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Neural Integration at the Mesoscopic Level:

the Advent of some Ideas in the Last Half Century

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Introduction

History seen by a professional historian, based only on the documented record, always incomplete and liable to bias, can be unreliable. Modern history seen by a protagonist must surely be among the most unreliable. My only excuse for this effort is that I was invited by the relevant Society committee. My reason for accepting is that I feel even the fragmentary part of neuroscience I can speak about is a human drama, romantic and exciting, and a flood on which we are floating, unable to dump the baggage of past biases. Our points of view, priorities, and positions on all the controversial issues and even the well established, noncontroversial ones, are not as rational as we would like to think but are strongly conditioned by where we came from.

I will depend mainly on selected vignettes of the way things looked when I was a student, a young postdoctoral fellow and an Assistant Professor, to compare with the way they look to me or to others now, in each of half a dozen mesoscopic domains. I mean by mesoscopic domains the middle levels - those in between the most basic subcellular or molecular and the higher levels of learning and cognition. The half dozen domains constitute of course, anything but a representative fraction of neuroscience. I believe, however, that they add up to a nontrivial segment of the big picture with respect to the integrative aspects of our science. Most of the fronts that grew into today's popular branches of our science are not represented but a small set of particular interest and probability of further surprises.

The Doctrinal Neuron as a Functional Unit

In 1936, when I was a graduate student at Berkeley, the **neuron doctrine** of Cajal (Jones 1994) was well established and accepted. There were still dissenters, mainly microscopists who said they saw fibrils or wisps of cytoplasm

passing from one neuron into another. But, on the whole, they were not very successful in convincing people. At most their material was regarded as exceptions to a well documented rule. Incidentally, Rafael Lorente de No never accepted that the third order giant fiber of squid is a confluence of processes from many neuron somata of ordinary size, a view otherwise generally accepted since it was first described by J. Z. Young in 1936. The confirmation of Cajal that came with electron microscopy many years later - not with the first good pictures but gradually, as more and more examples were described, was no surprise or upset.

The main contrasts with current views were in the physiology. Ever since all-or-none spikes became easy to record, at first in fine twigs of sensory nerves in frog's toes in the '20s, and for a long time, even today in some quarters - spikes were all. The neural activity of neurons **was** the action potential; that is, the only potential of action known was the all-or-none impulse.

In order to begin a list of names and dates, let me select McCulloch and Pitts (1943) to represent this period when the whole neuron was a unit and acted together throughout, when we had no idea of multiple forms of spikes like the simple and complex spikes of cerebellar Purkinje cells, or of spikes that don't invade the soma or dendrites or of spikes that fail at branch points or short of the axonal terminal, or of modulation of transmission.

Now, the fact had already been found by Katz in 1937 and Hodgkin in 1938 that axons can show something besides all-or-none spikes. This is the **local graded activity**, above the electrotonic spread from a nearby impulse in the axon. Although this local graded activity is without or before the explosive event of a nerve impulse, it is highly nonlinear with increments of subthreshold stimulating current. It was recognized immediately as a major claim, basic to our understanding or working model. But it was not instantly accepted generally. It was disbelieved and resisted by some, for years (including C.A.G. Wiersma who worked, as Hodgkin did, with crustacean peripheral motor axons). Only slowly did it work its way into the normal picture of axonal activity, especially near the terminals. It is now widely believed that spikes die out, at least often, if not generally, and much if not most transmitter release is under the control of this graded, local activity. This is often forgotten, however, even today.

The first direct demonstration of graded **junctional** potentials was accomplished intracellularly, in the neuromuscular junction, by Paul Fatt and Bernard Katz in 1951, followed in a few years by Eyzaguirre and Kuffler who found them in 1955 in a neuron, the crayfish stretch receptor. In the same year Coombs, Eccles and Fatt found them in the mammalian CNS. These were exciting discoveries and were instantly accepted. People realized a new set of questions was now opened up.

Even earlier, in 1947, D.A. Parry claimed to see **spikeless neurons** in an insect ocellus, that is neurons which transmitted normal signals of light reception to the central ganglia but without ever firing a spike. His work was forgotten and a long controversy ensued over the absence of spikes in the Limulus retinula cells. H. Autrum and D. Burkhardt (1960) settled the question for insects by recording spikes intracellularly from visual sense cells in flies, followed by Ken-Ichi Naka (1961) in bees. The most unequivocal spikeless neuron was given to us by S.H. Ripley, B.M.H. Bush and my former student Allan Roberts in 1968 from a large stretch receptor in the base of the legs of crabs. Although the sensory region of this large neuron is some 10 mm from the nearest central ganglion (in a small crab) and the graded receptor potential spreads along its large diameter axon with a great decrement, it is still adequate to mediate normal reflex movements. This anomalous neuron turned out, over subsequent years, to be a model of many.

Frank Werblin and John Dowling gave us spikeless bipolar cells in the vertebrate retina in 1969. We now know there are large numbers in the central ganglia of insects and in the retina of vertebrates. Spikeless neurons, it was recognized, opened up another can of worms; their discovery was cheered by some and grudgingly ignored by others, according to their predilections.

Years ago I placed my bet that they will be found in the brains of mammals, once we learn how to recognize them or to penetrate many cells with intracellular electrodes. That bet has not paid off yet, but it still holds. Even more common, I believe, will be found neurons that spike, but in between spikes exert a continuous and nonlinear graded influence on the next neuron or on neighbors. This can be either by continuous, graded release of transmitter which was suspected long ago and demonstrated by Katherine Graubard, John Raper and Dan Hartline in 1977 (Graubard et al. 1980), or it can be by electrical field effects.

Field effects are well established as a class of mechanisms but not well known in respect to requirements such as synchrony of sets of neurons or geometric relations with receiving neurons or effective portions of the frequency spectrum. Already early in the half century we are revisiting, electrical field effects were considered by some authors to be likely. In 1936 Alfred Fessard and in 1941 Ralph Gerard, one of the founders of the Society for Neuroscience, as well as Frederick Bremer in 1944 speculated that neurons can influence each other without impulses by graded signals and I agreed with them in 1945. Hansjochem Autrum (1952, 1953) in Göttingen, about the same time also invoked this idea in his theory of fast insect eyes. Psychologists thought they disproved the possibility of field effects by placing metal foil or grids over the rat cortex or rows of pins into the cortex, to shunt at least the gross fields. They reported no failure of rats to learn and to run mazes.

In 1956 Carlo Terzuolo and I imposed a very weak, subthreshold field onto a preparation of the stretch receptor neuron of a crayfish and saw changes in its tonic firing rate when the field was about as strong as some of the larger evoked potentials and spontaneous brain waves. This surprisingly high sensitivity was one of the main motivating factors that led me in 1960 to look into **electroreception** in electric fish, where still higher sensitivity, by orders of magnitude, must be found - small fractions of a microvolt per centimeter in the water around the fish. The fascinating story of the discovery of electroreception, which goes back before 1941, would take us too far afield in this essay (Moller & Fritzsch 1993). When my colleagues and I found the afferent axons for normal, physiological electroreception in the lateral line nerve of gymnotiform fish (Bullock et al. 1961), it soon became clear that specialized electroreceptor neurons can vary over an extremely wide range in their sensitivity to electric fields. Some can go down to a behaviorally significant gradient of 0.005μV per centimeter (Kalmijn 1988); others normally function with tens or hundreds of microvolts per centimeter (Bullock & Heiligenberg 1986). They are typically tuned to preferred frequencies from <10 Hz up to hundreds and even several thousands of cycles per second. The proposition that brain waves, evoked potentials and other kinds of normal electric fields might be, not only effects but also causes, not just the noise of the engine but in some degree and some places modulatory was put forward long ago, in the '30s and '40s (references in the preceding paragraph). It is still far from proven or delimited in terms of strength, frequency and places in the brain - but I am betting it will find its way into the popular working model of how the brain works any day now and it will become fashionable to study when, where and how!

I want to mention one other early claim that is closely related. Robert Gesell in 1940, in connection with his model of the neural control of respiration, proposed that the **neuron has DC polarization** along its axo-dendritic axis and that this exerts some influence in controlling

firing rate; a kind of self-generated field effect. This idea is still not solidly confirmed but seems to me quite likely, in some degree or formulation - and I look for it also to work its way into our orthodox view of how the brain works. As you see, I am choosing examples that happen to underline how much of history consists of unfinished stories on hold until the next push achieves an apparent closure, or adds another complication. These stories also underline how subjective are the attitudes, priorities and bets of the day, in this enterprise we like to think of as objective and reasoned!

The Controversy over Soup vs Sparks

The controversy over chemical vs electrical transmission at the synapse raged for years. For a long time it was just over peripheral junctions such as the neuromuscular junction and only slowly spread to include central synapses as well. The reason it raged for years was not that the evidence for electrical transmission was strong but that the arguments for chemical transmission were indirect and came from drugged preparations, often in a subnormal state. Evidence for electrical transmission was positive and required preparations in very good condition, although not really normal junctions - the ephapse, including crosstalk in nerves and spinal cord. One should mention Herbert Jasper and A.M. Monnier in 1938, Bernard Katz and Otto Schmitt in 1940, Arturo Rosenblueth in 1941 and Angelique Arvanitaki in 1942, as well as the finding of reflection from cut ends of nerves and tracts, that is the synapse-like crosstalk from some stimulated fibers in a nerve to others (Granit & Skoglund 1945). These are all special cases, not ordinary, normal synapses.

The best evidence for excitatory chemical transmission was negative evidence, that is, the direct demonstration that transmission could not be electrical. This was first announced in a dramatic meeting of the Royal Society on February 21, 1952 (Brock et al. 1952) by John Eccles, previously a champion of electrical transmission. He and his colleagues had penerated the motoneuron and found the hyperpolarization caused by inhibitory input; this could hardly be understood to result from arriving impulses of the same electrical form as excitatory impulses that cause depolarizing synaptic potentials. A specially favorable example was the squid giant synapse. I had found already in 1946 (Bullock 1946) that this was uniquely suitable for studying the unit synapse. With Susumu Hagiwara we penetrated both pre- and postsynaptic sides of this giant junction and found the clear absence of any sign of the prespike during the synaptic delay in intracellular recordings in the postunit (Bullock and Hagiwara 1957). The much earlier and classical evidence, namely findings of so-called transmitters in the soup around junctions, recognized to be "hypodynamic," and only when they were protected from cholinesterase by drugs that inhibited the esterase, was suggestive and quite sufficient to start a large following, but was not convincing that normal, healthy junctions worked this way.

The controversy simmered down only as two independent lines of evidence improved, in a succession of small saltations. The evidence for two kinds of electron microscopically defined synapses gradually became convincing - gap junctions and cleft junctions with subsynaptic apparatus. The pharmacological evidence for normal chemical transmitters became convincing and the physiological evidence accumulated that gap junctions work electrically. Although chemical transmission had the upper hand, Furshpan and Potter's beautiful demonstration in crayfish giant-to-motor synapses in 1957 was instantly hailed as proving that electrical junctions exist.

Now, I will guess that most workers today look back with some amusement at this long debate and regard the matter as finally settled. We understand today that there are chemical and electrical synapses and we look for advances in working out the molecular machinery of each. Well, I am ready to wager that the last laugh is some time off and our students will remember this stage, with amusement, as a short plateau or lull in the controversy. I am not ready to say where I think it will go - in which direction it will break out and change the issue; I have some bets but this is not where they belong. Among the names we should remember, even though I cannot detail here just which advance each was responsible for, or list all the worthies, are Otto Loewi, Sir Henry Dale and W. Feldberg, Paul Fatt and Bernard Katz, Sir John Eccles and Steve Kuffler, Carlos Eyzaguirre, and David Nachmansohn - each for distinctive contributions.

Integrative principles in small sets of neurons

Moving up a level of complexity, it was just over a half century ago that we began to see the integrative properties of synaptic transmission analyzed parametrically in a space whose variables are still increasing today. This domain overlaps with the physiological aspects of what Pasko Rakic named local circuits, the intimate functions between consenting neurons.

As the first example I turn to another way in which the neuron doctrine began to be refined from the McCulloch-Pitts all-or-none element. It represents one of the long list of "personality traits" of neurons, the elementary integrative property called **facilitation**, a junctional trait that was measured long before synaptic potentials were known. At a gross, reflex level we can recognize it in Sherrington's experiments on reflexes of mammalian limbs. But Carl Pantin at Cambridge in 1935 - actually at the Plymouth Marine Laboratory, quantitatively demonstrated it at the unit level in sea anemone nerve nets and in crustacean neuromuscular junctions, followed immediately by Kees Wiersma and Anthonie Van Harreveld (1938) in Holland, shortly before they came to Pasadena. The concept of facilitation was required by the evidence of a graded and local, highly nonlinear, subthreshold residue after an arriving impulse that failed to fire the postsynaptic unit but left it closer to threshold. This invisible, inferred change, whether pre- or postsynaptic, was found to be tuned to a preferred interstimulus interval. The preferred interval differs widely among the junctions of different muscles within sea anemones and among different neuromuscular junctions in crabs and crayfish and it was shown to have a decay with, sometimes, two or more time constants. Since this is an integrative form of neuronal action first found in neuromuscular junctions, quite unlike the familiar vertebrate type that transmits 1:1, Pantin and others spoke of crabs "thinking with their claws." As a crucial variable in determining output as function of input, it is remarkable that only slowly did it become a part of the everyday model of neuronal action people use although it was soon shown to be widespread.

Its opposite is a common **interval-dependent depression** of the postsynaptic potential, sometimes called antifacilitation or difficilation; it was also found to be widespread. My colleagues and I in the late '40s and '50s had a hand in all this, especially Donald Maynard (1955, 1967), Susumu Hagiwara (Hagiwara & Bullock 1957), Carlo Terzuolo (Bullock & Terzuolo 1957), and Akira Watanabe (Watanabe & Bullock 1960) in intracellular recordings from the squid synapse and lobster cardiac ganglion cells. These were very heady days, with exciting new parameters of neuronal integration coming along one after the other. Presynaptic inhibition and excitation, chemical *and* electrical transmission, sometimes in the same cell, different chemical transmitters in the same cell (contrary to Dale's Principle, as it was then

understood), slow postsynaptic potentials - lasting for seconds and even minutes (Laporte & Lorente de Nó 1950; Eccles and Libet 1961; Tosaka et al. 1968; Nishi and Koketsu 1968) not dependent on conductance increase and showing long-term modulation of one transmitter action by another transmitter (Libet & Tosaka 1970) are just some of them. Diversity in afterpotentials, in sensitivity to depolarization and in apparent neuronal codes are others. New integrative variables at the neuronal level are still being found in 1994. Besides these general neuronal parameters, back there in the 40s and 50s we were stimulated to widen our working model of the brain by related discoveries such as the first tonic receptors and sensory thresholds that showed hypersensitivity to light, sound, infrared, electric fields and olfactory stimuli.

Another of the principles that rose from the sea to astound us was **lateral inhibition**. This sprang full blown from the head of *Limulus* one day in 1953 thanks to discoveries of Keffer Hartline, Henry Wagner, and Tsuneo Tomita in a major step from their previous concentration on the primary receptor toward the central, multiunit interactions. It is difficult to exaggerate the excitement that followed the discovery of this process. It was immediately clear what an expansion in degrees of freedom throughout the nervous system it would represent, if lateral inhibition is not confined to the optic ganglion of a strange, living fossil for which there was still no evidence then that it can see. Fortunately evidence that lateral inhibition is indeed found widespread in the animal kingdom and in the brain came rolling in - not rapidly but steadily. The general feeling was, in a current phrase, "We needed that!" It reminds me of the similar sense of a sudden expansion of the degrees of freedom and an approach to the true complexity, compared with the simple and, so we thought, adequate mechanisms of a McCulloch-Pitts nervous system, when David Lloyd first gave us **direct neural inhibition**, in 1941, previously unknown except as refractoriness after excitation. And the same kind of saltation, multiplying all previous notions of the basic devices available to organized sets of neurons came with the demonstration of **presynaptic inhibition**, already touched upon. Eccles (1961) presented arguments for interpreting earlier observations of "remote inhibition" in mammalian spinal cord as direct transmitter action of inhibitory nerve endings near the synaptic terminals of excitatory axons and Dudel and Kuffler (1961) showed this in favorable crustacean preparations. In this as in other instances of major discoveries, it is humbling to read the literature just preceding, with extensive and sophisticated knowledge unaware of the vast change just around the corner. One wonders what surprise is in store for us tomorrow.

Two other principles are the **functional refractory period** and **nuclear delay**. These early insights have had less influence on everyday thinking than they should. They were due largely to Arturo Rosenblueth (1941, 1949a, b) or at least he articulated well what was a common understanding at the time. We need to be reminded of these concepts because many authors today tend to think in terms of the fastest recovery curve under maximal test stimuli and of the minimum synaptic delay of ca. 1 ms in the special case where a postspike can be attributed to a given prespike, usually requiring artificial synchrony of a group of presynaptic axons. The **functional refractory period** is the time it takes to recover excitability to a level where the normally available stimulus will excite. It is therefore not a fixed number but depends both upon the recovering membrane and the exciting event. And it is not a curve as the relative refractory period is, but a number that applies to that situation. **Nuclear delay**, as opposed to the minimum synaptic delay, is the time it takes for activity to emerge from a functional system, which is normally less than a brain nucleus but more than a monosynaptic junction receiving synchronized input. Nuclear delay is a useful concept when thinking about normal function even though it has no sharply defined input time or output time, both being barrages or bursts of spikes. The delay can be tens of milliseconds, even hundreds and thousands, when only one synapse is in series, obviating the common interpretation that, if 20 ms transpires, there must

be something around 15-20 synapses.

My next example is one of the forms of extra-synaptic transmission between neurons, a still little-known list of mechanisms and pathways that I predict will become a major category in our everyday model of how brains work, instead of the fringe phenomenon that it is respectable to ignore - as was true in turn for a list of curiosities that slowly became standard options for neurons in real animals with hair. I am thinking now of **slow electrotonic interaction**. This is perhaps still not widely familiar. It was shown already 30 years ago in lobster cardiac ganglion cells that a subthreshold, intracellularly imposed depolarization in a follower cell can accelerate the upstream pacemaker cell, if the depolarization lasts long enough - something like 50 ms. If it is repeated at suitable intervals, it soon entrains the pacemaker. A brief but stronger depolarization that reaches threshold and fires the penetrated follower cell does not accelerate or influence the pacemaker at all. The electrotonic