to a receptor density of only about 1.5×10^3 receptors per square micrometer of surface in both the nonendplate regions of adult denervated muscle fibers and in skeletal muscle in tissue culture (*4*). Thus, the receptor density at neuromuscular junctions is much higher than in extrajunctional membranes that are extremely sensitive to ACh. This difference has implications for the appraisal of denervation supersensitivity to ACh and of development and functioning of neuromuscular junctions.

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26 November 1971; revised 2 February 1972

Morphologic Alterations of Synapses in Electrically Stimulated Superior Cervical Ganglia of the Cat

Abstract. *Prolonged preganglionic stimulation produces marked ultrastructural changes in presynaptic endings, which develop larger zones of contact with postsynaptic dendrites. Profiles of such endings, compared to controls, have fewer synaptic vesicles, similar areas, and greater circumferences. These results are compatible with the hypothesis that synaptic vesicles become incorporated into the plasma membrane during stimulation.*

Biochemical and physiological data suggest that synaptic vesicles may be involved in the mechanism of transmitter release, although direct morphologic evidence is circumstantial (*1*). We investigated the morphology and amount of cholinergic vesicles at neuroneuronal synapses under conditions known to produce marked depletion of neurotransmitter substance. The preparation used was the intensely stimulated superior cervical ganglion of the cat, which had been treated with the drug hemicholinium-3 (HC-3) (*2*). We observed, in addition to changes in numbers of vesicles, unexpected changes in the morphological relation of presynaptic endings to postsynaptic processes in superior cervical ganglia subjected to prolonged electrical stimulation. These data suggest the mechanism of transmitter release that results from stimulation.

Both cervical sympathetic trunks of anesthetized cats were surgically exposed low in the neck, with one side serving as a sham-operated control while the other was placed upon a stimulating electrode. Saline or HC-3 was administered intravenously 5 minutes prior to stimulation. Supramaximal stimulation (as judged by contractions of the nictitating membrane) with monophasic rectangular pulses of 0.3-msec duration and 5- to 10-volt amplitude continued in interrupted trains at 20 to 32 hz for 150 to 190 minutes (*3*). At the end of experiments, stimulation was continued while cats were perfused intraaortically for 10 minutes with an aldehyde fixative (*4*). The ganglia (control and stimulated) were excised, processed simultaneously, and examined by electron microscopy (*5*).

Axodendritic synapses in nonstimulated superior cervical ganglia, in cats treated with saline or HC-3, had a typical ultrastructural appearance. Presynaptic processes were bulbous in shape with a smooth contour, contained spheroidal vesicles, and possessed characteristic membrane specializations (Fig. 1A). Our observations of control ganglia closely agree, qualitatively and quantitatively, with the interrelations reported for axon terminals and dendrites in normal superior cervical ganglia; these interrelations were derived from serial sections examined by electron microscope (*6*).

Visual examination of electron micrographs of synapses in stimulated ganglia, with or without HC-3, disclosed striking alterations in the ultrastructure of a subpopulation of axon terminals (*7*). Profiles of such axon terminals were irregular and crescent-shaped, contained fewer vesicles, had greater zones of apposition with postsynaptic dendrites, and had greater circumferences but similar areas (Fig. 1, B and C). These ultrastructural features apparently resulted from encircling of dendritic profiles by projections of axon endings. In some instances, axon terminals completely encircled dendritic profiles, increasing the zone of apposition to 100 percent. These larger zones of apposition were produced by increases in the nonspecialized portion of the plasma membrane with no obvious increase in the length of synaptic densities. Stimulation did not seem to alter the presynaptic profiles of smallest diameter (0.8 μm).

Measurements were made on electron micrographs in order to specify the extent and frequency of occurrence of the morphological changes. The degree of synaptic apposition for each synapse was expressed as the percentage of the circumference of the dendritic profile in apposition to the axon ending. This measurment was made on random electron micrographs, and frequency distribution histograms were made. The histograms indicated the more frequent occurrence, in stimulated ganglia, of synaptic profiles with a large percentage of the dendritic circumference in apposition to the axon ending. Table 1 shows mean differences between stimulated and control ganglia for this measurement. The effect of stimulation is statistically

Table 1. Effects of electrical stimulation on superior cervical ganglia of the cat. Paired comparison of stimulated and control ganglia in individual cats are given. Samples were approximately 20 to 40 random synapses in each ganglion.

Degree of synaptic apposition: percentage of dendritic circumference in contact with profile of axon ending (mean)			Synaptic vesicles per square micrometer of profile of axon ending (mean)		
Non-stimulated ganglia	Stimulated ganglia	Difference	Non-stimulated ganglia	Stimulated ganglia	Difference
		Saline			
16.87	30.81	13.95	65.0	41.1	23.9
24.63	46.92	22.30	66.0	27.2	38.8
		HC-3 (2 to 5 mg/kg)			
22.95	27.86	4.91	61.1	38.0	23.1
25.31	37.00	11.69	57.7	13.1	44.7
25.29	40.06	14.77	66.3	29.8	36.5
25.00	47.83	22.83	80.0	32.6	47.4
		HC-3 (1 mg/kg)			
24.57	37.86	13.29			
22.77	34.15	11.38			
		Cumulative means			
23.42	37.80	14.39	66.0	30.3	35.7

significant ($P < .004$, based on binomial probabilities) in experiments with saline or HC-3. Similar paired comparisons indicate that circumferences of axon endings in stimulated ganglia (treated with saline or HC-3) were on the average 20 percent larger than those in the respective nonstimulated controls.

To assess the decrease in synaptic vesicles, the number of vesicles in presynaptic profiles was counted and divided by the area of the profile. Table 1 also shows the mean number of vesicles per square micrometer of axon ending for each ganglion and the difference between the means for control and stimulated ganglia for each animal. Stimulation results in a significant decrease in the number of vesicles in animals treated with saline or HC-3 ($P < .02$, based on binomial probabilities). Since similar paired comparisons demonstrated no significant differences in mean areas of presynaptic profiles of the various groups, these measurements indicate that stimulation produces an absolute decrease in the number of vesicles in presynaptic terminals.

These observations made by electron microscopy demonstrate that preganglionic stimulation produces a marked increase in presynaptic axonal plasma membrane. This production of axonal membrane is specifically directed toward postsynaptic dendritic processes, and the added membrane engulfs dendrites and enlarges the zone of apposition between pre- and postsynaptic processes.

The resulting increase in the circumference of the axon terminals was not accompanied by a commensurate increase in the area of the profiles. It is unlikely that this apparently directed change in the shape of axon endings without a change in area could be due to a nonspecific mechanism such as simple osmotic swelling. Instead, the production of new membrane must have another explanation. This study correlates an increase in terminal axonal plasma membrane with an absolute decrease in synaptic vesicles (*8–10*). Our initial calculations indicate that the estimated quantity of vesicle membrane lost agrees with the amount of increase of membrane in axon endings, a result suggesting that stimulation produces fusion and incorporation of components of synaptic vesicles into presynaptic plasma membranes (*11*).

In stimulated ganglia that were treated with saline, we observed a 48 percent depletion of synaptic vesicles, which correlated closely with a 45 percent decrease (below initial levels) in contractions of the nictitating membrane. In contrast, in stimulated ganglia that were treated with HC-3, we observed a 59 percent depletion in vesicles but a considerably larger (98 to 99 percent) reduction in response of the nictitating membrane. Prolonged stimulation coupled with the administration of HC-3 produces a progressive decrease in the size of transmitter quanta, as judged by declines in the amplitude of miniature end-plate potentials (*12*). Therefore, the greater reduction in synaptic transmission in stimulated ganglia treated with HC-3 may be due to incomplete charging of vesicles with acetylcholine.

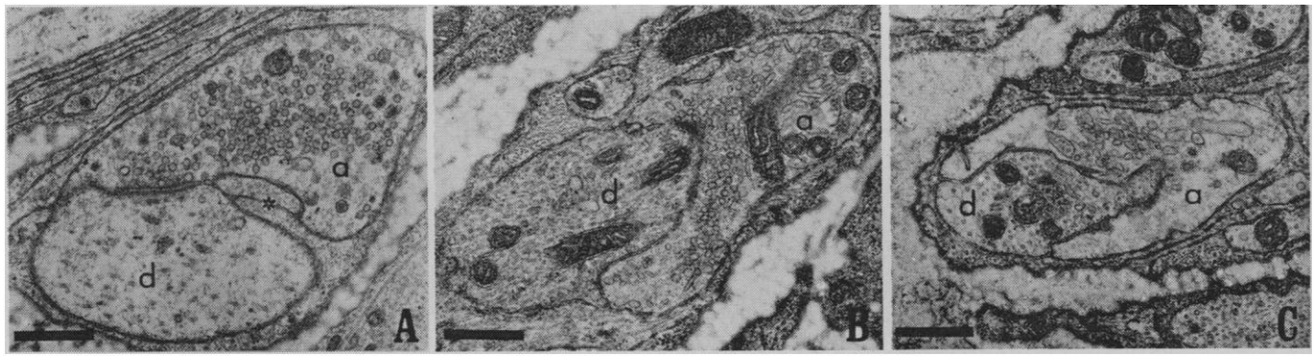

Fig. 1. Electron micrographs of axodendritic synapses in superior cervical ganglia of the cat. A nonstimulated ganglion is seen in A, and stimulated ganglia in B and C (*a*, axon ending; *d*, dendrite; *, cytoplasm of Schwann cell). In stimulated ganglia, with or without HC-3, a subpopulation of axon terminals (B and C) were crescent-shaped, contained fewer vesicles, and had greater zones of apposition with postsynaptic dendrites. Profiles of axon terminals that were more severely depleted of vesicles (C) had clearly greater circumferences but similar areas compared to controls. The bar is 0.5 μm. ($\times$ 21,000)

Thus, our data provide further evidence for a "vesicle hypothesis" (*9, 13*) and suggest that transmitter is released by exocytosis with at least temporary incorporation of vesicle components into plasma membranes (*14*).

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4. Fixation consisted of perfusion with a mixture of phosphate-buffered glutaraldehyde and formaldehyde followed by soaking of slices for 1 hour at 4°C in the same fixative and then postfixing in 1 percent O_sO_4. After slices were dehydrated in ethanol and embedded, large ultrathin sections were cut in the transverse plane.
5. Electron micrographs were systematically taken with a Siemens Elmiskop IA by recording fields at the corners of grid openings. For morphometric measurements, a series of random electron micrographs were assembled, and all axodendritic synapses that showed characteristic membrane densities were evaluated. Profile circumferences were measured with a map measurer, and profile areas with a planimeter.
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3 December 1971; revised 14 January 1972

Carbon Monoxide Concentration Trends in Urban Atmospheres

Carbon monoxide is a pollutant of urban air that has recently begun to attract increased attention (*1*). Although the concern about this gas at present is associated with vehicular exhaust, CO is not a new pollutant in man's environment. It is always produced during incomplete combustion and has long been artificially present in man's environment.

A number of studies of CO concentrations in the air of New York City have been made during the last 50 years (*2–6*). For purposes of comparison, the data of Table 1, which summarize the results of studies made between 1922 and 1967, are limited to those locations in the city at which vehicular traffic is heaviest. According to these data, the CO concentration near busy thoroughfares and intersections in New York City is not increasing and may actually have decreased since 1922.

The predominant modern source of CO is the exhaust gases from the internal-combustion engine. Approximately 97 percent of the CO in the air of New York City is currently attributable to this source (*7*). Prior to the advent of the internal-combustion engine, the major source was fuel burning for space heating, water heating, cooking, and the production of electrical energy. The types of fuel consumed in New York City during the past several decades have changed radically, the predominant shift having been from coal and manufactured gas (*8*) to oil and natural gas. The exhaust gases from the combustion of fuel oil and natural gas do not contain CO in significant amounts. The various fuels consumed in New York City during 1934 and 1965 are shown in Table 2 (*2, 9*).

Concurrently with changes in the types and amounts of fuel being burned, there have also been improvements in the combustion efficiencies of heating units. The coal- and wood-burning stoves that were present in many individual apartments 50 years ago have been replaced by larger, more efficient boiler units that provide heat and hot water for entire apartment buildings. Moreover, the coal furnaces that provided heat for tens of thousands of New York's private homes a half century ago have been replaced with oil or gas furnaces.

As an example which illustrates such changes in fuel consumption patterns and the corresponding reduction in CO emissions, let us consider the effect of the elimination of anthracite coal as a space heating fuel in one- and two-family dwellings. In 1934, 4.6×10^6 tons (4.1×10^6 metric tons) of anthracite were burned in such structures in New York City for space heating purposes (*9*). If we assume that 1 pound (454 g) of coal requires 8 pounds of air to produce 13,000 Btu (51,500 kcal) of heat (*10*), and that flue gas from coal-burning dwellings is approximately 1 percent CO (*11*), we estimate that 400,000 tons of CO were emitted from the one- and two-family dwellings that burned coal in 1934. The vehicular

Table 1. Carbon monoxide concentrations in New York City streets from 1922 to 1967.

Year	CO concentration (ppm)	Sampling condition	Reference
1922	100	Moderate-heavy traffic	(*5*)
1932	Range, 2–129; average, 32	Heavy traffic (average: 1900 cars per hour)	(*6*)
1966	Peak hourly average range, 19–95; average, 32	In-traffic sample, very heavy traffic	(*2*)
1967	Hourly average range, 1–17; average, 8	45th St. and Park Ave., continuous samples, heavy traffic flow (1000 to 3400 vehicles per hour)	(*3*)