Neurophysiology: United States-Japan Joint Symposium

Theodore H. Bullock

In June 1961 the Prime Minister of Japan, Hayato Ikeda, and President John F. Kennedy agreed to form a United States–Japan committee to strengthen scientific cooperation between the two countries. The Joint United States–Japan Committee on Scientific Cooperation, the United States delegation to which was appointed by the Department of State, has developed the guidelines for what is now called the U.S.–Japan Cooperative Science Program. Its principal characteristic is insistence upon mutuality in funding and in participation. Activities under this program are initiated after matching proposals in each country have been approved in both countries; each country pays for expenses of its own nationals wherever they go. The National Science Foundation, with the advice of panels of scientists, is responsible for coordinating U.S. scientific interest in the program and for providing operating funds in the U.S. In Japan, program coordination is shared among the Ministry of Education, the Japan Society for the Promotion of Science, and the Science and Technology Agency. Support is presently limited to research categories approved by the Joint Committee.

The first effort in the field of the nervous system and behavior, and the first, apart from planning meetings, in the area of biology, took place 5–10 March in Tokyo as a symposium on neurophysiology. Initiated and organized by Yasuji Katsuki, with U.S. coordination by T. H. Bullock, it brought together 10 American and 20 Japanese scientists. The meetings were unusually successful as international symposia, not only because of the excellent planning and thoughtful hospitality by the hosts, but also because neurophysiology has reached a high state of sophistication and because of commonality of interest among the Japanese and Americans. Many of the participants were already acquainted; most of the Japanese had worked in American laboratories. It cannot be said that there was no language barrier, but it was probably smaller than it would be in most other fields and countries.

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Neurophysiology has flourished in Japan, although the laboratories are not well supported and the number of career opportunities for brilliant young scientists is severely limited. It is one of the most popular branches of biology and its workers tend to be technically adept experimentalists, somewhat less concerned with theory. An important asset is the high development of technology and instrumentation in Japan. Another is the ready availability of a rich, semitropical fauna, both terrestrial and marine, that provides many species for study in home laboratories in Japan. Western scientists cannot obtain many of these species (for example, *Squilla*, cicadas, squid, *Onchidium*, horseshoe crab, many teleosts), or must travel to special stations to work upon them. It is clear that the time has arrived when American scientists should seek opportunities to work in Japanese laboratories in this, and doubtless other fields, for much the same reasons that they have traditionally gone to Europe.

I report only some highlights of the symposium, emphasizing Japanese contributions. One group of reports concerned sensory mechanisms. Y. Katsuki (Tokyo) reported the effects of electrophoretic application of ions from a glass capillary inserted through the basilar membrane to a position beneath the hair cells of the cochlea. Acetylcholine suppresses both the cochlear microphonic and the neural response, sometimes suppressing the former without affecting the latter! Katsuki concludes that the change of responses due to acetylcholine comes from influence on efferent endings rather than from afferent ones. In other experiments on the primary auditory neurons of the monkey, discharges elicited by a continuous pure tone near the best frequency were suppressed by a strong-tone burst with a lower or higher frequency. The latency of inhibition is shorter than 0.5 millisecond, thus excluding the possibility of reflex by efferent fibers and suggesting lateral inhibition. R. Galambos (New Haven) reported new complexities in the central responses to auditory stimuli. Such responses were recorded by electrodes implanted permanently in unanesthetized animals. The evoked potentials are more labile and widespread in areas outside the classical pathways than in anesthetized preparations; cortical-evoked responses persist after the classical pathway is severed in the midbrain. W. A. Rosenblith (Cambridge) also discussed central-peripheral relations in auditory physiology.

T. Tomita (Tokyo) announced a new technique of microelectrode penetration into single cells. The isolated retina of the carp is mounted on a platform which vibrates vertically a few microns at several kilocycles per second. A vertically held, slowly advancing pipette now records responses at the depth of the receptor layer; this has never been successful previously. Resting potentials of 30 to 40 millivolts and a sustained increase in polarization in response to light are obtained. There is no substantial area effect; spectral response curves are all monophasic. Tomita believes he is recording from inner segments of single cones. This is not the first time this has been claimed, but it is the latest claim and enjoys the advantage of the additional cautions that have resulted from the earlier claims of others. Kosuke Watanabe (Tokyo) has developed an opened eye-cup preparation of the carp with intact blood supply; this permits long-run experiments with fine micropipettes that record subthreshold graded potentials in response to light in the deeper layers of the retina. He has studied dark and light adaptation of the various types of the "s-potentials." One conclusion is that there is no contribution of the rod system to the clear change produced by dark adaptation in the shape of the s-potential to white light, and that this change is probably one of the properties of the cone system. G. Mitarai (Nagoya) reasserted the main results and conclusions previously reported with G. Svaetichin (Caracas) on the identification of different types of s-potentials of fish with different types of glia. The new study is on the carp and attempts to establish the lithiumcarmin method of localizing the electrode tip; most of the work is a confirmation of the reports based on Venezuelan fish. An additional feature is a description of monoamine oxidase as being low and confined to the feet of the Müller fibers during dark adaptation. However, after light adaptation, the monoamine oxidase invades all the glial system and the receptors. Another is that antidromic stimulation of the optic nerve causes a slow decrease in the membrane potential of the presumed glia and a graded increase of response amplitude to light. Kyoji Tasaki (Sendai) infers, on the basis of microelectrode insertions into the retina of the octopus, not one but two sources of potential in response to light among the retinal layers. The deep response spreads laterally and this worker suggests a role of axon collaterals of the receptor cells or of close membrane-to-membrane contact between adjacent cells found electron microscopically. K. Motokawa (Sendai) has undertaken experiments on color vision in man by electrical stimulation of the intact eye. In recording from single units in the optic tract of the cat, the latency of different types of units is affected in opposite ways by gross currents of different polarities traversing the bulb. Tract axons respond with a train of impulses to single electric shocks and the excitability is increased by an adapting light with one, and sometimes two, spectral maxima. Electrical stimulation also causes a slow potential in the retina.

F. Ratliff (New York) reviewed excitation and lateral inhibition in the eye of *Limulus*; current foci of interest include the threshold frequency of discharge of neighboring ommatidia, required to produce detectable inhibition in a given ommatidium, and the substantial and graded delay of inhibitory effect, which is apparently not due to conduction time but to synaptic processes. R. Kikuchi (Tokyo) has found effects of tetraethyl ammonium ions on the slow potentials and spikes of the horseshoe crab ommatidium that are not simply explained by the sodium theory. M. Sato (Kumamoto) reviewed excitation of the sensory terminal in Pacinian corpuscles, with emphasis on the summing of subthreshold mechanical stimulation applied at separate sites in the terminal. To mechanical stimuli of long duration, there is often a late, steady phase of small amplitude after the initial dynamic phase of the generator potential.

S. F. Takagi (Maebashi) discussed the origin of three forms of slow potentials in the olfactory epithelium of amphibians. The appearance of these responses in newts coming out onto the land and in frogs during degeneration of the sectioned olfactory nerve were correlated with histological observations.

Another group of contributions concerned membrane mechanism and the role of ions. T. Narahashi (Tokyo) has studied the relation of resting potential to action potential and excitability in lobster and cockroach giant axons. The curve relating spike amplitude or sodium current to membrane potential is shifted along the potential axis with many environmental agents; for example, increase in external calcium shifts the curve to lower membrane potential and loss of internal potassium in squid axons, although completely depolarizing, leaves the membrane capable of full-sized action potentials. He prefers to say that excitability is maintained as long as the membrane components are integrated in such a way as to favor conductance changes and that the resting potential is rather secondary to this. Cathodal depolarization increases the membrane conductance and finally inactivates through displacement of the membrane calcium, while anodal hypopolarization can restore excitability through reintegration of the membrane calcium. S. Hagiwara (Los Angeles) described the membrane properties of the giant muscle fibers of the barnacle, *Balanus nubilus*, revealed by controlling the external and internal ionic compositions. This is a nonsodium membrane, there being no change in active membrane potential with changes of external or internal concentration of that ion. The active membrane potential is determined by the ratio between the external Ca concentration and the internal K concentration; "all-or-none" spikes are possible only when the internal Ca is reduced by injecting a binding agent. A. and N. Takeuchi (Tokyo) applied the amino acids L-glutamate and γ-amino butyric acid electrophoretically to spots on the surface of crayfish muscle. At sharply localized sensitive spots, which coincide with the spots producing extracellular excitatory junctional potentials, L-glutamate (but not D-glutamate) produces transient depolarization (0.5×10^{-16} mole produces several millivolts). Desensitization begins in tenths of a second, not only to this compound but also to nerve impulses. Spots sensitive to γ-amino butyric acid coincide, but this compound causes little desensitization. Potentials induced by the compound reverse sign at the same membrane level as do inhibitory junction potentials. H. Grundfest (New York) emphasized the complexity and diversity of membrane properties of muscle fibers disclosed by electrophysiological, pharmacological, electron microscopic, and osmotic studies. The existence of a transverse tubular system with anion permselective characteristics in crayfish muscle and a functional relation between this and excitation-contraction coupling is adduced.

In a second, more general review, Grundfest documented the diversity of electrogenic manifestations in various excitable cells, giving evidence that Ca, Mg, and perhaps other ions in addition to Na, K, and Cl may play a role in electrical responses of spike and other forms not embraced in the Hodgkin-Huxley theory. He emphasized the necessity to extend understanding to those forms of response which can be considered as transducer potentials to chemical and other specific stimuli and which are not voltage dependent. A general view must also account for those electrically excitable responses which involve only K-activation, or Cl-activation, or K- or Cl-inactivation as well as the interplay in permutation of these fairly independent varieties of response.

Y. Oomura (Kanazawa) also noted diversity of this kind among giant ganglion cells of the gastropod *Onchidium*. If the axon of these unipolar cells is ligatured they are no longer excitable by synaptic or antidromic stimulation; but under direct depolarization, the membrane impedance decreased several hundredfold. Oomura believed this decrease must be attributed to activity of the soma membrane. Three types of cells can be distinguished by their response to acetylcholine. One is hyperpolarized with a reduction in membrane resistance due to increasing permeability to smaller sized anions. Another is depolarized with a large decrease in membrane resistance, and a third is depolarized with a small increase in membrane resistance. There is no considerable increase in permeability to sodium.

In another vein T. Araki (Kyoto) studied the role of calcium by extracellular injection of the calcium-binding agent, EDTA, close to the spinal motoneuron of the cat. He observed a steep reduction of the resting potential and of the spike potential with occasional spontaneous firing or repetitive discharge and plateau formation to single antidromic impulses. Recovery of spike height separately from resting potential suggested the recovery of a fraction of the sodium-carrying system, inactivated by calcium deficiency. Excitatory and inhibitory postsynaptic potentials were differently affected, the latter being more influenced with an initial increase and a later decrease in size.

In search of the inhibitory transmitter, D. D. Potter (Boston) and his collaborators have found that γ-amino butyric acid (GABA) is the only blocking compound out of ten extracted from peripheral nerves of lobsters which is in much higher concentration in efferent inhibitory than in excitatory axons. Study of the enzymes of GABA metabolism in isolated axons of the two types shows that inhibitory axons form GABA two to six times more rapidly than excitatory axons. Degradative reactions are catalyzed by both types of fibers, but quantitative aspects have not been worked out.

Contributions on the functional organization of the vertebrate central nervous system included that by M. Ito (Tokyo), who recorded intracellularly in cells of the lateral vestibular nucleus of Deiters. Besides small excitatory postsynaptic potentials resulting from stimulation of the vestibular nerve and of the spinal cord, there are inhibitory postsynaptic potentials apparently monosynaptically elicited from stimulation of the Purkinje cells of the cerebellar vermis.

H. Asanuma (Osaka) studied the changes in excitability of the cortex that were produced by repetitive antidromic stimulation of the pyramidal tract. Periods of early depression, facilitation, and late depression effect responses to stimulation of the skin as well as spontaneous unit activity and are correlated with the cortical potentials resulting from the antidromic pyramidal activation. He suggests that the effects are mediated by recurrent collaterals producing a cortical inhibition of importance in stabilizing cortical discharge and in fine control of corticospinal responses to sensory input.

T. Furukawa (Osaka) added to the complexity of the giant Mauthner cell in the teleost medulla by reporting an electrically transmitted, excitatory postsynaptic potential in the lateral dendrite in addition to the electrically transmitted inhibitory, and chemically transmitted excitatory and inhibitory postsynaptic potentials, which he and Furshpan had already shown. This model neuron is a veritable integrating center and decision unit, initiating startle responses as a result of complex converging input. M. V. L. Bennett (New York) reported several neural operations in the control of electric organ discharge in mormyrid, gymnotid, and malapterurid fishes. A group of "command" cells fire in synchrony, either spontaneously or from sensory or from higher central input, and are separated from the electroplaque effector cells by one or two interposed neurons, which may be mere relays or may perform some transformation, such as increased synchronization. Electrotonic connections are found between cells at each level and sometimes between levels; electron microscopy shows specialized contacts with fused membranes. Cells of one level may be electrotonically connected by their common electrotonic connection to the same prejunctional neuron. Several devices for increasing synchrony of effector discharge are employed. Electrotonic connections were also a theme of the contribution of A. Watanabe (Tokyo) on the cardiac ganglion of the crustacean *Squilla*. Slow waves come from dendrites or soma of pacemaker cells and trigger spikes in the axon of the same or of neighboring cells by electrotonic connections. Slow waves in particular spread from cell to cell and are little altered by the superposition of a spike. As in most such cases, it is not known whether the electrotonic connections are low resistance, electrical synapses or represent anastomoses.

The problem of what aspect of the temporal distribution of impulses in a train is significant in a given case (for example, most recent interval, average frequency over some period, variance of intervals, relative frequency of different intervals on each side of a mode, the succession or sequential correlation of intervals) was discussed by T. H. Bullock (Los Angeles). Naturally produced trains of impulses differ considerably and characteristically in each of these ways and are altered by environmental factors in definite, but still little understood ways. In given cases, it can be shown that one or another of these parameters is without significance or is able to carry information reflected in the output. Temporal microstructure can be employed in certain cases at the same average frequency, but the use of this variable in the nervous system appears to be severely restricted.

Several morphological papers attracted much interest from the physiologists. K. Hama (Hiroshima) classified several new and familiar types of electron microscopic contact between neurons in the rat cortex. The familiar forms he believed to be chemical were characterized by accumulated vesicles on the presynaptic side, the thickening of the apposed membranes, and the uniform and wide cleft between them. These are usually axodendritic and axosomatic. Newer types, known in lower animals and now found in the rat, include those with a very narrow cleft, less thickening, and less subsynaptic density on one side and fewer vesicles on the other; these are usually on the soma. In addition, some synapses have vesicles on the soma or dendrite side and dense material on the other; some have vesicles on both sides or the vesicles on the axon side may not be closely associated with the synapse, but on the other side they are, suggesting dendro-axonic and soma-axonic chemical synapses. Electrical connection between adjacent dendrites is suggested by cases of close apposition and fusion of dendrite membranes without accumulation of vesicles; this may occur over long distances and is also found between two glial processes. K. Uchizono (Tokyo) described various forms of synaptic contact from the frog sympathetic neuron and distinguished between smaller, clear-centered vesicles (believed to be cholinergic) and larger dense-centered vesicles (believed to be noradrenergic). He claims to see a remarkable change after intense stimulation of the vagus nerve in its (presynaptic) terminals on cardiac postganglionic cells. This change occurs both in the preterminal vesicles and in the cytoplasm of the soma. C. C. Hunt (Salt Lake City) found that section of preganglionic nerves of frog sympathetic ganglia produced electron microscopic changes within 24 to 36 hours, coinciding with the earliest functional changes. At this time spontaneous miniature synaptic potentials increase in frequency and then disappear. This process can be accelerated by repetitive presynaptic stimulation and is associated with failure of synaptic transmission. Unlike frog skeletal muscle, where the miniature potentials reappear as the nerve endings are replaced by Schwann cell processes, they do not reappear in the sympathetic ganglion cell. About 25 percent of the surface of the postganglionic cell is said to be covered by terminal endings without glial membranes intervening; about

4 percent of the surface has membrane thickenings, a condition suggestive of chemical synapses.

While neuroglial cells were a recurrent subject of discussion, they were the principal theme of two contributions. S. W. Kuffler (Boston) reported integrated electron microscope and electrophysiological and tracer studies of the giant glial cells in the leech. Here the glial resting potentials are at least as large as those of neurons and are similarly dependent mainly on a high internal K^+. They do not give active responses to stimulation or demonstrate marked drops in their normally high resistance. They are not essential for the short-term survival of neurons which continue to give normal impulses when glial cytoplasm surrounding them is removed. While there is little current spread between glial cells and neurons, current spreads readily from one glial cell to another. The important conclusion is that not the glia, but the narrow intercellular spaces which resemble those in vertebrates must be the path for the rapid diffusion of K^+, Na^+, sucrose, choline, and other small molecules; these substances can be shown to reach nerve cells without first passing through the glial cells. Kuffler calculates that if these spaces are 50 A or more, there should be no serious restriction on the movements of such molecules. R. Galambos (New Haven) reviewed the diverse sources of evidence concerning the physiological role of glial cells. He emphasized their participation in myelination and demyelination and their chemical changes with respect to RNA during learning in rats.

Earthquake Prediction

Research related to earthquake prediction was the subject of a conference held in Tokyo, Japan, 9–20 March 1964. The general tenor of the conference was that, while specific earthquake predictions are not now possible, a number of promising lines of investigation offer the hope that at some time in the future fairly detailed predictions can be accomplished. The lack of a satisfactory technique at present was demonstrated rather graphically after the conference when, without warning, a large earthquake devasted much of southcentral Alaska.

Generally speaking, the matter of earthquake prediction is taken much more seriously in Japan than in the United States where mention of the subject frequently draws comments of derision or hopelessness. Most Japanese think of the earthquake as one of a sequence of events, some of which precede the main shock and have properties which may very well be measurable and indicative of the earthquake to follow. Some Americans, on the other hand, tend to associate the term "earthquake prediction" with various schemes in the realm of astrology, or numerology, or some other form of mysticism or occultism, and thus overlook the possibility that sound scientific principles can be applied to the problem with some hope of success. The relative seismicities of the two countries must affect these attitudes; nearly all Japanese are continually reminded of the problem, while most Americans are not. The participants in this conference approached the problem with the gravity and thoughtfulness it deserves.

The opening session on the subject of general considerations was chaired by K. Wadati and a vigorous discussion, led by C. Tsuboi, developed and separated the optimists from the pessimists. It was pointed out that earthquake prediction is now a fact in the sense that we can predict where, although not when, most large earthquakes will occur. In response to the question of what sort of prediction is desired or required, two basic kinds of prediction evolved. One concerns the probability that potentially destructive earthquakes will occur in a given region, together with estimates of their effects, so that locations for man-made structures may be chosen wisely and the structures designed appropriately. Times of the order of the life of the building are of interest here and property damage is a principal concern. Much progress has already been made along these lines in certain areas. A second desired type of prediction would foretell the time of a large earthquake in a given area within minutes, or hours, or even days so that personnel might take proper precautions and injury or loss of life could be prevented. Less accurate predictions are also valuable; emergency supplies might be stored, for example, in areas where a shock is expected within months.

Many felt that prediction techniques might not be generally applicable; that is, one technique might work in one area, and a different technique might be suitable for another area. A parallel with the case of volcanic eruptions was demonstrated. Eruptions are now reasonably well predicted for a few volcanoes whose habits are well known, although different in each case. Because the relatively infrequent occurrence of destructive earthquakes in a given area may not provide sufficient data for thorough study by seismologists, it was emphasized that collection and storage of data must be done wisely now in order to provide the best possible information for succeeding generations.

In a session on crustal deformations measured by geodetic surveying, discussions centered on the past and proposed extensive triangulation and leveling surveys carried out in Japan on a scale rarely contemplated and never carried out in the United States. The value of such surveys was clearly demonstrated by numerous examples related to Japanese earthquakes. In fact, some well documented cases were reported where Japanese citizens without the aid of instruments made observations just prior to the shock of striking land deformation. Special leveling and triangulation surveys of the Mitaka Rhombus, a small-scale set of fixed points, showed deformation just prior to earthquakes. Emphasis was placed on the difference between California earthquakes and Japanese earthquakes. Movement in the former is largely horizontal, while movement in the latter is more often vertical.

Crustal deformations measured by tide gauges, strainmeters, and tiltmeters were discussed. One serious difficulty in all such measurements is that effects on the instruments by tectonic movements must be separated from those due to meteorological and oceanographic effects. There was discussion of the question of rate of strain accumulation; some feel it is rather uniform, others feel that it is somewhat sporadic. It was generally agreed that strain accumulation varies from place to place even in the same tectonic zone, such as the San Andreas rift zone. Related to this is the question, unanswered at this conference, of whether small shocks in an area mean strain relief or strain accumulation. While data on measurements of secular strain are sparse and generally not very reliable, the upper limits for secular strain in Japan, California, and New Jersey correlate well with the degree of seis-