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Electrical synapses between neurons synchronize gamma oscillations generated during higher level processing in the nervous system

by

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Editorial: A Tale in Two Academes

At the hour of depicting higher nervous processs and the psycho-physical nexus, the Anglo-American academe is by no means monolithic. Yet in general its views have long been consistent with a nervous system conceived as a system of commands transmitted by relays, as it was put by the celebrious definition by Louis Lapicque (1932) forwarded in the first volume of the *Nouveau Traité de Psychologie* edited by George Dumas. In contrast, the Argentine-German neurobiological tradition, foreign both to the influences of neuronism and behaviorism, evolved upon the turning-point models forwarded since 1906 by Christfried Jakob. As it locally is fairly known, these models in their earliest version presented the higher nervous dynam-

ics as taking place between "stationary waves" of neuroactivity kept by "reverberating microcircuits" in the gray, whose unsuitableness to account for long-term episodal memory – a prime concern for a neurobiology chiefly carried out in asylums, with a considerable proportion of dementized insanes – posed stimulating constraints since the beginning. When von Economo and Koskinas (1925; see in this journal's *Index* the articles devoted to their studies on Jakob's work) collated Jakob's and Cajal's contributions to the scientific description of the nervous gray, they mainly considered the less integrative, more anatomical contributions of Jakob and Cajal. The main disparity among these two scientists, however, was that, while Cajal embraced the application of the "system of commands by relays"'s view for the entire neurodynamics, Jakob rather kept beyond it a further level of integrative action to be physiologically as well as physically investigated. By the time, this further level was absent also from the descriptions of the integrative actions of the nervous system in the Anglo-American academe, so Jakob's appraissals of Cajal's contributions ("Santiago Ramón y Cajal: la significación de su obra científica para la neuropsiquiatría," *La Semana Médica* 34, 1935; "El significado de la obra de Ramón y Cajal en la filosofía de lo orgánico," *Humanidades* 26, 1938) addressed as well Cajal's views as those of his Anglo-American eponyms. While accepting a short number of different hierarchical levels for individual cells in the diverse functional organization of the neural tracts, Jakob in particular rejected chimaeras such as "psychical neurons" or "cerebral ducts for thought" (Cajal, *el cauce material del pensamiento*). Nevertheless, in Anglo-American academe Jakob was almost exclusively known by way of Economo and Koskinas' presentation, in spite of his well-known *Atlases* (quoted in several theses) and the exception made by some young people such as Donald Hebb and, in Holland, some Ariens Kappers' disciples (people from our tradition such as Ramón Carrillo, Manuel Balado and Braulio Moyano had worked for some time in Kappers' and German laboratories). Soon later, after 1942, when in peripheral nerves it was demonstrated that parallel axon's activity is reciprocally influenced by electric field effects, the electric-field interactions of the mentioned anatomo-physiological units within the vertebrate's neuropilar volume, *i.e.* among the "stationary waves entertained by reverberating microcircuits," were thoroughly discussed here. As Jakob mentioned it in 1949, even quantum effects in them were fleetingly considered. But it was in the 1960's, after Jakob's demise (1956), that the research of our neurobiological tradition – by then proceeding in comparative isolation, due to external and local circunstances – achieved the phylogenetic reconstruction (see Crocco, "¡Alma e' reptil! Los con-

tenidos mentales de los reptiles y su procedencia filética," *Electroneurobiología* 12: 1-72, 2004) needed to understand the complex physiological role of the electrical interactions among those units. It set the scenario to find out the localization of the causal operations involved in the psychophysical nexus, as required by the clinical observations of amnesia recoveries. In the Anglo-American academe, meanwhile, Cajal's system of commands by relays had instead become a solid prefiguration. Hodologies reigned without collateral effects. A few young researchers, among them the author of the present article Prof. Michael Bennett, started shaking them since the late 1950's – and their extensive work opened a new, wide scenario. It brings about the potential for a better reciprocal understanding of the work done in the two conceptual realms and, in the circumstances, Prof. Bennett's synopsis becomes of singular practical value. Let his word led us into the current landscape. MS

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Electrical synapses between neurons synchronize gamma oscillations generated during higher level processing in the nervous system

OUTLINE: The Neuron Doctrine transformed the 19th-century view of the nervous system, which saw the brain as a network of interconnected nerve fibers. A century later, the modern view holds the neuron as a discrete cell that processes information in more ways than originally envisaged. Intercellular communication by gap junctions, slow electrical potentials, action potentials initiated in dendrites, neuromodulatory effects, extrasynaptic release of neurotransmitters, and information flow between neurons and glia all contribute to information processing.

Neural information processing depends on communication between neurons. This communication occurs primarily at synapses, sites morphologically specialized for intercellular transmission. This statement avoids defining “specialized”, and the use of “primarily” allows for transmitter leakage and electric field effects without clear anatomical specializations. Moreover, glia may have time-varying influences on at least the slower neuronal oscillations.

Most if not all neurons express machinery for chemical transmission by secretion of a neurotransmitter from the presynaptic element that acts on a receptor in the postsynaptic element. Neurons also have genes to permit electrical transmission, *i.e.*, where an electrical potential gener-

ated in one cell affects a neighboring cell. Early in development most neurons form gap junctions, which constitute the common kind of electrical synapse, but only a minority do in the adult. Gap junctions are formed by connexins, a gene family of ~20 members in mammals. Cloning of Cx36, a (nearly) neuron-specific connexin allowed demonstration of the wide distribution of electrical synapses, particularly in sites where oscillations are prominent. Generally, electrical synapses mediate synchronization, but lateral spread and forward transmission of excitation also occur.

Although electrical transmission can be more rapid than chemical transmission, its speed of action is not necessary in generating gamma and related rhythms. (Electrical transmission may be required for the speed of high frequency "ripples", but the cellular basis of these externally recorded responses is as yet unclear.) Synchronization at low frequencies could be driven by chemical synapses, mutually excitatory or, less obviously, inhibitory. In the latter case, computer simulations show that reciprocal inhibition superimposed on tonic excitation can result in synchronous oscillation. Oscillation is driven by the tonic excitation (or can be an intrinsic membrane property). If one cell doesn't reach threshold when the others do, it is inhibited and then is ready to fire with the other cells in the next cycle.

Most cortical neurons must "choose" to release either the excitatory transmitter, glutamate, or the inhibitory transmitter, GABA. Gap junctions permit GABAergic cells also to be excitatory and to synchronize with other GABAergic cells more precisely than with inhibition alone. Inhibitory interneurons provide the pacemaker of the oscillations; principle cells and other downstream elements are synchronized, not by excitation but by inhibition. In the Cx36 knockout mouse, inhibitory interneurons are not coupled and oscillations in the external field are smaller but not absent. The continued oscillations depend in part on intrinsic membrane properties or tonic excitation, and reciprocal inhibition mediates some synchronization. The phenotype is benign, although results of cognitive testing have not been reported.

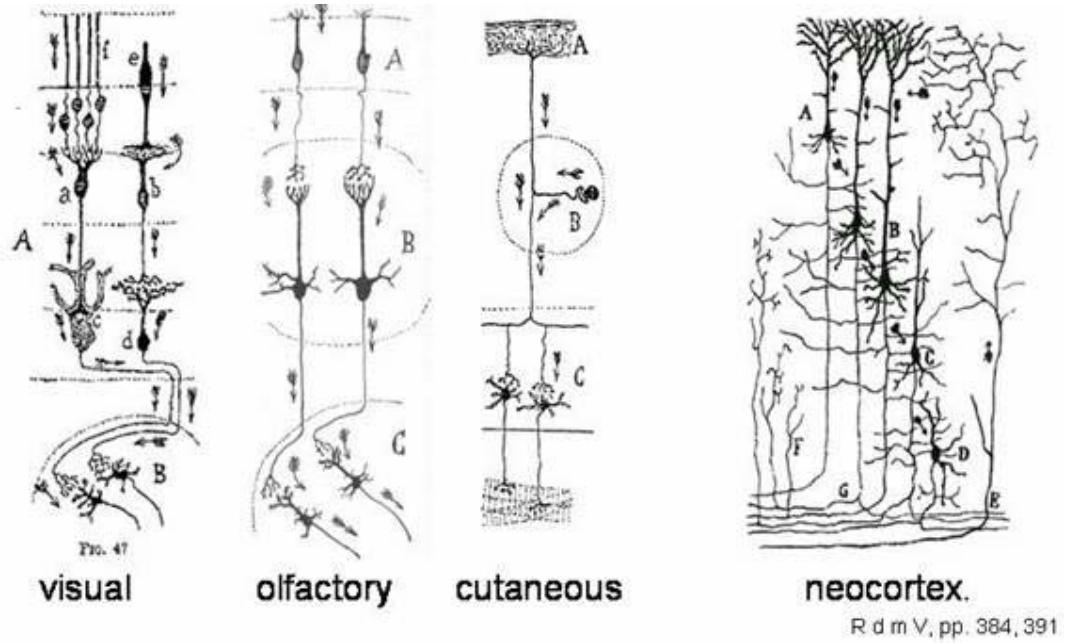
New methods of imaging and recording in vitro and in vivo make feasible characterization of microcircuitry of modules observed with earlier less inclusive methods. Neurons can express gap junction forming proteins, and electrical transmission is likely to be found wherever it is "useful." The relatively benign behavioral phenotype of the Cx36 knockout mouse indicates that synchronization of neurons by gap junctions confers a modest survival advantage in the laboratory, but quite likely a highly significant one in the real world.

1. Neuron Doctrine no longer encompasses important aspects of neuronal function

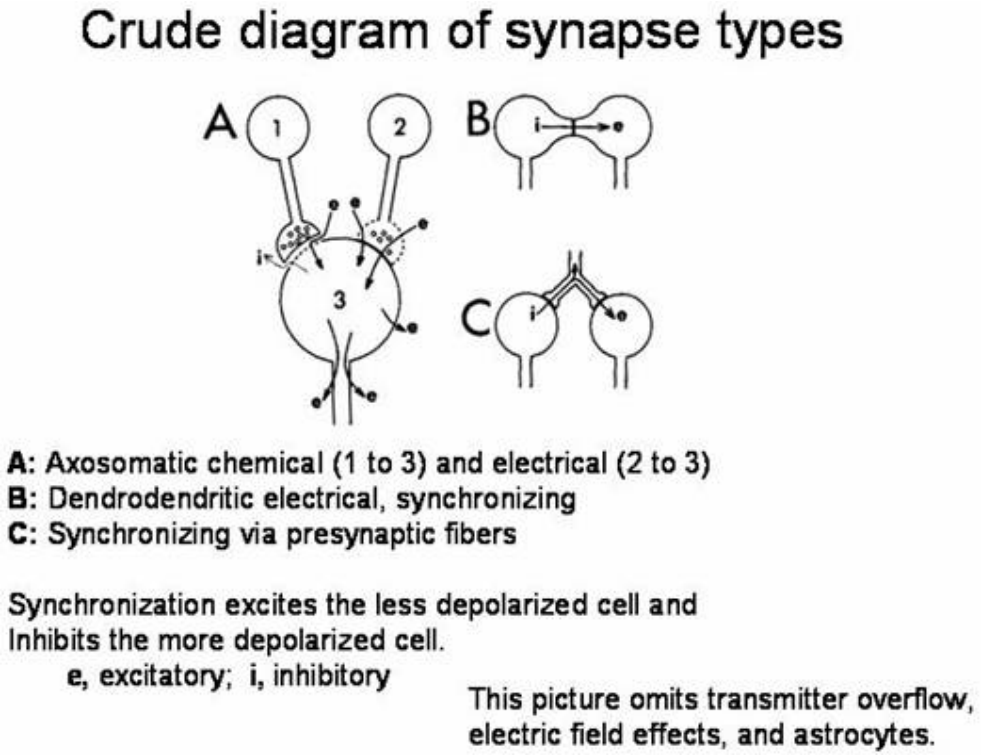
After a century, neuroscientists are rethinking the Neuron Doctrine, the fundamental principle of neuroscience. A modern view of brain cells allows a more proper if intricate perspective of how information is processed in the nervous system.

The formulator of the Neuron Doctrine was primarily the great Spanish anatomist and Nobel laureate Santiago Ramón y Cajal, arguably the Watson and Crick of neurobiology and author of *Recuerdos de mi Vida* (*Recollections of my Life*), which every student should read. Cajal argued that neurons interact at points of contiguity, later called synapses; he and others showed that there are no points of continuity as proposed by Golgi, one of the protagonists of the reticular theory. Neurons, Cajal stated, arise through differentiation of a neuroblast cell to become dynamically polarized: inputs are to dendrites, outputs are through axons.

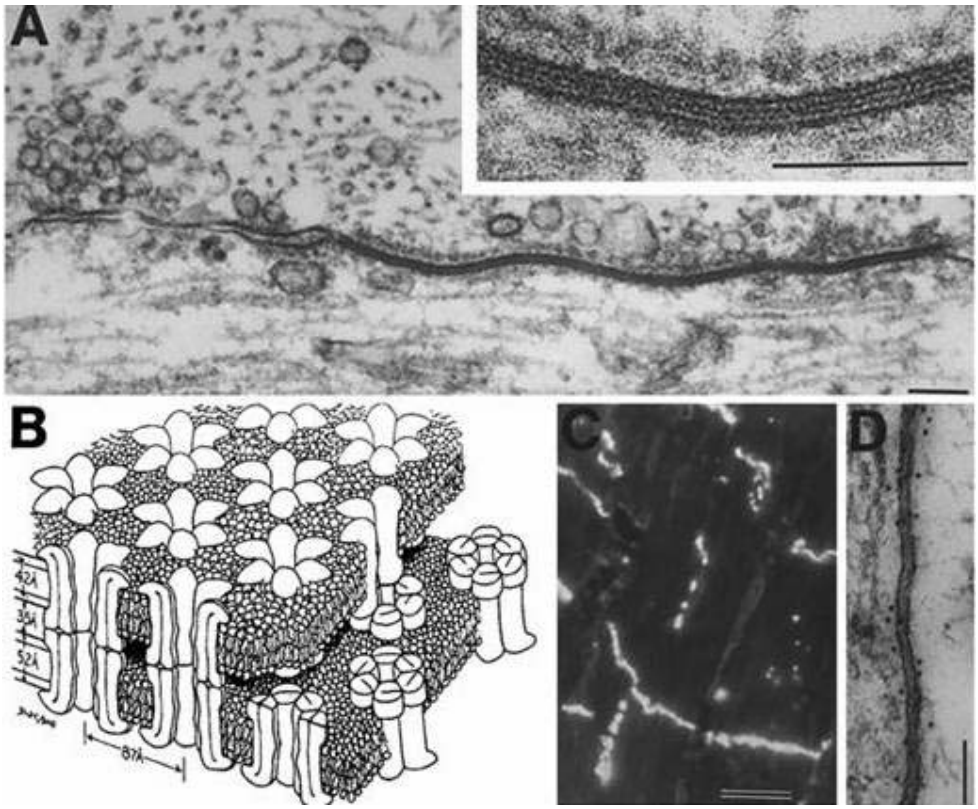
Separateness of neurons was an anatomical observation, at the time as much painstaking to generalize as rewarding. "Dynamic polarization" was ascertained on the basis of sensory inputs in visual, olfactory and cutaneous inputs and motor outputs, which data suggested a similar information flow in the neocortex.



One hundred years since its inception, an examination of the Neuron Doctrine indicates that it no longer encompasses important aspects of neuronal function. Technology and research have extended our knowledge far beyond the simple description that a neuron is an anatomically and functionally distinct cellular unit that arises through differentiation of a precursor neuroblast. And neurons are not the single functional units in the sense envisioned by early proponents of the Neuron Doctrine: non-neuronal constituents of the nervous system show a variety of unexpected participations in brain dynamics. If we are to understand complex, higher level neuronal processes, such as brain function, we need to explore beyond the limits of the Neuron Doctrine. We can no longer think the nervous system to function as a web of interconnected nerve fibers.



As physiological studies established that conduction of electrical activity along the neuronal axon involved brief, all-or-nothing, propagated changes in membrane potential called action potentials, it became often assumed that neuronal activity was correspondingly all-or nothing, action potentials spreading over all parts of a neuron. The neuron was regarded as a single functional unit: It either was active and "firing" or was not. This dogma began to erode with the advent of microelectrodes that could be inserted into neurons to record electrical signals.



Gap junctions, the most common form of electrical synapse

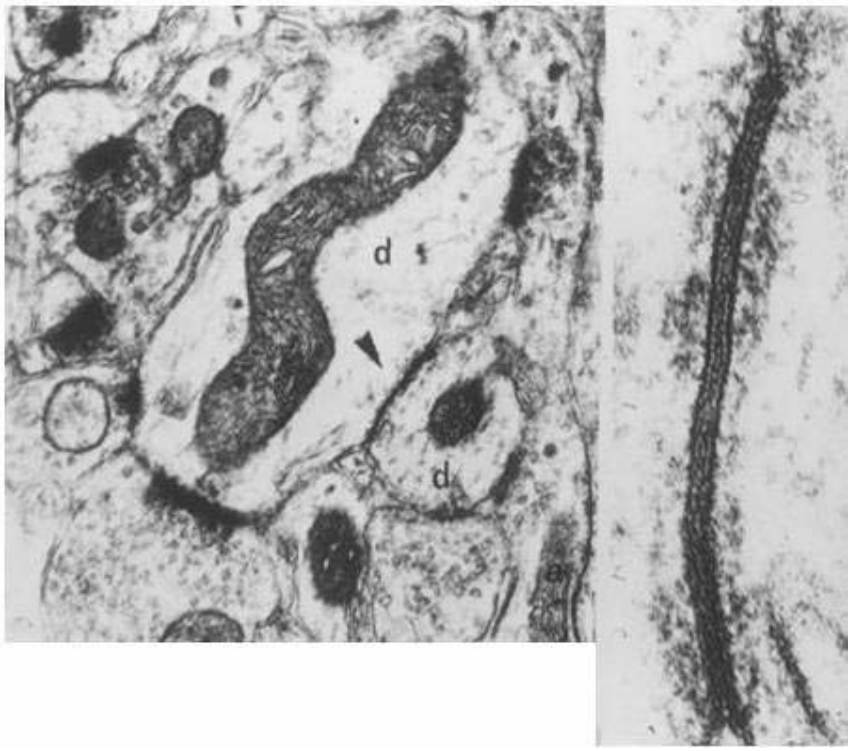
Before 1959, it was realized that much of the information processing by neurons involves electrical events that are graded in amplitude and decay over distance, developed in the past 50 years – notably single channel recording, live cell imaging, and molecular biology. Cajal wisely considered that "neuronal discontinuity… could sustain some exceptions" to the Doctrine's definition ("*la discontinuidad neuronal … pudiera padecer excepciones*"), as he wrote in *¿Neuronismo o reticularismo?*'s *Conclusión*.

Cajal also remarked that "It is clear that future techniques may contribute new and unsuspected arguments favoring the reticularist thesis, or other conceptions. A tiny improvement in a procedure's yield, or a histological discovery of general reaching, may force us to modify our conclusions. Nowadays, however, such revision does not appear either in close proximity or even as probable. We can therefore still adopt, without reservations, the brilliant doctrine of His, Forel, and Kölliker" ("*Claro es que la técnica del porvenir puede aportar argumentos nuevos e insospechados en favor de la tesis reticularista o de otras concepciones. Una pequeña mejora en el rendimiento de un método, o un descubrimiento histológico de alcance general, pueden obligarnos a modificar nuestras conclusiones. Mas hoy por hoy esta revisión no parece próxima ni probable. Podemos, pues, adoptar aún,*

sin reservas, la genial doctrina de His, Forel y Kölliker …"; Cajal, in *¿Neuronismo o reticularismo?*'s opening).

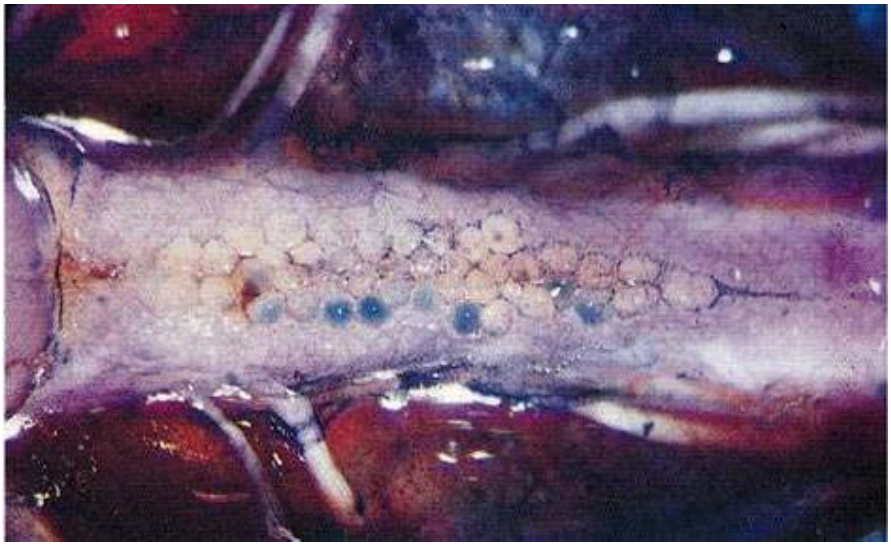
Even so, he could not have foreseen the presence and role of neuronal gap junctions as one of these exceptions. Furthermore, gap junctions have been described between neurons and non-neuronal cells such as astrocytes, a somewhat controversial finding not either conceived in the original Neuron Doctrine.

These assemblages of protein pores (B, above) form small aqueous channels of limited selectivity that connect neurons, providing cytoplasmic continuity. Their collections appear as regions resembling the active zones of chemical synapses, although there is no chemically mediated signal transmission and response. We now know that although only a minority of neurons form them in the adult, gap junctions are widespread in the mammalian nervous system and function to synchronize neuronal firing.

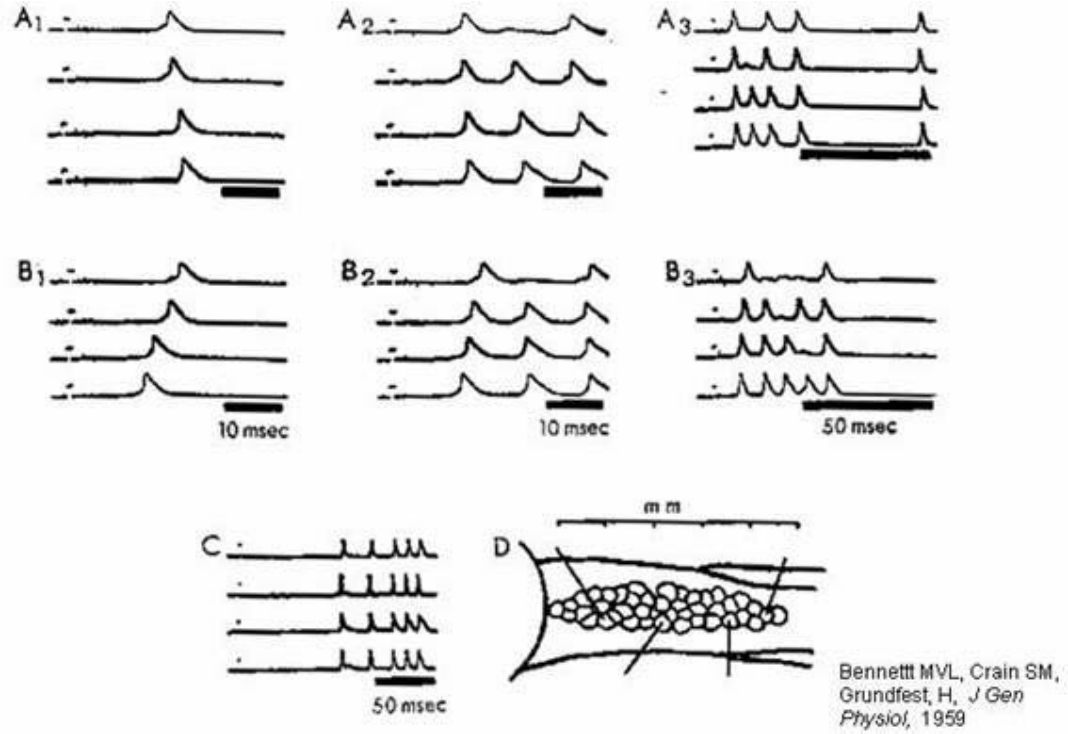


Dendrodendritic gap junctions in primate neocortex
(J. J.Sloper)

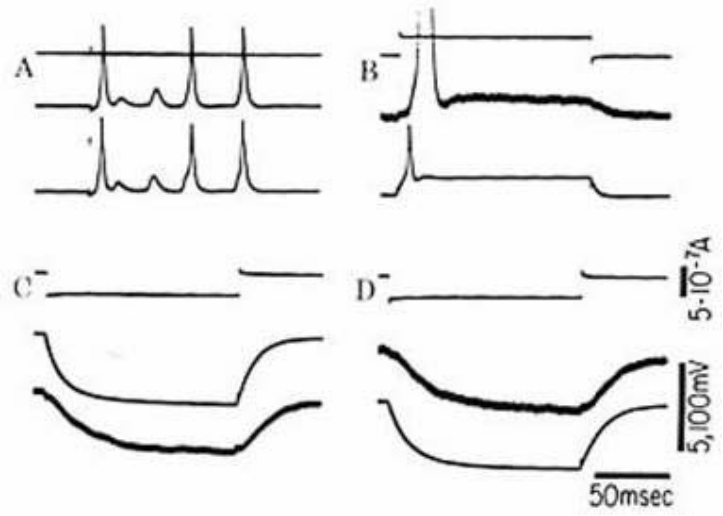
They constitute electrical synapses that couple groups of cells into functional syncytia—in this sense, the reticular concept, reinvoked.



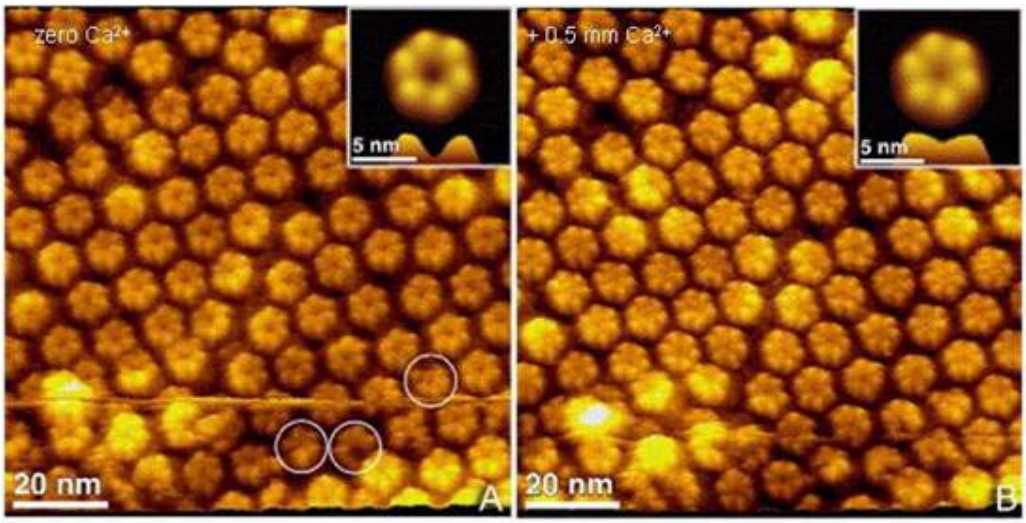
Supramedullary neurons of the puffer, *Spheroides maculatus*. (Freud studied similar neurons in the lamprey: Freud, S., "Über den Ursprung der hinteren Nervenwurzeln im Rückenmark von Ammocoetes (*Petromyzon Planeri*)," *Sitzungsbericht der kaiserlichen Akademie der Wissenschaften* LXXV, III Abtheilung; eds. Karl Gerold's Sohn, Vienna, Jänner bis Mai 1877).



Synchronization of neuronal firing by electrical coupling. ***D*** shows the placement of electrodes in the fish anatomy (previous figure). Synchronization depends on electrical coupling: see (next graph) responses to stimulating electrode.



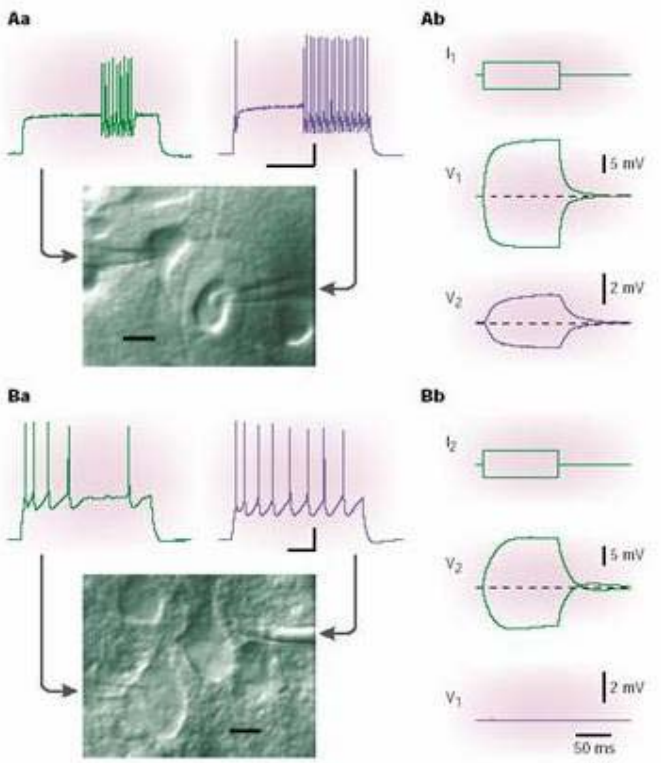
Although gap junctions can behave as simple electrical resistances between connected cells, an electrical impulse in one cell by no means inevitably propagates to the other cells with which it shares gap junctions. In fact, a channel within a gap junction is not necessarily open, and an entire gap junction may not transmit electrical current until it is appropriately modified in response to transmission from chemical synapses of the same, "presynaptic" neuron. This modulation of channels provides electrical synapses at gap junctions with the plasticity long considered an exclusive province of chemical synapses at axon-dendrite junctions (6).



Atomic force microscopy of a split open junction shows hexameric composition. Elevated Ca^{2+} causes the apparent pore in the center of the hemichannel to close.

2. Some recent experimental results

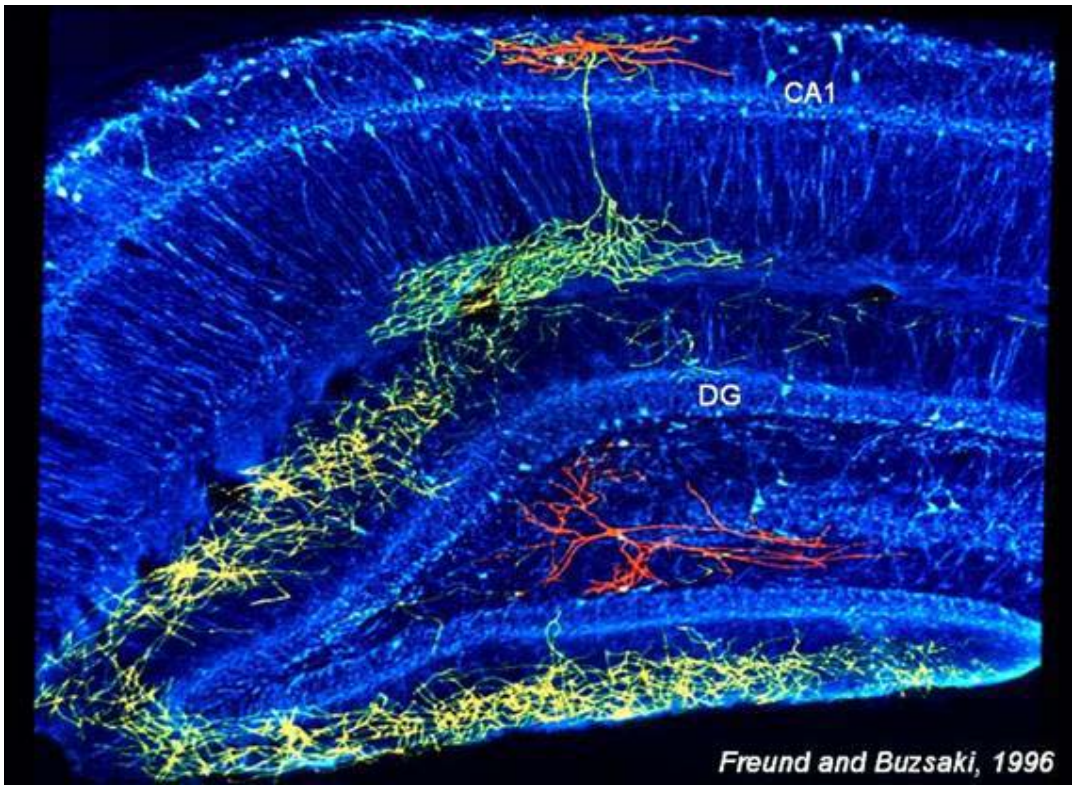
A plethora of neuromodulatory substances, such as amines and neuropeptides, can reconfigure neuronal circuits into different patterns of functional connection, capable of a variety of activity patterns (8). Such neuromodulation remodels neuron behavior and circuitry within minutes and hours rather than on the millisecond time scale typical of electrical impulse transmission. In addition, neuromodulatory substances can act at multiple sites on the neuron, including the axon. For example, some crab (9) and lobster (10) axons have receptors to amines such as dopamine, serotonin, and octopamine. When these amines are applied to the axons, these areas can spontaneously initiate action potentials in a nonclassical mode of integration.



Electrical coupling between a pair of fast-spiking (FS) GABA interneurons (above) and absence of coupling between pyramidal cells. Recording pipettes are obvious. When either cell is depolarized or hyperpolarized, attenuated and slowed potentials are recorded from the other cell. Demonstration by Hestrin and Galarreta, *Nature Rev. Neuroscience* 2, 524-433, 2001.

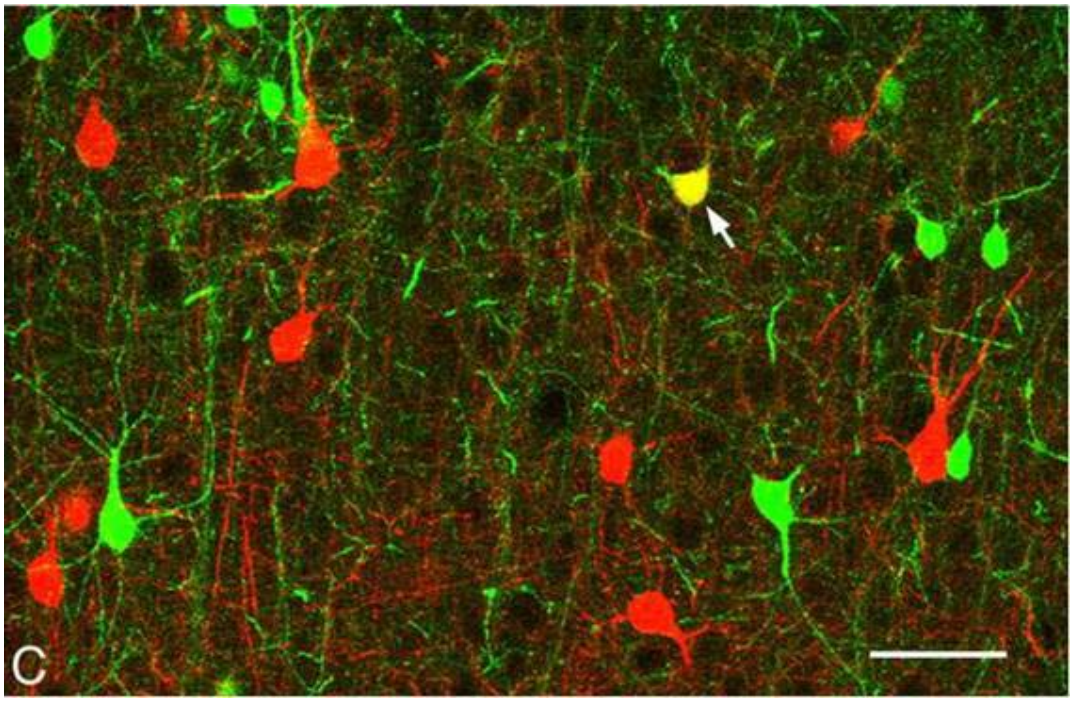
Research during the past ten years has shown that in many neurons, action potentials can travel backward from the axon and soma regions into the dendrites. Moreover, under certain conditions action

potentials can be initiated in dendrites, remaining local or sometimes propagating into the soma to initiate single or multiple spikes of activity in the axon. The functional complexity of dendrites and the roles they play in synaptic integration and plasticity are well beyond what could have been deduced from Cajal's anatomy or from later somatic recordings. Finally, the function, origin, and diversity of non-neuronal cells eluded Cajal, because a staining method, which revealed neuronal structure with brilliant clarity, left major classes of non-neuronal cells invisible (including microglia and oligodendrocytes). We now know that some of these non-neuronal cells partake in neuroactivity, through a complex superposed system, mentioned below, whose time scales are slower than those of neuronal exchanges.



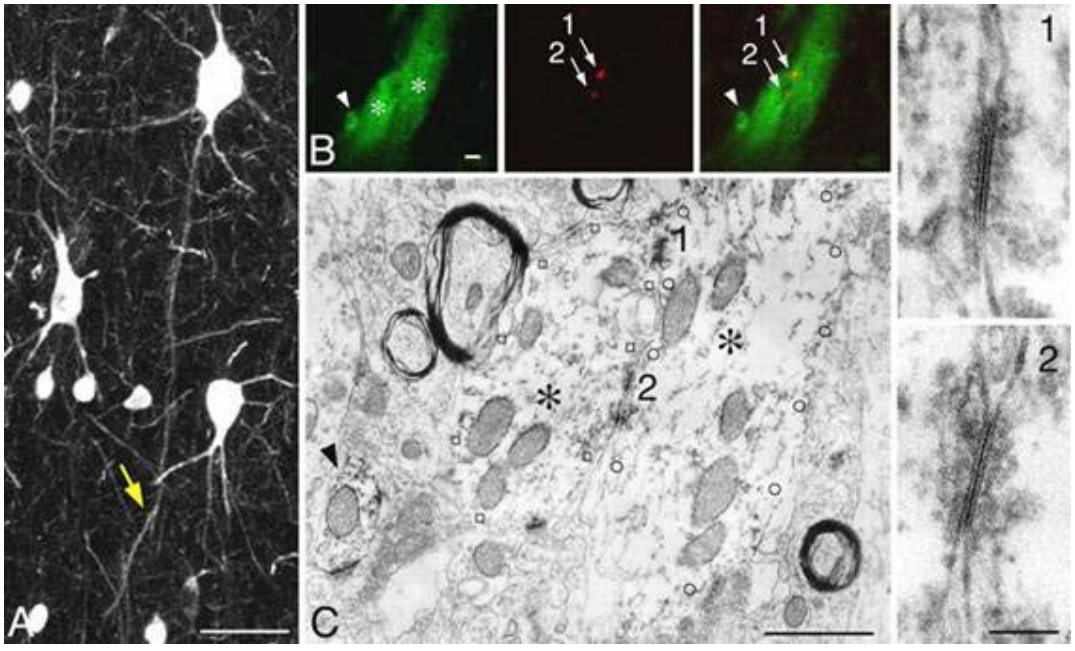
Hippocampal inhibitory axons (yellow and green) can synapse on many neurons. Cell body and dendrites shown in red.

Dendrites contain a mosaic of voltage-gated ion channels (13). The types, densities, and properties of these channels are very diverse among classes of neurons (and even within a single class), and these channels regulate, on wide-ranging time scales, how a neuron responds to the thousands of incoming synaptic events that impinge on its dendrites.

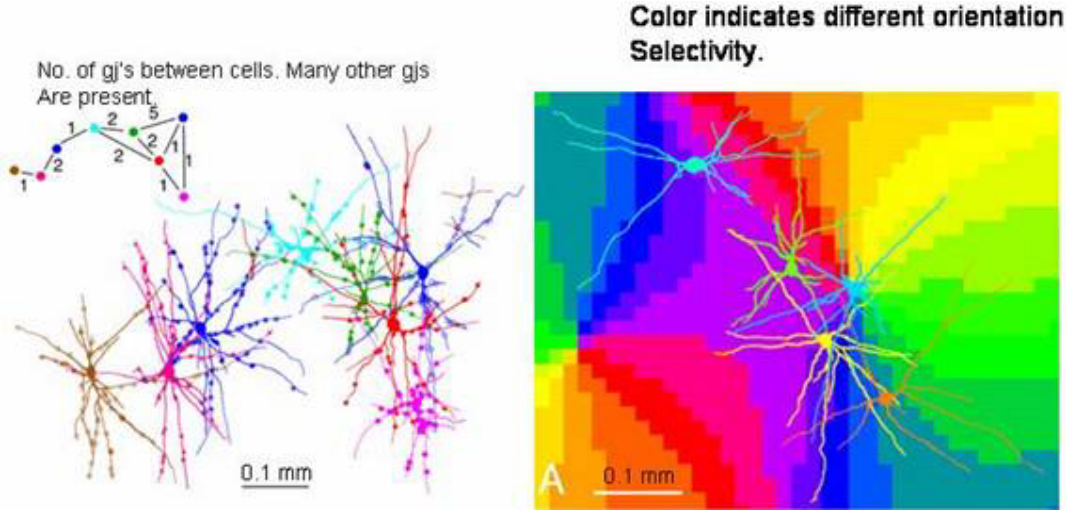


While parvalbumin (PV) is red, showing fast-spiking (FS) neurons on the above image; calbindin (CB) is green, showing low-threshold-spiking (LTS) cells. Neocortical inhibitory (GABAergic) interneurons are mostly either PV- or CB- positive. Source: Fukuda, T., Kosaka, T., Singer, W., Gauske, R. A., "Gap junctions among dendrites of cortical GABAergic neurons establish a dense and widepread intercolumnar network," *J. Neuroscience* 26, 3434-3443, 2006.

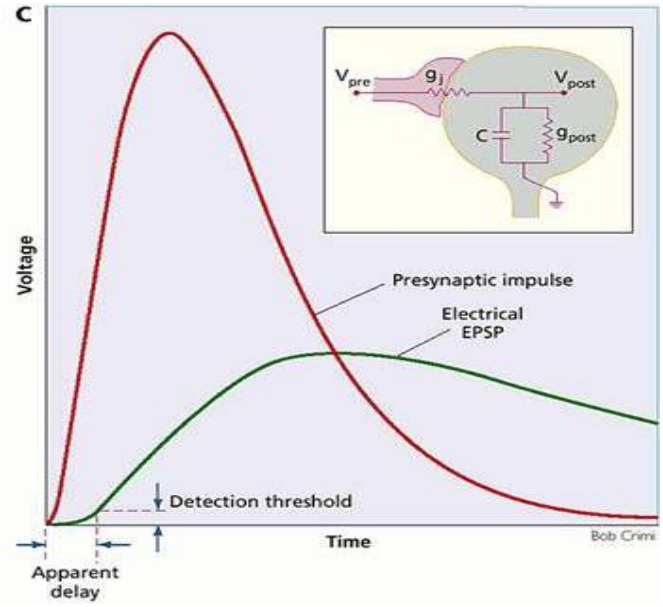
In the figure below, those tiny Cx36 immunoreactive spots (red) between PV neurons are actually gap junctions:



The dendrites of individual inhibitory neurons extend across the borders of orientation columns, as shown in 2006 by Fukuda, Kosaka, Singer, and Gauske ("Gap junctions among dendrites of cortical GABAergic neurons establish a dense and widepread intercolumnar network," *J. Neuroscience* 26, 3434-3443). It demonstrates that in visual cortex inhibitory dendrites extend long distances to make gap junctions with other inhibitory neurons. Thereto, these dendrites cross borders between functional domains.



Now, we should consider that the charging time of the postsynaptic capacity introduces delay at electrical synapses; another way of describing this feature is saying that they act as low-pass filters. Then there is further delay in reaching threshold and propagating the action potential to the next cell. (One may smile mulling over the fact that Helmholtz was right, nerve conduction velocity being surely finite even here.)



Explanation in next page.

(Previous page) Gap junctions in conjunction with postsynaptic capacitance behave as low pass filters. In the box, the equivalent circuit is given. The junctional conductance, **gj**, connects the presynaptic cell to the postsynaptic conductance, **gpost**, and capacitance, **C**, in parallel. The curves show calculated presynaptic impulse and postsynaptic potential for a reasonable ratio of impulse rise time to coupling time constant, but with a DC coupling coefficient of unity. The postsynaptic potential is attenuated and slowed. The slowing introduces a measured synaptic delay.

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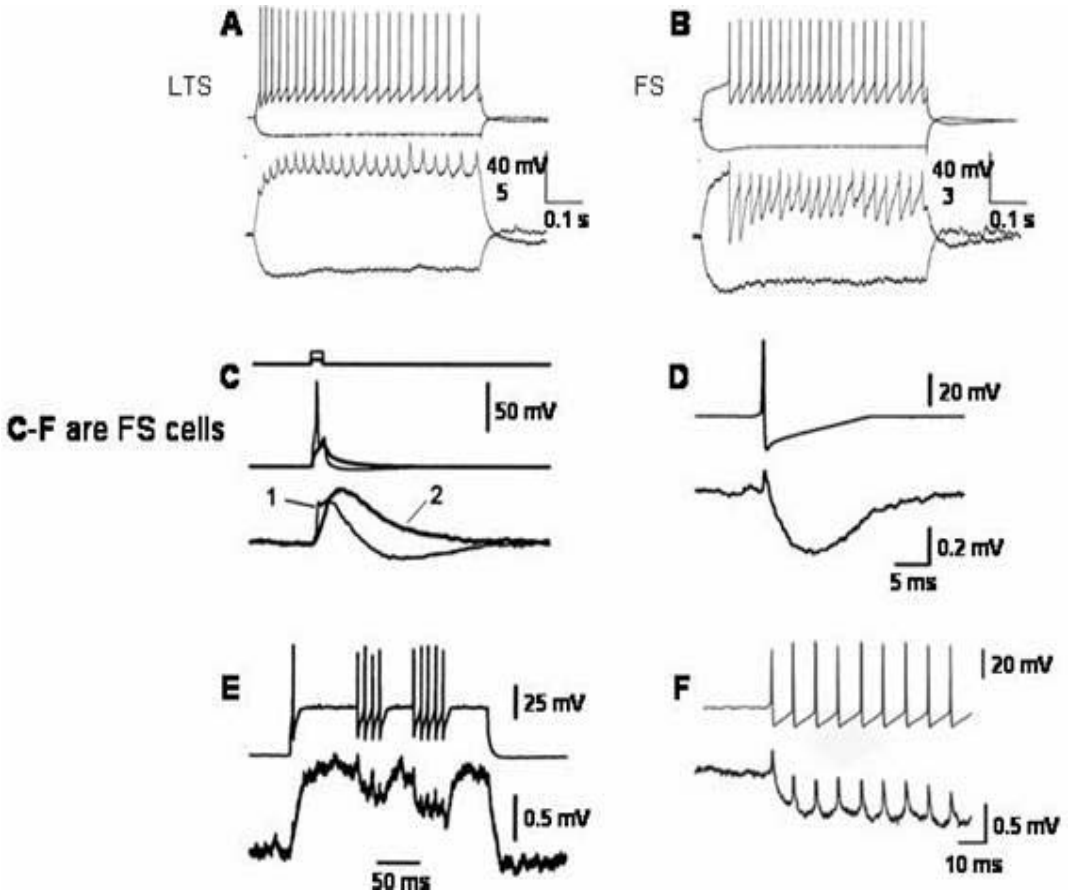
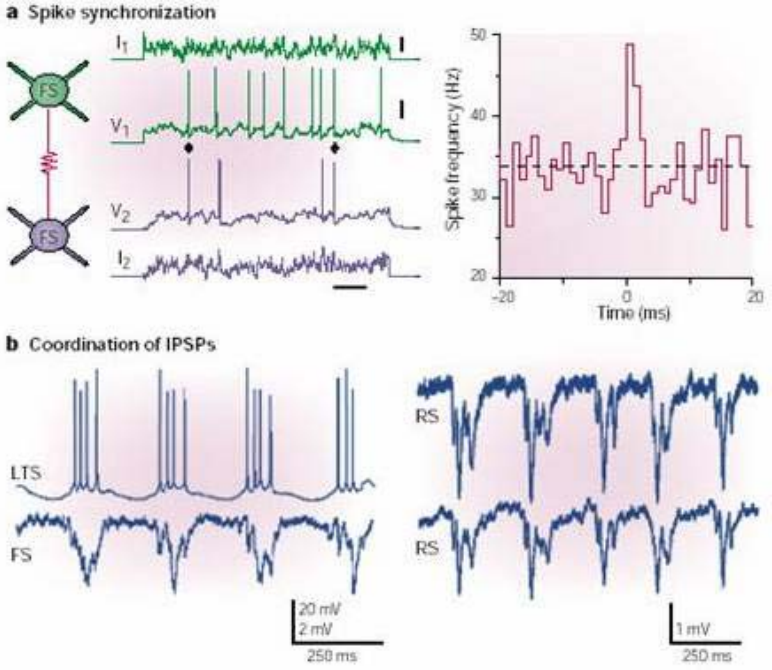
The transmitted spikes can led to net inhibition, depending on the afterpotential, as shown in the following results

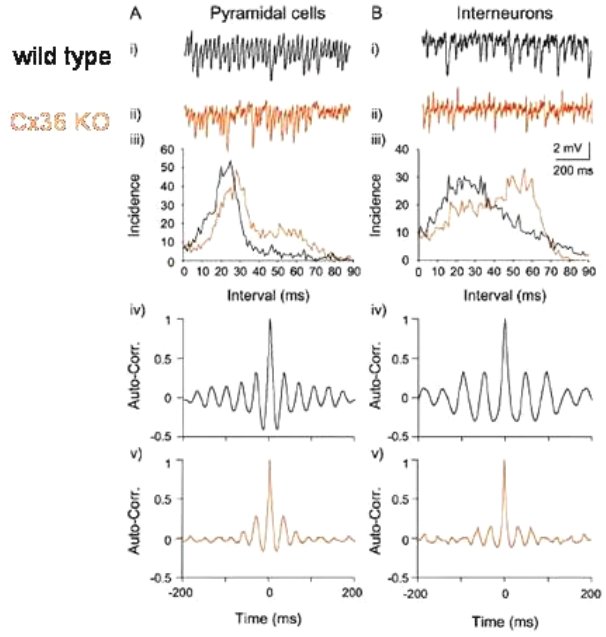


Low pass filtering at interneuronal electrical synapses: hyperpolarizing afterpotentials may lead to inhibition (A) Electrical PSPs of coupled LTS (low threshold spiking) interneurons summate so that depolarization increases during a burst of impulses. (B) Electrical PSPs of coupled FS (fast spiking) interneurons have an initial depolarizing spikelet, but the afterhyperpolarization then decreases the depolarization to below the level at which presynaptic firing began. (A) and (B) from Deans, M.R. *et al*., *Neuron* 31, 477-485, 2001. (C) Electrical PSPs from brief depolarizations just suprathreshold and subthreshold for an impulse in one of a coupled pair of FS interneurons. The subthreshold presynaptic depolarization (2) causes a monophasic and slowed postsynaptic depolarization. The suprathreshold stimulus (1) causes a biphasic postsynaptic potential due to transmission of the afterhyperpolarization; the depolarizing phase decays more rapidly in (1) than in (2). (D) Averaged postsynaptic responses of single presynaptic impulses evoked by a steady depolarization. These impulses have a greatly increased afterhyperpolarization measured from the depolarized potential just prior to the impulses compared to those initiated by a brief stimulus. The resulting PSP has a large negative going, inhibitory component. (E) Postsynaptic responses in a coupled pair of FS neurons. When the stimulated cell generates a burst of impulses, the postsynaptic response has an initial depolarizing spikelet followed by relative hyperpolarization with smaller superimposed spikelets. (C)–(E) from M. Galarreta and S. Hestrin, *PNAS* 99, 12438-12443, 2002. (F) An expanded sweep of a similar burst to those in (E) showing the spikelets superimposed on the hyperpolarization. (F) by the same, *Nature Rev. Neurosci*. 2, 524-433, 2001.

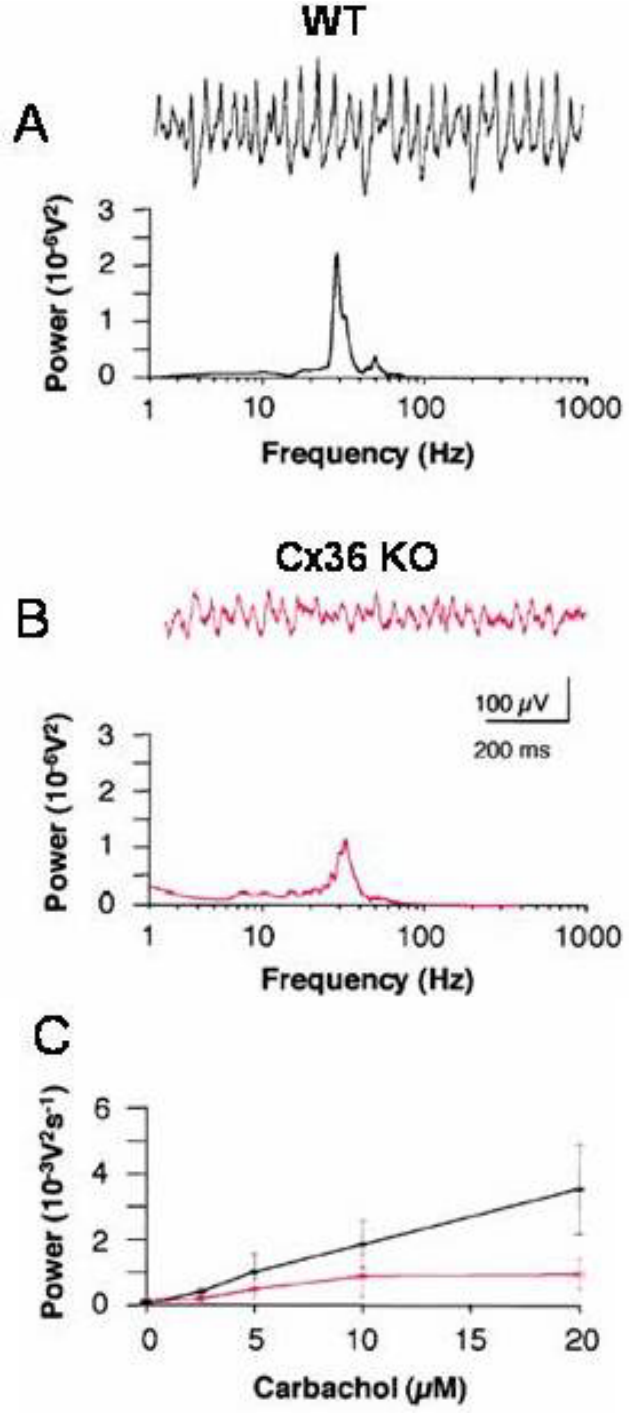
As also shown by M. Galarreta and S. Hestrin in *Nature Rev. Neurosci*. 2, 524-433, 2001 (next image), the coupling of interneurons synchronizes IPSPs in follower cells, thereby establishing an activity that is electroencephalographically recorded as gamma rhythm. LTS (low-threshold spiking) cells inhibit FS (fast-spiking) cells and RS (regular spiking) pyramidal cells. Cross-correlogram is in the upper-right quadrant:



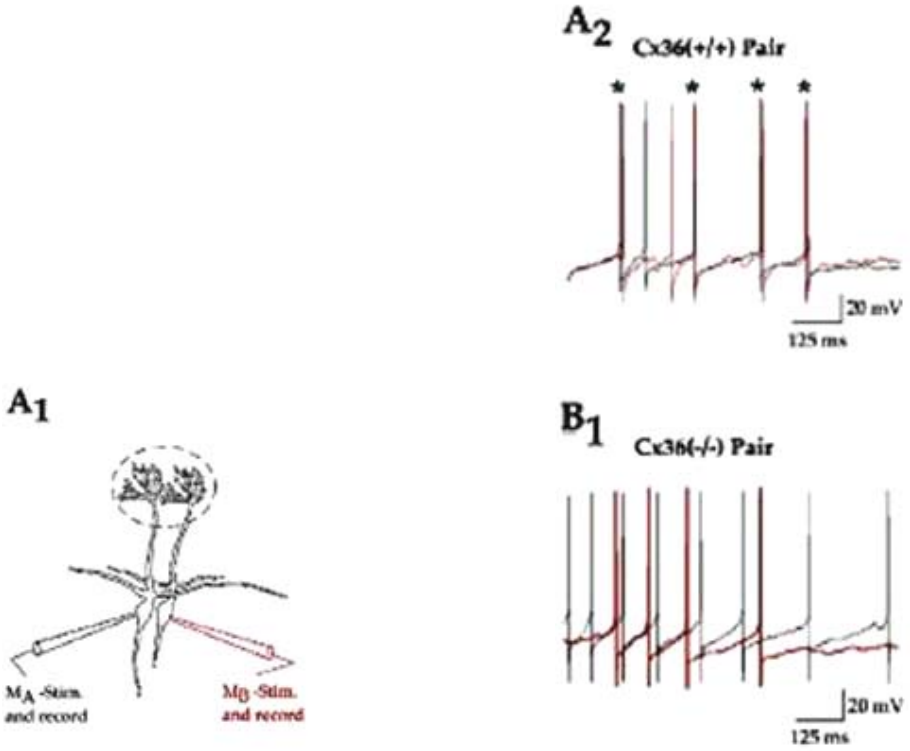
Cx36 synchronizes ocillations in the gamma band with little effect on frequency, as shown by S. G. Hormuzdi and H. Monyer in *Neuron* 31, 487-495, 2001:



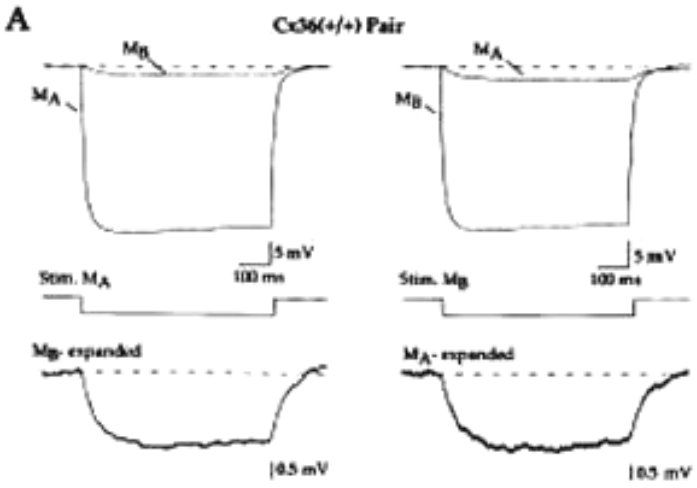
On brain slices of the Cx36 KO mouse, with the gamma induced by carbachol, bath-applied drug and extracellular recording, Hormuzdi and Monyer (*loc. cit*.) observed that the gamma power is reduced, but the frequency is the same:



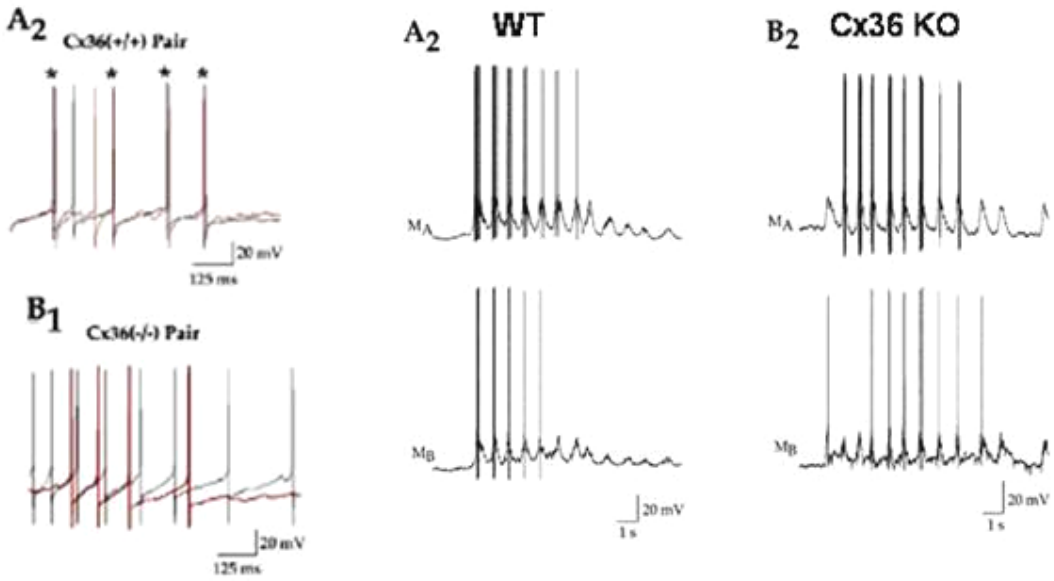
As observed by J. M. Christie *et al*. (*Neuron* 46, 761-772, 2005), olfactory bulb mitral cells with dendrites in the same glomerulus are coupled and tend to fire syncronously:



No synchronization in the Cx36 KO mouse.



Yet, as communicated by the same source, low frequency oscillations of mitral cells do not require Cx36 for synchronization of bursts if Glu uptake is blocked:



How do we know that the observed coupling is mediated by gap junctions? For voltages small enough that voltage gating of gap junctions is negligible, coupling via (nonrectifying) gap junctions has the same electrical characteristics as that mediated by a small region of cytoplasmic continuity, *i.e.*, the junction acts like a decrease in conductor diameter that decreases longitudinal conductance.

Morphological data can demonstrate gap junctions between classes of cells that are coupled physiologically, but marking a pair of coupled cells and then demonstrating gap junctions between them by electron microscopy is difficult. More indirectly, gap junction mediation of coupling between interneurons is indicated by sensitivity to blocking agents, which include heptanol, octanol, halothane, carbenoxolone, α-glycyrrhetinic acid, anandamide, oleamide, and fenamates. Cytoplasmic continuity, where tested, is unaffected by these agents.

Another indication of coupling via cytoplasmic continuity, rather than via gap junctions, is cell-cell passage of larger molecules that do not cross gap junctions, such as fluoresceinated dextrans; injection of a combination of gap junction- permeant and -impermeant molecules into cells in principle enables one to distinguish between the two mechanisms. The possibility that inhibitory interneurons are coupled by cytoplasmic continuity seems excluded by the (near) absence of biocytin or Neurobiotin coupling.

3. Connexins and pannexins

A bird-eye view on connexins may be in order here. Connexins, the proteins forming gap junctions, are encoded by a gene family with at least 20 members in mammals. They are commonly named by their predicted molecular mass to the nearest kDa, with a prefix for species where necessary. A later connexin with a kDa number already occupied gets an additional significant figure, as in Cx30.1. Given that the human genome and much of the mouse genome have been sequenced, few additional mammalian connexins are likely to be found. Connexins (as members of a gene family) have conserved sequences and exhibit a common membrane topology. Different connexins assemble to form junctions that differ in single channel conductance, gating, permeability depending on both size and charge, and temporal and spatial patterns of expression. At a gap junction, each cell provides hemichannels or connexons that dock one to one with hemichannels in the other cell. Hemichannels are hexamers, homomeric if they are comprised of one kind of connexin and heteromeric if they are comprised of more than one kind. Co-expression of multiple connexins is common in cells, and heteromeric hemichannels do occur, although their prevalence and stoichiometry are poorly known.

At least ten connexins are expressed in the mammalian central nervous system but with differing cell specificity. Cx36 is the principal neuronal connexin in the adult. Cx45 is strongly expressed in the brain at the mRNA level for the first two weeks of development and is largely absent in the adult except for hippocampal CA3, thalamus, and cerebellar granule cells. Studies by freeze fracture replica immunolabeling (FRIL), which are precise but at this time still limited in number of cell types examined, indicate that the studied neurons do not express Cx30, Cx32, or Cx43. Other methods of mRNA and protein detection provide evidence for expression of Cx43 and/or Cx45 by olfactory neurons, mitral cells of the olfactory bulb, locus coeruleus neurons, and motoneurons. Horizontal cells, which are extensively coupled probably in all vertebrates, do not express Cx26 or Cx36 and may be coupled by Cx57. High-frequency discharges in hippocampus, which appear to be mediated by electrical synapses, persist in the Cx36 knockout animal; these data support the existence of one or more additional connexins expressed by neurons.

An intriguing possibility is the existence of yet another class of proteins that form gap junctions in mammals. In protostomes, such as *C. elegans* and *Drosophila*, gap junctions are composed of members of

a gene family that is unrelated to connexins but exhibits a surprising degree of evolutionary convergence. The absence of connexins in protostomes can be asserted with some confidence now that the genomes of *Drosophila* and *C. elegans* have been sequenced. To those of us who had come to regard connexins as our gap junction family, it was a bit of a shock when data mining in the human genome disclosed three homologs of the worm and fly gap junction genes, a family evidently unrelated to the connexins.

These genes were originally termed innexins for invertebrate gap junction forming proteins, an inappropriate name at the time (1998) given that ascidians, which are invertebrates, had gap junctions with Cx32-like immunoreactivity. Y. Panchin *et al*. ("A ubiquitous family of putative gap junction molecules," Curr. Biol. 10, R473–R474, 2000) proposef the name "pannexin" for universal (pan) nexus (connection) protein for both the mammalian and invertebrate proteins in this family, a proposal resisted by others, who retain innexin for the protostome line of bilaterally symmetrically animals (nematodes, mollusks, annelids, arthropods) and (ignoring the etymology) use pannexin for the vertebrate homologs. Two of the rat homologs are expressed in the CNS and form gap junctions when expressed in *Xenopus* oocytes. Functionality in the CNS remains to be determined.

Although differences in the sequence indicate that connexin-based and pannexin-based gap junctions are separate evolutionary adaptations, there is a remarkable degree of functional convergence including permeability to molecules of ~1 kDa and block by many of the same pharmacological agents, by low cytoplasmic pH, and by high cytoplasmic Ca^{2+}. Dual mechanisms of gating by transjunctional voltage are found in both classes. In pannexin-based junctions, the channel diameter is a little bigger, the gap is a little wider, and the number of channels per unit area is a little lower. Although the role of pannexins in mammalian tissues is still unknown, conservation of function has been established for many proteins with real homologs (not analogs) in both insects and mammals.

What is the survival value of multiple connexins? There are the obvious functional differences in permeability, gating, and posttranscriptional regulation of formation and degradation. Formation of heterotypic gap junctions can be prevented through expression of incompatible connexins, although many cells expressing compatible connexins do not form junctions. Differences in transcriptional control may be more important than the functional differences in the connexins themselves.

4. A recently-achieved panorama

It is indeed ironic that the fundamental tenet of the Neuron Doctrine – polarized communication between neurons by action potentials – is heavily influenced by non-neuronal cells. Namely, by the constituents of the nervous system that form the myelin sheath around axons and organize ion channels into periodic clusters along the axon, features that facilitate action potential propagation. We do not yet know how the spatial distributions of individual ion channels in the surface membrane of dendrites are established, how this variable localization changes in response to incoming synaptic inputs and output firing patterns, and how the channels dynamically regulate excitability during different behavioral states. Yet we do know that non-neuronal cells act upon them. Besides, they work in a myriad other ways. Myelinating glia do not fire action potentials, but they can detect impulses in axons through membrane receptors that bind signaling molecules. These include ATP and adenosine that are released along the axon, and also potassium that is released during intense neural activity.

This axon-glial communication violates the Neuron Doctrine in two ways. Information is communicated between cells at sites far removed from chemical synapses, and it propagates in a transduced form through cells that are not neurons. In response to neural firing, glia communicate with other glia by chemical signaling and gap junctions rather than by electrical impulses. Chemical synapses have been detected between neurons and a class of glia (oligodendrocyte precursor cells), undermining a defining feature of neurons. However, the functional importance of this neuron-glia interaction is as yet unknown.

Other interesting facts whose import has not yet been fully elucidated are the following:

1. We now know that during vertebrate embryonic development, glia can give birth to neurons, challenging Cajal's conclusion that neurons develop only from neuroblasts.

2. Astrocytes are now known to communicate among themselves by means of glial transmitters and neuromodulators as well as by gap junctions.

3. Moreover, astrocytes can detect neurotransmitters that are released from neuronal chemical synapses. These transmitters are delivered via synaptic vesicles into the synaptic cleft and diffuse to perisynaptic astrocytes.

4. Additionally, neurotransmitters can be released outside the synapse and detected by perisynaptic glia.

5. In response, astrocytes can regulate communication between neurons by modifying synaptic transmission through the release of neurotransmitters and neuromodulators.

As these five facts intimate, there may be a "parallel" or rather overlapping system of information processing that interacts with neuronal communication but propagates over much slower time scales through a functionally reticular network of non-neuronal cells. This functional reticulum results from gap junction coupling and the omnidirectional communication that is mediated by chemical messengers released from astrocytes over much slower time scales.

5. What would Cajal have said?

As cloning of neuron-specific connexins, increased capability of visualizing cells within brain tissue, labeling of cell types by transgenic methods, and generation of connexin knockouts have spurred a rapid increase in our knowledge of the role of gap junctions in neural activity, many new questions arose. Yet one should not lose view of the most basic, fundamental ones. So, why electrical synapses, then?

Factors such as speed of action and shorter conduction delays are important in some escape systems where time is of the essence, what is only true in small animals. Speed of action and shorter conduction delays also are important in precise synchronization. This is not obviously true of gamma waves. Such features are potentially important in increasing phase velocity – but action only over short distances. Whereas electrical synapses are so good for synchronization, in contrast chemical communication, whose diffusion is spatially limited and slow, is important in development. As for phylogenetic reasons, electrical synapses may have used to be good for something and we have not figured how to get rid of them. The fact is, that many mammalian neurons do form electrical synapses.

At most sites, the function is to synchronize, *i.e.* the neurons are quasi reticular. So, what would Cajal have said? " … [I]n accepting the most exaggerated syncytial hypotheses … eveything that the physiologists, during 50 years of dogged and fruitful investigation, have taught us concerning localization in the nervous centers is left without an explanation. We would therefore precipitate into chaos, into a discouraging nihilism …" ("… *aceptando las hipótesis sinciciales más exageradas, y extendiéndolas a todo el sistema nervioso, quedan sin explicación todos los reflejos musculares limitados, así como las impresiones sensoriales concretas (cromáticas, acústicas, tactiles, espaciales, et-*

cétera), y en fin, todo cuanto durante los cincuenta años de porfiada y fecunda investigación nos han enseñado los fisiólogos acerca de las localizaciones en los centros nerviosos. Caeríamos, pues, en el caos, en un nihilismo desalentador… ", as he wrote short before starting *¿Neuronismo o reticularismo?*'s *Conclusión*). But on the next page, he adds: "I am neither exclusive nor dogmatic, I am proud of retaining a mental flexibility which is not afraid of corrections. Neuronal discontinuity … could sustain some exceptions." ("*No somos exclusivos ni dogmáticos. Tenemos a gala el conservar una flexibilidad mental que no se avergüenza de rectificaciones. La discontinuidad neuronal, evidentísima en innumerables ejemplos, pudiera padecer excepciones*", Cajal's words in *¿Neuronismo o reticularismo?*'s *Conclusión*). Gap junctions, indeed, connect cell cytoplasms on a molecular scale, ~ 1 kDa or 1,5 nm to form a functional syncitium; the squid giant axon and some septate axons are exceptions.

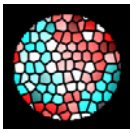
One may still mull over what Cajal said in the Nobel Prize award ceremony: "Finally, the prize for Peace was awarded to the American Theodore Roosevelt. This decision produced great surprise, specially in Spain. It is not the acme of irony and humor to convert into a champion of pacifism the man of the most impetuously pugnacious temperament and the most determined imperialist that the United States have ever produced?" (p. 550). And, on Golgi (p. 553): "What a cruel irony of fate to pair, like Siameses [sic] twins united by the shoulders, scientific adversaries of such contrasting character!"

Further panoramas of the topic:

Santiago Ramón y Cajal, *Histology of the Nervous System of Man and Vertebrates,* N. Swanson, L.W. Swanson, trans. (Oxford Univ. Press, New York, 1995).

Michael V.L. Bennett and R. Suzanne Zukin, "Electrical Coupling and Neuronal Synchronization in the Mammalian Brain," *Neuron* 41, 495–511, February 19, 2004.

Theodore H. Bullock, Michael V. L. Bennett, Daniel Johnston, Robert Josephson, Eve Marder, R. Douglas Fields, "The Neuron Doctrine, Redux," *Science* 310, 791-3 (2005).



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