounced for the complex than for the simple reaction time. Five subjects made one error, one subject two errors in brightness discrimination (marked with numbers in fig. 2).

Some innervation patterns in parkinsonian patients

The following curves were obtained from patients with pronounced akinesia. The curves of figure 3 are from a patient with akinesia and a discrete cogwheel phenomenon in the wrist. The volitional activity was superimposed on a background activity. Signs of reciprocal activity were observed in the simple reaction

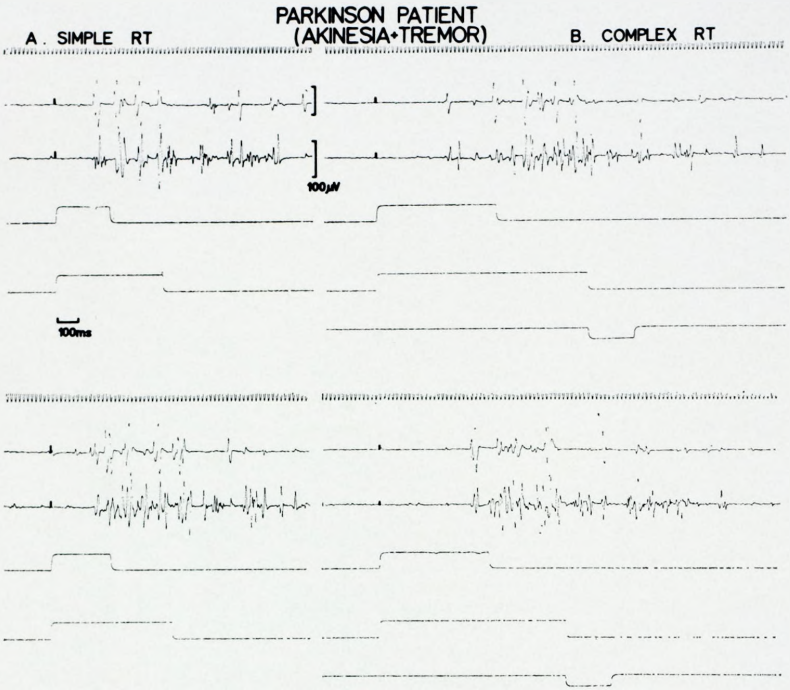

FIG. 4. RT experiment in a parkinsonian patient with akinesia and tremor. Age 58 years. For explanation, see text.

movements (*A*). The alternating bursts of the triceps electromyogram are the equivalent of an action tremor. When the complex reaction (*B*) was performed, there was almost no increase of biceps activity, but an inhibition of triceps background activity was observed. Due to a lack of synchronization and due to the pre-existing background activity, it was not possible to measure in each trial the electromyographic latency.

Figure 4 shows the curves recorded from a patient who had akinesia and an intermittent resting tremor. Voluntary movement induced an action tremor with a tendency toward synchronization of the agonistic and antagonistic bursts. Finally, the curves of a patient whose prominent symptom was a resting tremor are presented in figure 5. Rigidity and akinesia were not pronounced. The resting tremor, consisting of alternating bursts, is shown in *A*. When the patient performed a voluntary movement, an action tremor with synchronized bursts in agonists and antagonists, appeared. Interestingly enough, the complex reaction (*C*) of this patient was also prolonged.

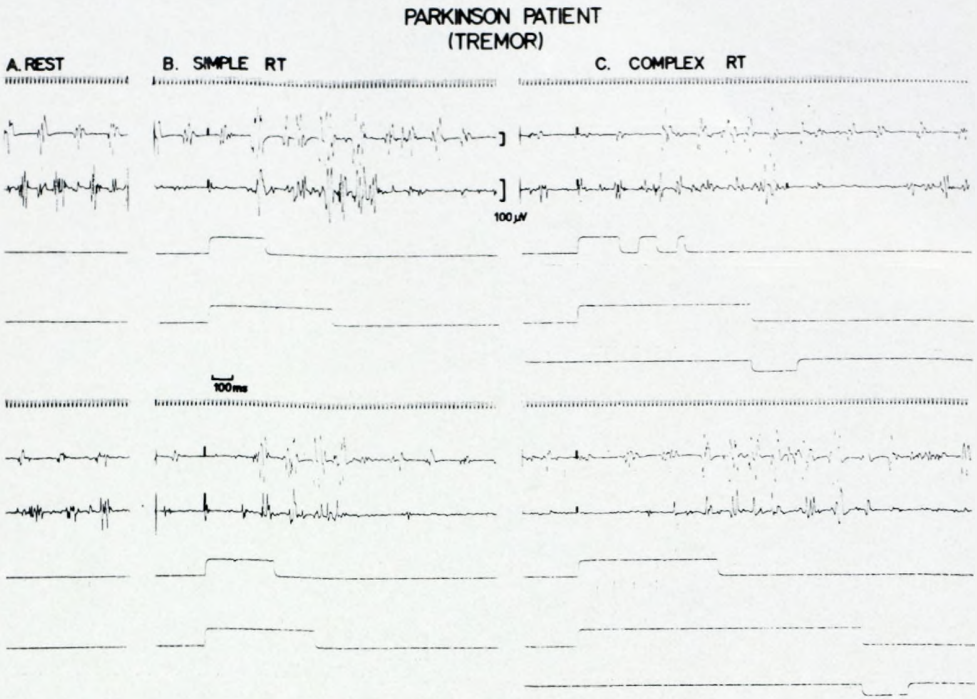

FIG. 5. RT experiment in a parkinsonian patient with tremor as a dominant symptom. Age 63 years. For explanation, see text.

In view of the marked fatiguability of purposive movements in parkinsonian patients, we have plotted movement latencies (simple reaction) sequentially. The histograms of four control subjects are compared in figure 6 with the histograms of four patients with marked akinesia. The variability of the movement latencies was greater in the patients than in the controls; there was, however, no tendency for longer latencies towards the end of the session. A preliminary statistical analysis considered all parkinsonian patients regardless of age or severity of illness. The number of controls tested under the same experimental conditions was smaller, and the ages did not cover the whole range of the parkinsonian patients. The mean latencies for both groups are listed in table 1 together with the level of significance. There were no more discrimination errors in patients than in the control subjects.

DISCUSSION

The results of this experimental series have confirmed our previous results with respect to the patterning of muscle activity during rapid volitional movements in normals and parkinsonian patients. The typical reciprocal pattern with a pronounced initial synchronization of the biceps activity contrasted with a prolonged period of voluntary activity in parkinsonian patients. The onset of

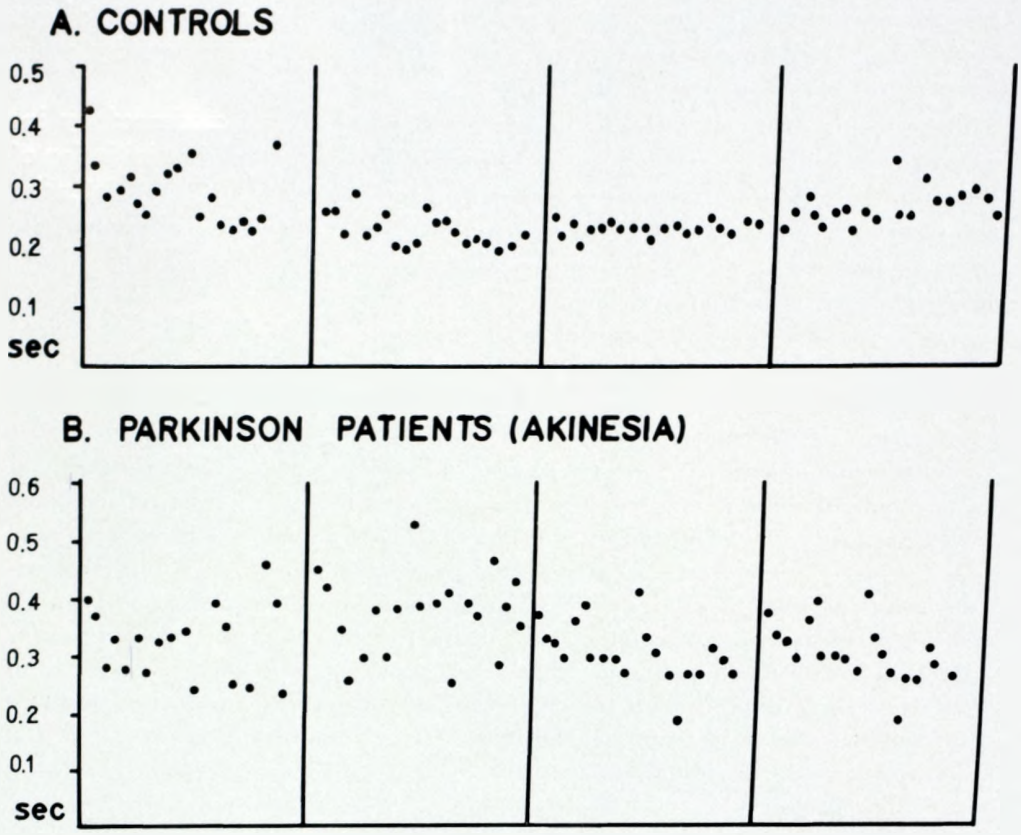

FIG. 6. Histograms of movement latencies for simple RTs. The RTs are plotted in sequential order (20 trials each) for four controls (*A*) and four parkinsonian patients with pronounced akinesia (*B*).

TABLE 1

Statistical analysis of data

		Controls (N = 10)	Parkinsonian patients (N = 17)	Mean difference	Level of significance*
M. biceps EMG-latencies (msec.)	Simple RT	179	210	31	$P > 0.05$
	Complex RT	290	323	33	$P > 0.05$
Movement latencies (msec.)	Simple RT	270	321	51	$P > 0.05$
	Complex RT	434	581	147	$P < 0.05$
Contact latencies (msec.)	Simple RT	396	568	172	$P < 0.01$
	Complex RT	651	1052	401	$P < 0.01$

* Wilcoxon Rank Sum Test (two sided).

electromyographic activity was sometimes hard to differentiate from the preexisting background activity. Several cases did not reveal reciprocal activation but instead had pronounced synchronous, rhythmic activity in both agonist and antagonist. The abnormal findings were variable from patient to patient. This is

not surprising in view of the variability of the clinical symptomatology. We have already discussed these findings with respect to similar observations by other investigators (Wiesendanger *et al.*, 1967).

The slowing of movements in parkinsonian patients was evidenced by increased reaction times. Preliminary statistical analysis revealed that initiation of the simple reaction movement was not slowed, as evidenced by EMG latencies and movement latencies. When the reaction involved a visual discrimination, the onset of movement was delayed in parkinsonian patients, although the EMG latencies were not significantly longer than in normals. This is probably due to the lack of muscular synchronization in the agonist, since a longer time is required to overcome the inertia of the limb. The movements of parkinsonian patients were slowed in both simple and the complex reactions, but the slowing was more pronounced for the complex reaction.

Based on an individual case of a 40-year-old patient with a severe akinesia and a pronounced discrepancy between a normal simple and a prolonged complex reaction time, we have postulated that the slowing of movements in such patients may be due to a prolonged time required for visuo-motor integration. (Wiesendanger *et al.*, 1967.) The present study confirms that the difference between normals and parkinsonian patients is greater for the complex reaction than for the simple reaction. This implies that visual discrimination adds consistently to the slowing of parkinsonian patients. The difference would probably be more evident if the analysis were expanded to a larger number of patients suffering from akinesia as the leading symptom.

SUMMARY

Simple and complex reaction times were measured in normal subjects and parkinsonian patients. In response to a visual signal, the subject had to perform as quickly as possible a flexion movement of the forearm. The complex reaction included a visual brightness discrimination. The electromyographic latencies of the biceps and triceps muscles, the mechanical movement latency of the forearm and the latency to the contact with the target were measured. The comparison of the average values revealed that the start of the simple reaction movement was the same for normals and for parkinsonian patients. The movement latencies of the complex reaction were slightly longer in parkinsonian patients. The contact latencies in parkinsonians were longer for both the simple and the complex reaction, but the increase was more pronounced for the complex reaction movement. Several abnormal patterns of voluntary activation in parkinsonian patients were described. It was concluded that a prolonged visuo-motor integration contributes to bradykinesia.

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