Excerpts from

The NEURONS and NEURAL SYSTEM:

a 21\textsuperscript{st} Century Paradigm

This material is excerpted from the full $\beta$-version of the text. The final printed version will be more concise due to further editing and economical constraints.

A Table of Contents and an index are located at the end of this paper.

A few cross-refs. have yet to be defined and are indicated by “xxx.”

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Neural Concepts

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20.1 Introduction

In developing the role of neuroaffectors in the neural system, references kept appearing to the enteric and cardiac systems containing mini-brains. On closer examination, these mini-brains were actually complete neural systems. They contained their own dedicated sensory neurons and their own neuroaffectors along with a full complement of stage 2 through stage 6 elements. The stage 5 mini-brain utilized this collection of circuits to operate virtually independently of the parent system, accepting and providing signals to the autonomic system only minimally.

As investigation continued, it became clear that the uterine system also contained a mini-neural system, and acted largely independently within the overall neural system.

Recognizing the completeness of their neural capabilities makes it obvious how an animal can continue to survive major injuries to its spinal column. These mini-neural systems continue to perform normally and independently as long as they are provided an acceptable environment by the parent organism. They only receive advisory commands via the vagus nerve which departs from the spinal chord very near the base of the cranium.

Investigation into the cardiac system uncovered an apparently unique situation wherein the "myocytes" were more than mere muscle tissue; they actually incorporated significant neural capabilities far beyond those of any other muscle tissue. This led to their being redefined as cardiocytes in this work, cells exhibiting the functional capabilities of neuro-myocytes.

The delineation of the di-amino acid, lysine, as a positively charged amino acid providing power to the cardiocytes of the heart expands on the role of the polar amino acids in the neural system. The negatively charged amino acids power the majority of the neurons of the system with a combination of the negatively charged and positively charged cooperating in the powering of the neural portions of the cardiocytes.

The expanded roles of the cardiocytes and the additional electrostenoletic process providing positive electrical potential to certain neural types may be present in the other visceral systems but this has not been confirmed.

The following material will focus primarily on the cardiac system, with only a placeholder for the uterine system. The uterine system clearly operates on a time scale (an event driven nine-month mono-cycle) significantly different than the majority of the neural system.

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1Released: 2 August 2016
Figure 20.1.1-1 Arrangement of blood vessels and lacteals in an intestinal villus. From McNelly in Stacy & Santolucito, 1966.
20.1.1 The afferent neural system appears to be minimal

The following material will focus on the efferent neural system and its interface with the motor and glandular elements associated with the visceral system. The paucity of material regarding the afferent neurons associated with the visceral system suggests they play a minimal role and probably lack direct projection to the CNS.

20.2 The enteric neural system (ENS)—a semiautonomous system

The internal chemoreceptors associated with sensing the chemical content of ingested substances, the enteric sensory system, are parts of a very complex sensory-motor system that operates largely autonomously in conjunction with an extensive musculatura of the gut. Costa et al. describe the operation as quasi autonomous using caricatures to describe a variety of specialized neurons.

Figure 20.2.1-1 provides an overview of the human gastro-intestinal system.

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Figure 20.2.1-1 A caricature of the human gastrointestinal system. The graphic shows the system divided within the esophagus into an upper portion that employs striated muscle and the lower portion that employs smooth muscle. The striate muscle is largely under reflex (not voluntary) control. The smooth muscle and the neural system controlling it operate largely autonomously. The location of the cardiac and pyloric sphincter muscles are shown for orientation. Adapted from Stacy & Santolucito, 1966.
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As noted in the caption, the majority of the gastrointestinal system operates autonomously. The human system employs a neural enteric subsystem with a complexity comparable to the CNS of a cat. While operating largely autonomously, the subsystem does connect to the CNS via the vagus (parasympathetic) nerve and the splanchnic (sympathetic) nerve. Whether this system should be considered as part of the PNS in humans, or as a nominally separate neural system, containing over 0.5 billion neurons, employing all of the stages of the parent system, but nominally subservient to the parent, remains open for discussion.

The extended length of the intestine of mammals (about eight meters in adult humans) requires the employment of stage 3 signal projection neurons to achieve reasonable neural efficiency.

It is interesting to note, the enteric neural system of mammals may contain a type of neuron normally associated with fish, reptiles, and other lower forms. It is a specialized analog neuron analogous to the giant axon of the squid and other species used to control the rhythmic motions involved in swimming. In this case the inverse physiology is described as peristalsis, moving the chyme through the alimentary canal instead of the animal through the surrounding medium.

The vagus nerve interfaces primarily with the sensory and motor elements of the musculatura surrounding the alimentary canal. Elementary discussions indicate it does so through the celiac, or superior mesenteric, ganglion. It controls the operation of the lower esophagus, the stomach, the small intestine and the large intestine. More detailed discussions indicate the presence of individual ganglia associated with each major portion of the intestinal tract. The celiac ganglion (stomach and major glands), the superior mesenteric ganglion (small intestine) and the inferior mesenteric ganglion (large intestine) are three principle ganglia. These ganglia taken as a group are frequently labeled the “gut brain” or the “little brains along the intestine.”

The Splanchnic nerve interfaces primarily with the neurons within the submucosal layer of the intestine and the finer elements of this layer generally described as the intestinal villus.

The enteric neural system is a major component of the autonomous neural system. It is also described as a major portion of the tenth cranial, or vagus, nerve. Both the sympathetic and parasympathetic portions of the autonomous system play important roles in the enteric system. The two-way operation of the autonomous neural system peripheral to the major ganglia is seldom developed in the older literature. But it is a major feature leading to the description of these ganglia as the gut brain. The sensing and control functions form numerous reflexive arcs at the level of the ganglia that operate without CNS participation.

Prior to 1990, the sympathetic portion was described as adrenergic (based on noradrenaline as the putative neuro-affector), and the parasympathetic portion was described as cholinergic (based on acetylcholine as the putative neuro-affector). It is now recognized that nitric oxide is the primary neuro-affector associated with the parasympathetic portion. The “cholinergic neural fibers” of old texts are more appropriately described as nitronergic neural fibers. The lack of identifiable end-plates on the target cells (typically smooth muscle) is explained by the soluble transport properties of nitric oxide.

It is frequently noted that a mammal can exist without a functional (adrenergic) sympathetic system (if adequately protected from its environment). However, it cannot survive without a functional (nitronergic) parasympathetic system. It is the extractor, conserver, and restorer of both the tissue and the energy supplies of the body.

Figure 20.2.1-2 describes the neural-motor aspects of the intestines.
Gautman Naik of the Wall Street Journal reported on the work of Nestle SA in seeking to understand the enteric system on 25 January, 2011 under the title, “Your Stomach has a mind of its own.” It included a descriptive graphic. They noted the enteric sensory system involves about 500 million sensor neurons (approximately the number of neurons in the brain of a cat). These neurons operate in conjunction with the musculatura of the gut to process ingested substances highly autonomously. Only irregular situations are reported to the CNS, sometimes as a warning of a regurgitation to follow.

Figure 20.2.1-2 Caricature of the neuronal-motor aspects of the enteric subsystem. The distinct nerves and plexus of the intestine are shown, along with the distinct layers of the musculatura. Expanded from Naik, 2011.
Figure 20.2.1-3 shows the intestinal villus forming the intestinal mucosa. The highly folded character of the villi greatly increase the effective absorption area of the intestine. It is important to recognize the separate arterial-venal and lacteal fluid networks serving the digestive process. Not all of the food ingested passes into the blood vessel system initially. Much of the food passes into the portal venal system leading to the liver. Some of the food (particularly the fat portion) passes into the lacteal system that empties into the thoracic duct, which ultimately empties into the venous system in the upper thorax. There are multiple mechanisms supporting the transport of foodstuffs across the membranes separating the intestinal lumen from the fluid systems.
Figure 20.2.1-3 Arrangement of blood vessels and lacteals in an intestinal villus. From McNelly in Stacy & Santolucito, 1966.
20.2.1 The character of the Enteric System

The enteric system is sufficiently different from other elements of the neural system that Langley subdivided the neural system into three parts, the sympathetic, parasympathetic and enteric subsystems during the 1920. He was addressing primarily the response subsystems and did not address the additional sensory subsystems.

Descriptions of the enteric neural system remain quite sparse. As noted in a 1988 review by Bornstein & Furness,

“Neither submucous ganglia, nor intestinal secretomotor reflexes are mentioned in the majority of the textbooks of physiology; because it has been realized only very recently that the submucous neurons may have important influences on whole body water and electrolyte balance.”

Descriptions in texts and journals prior to 1990, do not generally recognize the role of nitric oxide in the operation of the enteric system. Most of the following cited books do not even include nitric oxide in their index. They have generally assumed the system employed the common adrenergic and cholinergic neuro-affectors rather than the nitronergic neuro-affectortailored for the control of smooth muscle.

The text by Texter, et al, of 1968 is primarily of historical interest. It does contain some pressure data within the intestinal tract, and does discuss the characteristics of the sphincter muscles. However, it has little data on the musculatura or neural system. They do include an interesting figure on the affects of acetylcholine and adrenaline on the taenia coli from Bulbring (1962). Unfortunately, the interpretation is based on the ionic (K+ & Na+) hypothesis of the neuron.

Friedman’s text of 1975 is essentially of historic interest. It does contain some information on pressure levels within the intestine but little on the muscular or neural operation of the intestine.

Sernka & Jacobson met the minimal requirements of an introductory book on the physiology of the gastrointestinal system in 1979. However, they fail to recognize the difference between striate and smooth muscle and the role of nitric oxide in stimulating smooth muscle. Their chapter on neural regulation is remarkably short and contains only one caricature of the system.

Karczmar, Koketsu & Nishi reviewed the history of the chemical versus electrolytic neurotransmitter in 1986. While they remain proponents of the chemical theory (page 13), their text presents extensive electrical data on the function, including excellent evidence for the unique operating characteristic of the neuron and synapse clearly describing an internal electrical diode (pages 87, 112, 165 & 166).

Furness & Costa struggled in 1987 to define the basic functionality of the enteric system by

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addressing the definition of neurotransmitters. They used the highly conceptual definition that, “Any substance that is released from a neuron and has an effect on that neuron or on a cell near the site of release can be regarded as a neurotransmitter.” Their subsequent discussion in Chapter 9 is excessively wordy and lacks a specific framework to explain the variety of behavioral responses to pharmacological intervention.

Brown noted in 1991, “The classical view of chemical transmission was that an individual nerve cell only released a single transmitter substance and that the effects of that substance depended on the particular receptor on the post synaptic cell.” He obviously provided a more specific definition of a neurotransmitter, but without addressing the neurotransmitter’s character.

The critical review by xxx in 1990 chronicled the revolution in thinking relative to the enteric system. In 2000, the journal, GUT, provided a special supplemental issue on the enteric system including a broad range of recent studies presented at a workshop. A major element in this transition in thinking was provided by Brehmer in 2006. While his study was largely oriented toward anatomy, he did define the geometric configuration of a variety of neurons within the enteric system in detail. These definitions included types of neurons not previously associated with the enteric system.

Brehmer opened his discussion of the enteric system with a quotation;

“The plexuses of Auerbach and Meissner are peculiar to the gut; they extend from the beginning of the unstriated portion of the oesophagus to the end of the rectum. They have usually been considered to belong to the sympathetic system, but it appears to me preferable to place them in a class by themselves. We may speak of them as forming the enteric nervous system. (Langley 1900)”

This discussion will adopt the above position.

Texter et al. have described the intrinsic innervation of the gastrointestinal tract. It is secured by the myenteric plexus of Auerbach, which has mainly a motor function, and by the submucosal plexus of Meissner, which is mainly sensory. The post ganglionic fibers form a fine meshwork of nonmyelinated nerves enveloped by a Schwann syncytium and distributed throughout the muscle layers. The “syncytium” performs the same function as myelination. It reduces the capacitance between the axoplasm of a neuron and the conductive fluids of the surrounding milieu.

The enteric system employs several features that distinguish it from, and isolate its operation from, the rest of the bodily systems. The fundamental structure and operation of smooth muscle of the enteric system separates it from the striate muscle of the skeletal motor system. Whereas striate muscle can be thought of as reacting positively to stimulation (excitation causes contraction), smooth muscle relaxes under stimulation. Because of this feature alone, the enteric neural system employs a different operating logic than does the rest of the CNS. The difference is so large, it is appropriate to consider the enteric neural system as a specialized “controller,” equivalent to a computer controller (such as a game chip or other graphic controller) operating under the supervision of a general purpose computer (such as a PC).

The “negative” operating mode of the smooth muscle system is supported by a unique neurotransmitter, nitrogen oxide, that has no recognized in-vivo affect on striate muscle. Conversely, acetylcholine, an important cholinergic neurotransmitter of the striate muscle system, has no


significant role in the in-vivo operation of the smooth muscle system. In-vivo is emphasized to indicate, the effect of these agents may be measured in the in-vitro situation, but this situation is irrelevant to normal mammalian operations. Boeckxstaens et al.\textsuperscript{12} have stressed this point in the title of their 1990 paper, “Non-adrenergic non-cholinergic relaxation mediated by nitric oxide in the canine ileocolonic junction.” They continued in 1994\textsuperscript{13} using the title, “Pharmacological similarity between nitric oxide and the nitrergic neurotransmitter in the canine ileocolonic junction”. More recent work has demonstrated that nitric oxide is the nitrergic neuroaflctor within the enteric system.

This emphasis on the nitronergic (the term superceding nitrogic in recent literature to emphasize the presence of oxygen) operation of the enteric system was also emphasized in Brehmer’s concluding remarks.

“an important conclusion from the above studies is that interspecies differences hamper a direct transfer of knowledge from one species to another. There are some general similarities (e.g. ascending cholinergic and descending nitrergic pathways occur in all species) but there are a number of differences in detail. This is true for neurochemical properties and probably also for morphological features of enteric neurons.”

Nitric oxide has frequently been described as hormone-like. This is because it is not excreted within the axon pedicle/muscle end-plate space. It is released from the pedicle of the axon into the general area of smooth muscle tissue and is able to diffuse so as to affect more than a single muscle fiber. Because it is able to diffuse through muscle tissue and into the blood stream when associated with muscle tissue of the cardio-vascular system, it is important that nitric oxide not affect striate muscle. The chemistry of nitric oxide within the neural system is developed in detail in Chapter 16, Neuro-affectors.

Many authors have noted the modular character of the intestines. This modularity is difficult to observe physically but is obvious from physiological observations. Stacy & Santolucito (pg 308) have noted, “The smooth muscle involved is generally quite featureless in its appearance, but it has some remarkable properties.” Figure 20.2.1-4 shows a typical image that is representative of extensive section of the human intestine as observed during colonoscopy.

While not obvious from the physical appearance of the inner intestinal wall, the intestine is known to support a variety of peristaltic motions in different modules at the same time. These motions suggest a modular neural system supporting these modular motions. Such a modular neural system is compatible with the known arrangements of individual ganglia along the intestine.

While the transport of nutrients across the epithelium wall of the intestine is not a focus of this work, it is useful to note the multitude of methods used to achieve this transfer of nutrients across the epithelium wall of the intestine during colonoscopy. The white streaks are reflections of the light source by the moist surface.

\textsuperscript{12}Boeckxstaens, G. Peickmans, P. et al. (1990) Non-adrenergic non-cholinergic relaxation mediated by nitric oxide in the canine ileocolonic junction \textit{Euro J. Pharm} vol 190(1-2), pp 239-246

\textsuperscript{13}Boeckxstaens, G. De Man, J. et al. (1994) Pharmacological similarity between nitric oxide and the nitrergic neurotransmitter in the canine ileocolonic junction \textit{Euro J. Pharma} vol 264(1), pp 85-89
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beyond the physical mixing associated with the musculatura. Transport is generally divided into simple passive, facilitated passive, active, coupled active and pinocytosis (endothelial membrane engulfment of small particles, typically fats).

20.2.2 The musculatura & inner lining of the enteric system

The enteric system consists primarily of smooth muscle, frequently with a fine mesh of neural tissue embedded in it. This combination has made it difficult to explore and determine the properties of smooth muscle alone. Texts prior to the 1990's (including Texter et al.) typically provide a "common wisdom" based more on folklore than fact or simple morphological evidence. Even recent texts tend to lack precision in discussing the innervation and neural stimulation of smooth muscle. They frequently confuse (pulse) action potential and (analog) generator potentials. They seldom recognize neuro-affectors such as the short-lived "localized hormone" nitric oxide that do not require the intimate contact between a neuro-affector neuron and the muscle tissue. Currently, Wikipedia provides a good overview of the latest understanding of morphology and physiology of smooth muscle (but largely without considering its electrolytic properties). While the article mentions epinephrine, norepinephrine and carbon dioxide, it does not mention nitric oxide. As is normal, the article is without author attribution although some citations are provided.

Texter et al. provided a readable description of all elements of the gastrointestinal tract. Their anatomical description of the gastric mucosa is useful. "The surface of the adult gastric mucosa is about 850 sq. cm. in men and 783 sq. cm. in women. Each square centimeter of the surface is studded by the opening of about 100 gastric pits. The free surface and the walls of the gastric pits are lined by columnar epithelium. The gastric mucosa is divided into three zones based upon secretory function." and, "The gastric glands number about 35,000,000 and are composed of four types." The gastric glands are simple, branched tubules arranged perpendicular to the local surface." All of these structures are far too small to be seen by conventional medical endoscopy.

They note the resting potential of the cells of in-vivo smooth muscle are typically at ~50 mV relative to the surrounding medium. It may settle at ~70 mV after extended rest. Smooth muscle differs from striated muscle in not exhibiting end-plates. The smooth muscle appears to be affected primarily by neuro-affectors acting as (local) hormones. In a later paragraph (page 62), they provide somewhat different information. They indicate the resting potential for nerve cells is about ~70 mV, for striated muscle about ~90 mV and for myocardial tissue about ~80 mV.

They also note (page 63), "A characteristic of visceral smooth muscle, which allows it to be differentiated from striated muscle, is its ability to contract, usually rhythmically, in the absence known external stimuli." "These rhythmic changes—the slow waves or basic electrical rhythm—of myogenic origin persist after anatomic and pharmacologic denervation." It is not clear whether their denervation included destruction of the plexus of Auerbach embedded in the syncytium of the tissue of interest, or only of efferent neurons from higher ganglia.

In 2000, Costa, Brookes & Hennig provided a transitional paper based on the earlier work of several of the authors and the rapidly evolving new information about the enteric system. The paper necessarily contains many partially formed concepts of enteric operations. Brehmer has provided a more definitive analysis of the musculatura and their neural support within the enteric system.

H. Kuriyama was active throughout the period 1960-2003. His writings reflect the introduction of nitric oxide into the discussions of smooth muscle in the late 1990's and early 2000's.

In 2003, Circulation Research (sponsored and published by the American Heart Association) provided a thematic review series associated with smooth muscle operation. The article by Rybalkin et al. appears relevant to an understanding of the operation of smooth muscle in

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particular, including the key roles played by nitric oxide and the PDE family (phosphodiesterases) in the control of the key substance, cGMP. It also addresses the role of nonadrenergic/noncholinergic (NANC) neurons, i.e., nitric oxide releasing neurons. Unfortunately, his text provides little support for his figure 2, and it only presents a caricature of the processes initiated by nitric oxide. The paper does not highlight any type of syncytium present in smooth muscle. In an accompanying paper, Friebe & Koesling discuss the role of NO as a signaling molecule—a neuro-affector in the parlance of this paper. For many years, NO was known only as the “endothelium-derived relaxing factor” (EDRF). From a signaling perspective, this source was the neurons within the endothelium. Circulation Research has a vast number of recent articles related to smooth muscle physiology.

20.2.2.1 The multiple rhythms of peristalsis

Keihn et al. have addressed the many rhythms found in intestinal peristalsis.

20.2.3 The neural components of the enteric system

Costa, Brookes & Hennig described a series of conceptual enteric neurons without going into detail on their operation. Their labels are generally associated with the related motor functions rather than the actual neural type from a topological or functional perspective. They do suggest a percentage of the total number of neurons for each identified type and clearly differentiate between ascending and descending neuron types. They cite an earlier paper describing the neuro-chemical classification of these neurons in the guinea pig. Brehmer (2006) has gone farther in developing the specific types of neurons in this system in conjunction with his anatomic analyses. As noted above, Brehmer takes the position that the enteric neural system is distinct from the sympathetic nervous system based first and foremost on physiological considerations. He noted that in 1921, Langley divided the autonomic nerves into three groups, sympathetic, parasympathetic and intestinal. While the broader community did not follow his lead, and the field largely stagnated; the field was resuscitated by the return to Langley’s concept beginning with Furness & Costa in 1987. Furness expanded on the 1987 paper in 2006.

20.2.3.1 The 2004 paper of Furness et al.

Furness, Jones, Nurgali & Cleric provided an extensive paper in 2004. The paper contains a considerable amount of data but clearly shows the participation of a committee in its preparation. The opening sentence of its abstract, asserting their “Intrinsic primary afferent neurons (IPANS) of the enteric nervous system are quite different from all other peripheral neurons” does not appear well supported. The primary reason is that the paper lacks a theoretical, or even working framework to support the assertion. As an example of the problems of the paper, their figure 5 asserts it is a diagrammatic representation (lacking any scale) of an action potential. In fact, it describes the typical stage 1 sensory neuron response (the quantum mechanical E/D response) as found throughout the mammalian neural system (Section xxx).


The paper includes several definitions of terms, many of which appear to need further, or clearer, specification.

After describing an IPAN as an “intrinsic primary afferent neuron,” they go on to note such neurons occur in series leading to the CNS and that the first of these neurons in a series are designated as “primary afferent neurons.” It is clear from these statements that an IPAN is not a primary afferent neuron, or initial primary afferent neuron, although it includes such a type.

They continue and say, “As expected of primary afferent neurons, the responses of IPANs are graded with stimulus strength.” Lacking an adequate framework, this statement can be misleading. While the initial stage 1 “primary afferent neuron” and potentially stage 2 secondary afferent neurons exhibit analog waveforms of graded stimulus strength, the stage 3 neurons encode the analog information into a stream of pulses described as action potentials. The stage 1 neurons provide analog generator potentials at their axons. The stage 2 neurons exhibit analog waveforms at their axons that may be significantly modified from the generator potentials at the dendrites of the neurons.

They did note that many of their so-called IPANs may sometimes function as nocioreceptors. While a majority of the signals prepared by the stage 2 signal processing neurons are employed in reflex arcs within the enteric neural system, some signals are passed up the neural system to the CNS. Most of these signals are only perceived under conditions of stress in the system, and these signals can be reasonably defined as a nociceptive response even if not created by nocioreceptors separate from other sensory neurons.

Similarly in describing figure 2, they make a superficial statement about the gastrointestinal tract involves three systems, primary afferent neurons, entero-endocrine cells and immune cells.” This statement mixes primary afferent neurons with undefined “cells.” All sensory functions are performed by their defined primary afferent neurons. Subsequent entero-endocrine and immune signal pathways depend on these primary afferent neurons. The following paragraph appears to lack adequate clarity based on their earlier definitions,

“Two broad classes of primary afferent neurons are associated with the gut: IPANs with cell bodies, processes and synaptic connections in the gut wall and extrinsic primary afferent neurons (Fig. 3). Extrinsic primary afferent neurons have cell bodies in nodose and jugular ganglia (vagal afferent) in dorsal root ganglia (spinal afferents).”

It appears the terms may have been interposed. A simpler, alternate, wording might be as follows,

Two broad classes of IPANs are associated with the gut and its interface with the CNS: the primary afferent neurons with cell bodies, processes and synaptic connections in the gut wall, and extrinsic (outside stage 2 & 3) IPANs. The extrinsic IPANs have cell bodies in nodose and jugular ganglia (vagal afferent) or in dorsal root ganglia (spinal afferents).

The explanation of, and caption to, figure 3 further confuses their description. They define, “Two classes of intrinsic primary afferent neuron (IPAN) have been identified: myenteric IPANs that respond to distortion of their processes in the external layers, and via processes in the mucosa, to changes in the luminal chemistry, and submucosal IPANs that detect mechanical distortion of the mucosa and luminal chemistry.” They are clearly speaking of primary afferent neurons (as they defined them) and not secondary and tertiary IPANs. Both of their defined classes respond to mechanical distortion and luminal chemistry. In the real world, one type of primary sensory neuron responds to mechanical distortion and a second type responds to luminal chemistry (Section xxx).

Their section 2.4 continues the confusion between IPANs and primary afferent neurons. The location of the neurons they describe as Dogiel type II morphology is clearly that of primary afferent neurons, not all IPANs. They also adopt the terminology of Hirst et al. 1974 that has not received general acceptance, and wide usage, within the electrophysiology community.

A comment on page 148 is illuminating. “The electrophysiological properties of IPANs in the guinea pig are influenced by the recording conditions.” Obviously, the measured
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electrophysiological properties of the IPANs are frequently impacted by the test protocol. However, the intrinsic in-vivo properties of the IPANs are stable in the absence of interference by the test protocol. It is the duty of the investigator to insure his protocol does not distort the underlying cell performance, or to note the effect of his interference. They do note a variation in “input resistance” of from 200 MegOhms measured by intracellular electrodes and about 500 MegOhms measured with whole cell patch recording (itself a type of intracellular recording). The input resistance is apparently the impedance between the axoplasm and the adjacent milieu outside of the neuron. The input resistance of the dendritic and poditic compartments would be expected to be quite different.

The data they provide after this comment is generally inadequately defined. Some is collected by probing inter-neural space and some is obtained by intraneural measurements.

Their characteristics of a typical action potential (as generated by a stage 3 neuron, ortertiany IPANs) on page 148 is useful but their location of the source is less precise. Citing Jones et al. 2003, “The action potential in the IPAN soma (presumably the Hillock) has a large amplitude (about 80-90 mV, measured by intracellular electrodes) and a half width of about 2.5 ms at 33 C; at 37 C, the half width is about 2.0 ms.” These values agree with other data for stage 3 neurons (Chapter 9). Their data for resting potentials (presumably the quiescent axoplasm potential in the absence of stimulation) is less precise.

The opening line of section 2.8 appears to need attention. It speaks of “all IPANS” of the guinea pig as projecting to the mucosa, when it is clear speaking of one of their forms of myentric IPANS.

Finally, the opening sentence of their Conclusion section appears poorly worded. The first word should not be the broad term IPANS as they define them. The sentence should read “Primary afferent neurons have pivotal roles in the gastrointestinal tract as the first neurons of intrinsic reflex pathways . . .” In this revised form, the sentence is largely self-evident.

20.2.3.2 The Brehmer (2006) paper

Brehmer presents the goal of his paper in the summary on page 73. “We follow two aims. First, we have presented an overview on the chemical coding of the morphological neuron types described by Stach in the pig intestine. In doing so, we have pointed out the difference between the definitions of type I neurons given by Dogiel and Stach. Second, we have attempted to provide a basis for the morpho-chemical classification of human enteric neurons as revealed by their immunoreactivity for NFs and several neuroactive substances or related markers.” He does not employ any electrical waveform information in his categorization of this large number of neurons (one-half billion).

Brehmer has defined the ENS as consisting of “(1) enteric neurons whose cell bodies lie within the wall of the gut, irrespective of the location of their axonal endings (some neurons project outside of the gut); (2) axonal endings of extrinsic neurons (sympathetic and parasympathetic efferents as well as afferents whose cell bodies lie outside the above mentioned organs); and (3) enteric glial cells.”

He goes on, “The histological description of the ENS is that of enteric plexuses. [Although linguistically incorrect, ‘plexuses’ is used as the plural form of ‘plexus’.] These are nerve networks lying within the gut wall, of which there are two general types: (1) ganglionic plexuses contain clusters of neuronal cell bodies, denoted enteric ganglia, and interganglionic nerve fibre strands connecting the ganglia in various directions. Shapes of ganglia as well as the thickness and orientation of connecting strands determine the architecture of a given network; (2) non-ganglionic plexuses consist of nerve fibre strands commonly containing only axons and glia cells. The various plexuses are richly interconnected with each other. Thus, the (gut) ENS as a whole has a multi-layered tubular shape consisting of several interconnected network levels. The architecture of both ganglionic and aganglionic plexuses varies between species and shows interregional differences in the gut of a given species (e.g. Irwin 1931).

Concerning its fine structure, the enteric nervous tissue resembles more the central than the remaining peripheral nervous system. It contains only nerve and glia cells surrounded by a basement membrane but is devoid of connective tissue (although exceptions have been
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described by De Souza et al. 1988) and it is not entered by blood vessels. The border between nervous and other tissues has been considered to be a blood-plexus-barrier (Gershon and Bursztajn 1978), although other authors presented results contradicting this concept (Jacobs 1977; Gabella 1982). The fact that drugs that do not enter the CNS, such as hexamethonium, are effective in the ENS in vivo, indicates that any barrier between the blood and the ENS is not as secure as that of the CNS."

Brehmer identifies two major ganglionated enteric plexuses, the myenteric and the submucosal. He subdivides the submucosal into at least three subgroups.

He notes, “In contrast to the myenteric plexus which extends as a ganglionated network from the beginning of the oesophagus to the internal anal sphincter, submucosal ganglia are rare or even absent from the oesophagus and stomach (Furness 2006; Neuhuber et al. 2006).”

Brehmer then describes the non-ganglionated enteric plexuses and notes that these plexuses frequently contain no nerve cell bodies, only extended groups of axons.

Brehmer generally follows the morphological classifications of Dogiel, who worked in the late 19th Century. Dogiel supported the position that “All neurons were described as having a number of dendrites and a single axon (the general view at that time). Dogiel’s criteria for distinguishing the neuron types included the shapes and lengths of the dendrites as well as the shapes of the dendritic endings.” This work also supports the single axon concept at the functional cellular level, without limiting the arborization or number of pedicles along that axon. He compared Dogiel’s work with the more recent work of Stach before presenting his own findings. It is noteworthy that these individuals were mainly concerned with the profile topography of the neurons and not their physiology, internal structure or even whether the axons were myelinated or not.

Section 3.1 of Brehmer’s paper continued to confirm the enteric neurons were not cholinergic in character. “It has been shown previously that the commonly used antibodies against choline acetyl transferase (ChAT), which gave efficient results in labelling central cholinergic neurons, resulted in rather weak staining in the ENS of various species (Furness et al. 1983a; Schemann et al. 1993; Porter et al. 1996; Sang and Young 1998; Hens et al. 2000).”

Brehmer took pains to differentiate between Dogiel type I and Stach type I neurons before presenting his own histology-based discussion. In his extensive and valuable Section 3, he frequently employed the expression “on the other hand.” He was generally unable to draw clear conclusions. After defining three types of enteric neurons, he then defined a fourth with the opening statement, “These neurons were defined as multidendritic, uniaxonal neurons (as are type I and III neurons), displaying mainly short dendrites (as type I neurons; for quantitative explanation of the term ‘short’ see Sect. 3.6) with tip endings (as type III neurons) and axons running into disrupted interconnecting strands.” Such a definition is obviously short of a clear delineation between his neuron types. No functional or electrolytic criteria were employed.

Before describing his type IV neurons, he described his type III as follows. “However, both morphologically and chemically, the population of type III neurons as presently defined, seems to be heterogeneous.”

Brehmer continued by discussing the largely irrelevant subject of cell nucleus location. “Already in the 1930s, it was observed that the position of enteric neuronal nuclei are frequently eccentric and it has been supposed that this is a non-pathological feature (Ito 1936; Ito and Nagahiro 1937). Stach (1982b) described this feature as typical for special neuron types.”

Brehmer’s description of a type V enteric neuron is of particular interest. He notes these neurons tend to occur in tight groups with the dendrites of at least most of them forming a tangle. “This very peculiar neuron population exists in two forms, as single type V neurons and as aggregate type V neurons (Stach 1985, 1989). Similar to type I, III and IV neurons, they have been defined as multi-dendritic and uniaxial. However, frequently the ‘multi-dendritic appearance is reduced to very few, sometimes only one, albeit very long and extensively branched dendrite(s). The axonal projection pattern of type V neurons is mainly directed anally. They display, similar
to type IV neurons, a polar arrangement of their dendrites and an eccentric nucleus. Their preferred location along the pig small intestine is the ileum.” “A very conspicuous morphological feature of ileal myenteric ganglia are the type V neuron aggregates (Figs. 9B, 10, 11). These are accumulations of 2–12 (seldom even more) type V neurons. Their dendrites extend mostly towards the centre of the aggregates whereas the somata, typically with peripherally displaced nuclei, are located circumferentially around the tangles. Among their other morphological characteristics, single type V neurons have some similarities to both type IV and type II neurons.”

In this case as well, Brehmer followed the “on the other hand” approach. He noted, “Due to their few but long processes and their smoothly contoured somal outlines, type V and type II neurons display some similarities in shape. This similarity in shape is historically illustrated in both human, by Cavazzana and Borsetto, and pig, by Gunn, who depicted type V neurons but misidentified them as type II neurons. “The morphological decision of whether a process is an axon or a dendrite is thus crucial for the differentiation of type II and type V neurons.” The differentiation of these types would be much easier if electrical measurements of the axonal waveforms were collected. [xxx develop here the possibility that the type V is the rhythmic signal generator for peristalsis (like the squid swimm neuron)–alternately in section 20.2.3.2 below. ]

Brehmer identified a type VI enteric neuron as well and noted, “Type VI neurons are a nitric population as has been demonstrated with both NADPHd reactivity combined with silver impregnation and immunohistochemistry.” He also added comments concerning some immunohistological techniques discussed earlier. “Interestingly, they were immunonegative for cChAT but 95% of them were positive for pChAT.” There staining techniques did not lead to unequivocal results.

Brehmer also identified a type VII enteric neuron. “These dendritic, uniauxonal neurons have been originally described in material from human, pig and dog (Stach et al. 2000). Type VII neurons are a very peculiar population, i.e. they are restricted to the duodenum and proximal jejunum and represent a very small population. The somata of these neurons are commonly larger than those of the surrounding other types (e.g. types I, II, III) and display frequently frayed outlines as visualized by staining for the cytoskeletal marker NF. This is in contrast to the outlines of the somata of other neuron types which are smoothly contoured between the origins of processes.” It must be noted here that these observations are based on light microscopy the resolution of which is generally inadequate for describing the surface detail of individual neural cells.

[Brehmer defines NF as neuro-filament in his text, although it appears he uses this as a shorthand for a specific staining procedure probably defined more clearly in Vickers and Costa 1992. He notes, “Furthermore, both silver impregnation and NF immunostaining are unable to visualize the axonal morphology distant from the soma, i.e. axonal branching and terminal axonal fields.”]

Brehmer identified an additional mixture of neuron types in Section 3.9 but provides negligible details. One of particular interest is his giant neurons. “Giant neurons are, by number, surely the smallest population known so far. They have been found exclusively in the most oral part of the duodenum (up to several centimetres beyond the pyloric sphincter) in human, pig and dog. They have long, branched, tapering dendrites and an axon running mainly analy in pig and dog (in human also longitudinally but the direction was unknown). Their function is unclear.” These giant neurons are clearly candidates to perform the same function as the mislabeled “giant axons” of squid. See Section xxx of “Processes in Biological Vision” for a discussion of the giant neuron of squid.

In his Section 4.1, Brehmer reiterated that his data was based on profile topography at the light microscope level and chemical staining (with a few electron microscope images of certain details). He then stated, “Any nomenclature has to consider both historical aspects and actual requirements. In detail, inclusion of these two factors into a conclusive classification system is difficult.” Then he notes in his Section 5, “Thus, for interspecies comparisons, the chemical coding of neurons is only of limited value.” It should be clear that electrical measurements must be added to this mix if unequivocal and demonstrable delineation of neurons is to be achieved. The rest of his Section 4 presents a description of his seven type classifications based primarily on
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human samples. They are generally not divided into unique classifications, nor do they align totally with the data of earlier sections describing Stach’s definitions, at the detailed level.

While Sections 5 & 6 of Brehmer make interesting reading, and provide a variety of antidotal information, the discussion does not lead to defendable conclusions. The formal Conclusion consists of one paragraph. An extensive bibliography is provided as well as an index. He asserts there are 14 different neural types in the enteric system and provides citations but does not summarize them or provide any electrical waveforms. It should be noted that the content of Section 5.2 may have been abridged in the editorial process. There are many references to subsections of Section 5.2 that do not appear in the paper as published.

Brehmer did not address whether his neurons were myelinated or not. His focus was primarily on the dendritic structure of the neurons and the (functionally irrelevant) physical location of the nucleus of the neuron. A scale is provided in some images but the axon is frequently truncated by the image border. At one point he notes the constant diameter of a specific axon. While noting that many of histotypes of neurons are multidendritic, the text and imagery provided does not clearly determine whether any of the neurons exhibit bilateral arborization. Typically, bilateral arborization involves one dendritic tree entering the peak of the neuron (opposite the axon hillock) and the second (poditic) tree entering the peripheral edge of the neuron (near the base of the hillock). Brehmer does note several type VI neurons with “so-called axonal dendrites which arise from the prominent axon hillock and the proximal axonal segment of these neurons (Stach 1989).” In discussing his “spiny (type I) neurons,” he also notes, “Their dendrites emanated radially from the perikaryon, some of them branched, partly multiple, but in general the side branches were short.” Both of these groups of neurites are likely to be podites rather than dendrites.

In Section 4, Brehmer makes some suggestions as to the functional role of some of the neuron types based primarily on his staining results, some gross histology (the direction axons proceed after emanating from the soma relative to the length of the intestine) and whether the neurons are cholinergic or nitronergic. He suggests his type II neurons are human IPANs (Intrinsic primary afferent neurons). He suggests his “stubby type I” neurons may be either ascending motor or interneurons (the support in section 5.2.2 & 5.2.3 is missing). He suggests his “spiny type I” neurons may be descending motor or interneurons (again the support sections are missing). He suggests his type V neurons may be descending interneurons with long branched dendrites. He notes his type III neurons may not be a homogenous family. Their major feature is they belong to a non-nitrogenic phenotype. His “dendritic type II neurons” are described as primarily afferent neurons. He drew no conclusions as to the role of his “spiny neurons with main dendrites or human type VII neurons.”

20.2.3.3 Other papers containing recorded waveforms or definitions

Brehmer cites a number of other papers reporting electrical waveforms for enteric neurons but does not address the information in his paper. Some of the citations refer to intracellular recordings that are difficult to interpret precisely [xxx].

Physiological Reviews presented an early special issue on smooth muscle, including their electrolytic characteristics in 1962 as a supplement. It contained important articles and subsequent critiques of the articles by Bulbring, by Bozler and by Prosser. The Bulbring paper contains many electrical waveforms. However, their interpretation in the paper reflects the early time period of this work. In some cases, it is not clear whether the waveforms were obtained from smooth muscle or the accompanying Plexus of Auerbach. One figure will be presented and reinterpreted below. Bozler had described multiple types of smooth muscle with distinct functional properties. He describes one type, including the vascular smooth muscle, the sphincter of the iris and muscles of the urinary bladder, as acting very similarly to striate muscle. He defines a second type (including visceral smooth muscle) that exhibits automaticity and is therefore similar to cardiac muscle. He notes the functionality of this second type is similar to cardiac muscle in that groups of cells act as single units. Prosser asserts that visceral and vascular muscle are so different from other nonstriated muscle that they can be considered the only true smooth muscle. He suggests the cell-to-cell communications is electrical, not chemical, and provides many electrical parameters of the cells. His values appear to be global
in character and no figures were provided to help interpret his text. He does not describe his protocols in detail. These early papers deserve further review.

Brookes et al. reported on in-vitro intracellular recordings from cells in the myenteric plexus of the human colon. They recorded a variety of analog and pulse waveforms from both glia and neurons. The excitation was primarily parametric (introduced into the axoplasm by the electronics of the test set). They noted a particular problem that should be documented by all investigators if true. “On penetrating a neurone with a recording micro-electrode, a drop in resting potential of 30-50 mV was recorded that gradually stabilized over the next 2-5 min.” This effect is probably due to the limited input impedance of their test equipment (which pre-dates modern MOS-FET type amplifiers). Such a change in resting potential cannot be associated with the in-vivo resting potential of the neuron.

Comelissen et al. have provided considerable data on the action potentials of the enteric system of the pig as they are affected by various pharmaceuticals.

Nurgali et al. have provided some waveforms related to the mouse colon. The combined analog and pulse waveforms are worthy of further study.

As seen below, more recent work tends to minimize the ability of smooth muscle to generate electrical activity spontaneously.

**20.2.3.4 The effect of acetylcholine and adrenaline on taenia coli neurons**

Bulbring has provided a graphic describing the effect of acetylcholine and adrenaline on the smooth visceral muscle of the taenia coli of guinea pig using intracellular recording techniques. Unfortunately, the voltage measurements are only relative using a capacitive coupled oscilloscope probe. Figure 20.2.3-1 shows her data. She notes in A: Acetylcholine depolarizes the membrane, initiates or accelerates spike discharge, and thereby produces an increase of tension (upper line). She notes in B: Adrenaline stops the spike generation and increases the membrane potential. The upper trace shows the decrease of tension after adrenaline.

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Unfortunately, Bulbring did not provide any discussion of the protocol used to obtain these waveforms and indicated they were unpublished data from H. Kuriyama. No mention of the plexus of Auerbach appears in the paper. Both in the paper and the subsequent discussion, the problems of the chemical theory of the neuron are surfaced (pages 167 & 175). The analysis of Bulbring can be carried further based on the Electrolytic Theory of the Neuron [xxx cite my Processes or other work] and the assumption the pharmacological was applied topically to the neurological material within or on the sample muscular tissue. In the upper frame, the affect of acetylcholine is clear. It has changed the electrical parameters of the emitter-to-base circuit of the Activa within the neuron. The change is so great, the neuron is generating action potentials with a spacing below that for which it was designed. An obvious result is the instability of the baseline and the reduced amplitude of the individual action potentials. The excessive elevation of the resting potential indicates the emitter-to-base (dendrite-to-podite) potential was raised (made more negative). The neuron and its associated muscle fiber return to normal operation within about 60 seconds.

In the lower frame, once it reaches the surface of the neuron, adrenaline has effectively inhibited the generation of any action potentials by the neuron for 36 seconds. The quiescent potential of the axon is held at a slightly more negative level during this interval, indicating the emitter-to-base (dendrite-to-podite) potential of the Activa within the neuron has been reduced to a sub-threshold value that prevents action potential generation.

20.2.3.5 An expanded generic neuron with multiple receptor sites

Reviewing the complex operation of the chemical-sensing neurons, and the effect of a variety of hormones on their operation, suggest an expanded generic neuron is needed. While it appears the class 1 neuro-inhibitors can occupy the same receptor site as glutamate and interfere with the electrostenolytic process (they have the same d-value in Angstrom), the majority of the class 2 inhibitors (including the catecholines; epinephrine, norepinephrine & dopamine) exhibit a higher d-value incompatible with the electrostenolytic receptor sites. The d-value of this group is 2.6 Angstrom and is associated with their 1,2 cis-glycol group. It appears that these class 2 neuro-inhibitors affect the individual neuron through a separate and distinct receptor site(s). Within the CNS, the pharmacology community has described at least four distinct receptor sites for dopamine. Figure 20.2.3-2 shows such a neuron.

Figure 20.2.3-1 Effect of acetylcholine and adrenaline on taenia coli of guinea pig. Intracellular records. Credited to Kuriyama in Bulbring, 1962.
The Viscera 20-  21

The previous generic neuron supported electrostenolytic receptor sites on type 2 lemma associated with all three plasmas, the dendroplasm, podaplasm and axoplasm. This figure has been expanded to include potential catecholamine receptor sites on additional areas of type 2 lemma associated with each plasma and labeled \( L_d \), \( L_p \) and \( L_a \) respectively. All three areas need not be present on every neuron.

The discussion of the previous section suggests a receptor site separate from the electrostenolytic site for the catecholines affecting stage 3 neural tissue of taenia coli of guinea pig. The action potential waveforms reproduced from Kuriyama by Bulbring are clearly generated by stage 3 neurons. In the absence of other data, it must be assumed the catecholamine was applied topically to the stage 3 neuron and not to stage 2 neurons antidromic to the stage 3 neuron. Otherwise, the analysis of this paragraph does not apply. Except for the excessive pulse rate, and resulting reduction in action potential pulse amplitude, it does not appear the adrenalin or acetylcholine impacted the axoplasm potential via either the catecholamine site labeled \( L_d \) or an acetylcholine site on the axolemma. In the simplest scenario relating to the dendrite-to-podite potential, the podite terminal is essentially at ground...
(surrounding extra-neural environment) potential. In this case, the acetylcholine would have cause the dendroplasm potential to become more negative by effectively raising the impedance of the plasma wall (reducing any current leakage through the wall). Under the same poditic potential, the adrenaline obviously caused the dendroplasm potential to drop by effectively lowering the impedance of the plasma wall (increasing the current leakage through the wall). This caused the resting potential of the axoplasm to fall closer to the negative supply potential and inhibited any action potential generation in the output (axon) circuit of the Activa. In both cases the topical dosage caused a 45-60 second disturbance in the normal operation of the stage 3 neuron. These disturbances are clearly reflected in the tension of the associated muscle (the slowly varying line). Rather than holding the poditic potential at zero and raising the impedance of the dendrolemma, the same effect can be achieved by lowering the impedance of the podalemma when it is at a non-zero (marginally negative) potential. [xxx may want to add a circuit diagram here or cite an earlier section]

Within the CNS area protected by the blood-brain-barrier, the effect of dopamine, and any locally generated epinephrine, on any stage 3 neurons would be expected to mirror the effect of adrenaline on the stage 3 neurons of the PNS.

The effect of these neuro-modulators can be expected to be similar for stage 1 and stage 2 neurons populated with similar receptor sites. The resting potentials would be changed but no action potentials would be generated. Since the neural system is direct coupled, changes in the axoplasm resting potential can have significant impact on the performance of orthodromic neurons.

20.2.3.6 Stereochemistry of the catecholines

Dowd has diagramed the catecholines from the perspective of the nitrogen element without maintaining charge neutrality in the individual molecules. Figure 20.2.3-3 shows his figure. Epinephrine is shown in the less active (+)Epinephrine form. To achieve electrical neutrality a hydrogen must be removed from one of the hydroxyl groups of each molecule (or the methyl group in the case of acetylcholine). By removing a hydrogen from epinephrine, the more active (-)Epinephrine form is obtained. In this form, the molecule is susceptible to coordinate bond pairing of the two adjacent oxygen atoms through hydrogen bonding (Sections 8.5.1 & 8.5.5). The distance between the hydrogen bonds, its d-value, is 2.6 for its 1,2 cis glucol group. It also exhibits a dipole moment of 3.54 Debye. This arrangement, and its d-value, is very similar to that used in the taste and smell receptors to sense “sweet” compounds. The electrostenolytic process also uses coordinate bond paired but with a different d-value.

The structure of the epinephrine, norepinephrine and dopamine members of the catecholines suggest,

+ a first order stereochemical characteristic related to the glucol group.
+ a second order stereochemical characteristic related to the ring structure containing the glucol group.
+ a third order stereochemical characteristic related to the nitrogen removed from the ring by two carbons.

The diagrams suggest a dipole moment for


The shinagawas have published calculated dipole moments for several catecholines using the CNDO/2 and the MINDO/3 methods. Park has provided a series of calculated dipole moments for dopamine in three different configurations. The range is from 2.88 to 3.62 Debye.

The dipole moment is readily measured for materials in solution. However, it is the dipole potential that is important in the electrophysiology of the neurons. The dipole potential is easily measured for liquid crystalline monolayer of the catecholines on the surface of a conducting fluid (Section 8.4.4.2.1). It is not clear these measurements have been made to date. A search of the journal, Langmuir, produced no useful data as of 2011.

### 20.2.3.7 The effect of the catecholines on an expanded generic neuron

At rest, the electrolytic state of the generic neuron is an equilibrium condition where the surrounding environment is important (specifically the concentrations of glutamic acid and GABA are critical). It is likely that the local concentration of the catecholines is also of critical importance. If catecholines are routinely in the vicinity, the equilibrium conditions of the expanded generic neuron will reflect these concentrations. The relative occupancy of the catecholamine receptors, the relative occupancy of the electrophysiology receptors, and the nominal electrical potentials within the neuron will be relevant. As in the case of taste and smell, the dipole potential of the catecholines can shift the resting potentials of the various compartments of the neuron to which their receptors are attached.

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An important area of further investigation is to determine whether the receptor site for the catecholines is similar to that for glutamic acid (Option A; no amplification of the potential associated with glutamate to GABA conversion) or to that for taste and smell sensing (Option B; amplification of the dipole potential presented to the interstitial space between the inner and outer leaves of the type 2 lemma). The latter approach could significantly increase the sensitivity of the neuron to the catecholines. Figure 20.2.3-4 illustrates these options.

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The lemma configuration shown in option B is described more completely in Figure 8.5.1-16, The electrophysiology of the gustatory “sweet” microvilli MOD. The lemma is predominantly composed of type 1 lemma where both the inner and outer leaf are insulating, type 2 lemma where both leaves are semiconducting and forms an active electrolytic amplifying device, an Activa, and a region of type 4 lemma consisting of a semiconducting leaf facing the mucosa and an insulating leaf facing the dendroplasm. The type 4 lemma forms an electrically conducting path through the outer leaf to the electrically conducting hydronium forming a connection to the base terminal of the Activa.

Both options suggest the participation of the catecholamine neuro-modulators was part of the fundamental design of the neural system. Option B is more economical with respect to the transport of the neuro-modulators within the endocrine system. In both cases, the receptor site is ideally suited to coordinate pair bonding modulators containing a 1,2 cis-glucol group with a d-value of 2.6 Angstrom, suggesting that acetylcholine is not effective at these sites.

The stereochemical requirements of the catecholamine receptor sites probably requires the presence of the glucol group in a ring structure in order to discriminate against other 1,2 cis-glucol containing molecules.

Option A appears to require the neuro-modulator to contribute energy to the neuron continuously during its active role in order to change the electrical potential of the appropriate...
neural plasma. This is not a likely scenario. Option B only requires the neuro-modulator to maintain a change in the potential at the base of the Activa (a very high impedance point) to cause a continuous flow of charge into the plasma in order to cause a change in the potential of that plasma (a relatively low impedance point). Operationally, option B appears to be the more likely scenario.

The means by which the modulators are deactivated and released from the receptor sites after a period of time remains to be discovered. The mechanism may be similar to that used to generate nitric oxide at locations on the axolemma of stage 7 neurons sensitive to molecules of different d-values and stereochemistry (Section 7.xxx).

Option B offers an interesting insight. A neuron with a dendritic 1,2 cis-glucol receptor site located inside the body could be sensitive to excitation by a catecholamine modulator while the same neuron with its dendritic portion outside the body (specifically within the oral cavity) could be sensitive to a wide range of sugars and pseudo-sugars.

20.2.3.7.1 The dipole potential of the catecholamines

Section 8.4.4.2.1 has discussed the measurement of the dipole potential of the phospholipids. Dipole potentials (as distinct from dipole moments) have not been measured for most biological materials. However, in the case of the phospholipids, the values are large—in the range of -280 to -500 mV for a material with a dipole moment of 19 Debye. These voltages are much larger than the typical dynamic range (~20 mV) of an Activa embedded in the lemma of a neuron as suggested in Option B.

The available dipole moment values for the catecholamines are few. They range from 3.54 Debye for epinephrine (Boone, 2003) to 2.88 to 3.62 Debye for various optically active configurations of dopamine (Park, 2000).

The dipole potential is given by the dipole moment of a molecule divided by the mean distance between the charge centers within the molecule. Using the ratios above, the estimated dipole potential available to influence the base potential (a high impedance point) of an Activa in the common base configuration is about 14 to 20 mV. This is enough to significantly impact the current flow through the Activa.

20.2.3.8 Peristaltic signal generation

The literature has suggested peristaltic drive signals are generated in the enteric neural system conceptually by a syncytial network of neurons. This concept has apparently arisen from a similar concept suggested for generating (at least) the mammalian heart beat. This work suggests an alternate methodology based on the system used in many animals and fish to create the rhythmic motions of swimming. The best known of these is the methodology used in the squid. The methodology involves a hybrid form of the common neuron based on the electrolytic theory of the neuron. The proposed neuron operates in the analog domain and involves diffusion, as opposed to the propagation used in stage 3 pulse neurons. By employing an extended length axon with pedicles arranged periodically along its length, diffusion of a signal within this single neuron can generate a series of time related drive signals. As in the squid, the rate of electronic diffusion can be varied by changing the capacitive loading of the axonal wall. This is accomplished by changing the potential of the axonal fluid in adjacent neurons surrounding the primarily peristaltic signal generator. Figure xxx illustrates this technique.

Costa et al. (2000) do note the “neurally mediated migrating motor complex” is controlled by the content of the intestine within a given module.

The primary candidates for the peristaltic signal generation neurons, lacking any electrical waveform data, are the giant neurons or the Type V neurons of Brehmer. These were described briefly above. The small number of giant neurons, as a percent of the total enteric population is noted. Alternately, the bunching of neurons around the type V neurons, with their dendrites forming a tangle is suggestive of a main neuron operating in analog mode and forming distinct drive signals are periodically placed pedicles along its axon. The surrounding neurons would perform the same electrical environment changing function that they do with respect to the
extensively studied giant neuron of the squid.

20.2.4 Block diagram of the enteric system

Figure 20.2.4-1 illustrates the complexity of the enteric system. It can be divided into a neural system component and a largely muscular and enzymatic component.

The dual anal sphincters, while shown in series, are not in series, morphologically. They are arranged coaxially so that the limited tension provided by the autonomous sphincter can be enhanced by the somatic sphincter (Texter et al., pg 136).

20.2.4.1 The muscular and enzymatic elements of the enteric system

The intestinal tract is divided into a series of functional sections, although these sections are not always identifiable anatomically. The major physical elements are separated by distinct sphincter muscles. These elements frequently exhibit different pH levels that largely control the activity of the enzymatic components.

As noted above, the wall of the intestine consists of three major muscular layers, each with its associated neural plexus of sensory and neuro-affectoeffect neurons. The inner layer consists of fibers of smooth muscle arranged longitudinally. The middle layer consists of fibers of smooth muscle arranged circularly. The outer layer consists of fibers of smooth muscle arranged longitudinally.

The inner layer appears designed to cause the intestinal villi to continuously move relative to the contents of the intestine in order to increase the efficiency of enzymatic contact and diffusion with the contents.
The middle, or circular, layer appears designed to participate in at least two actions. It supports the squeezing of the contents over significant distances to support compression (potentially supporting diffusion of fluids out of the bolus) and probably extrusion into the next alimentary section. It also supports the periodic squeezing of the content into boluses that are subsequently transported anally by the periodic contractions of the longitudinal outer muscle layer as illustrated in Figure 20.2.4-2. This is the fundamental peristalsis of the intestine. The rates of peristalsis for separate sections of the intestine, and even sections of separate sections of the intestine are not same. Note the two rates given for the for and aft sections of the colon in the previous figure. Recall, the relaxed condition is not the quiescent condition for smooth muscle.

The musculature of the intestine, particularly the stomach and esophagus, can operate in an over-ride mode (such as vomiting) when the sensory system detects an unacceptable condition within the intestines. This condition is also reported to the CNS via the IPAN neural channels generally before the vomit reflex is initiated.

20.2.4.2 The organization and operation of the enteric neural system

The neurons of the enteric system are organized much like the parent CNS. The major ganglia contain large numbers of neurons operating in the analog mode to accept signals from the sensory neurons imbedded in the inner intestinal wall and prepare both instructions for the motor portion of the neural system and reportsto be forwarded to the CNS via the IPAN channels. Where distances exceeding two millimeters are involved within the enteric system, stage 3 projection neurons are used to transfer signals within the ganglia. The major instructions generated by the stage 4 neurons are transferred to the specialized peristalsis generator neurons. These have historically been conceptually labeled syncyclic neurons. This work will develop the concept that the peristalsis generator contains a primary, and highly tailored, analog neuron used to generate multiple time sequential waveforms that are then applied to the vast array of stage 7 neuro-affector neurons interfacing with the musculatura of the intestine.

All of the stage 4 and stage 6 neurons of the enteric system operate analogously with those of the similar stage CNS neurons. They are interconnected by synapses that are active electrolytic circuits and the signals between the neurons are carried by electrical currents (electrons and/or holes) and not chemicals (Section 2.4).

In the absence of any waveform data, Brehmer has identified the stage 4 and stage 6 neurons of the ganglia as his non-nitronergic type III, type V and “dendritic” type II neurons.

In the absence of any waveform data, Brehmer has identified the IPAN neurons as his type II and “stubby” type I neurons. His assertion that they are cholinergic neurons is irrelevant to the actual operation of these neurons. The IPAN are sensory signal channels and are independent of the sympathetic system where the term cholinergic is typically used.

The stage 7 neuro-affector neurons can have exceptionally long axons because of the remarkably long distances involved in intestinal sections. The distance between the pyloric sphincter and the ileum/cecum valve (7-8 meters) is typically three to four times the height of the human containing it. These axons contain conventional active Nodes of Ranvier at intervals of about 2 millimeters and propagate electrolytic signals. However, their pedicles are modified to interact only with smooth muscle. The pedicles release Nitric oxide, derived from arginine, as
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the neuro-affector. The smooth muscle does not support end-plates like striate muscle. As a result, the nitric oxide is released into the milieu in which the smooth muscle is embedded. The nitric oxide is thus able to excite multiple smooth muscle cells within a short range. Thus, the action of the nitric oxide resembles that of a hormone operating over a very short distance.

In the absence of any waveform information, Brehmer has identified the nitronergic neuro-affector neurons as his “spiny” type I neurons.

Brehmer has attempted to associate the location of the enteric ganglia with either the beginning or the ending of a specific intestinal segment. Based on this association, he describes the neuro-affector neurons as having a preference for extending their axons orally or anally. Other investigators ignore this concept and show the neuro-affector neurons as radiating toward their respective target smooth muscle tissue. Following the dictum that “form follows function and the available packaging space,” it appears any directional preference for axons of neuro-affector neurons is secondary and largely coincidental.

20.2.4.2.1 EMPTY Details of peristalsis signal generation

20.3 The cardiac system

[xxx settle on cardiocyte waveform and cardiocyte potential as distinct waveforms separate from nodal & Purkinje waveforms ]

A key feature of the cardiac system is its extremely high reliability. To live 70 years, a person’s heart must beat about two billion times without significant variation in performance. In total, it pumps about xxx liters during that period.

The cardiac system has been optimized to the point it is difficult to define the character of cells forming it. Various names have been used for these cells based on the perspective of the author, including myocardiocyteal muscle cells. Many of the cells have historically been defined generically as myocytes. However, the ability of most of the cells of the heart to project neuron-like electrolytic waveforms makes this designation questionable. These myocytes might more properly be described as cardiac neuro-myocytes, or just cardiocytes. Wahler has noted the unique character of the cardiac cells28;

“Cardiac cells have certain properties in common with other excitable cells, such as nerve and skeletal muscle cells. However, the behavior of cardiac cells also differs from the behavior of nerve and skeletal muscle cells in some important respects.” Synopsizing further, nerve and skeletal muscle action potentials are relatively brief compared to that of cardiac cells. In cardiac cells, action potentials are more complex in waveform and generally much longer in duration. In addition, unlike from nerve and skeletal muscle cells, action potentials from different regions of the heart vary substantially in shape.

There are two primary types of cardiac cells. One cell type (fast response) is found in the working cells of the atria, ventricles and the specialized cells of the His-Purkinje network. The second type (slow response) is found in sinoatrial (SA) and atrioventricular (AV) nodes.

Every cardiocyte incorporates one active amplifier, an Activa, as defined earlier in Chapter 1. Each cardiocytes exhibit a neural feature unique to those cells within the neural system. The cardiocytes employ two distinct sources of electrical energy, two electrostenolytic processes. The second electrostenolytic process connects to the podaplasm of the internal amplifier, the Activa. This source accounts for the maximum positive excursion of some of the cardiocyte output waveforms. It is also critically important to the operation of the animal heart. It

introduces the interval-duration relationship that narrows the excitation pulse as the pulse rate goes up (Section 20.3.5.4). It may also play a significant role in biasing the resting potential of the axoplasm of the cardiocyte in order to optimally control the release of Ca++, and potentially other chemical agents within the sarcomeres of the cardiocyte.

Key features of the cardiac system are its typical resting pulse rate and its maximum rate under stress. The typical resting rate is species dependent, ranging from 500-600 ppm in mice to 70 ppm in humans and 28 ppm in elephants. The rates between members of a species also show significant ranges. Young adult male distance runners frequently have resting heart rates in the low 60's. Figure 20.3.1-1 shows the typical range of human heart rate activity as used in many exercise programs. Human pulse rates exceeding 200 ppm are rare, even among the young.

Attempts to model the neural elements of the cardiac system based on chemical principles have been frustrating within the neural community for a very long time. Efforts to model the neural elements mathematically have been even less rewarding. Nickerson & Hunter have described the situation in 2010. They note, “Beginning with the initial four ODE (ordinary differential equation) models from Noble in 1961, models are now being developed with many tens of differential equations requiring hundreds of parameters and integrating multiple aspect of cellular physiology. Such increases in complexity inevitably result in significant barriers to the use of the models by independent scientists.” The intent of this work is to describe deterministic models not requiring the solution of any family of differential equations and involving less than a dozen measurable parameters.

The basic problem for the community has been the assumption that the ODE were continuous over an extended time interval. The empirically measured waveforms involved several discontinuities that have not been generally recognized and that cause the ODE to contain many unnecessary harmonics. In fact, the intrinsic biological oscillator is a switching type relaxation oscillator employing only simple exponential functions, except over short intervals when saturation is encountered that leads to switching. The resulting descriptive equations based on switching are both simpler and deterministic.

20.3.1 Gross morphology and electrophysiology of the cardiac system

Figure 20.3.1-2, from Boron & Boulpaep (page 484), provides a good illustration of the human heart with an overlay of the neural paths.

Canale et al. have provided a text focused on the cardiac muscle. While dated, it does provide considerable reference material.

The medical community has long described the cardiac neural system as the “conduction system.” At the more detailed level, it will be seen that “conduction,” in the electrical engineering sense, plays a very small role in the cardiac neural system. Its operation is dominated by stage 3 and stage 7 neurons employing electromagnetic signal propagation at speeds of four meters/sec along the insulator formed by the axolemma rather than ionic conduction in the axoplasm fluid.

That community also speaks generically of myocytes to describe both muscle cells and neural cells. The physiology community seldom differentiates between muscle and neural cells in the cardiac system. They rely upon the chemical theory of the neuron and do not seek to determine if the neural cells are multi-compartmented.

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Canale, E. Campbell, G. et al. (1986) Cardiac Muscle. NY: Springer-Verlag
The performance of the muscle tissue of the heart is significantly different from that of skeletal muscle. Figure 20.3.1-3 shows the percent of maximum active tension versus sarcomere length for the two muscle elements. Of particular interest is the earlier and more rapid rise in the passive tension in the cardiac tissue. This property is attributed to the much higher rigidity (less elasticity) of the protein titin which acts as a mechanical equalizer between the parallel fibers of the sarcomere and the intercalated disks of the heart. This element is discussed more fully in Section 20.3.1.3.

Ter Horst & Zipes have provided a text on the neural system interface with the heart. They express the idea that the cardiac system includes a mini-brain similar to that of the enteric system. While many texts suggest the absence of neural tissue within the morphology of the heart, they suggest “a significant proportion of the heart (up to 30% of the volume of some cardiac tissues) contains neural tissue.” “The importance of the intrinsic nervous system is often overlooked.”

The fact that as much as 30% of the volume of the cardiac tissue is of neural origin is critically important. If correct, the cardiac system is similar to the enteric system in having significant regions of neural tissue scattered throughout the cardiac system.

Much of the neural tissue of the cardiac system may be unresolvable using light microscopy.

It is critically important to uncover the detailed histology, cytology and architecture of the neural/muscular structure of the heart before any firm conclusions can be made about the electrical performance of the heart.

**The specific question is**, do the muscle cells exhibit active electrolytic amplification or are they driven by associated active neurons?

Iaizzo & Laske have provided an introduction to the subject of the myocytes of the heart,

“...all myocytes within the heart have the capacity to conduct the cardiac impulse. A population of myocytes is specialized to generate the cardiac impulse and then to conduct it from the atrial to the ventricular chambers. This population has become known as the conduction system.”

They describe a complex neural interface consisting of efferent, afferent and intra neural paths, Figure 20.3.1-4. They also introduce a broad range of “neurotransmitters” into the discussion, including the recent emergence of nitric oxide as a significant non-adrenergic non-cholinergic (NANC) participant. “Nitric oxide is now thought to play a crucial role as a neurotransmitter and ...

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neuromodulator, both in the peripheral and central nervous systems. (page 32). This work will refine the role of nitric oxide to that of a neuroaffecter. They suggest that “noradrenaline (NA) and acetylcholine (ACH) are the predominant ‘classical’ transmitters utilized by the mammalian heart.” They assert, “The predominant neurotransmitter released at cardiac sympathetic nerve terminals is noradrenaline, with important excitatory influences on the modulation of cardiac function.” “The predominant neurotransmitter released at the pre-ganglionic and postganglionic vagal nerve terminals is acetylcholine.” It is difficult to determine how much of the above information is from third parties, and potentially archaic. As earlier in this work, the chemicals released by a stage 7 neuron will be described here as neuroaffecters rather than neurotransmitters. Most of the other “neurotransmitters” described by Ter Horst & Zipes will be considered neuromodulators in this work.

They note, “branches of the same efferent postganglionic nerve can innervate both the endocardium and the adjacent myocardium. This type of branching nerve fiber possesses numerous varicosities. It is these terminal swelling that, in effect, represent the neuroeffector junction of the majority of cardiac nerve fibers. They are structurally different than the fixed neuromuscular junctions, or synapses, observed in skeletal muscle.”

They assert, “Despite the fact that numerous investigators have studied the anatomy and electrophysiological properties of intra-cardiac neurons, little is known about the overall contribution, in terms of the proportion of intrinsic nerve fibers, to the overall pattern of cardiac innervation.” And, “To date, there have been no investigations into the quantitative extent (or density) of the intrinsic nervous system within the intact heart.”

“Exquisite micro-dissections of the intra-cardiac nervous system of humans have recently been reported. These permitted the intra-cardiac ganglionated plexus of the human heart to be localized to approximately five atrial and five ventricular regions.”
Figure 20.3.1-4 Schematic illustrating possible extrinsic and intrinsic cardiac innervation. Within the intrinsic cardiac ganglia, note that some sympathetic and parasympathetic efferent projections are depicted as fibers of passage, whereas others terminate on cell bodies. The extrinsic ganglia consist of the stellate ganglion, the middle cervical ganglion and the mediastinal ganglion. Two separate afferent populations are represented: one whose cell bodies are located extrinsically and a second population whose cell bodies are situated within the intrinsic ganglionated plexus. From Ter Horst & Zipes, 2000.
20.3.1.1 Description of the muscle tissue of the cardiac system

Cardiac muscle tissue is found only in the heart and surrounding the mouths of the great veins which enter it. Like voluntary muscle, its contractile elements show transverse striation; like visceral muscle, its fibers possess centrally placed nuclei, and further, it too has an autonomic innervation.

Hill provided a seminal set of papers on muscle in 194933. Shortly after, Huxley & Taylor made a significant comment on page 399 of the Hill work, “A. V. Hill has shown that ordinary diffusion of a chemical substance is too slow to explain the rapidity with which the contraction in the interior of a striated muscle fibre is set off by the action potential, which is a change in the potential difference across the surface membrane; some more rapid link must therefore exist34.”

A full volume of the Journal of the Royal Society appeared in 1964 dedicated to the biological muscle. The first of several articles by Huxley in that volume provides a great deal of dimensional information at the protein level and discusses ATP utilization within sarcomeres of striate muscle35. A paper by Caldwell in the same volume provides background on the role of ionized calcium in muscle contraction36. A paper by H. E. Huxley in 1973 (25 years after Hill’s work) provided a great deal of information and remains the defining work on the molecular basis of muscle tissue37. Figure 20.3.1-5 from H. E. Huxley shows the schematic arrangement of muscle at increasing levels of detail.

There is confusion in the literature as to the histology of the cardiac muscle tissue. While it is frequently described as smooth muscle in introductory texts, the individual cells are quite small (~85 microns versus 1 to 40 mm for skeletal muscle). Under adequate magnification and illumination, the muscle is clearly striated as shown above and in Figure 20.3.1-6 from Lingappa & Farey\textsuperscript{38}. Huxley has provided excellent electron micrographs at magnifications from 900x to 148,000x illustrating the features of these components.

A slightly expanded view of (a) would show very close positioning between adjacent cells (intercalated disks). Intercalated disks provide a strong mechanical connection between myocytes and they are generally considered to also contain synapses between the cells.

Katz has released a very comprehensive volume, 5th edition and considerably updated, on the histological and chemical/mechanical physiology of the heart. It contains little on the neurological aspects of the heart.

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Smaill & Hunter have recently reported on finite-element modeling of the heart during contraction and illustrating the spiral “weave” of the muscular tissue⁴⁰. Stanfield (page 371) illustrates the spread of electrical signals through the heart to optimize pumping efficiency, noting the need for atrial contractions to be completed (transferring blood to the ventricles) before ventricular contractions begin.

20.3.1.1.1 Work performed by the heart

Katz has described the work accomplished by the heart in normal operation. This will become an important subject when defining the details of the myocytes performing this work. It is seldom addressed in conjunction with laboratory investigations into the electrophysiological performance of the myocytes and the heart. Katz develops the basic concepts of work in his chapter 11 using a linear analogy involving tension and distance. He relates work to each phase of the operating cycle of the heart schematically without focusing on the positive and negative aspects of the work involved. He then presents a more complex, but realistic, diagram based on pressure and volume. He stops short of defining the well known relationships (Green’s Law) leading to the net work accomplished per cycle by the left ventricle until his chapter 12. He then recognizes the net work of the heart as being equal to the area within the pressure-volume diagram of Figure 20.3.1.7.

The cycle starts with mitral valve closing (MVC) while the ventricle is at rest, and proceeds counterclockwise around the loop. Pressure increases rapidly since blood is non-compressible. The aortic valve opens (AVO) when the ventricle pressure exceeds that of the aorta and the ejection phase begins. The aortic pressure begins to rise, crests and then falls due to blood flowing from the arterial system into the venous system. The ejection phase ends formally when the ventricular pressure falls below the aortic pressure and the aortic valve closes (AVC). Pressure in the ventricle falls rapidly until the mitral valve again opens (MVO). The ventricle then fills from the atrial chamber until the pressure in the two chambers equalizes and the mitral valve again closes (MVC).

To understand these relationships with respect to time, a Wiggers diagram is used as in Figure 20.3.1.8. The major work associated with pumping begins when the ventricular pressure first exceeds the aortic pressure shown on the left (dotted line), from AVO to AVC in the previous figure.

Figure 20.3.1.7 Pressure-volume diagram of the left ventricle. See text. The two ventricles have similar diagrams, except the pressures in the right ventricle are considerably lower. From Katz, 2011.
Figure 20.3.1-8 The cardiac cycle as a Wiggers diagram showing seven phases of left ventricular systole. By convention, the cycle begins with the onset of ventricular systole. The top three curves represent aortic pressure (upper dotted line), left ventricular pressure (solid line), and left atrial pressure (lower dashed line). The solid line below these pressure curves is left ventricular volume. From Katz, 2011.
20.3.1.2 Terminology related to the cardiac system

The terminology of the cardiac system is continuing to evolve. Katz reviewed his changes in the Preface to his 5th Edition. Interestingly, he changed the terminology he used to describe short-term and long-term responses from phasic and tonic respectively to functional and proliferative. Proliferative is the adjective form of the verb proliferate; “To cause to grow or increase rapidly. He also reviewed some of the changes in fundamental concepts that have occurred since his first edition in 1977. He notes, “Much of the ‘mature’ science has been condensed and material that no longer seems important has been removed.”

The physiology of the cardiac system has introduced some terminology not used elsewhere in the neurosciences. The use of the term varicosities to describe unresolved neural features is an example. These varicosities appear to be conventional axon pedicles arranged at the end of very short axon branches of a continuing axon. Stanfield has indicated the presence of these varicosities along cardiac muscle is similar to that of smooth muscle (Table 12.2) but does not illustrate them. The table describes the varicosities as diffuse without further discussion. It may refer to their lack of definition under light microscopy or their paracrine character (lacking specific contact with individual myocytes). The table does suggest epinephrine is the mechanism of hormonal control in cardiac muscle. However, the potential role of nitric oxide is only mentioned briefly in her text (page 212). She notes that nitric oxide is not stored in vesicles associated with the typical synapse of the chemical theory of the neuron. This work asserts nitric oxide is formed on the exterior lemma of the axon and released upon formation as a paracrine neuroaffecter.

It is proposed that Figure 20.3.1-9, adopted from her text, correctly describes the neural/muscular interface for both smooth and cardiac muscle, as found in the cardiac system. The neurons of the mini-brain of the cardiac system interfacing with cardiac muscle may emulate but are not truly a part of the autonomic neural system. They are under the control of the mini-brain itself, which is responsive to the autonomic system of the primary neural system. The neurons interfacing with cardiac muscle appear to act as stage 7 paracrine neuroaffectors releasing appropriate agents to the intercellular space adjacent to the myocytes.

A second term that can be defined more precisely is “conduction system” when referring to the neural tissue within the cardiac system. Major parts of this system consist of the SA and AV nodes, the “bundles of His,” and the “Purkinje bundles” within the cardiac system. Lesser neural paths are not typically named. The bundles of His appear to be conventional stage 3 projection neurons feeding into the Purkinje bundles and subsequent individual neurons.

The source of the bundle of His is the atroventricular (AV) node, the secondary source of timing signals controlling the cardiac system and constituting, along with the sinoatrial (SA) node, the major central nervous system of the cardiac mini-brain.

If the cardiac system employs a mini-brain and an extensive system of neural pathways interfacing with the individual myocytes via paracrine techniques, the concept of myogenic operation within the cardiac system is unrealistic. The myocytes are driven by neuroaffectors just like all other muscle tissue.

Figure 20.3.1-9 Proposed neural/muscular interface in both smooth and cardiac muscle ADD. The varicosities represent pedicles of stage 7 neurons employing signal propagation and releasing neuroaffecter agents. Adapted from Stanfield, 2011.
20.3.1.2.1 Solutions used to support cells during experiments

Several specialized solutions have appeared in the literature for culturing cells or maintaining cells in-vitro.

Claycomb has defined his solution for use in culturing the HL lines of cardiac myocytes. Xiao et al. have used a modified Claycomb medium. Xiao et al. also described their version of a Tyrode solution and a number of variants designed to isolate specific ionic currents

20.3.1.3 Description of the myocyte

The small size of the typical myocyte makes determination of their internal structure difficult. They clearly contain a nucleus and are known to contain considerable amounts of mitochondria relative to their size. This mitochondria supports the intermittent contraction of the cell through the interaction of myosin and actin fila ments within sarcomere as typical in other muscle tissue. Swynghedauw has provided important information about the myocytes of the cardiac system in an expansive review with 527 citations. The paper focuses on energy usage and cellular failures leading to necrosis. He provides an interesting heat profile suggesting much of the heat generated in a sarcomere is generated during the recovery interval following an action potential. His conceptual figure 2 suggests the sarcoplasmic reticulum is enclosed within the sarcolemma rather than the larger myocyte lemma. Gerdes & Capasso have also provided a valuable review. They note, “The ratio of myocyte length to myocyte width is approximately seven in normal adult rats, hamsters, guinea-pigs, cats, and humans, but increases markedly in failing human hearts.

Tseng et al. of 1987 investigating canine myocytes give, “For apparent single cells, the width was 20-33 microns and the length was 110-140 microns.” They also provided the following passive values

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Passive Membrane Characteristics of Canine Ventricular Myocytes and Multicellular Canine Ventricular Tissues

<table>
<thead>
<tr>
<th>Passive membrane characteristics</th>
<th>Isolated myocytes*</th>
<th>Multicellular tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input resistance at RMP</td>
<td>61.23±18.0 Mohm</td>
<td>3.0 ms,** 3.2 ms/</td>
</tr>
<tr>
<td>Membrane time constant at RMP</td>
<td>3.033±1.14 ms</td>
<td>3.0 ms,** 3.2 ms/</td>
</tr>
<tr>
<td>Input capacitance I</td>
<td>54.4±18.2 pF</td>
<td>80.43±16.2 pF --</td>
</tr>
<tr>
<td>Surface area from input capacitance I</td>
<td>54.4±18.2 X 10^{-6} cm²</td>
<td>54.4±18.2 X 10^{-6} cm²</td>
</tr>
<tr>
<td>Input capacitance II</td>
<td>80.43±16.2 pF --</td>
<td>54.4±18.2 X 10^{-6} cm²</td>
</tr>
<tr>
<td>Surface area from input capacitance II</td>
<td>80.43±16.2 X 10^{-6} cm²</td>
<td>80.43±16.2 X 10^{-6} cm²</td>
</tr>
<tr>
<td>Specific membrane resistance</td>
<td>3.253±1.15 X 10^3 ohm-cm²</td>
<td>3 X 10^3 ohm-cm²,** 5.3 X 10^3 ohm-cm²</td>
</tr>
<tr>
<td>Space constant</td>
<td>1.0-1.1 or 1.5-1.6 mm//</td>
<td>0.8 mm*, 1.2 mm//</td>
</tr>
</tbody>
</table>

* For all parameters (excluding space constant), n = 6 and mean ± SD is given.
** From Ikeda and Hiraoka (1982).
// The shorter values result from setting Rt -- 200 s the longer values result from Rt = 100 fl.cm.

Figure 20.3.1-10 from Katz attempts to highlight the portions of the myocyte dedicated to mechanical contraction.
the figure is focused totally on the elements related to contraction of the cell. It shows two examples of reticulum within the conceptual drawing. Frame B can be extended to show the nucleus of the cell, and both the gap junctions and the intercalated disks on the surface of the cell that interface electrolytically and mechanically with other cells. What has not been found in the record is any knowledge of the internal partitioning of the myocyte into electrically isolated compartments, or any mapping of the electrolytic potential of the
The interior of a myocyte. This information would help determine whether the myocytes of the cardiac system were a hybrid containing active neuronal and muscular components. Besides the reticulum shown, there may be more. In addition, the various components may also exhibit electrically insulating properties and effectively create electrolytically isolated compartments within the cell. The frontpiece of a text by Opie shows an alternate view of the above figure that may provide additional perspective.

Figure 20.3.1-12, from Opie and credited to Moravec, Katz provides a two-dimensional plan view of the structural form and interlinking of the sarcomere.

The site where the myosin and actin filaments overlap is called the anisotropic (A) band, which shows up darkly on electron microscopy, in contrast to the lighter zones of isotropic (I) bands on either side, which contain only actin filaments. These bands have different light-scattering properties, the A bands being more powerful in this region than the I bands. In the center of each A band there is a relatively clear zone known as the H zone (abbreviation for the German helle, clear). Here, only myosin is present because the overlapping of the thin and thick filaments falls short of this region. Each H zone contains a central dark region, the M line, which contains M-line proteins that appear to extend across the filaments as if to hold them in the incorrect anatomical position. This two-dimensional picture should not disguise the fact that the Z line is really a “Z disk.” The previous oblique view from Katz includes an end view of a sarcomere. The cross-section of the sarcomere at the molecular level is shown in Figure 20.3.1-12. The thick filaments are composed largely of myosin polymers and a huge cytoskeletal protein called titin. The central regions of the thick filaments contain several additional cytoskeletal proteins. Cross-bridges that project from the thick filaments and interact with the thin filaments represent the heads of myosin molecules.

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Figure 20.3.1-12 Schematic cross sections at three regions of the sarcomere. Top: A; In the A-band thin filaments lie at the trigonal points in a hexagonal array of thick filaments. I; In the I-band, where thick filaments are absent, the thin filaments are less ordered. M; In the M-band at the center of the A-band, thin radial filaments made up of myosin-binding protein C connect adjacent thick filaments. From Katz, 2011. Bottom: Cross-bridge contraction phase concept. Schematic of interaction between myosin head and actin filament during contraction. See text. From Opie, 1998.

Other sources suggest the thin filaments in the I-band retain their orderliness (compatible with the A and M-bands) more than presented by Katz. H. E. Huxley has provided a more detailed analysis of the arrangement (page 318) showing how different slices through the fibril present apparently different arrangements.

The conceptual action of the cross-bridges is shown in the bottom frame from Opie. “The myosin head flexes to interact with one of the actin units (black). During flexion, this unit is moved along (new position indicated by dotted circle). Then the myosin head relaxes, thereby being able to restart the cycle. There are many different myosin heads interacting, some in flexion and some in extension, the whole process occurring throughout the period of contraction during the availability of calcium ions.” Why the myosin head would act in a cyclic manner in the presence of nominally constant calcium concentration is yet to be explained.

Finkelstein & Ptitsyn have provided some additional caricatures of the “walking” of myosin along actin.

Only recently, the term liquid crystal has entered the language of the myocyte. The sarcomere is a clear example of a liquid crystalline structure with obvious similarity to the liquid crystalline structure of the piezoelectric structure found in the sensory neurons of

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Descriptions of the fundamental operating mechanism of the sarcomere have not appeared widely in the cardiac literature. What is the fundamental mechanism that causes the sarcomere to contract? The assertion that ionic calcium, by its presence, causes the sarcomere to contract is not an adequate answer at the theoretical, or basic research, level.

### 20.3.1.3.1 The chemical explanation of contractile function

Beginning in the 1950's, the theories of sarcomere contraction have been based on calcium ions stimulating one or more of the proteins, myosin and actin. The theory of the fundamental mechanism has changed over the years (Katz, page 33). Most of the current concept is based on work reported during the 1960's (Katz, page 143). The comments of H. Huxley in 1973 describe the limits of the theory of contraction based on the chemical theory of the neuron alone (page 377). The field remains largely conceptual to this day.

Katz gives an extensive account of the contractile mechanism in myocytes, based on the chemical theory of the neuron. His discussion does not attempt to relate the chemical environment within the myocyte to the concurrent electrical environment. He does note the presence of several lemma within the external lemma of a myocyte. He notes the difficulty of exploring the thermodynamic cardiac efficiency (page 37) due to the lack of detailed information concerning the chemical processes causing contraction. Thus, the mechanical efficiency is usually used, the ratio between useful work and the enthalpy changes during substrate metabolism. His only mention of glutamate is in the context of the overall citric acid cycle. No discussion is provided relating to its use by myocytes to provide electrical polarization. The glutamate loop of the citric acid cycle is not addressed. While chapter 2 discusses many aspects of energy production within the biological system, little is said about chemical energy consumption directly, and specifically, tied to contraction. Chapter 10 employs a number of caricatures related to myocyte contraction, but no specific equations. Similarly, chapter 13 employs endless caricatures of ion channels through the myocyte membrane.

Stanfield takes a somewhat different view as presented conceptually in her figure 13.14. She shows current from the synapse initiating the flow of Ca++ from the extracellular fluid. This places the current through the synapse as the controlling mechanism in cardiac muscle contraction. She then asserts that this Ca++ induces additional Ca++ to be released from the sarcoplasmic membrane. She gives this action the name, “calcium-induced calcium release” (page 374). This asserted mode of operation seems more than counter-intuitive.

A more likely scenario is that the charge passing through the synapse causes the release of molecules containing charged calcium ions and some other negative ions that enter the myoplasm in ionized form. The calcium ions then cause contraction within the sarcomeres until the ions are sequestered by some other agent. Following the sequestration, the calcium ions and their associated ions are returned to the sarcoplasmic reticulum for reuse. The calcium ions and negative partners passing through the myolemma are used to make up for any small loss of calcium in the above operational loop. The small size of the makeup component relative to the major components involved in contraction are in agreement with the calculations of Katz (page 170). Katz has noted the protein molecule calsequestrin contains 18 to 50 calcium binding sites (page 165).

Boron & Boulpaep have omitted any discussion of chemical synapses between myocytes (page 231), attributing all communications to “gap junctions.” “When an action potential is initiated in one cell, current flows through the gap junctions and depolarizes neighboring cells. If depolarization causes the membrane potential (Vm) to be more positive than threshold, self-propagating action potentials occur in the neighboring cells as well.”

The field of myocyte contraction chemistry is an active one. Yamasaki et al., Balnave et al.
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and Swartz et al50. are worthy of further review. Swartz et al specifically note the importance of other proteins, and the possibility that calcium alone is not totally adequate as a stimulant, in the contraction mechanism. Katz indicates six proteins are necessary to perform the process of contraction (page 89). “All utilize chemical energy released during ATP hydrolysis to perform mechanical work.” This suggests that a change in valence by ionized calcium is not a significant participant in the energy equation of contraction.

The comment by Katz on page 97 sets the role of calcium, “… actomyosins reconstituted from highly purified actin and myosin were found to remain active after powerful calcium chelators lowered ionized calcium concentrations almost to zero. This surprising observation stimulated a search for the calcium receptor of the contractile proteins that ended with the discovery of the roles of tropomyosin and the troponin complex.” This may not be the end of the story.

Katz has recently presented an extensive group of caricatures of the role of calcium within the myocyte, including its apparent storage within a closed sarcoplasmic reticulum. He presents an intricate set of gates leading into and out of this reticulum. While outside of the reticulum, the calcium ions are shown affecting the sarcomeres of the myocyte.

Katz has defined five separate “pools,” or compartments as critical to the operation of the sarcomeres (page 167). He has also indicated the flow of calcium ions to and from the reticulum is controlled by a variety of proteins, calsequestrin, sarcalumenin, calreticulin, and a histidine-rich calcium-binding protein. These names are related to their putative function, not their chemistry. He names additional proteins on page 160.

Katz describes the roles of these proteins and calcium by flowing commentary and caricatures but few equations or experimental demonstrations. His largely tabular discussion of the rates of calcium flow between some of these pools (page 170) reported by various investigators is less than convincing. In discussing his mathematical scenario; he notes, “The actual difference in the intact heart is much greater because calcium uptake into the sarcoplasmic reticulum slows significantly as cytosolic calcium concentration decreases during diastole.”

20.3.1.3.2 An alternate explanation of contractile function

Actin is the principle piezoelectric molecule in the sensory neurons of hearing. Its role there is to create a potential when mechanically stressed, just the opposite of its role in the myocyte. Interestingly, its role in hearing is discussed without considering the presence of calcium ions in the vicinity. The mere mechanical stress on the molecule generates an electrical potential. This subject has been reviewed and developed in Fulton51. It is reasonable to assume that a normally reversible piezoelectric effect can account for the movement of actin in response to the application of an electrical potential.

Several of the caricatures in Opie, including the bottom frame above, are compatible with Actin performing as a piezoelectric transducer in a matrix of myosin. Figure 20.3.1-13 is reproduced to illustrate a point. If the figure is drawn to show the myosin heads in intimate contact with the actin filaments, the figure suggests several mechanisms for contraction, including the ratchet action suggested by Opie. First, the actin filaments could contract, dragging the myosin heads of myosin (or cross links between myosin and actin) closer to the Z-lines. Second, the actin filaments (alternately, the myosin filament) could rotate causing

the myosin heads in contact with the actin to be drawn closer to the two Z-lines. Either of these mechanisms are consistent with the actions of actin as a piezoelectric molecule. The latter two mechanisms do not require an intermittent cycling in the presence of a constant potential (or calcium ion concentration). They suggest a contraction proportional to the amplitude of the electrical potential (or calcium ion concentration) present.

H. E. Huxley has provided more detailed information on the 3-dimensional arrangement of the heads of the myosin molecules of the thick filaments (page 323). Huxley noted, “the A-band remains apparently constant in length to within ±0.075 microns (i.e., any changes in length are less than the resolving power of the light microscope), over the range of sarcomere lengths used, 2.3–3.3 microns. The I bands were, of course, not constant in length, and the H-bands, too, were found to be much longer in stretched muscle.”

Katz shows an alternate, and ever more complex, structure for the sarcomere (pages 110-113). The I-band region is shown consisting of the proteins titin and telethonin, instead of actin. The Z-line is shown in an expanded caricature.

20.3.1.3.3 The character of the fundamental contractile mechanism

The character of the contractile mechanism within the myocyte has long been recognized at the first order level. A major volume on muscle tissue was produced by the Royal Society
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(London) in 1964\textsuperscript{52}. It included some of the earliest electron micrographs of muscle tissue as well as the results of early electrophysiological experiments. A highly structured array of two interlaced proteins, actin and myosin, slide over each other between the relaxed and contracted states of muscle tissue. These sarcomeres (the arrays) make up about 50\% of the volume of a myocyte. Most of the remaining volume is occupied by mitochondria. About 3.5\% is allocated to the sarcoplasmic reticulum. The first order sarcomere is shared among all types of muscle tissue.

The sarcomeres of one myocyte are interconnected with the sarcomeres of an adjacent myocyte through the intercalated disks described by Katz on pages 116-118.

Katz reverts to the simpler caricature of the sarcomere, as in the above Opie figure, when discussing the contraction of cardiac muscle on pages 133 to 135. Figure 20.3.1-14 shows two of the set of caricatures of Katz. The captions have been quoted verbatim where significant. The actin filaments move toward each other axially and begin to slide past each other at 2.0 microns, resulting in the “double overlap” condition. At contraction lengths between 1.7 microns and 1.6 microns, a deforming of the Z-line structure is encountered and a significant reduction in net tension results. This is attributed to severe calcium overload. Nominal operation is limited to the sarcomere length from 3.65 to 1.7 microns. The exact character of operation in the 1.6 to 1.7 micron region remains under investigation.

Eyzaguirre & Fidone have provided additional dimensions for this structure in striated muscle (page 47. The sarcomeres have a typical diameter of 10-100 microns. They contain multiple myofibrils which are 1-2 microns in diameter. Inside the sarcolemma, interposed between myofibrils, there is a predominantly longitudinal tubular system that does not communicate with the exterior of the fibril; the sarcoplasmic reticulum. The thick filaments of the myofibrils form a hexagonal array with a spacing of 400-450 Angstrom. The thick filaments are of myosin (mol. wt. 500,000) and the thin filaments are actin (mol. wt. 60,000). Tropomyosin (est. mol. wt. 70,000) is also a constituent of the thin filaments. Troponin is present and has the capability to bind large amounts of Ca\textsuperscript{2+}. It is believed the troponin and tropomyosin form a complex and bind to the thin filaments with a periodicity of 400 Angstrom.

\textsuperscript{52} (1964) J Royal Soc (Lond) series B vol160(981)
The proteins, actin and myosin, have been extensively studied but little has been said concerning their liquid crystalline character. Katz has noted their appearance under polarized illumination, clear signs of their liquid crystalline character. The darkly staining striations contain a parallel array of thick and thin filaments that strongly rotate polarized light.
and so are highly birefringent (anisotropic), hence their designation A-bands. The lightly staining striations, which contain only thin filaments, are less birefringent (more isotropic) and so are called I-bands. “The thick filaments are composed largely of myosin polymers and a huge cytoskeletal protein called titin.”

Liquid crystalline materials are highly sensitive to electrical fields (e.g., the screens of cell-phones and other consumer electronics involving flat screens). It is likely the electrical potential associated with the axoplasm of a myocyte plays a role in contraction of these proteins.

The conventional understanding is that calcium ions play the major role in controlling sarcomere contraction. Katz (pages 168 & 274–284) has provided a large set of caricatures describing the movement of calcium within the myocyte during the various phases of contraction. Katz also provides a group of caricatures (pages 98–99) to explain the role of calcium in the response of the contractile proteins. He discusses the biochemistry of contraction briefly on pages 100–104). No overlap could be found in Katz between the electrolytic and chemical mechanisms of the myocyte. Whether the potential controls the release of calcium, or more directly controls the contraction of the sarcomere is not addressed.

Eyzaguirre & Fidone have written that the release of calcium ions is subject to electrical stimulation. They cite the early work of Taylor & Huxley with a frog muscle reported in 1955. They showed using high-speed cinematography that, “The stimulating pipet (2 micron diameter) evoked contraction of the fiber only when applied to the Z-bands; and this induced contraction of the I-bands.” They went on, “It is not yet clear whether or not (1) the action potential propagates along the membrane of the T tubules and (2) the sarcoplasmic reticulum is depolarized to induce contraction. It is clear, however, that the electric signal induces release of Ca++ from the sarcoplasmic reticulum where it is bound at rest. During contraction Ca++ is released and moves into the sarcoplasm, to be accumulate in the A–band. The intracellular calcium concentration falls quickly, probably due to fixation of the ion to troponin.”

The application of a potential between the Z-bands to achieve contraction is reminiscent of the complementary situation in the sensory neurons of the hearing modality. There, a compression of the piezoelectric filaments of actin generate a potential between the ends of the filaments.

20.3.2 The waveforms of the cardiac system

Cabo has provided the oversimplified remark, “The morphology (?form) of the cardiac action potential is more complex than that of skeletal or nerve cells, and consists of five different phases.” This difference necessitates distinguishing between the highly standardized action potentials of stage 3 signal projection neurons, the generic generator potentials of the sensory neurons, and the family of waveforms historically described as action potentials found in the cardiac system. The former action potentials are generated by a well defined relaxation oscillator of the switching type (switching occurs at the peak of the waveform and the generator waveforms are generated by a well behaved transconduction mechanism exhibiting two distinct time constants. The later waveforms of the cardiac system are generated by unknown (potentially multiple) mechanisms.

Like Cabo and other recent authors, Lederer has adopted the practice of adding the adjective when discussing “cardiac action potentials.” This practice will be followed.

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The Viscera 20- 51

Figure 20.3.2-1 from Lingappa & Farey shows an actual selection of cardiac system waveforms keyed to their location in the human heart. The variety of waveforms is significant. The resting potential of these cells ranges from −60 mV to −90 mV depending on cell type; around −60 mV in cells of the sinoatrial node (SA node), and around −90 mV in atrial and ventricular cells. They note, “the action potential generated in cardiac muscle is quite distinct from that of other muscles, lasting 3 to 15 times longer, . . .”

It is worth noting that Lingappa & Farey incorporate neural waveforms in their 2000 text on Physiological Medicine that are at least forty years old. This suggests basic research, as separate from applied (read clinical) research, in this field has moved forward at a glacial pace. In their 2nd Edition of 2000, Podrid & Kowey note the focus on clinical research (page 3).

It is also important to note these waveforms were not acquired using patch clamp techniques, which were only developed in the mid 1970’s by Sakmann & Neher. These are most likely extra-neural waveforms acquired using electrodes significantly larger in diameter than individual myocytes and neurons. As a result, these waveforms are likely to represent the sum of multiple neural waveforms.

Grant & Carboni, writing more recently, segregate the waveforms associated with the nodal

cells from the atrial, ventricular and other muscular cells\textsuperscript{56}.

Boyett et al. cite Boyett, et al. of 1999 and note that, while these waveforms may be typical, the variation in the waveforms at each of these various locations is significant\textsuperscript{57}. Focusing on the SA node, they describe the significant variation in these waveforms with location.

Unlike this work, they treat any repetitive waveform as an action potential.

Lederer (page 484) presents a similar figure (without attribution) but including the resting potentials of the individual waveforms showing they originate in different types of material.

The SA node and AV node waveforms may actually be generated by a simple switching type relaxation oscillator as in stage 3 neurons (except with different time constants). The shape of the other waveforms suggest they are created in a cell containing an active source and driven from an external source.


Figure 20.3.2-2 illustrates the two fundamental waveforms associated with cardiac tissue. The bottom waveform is typically associated with the SA & AV nodal tissue. It represents the output of a free running relaxation oscillator as discussed more fully in Section 2.3. The waveform is repetitive. Note the slope of the phase 4 segment at lower right has the same slope as the segment at lower left. The region below the threshold potential line represents the active region of the Activa within the cell. The region above the line represents the pulse generation region. The Activa within the cell operates in a positive internal feedback mode during phase 0 (a region of high transconductance, $g_m$). The Activa enters cutoff at the peak of the waveform and the phase 3 region of the waveform is a period of repolarization. In the phase 4 region, the dendroplasm to podaplasm potential is rising slowly, under the control of its time constant, until it reaches the threshold potential of the circuit. At threshold, the internal feedback becomes greater than 1.0 and the waveform re-enters phase 0. The two regions marked phase 4 are continuous in the interval between pulses. The region including phase 3 and 4 is called the diastolic interval in medical texts. The phase 0 region occurs during the systolic interval. Systole is defined as the contraction and emptying of the chambers of the heart. Diastole is defined as the relaxation and refilling of the chambers. To avoid confusion, it is proposed the terms systolic and diastolic should be reserved for discussing the contraction and dilation of the gross muscle tissue and not used when discussing the electrical waveforms at the cellular level.

While this cardiac action potential waveform has the typical shape of that generated by stage 3 neurons of the parent system, the duration of the pulse is significantly longer.

The top waveform is typically associated with the bulk of the cardiac tissue. The Activa within the cell is biased to act as a driven (not free-running) oscillator. The Activa within the cell is at a quiescent value or resting potential (RP) within its active region during phase 4 until it begins to respond to a stimulation. Upon stimulation, the axoplasm potential begins to depolarize in response to the stimulation until it reaches the apparent threshold potential (TP) of the axoplasm. When it is stimulated sufficiently to cause the dendroplasm to podaplasm potential to exceed its actual threshold value (not shown), phase 0 begins. During this phase, the internal feedback is positive and its net value exceeds 1.0. As a result, the axoplasm potential depolarizes rapidly. The nearly vertical rise in the axoplasm potential is indicative of the high transconductance, $g_m$, of the circuit during this interval. The Activa enters saturation at the peak of the waveform. The internal positive feedback collapses and the circuit is said to enter cutoff. During cutoff, the circuit is repolarized by the flow of electron current from the electrostenolytic power supply entering the axoplasm.

Under nominal conditions, the repolarization process follows the curve marked phase 3. However, under stressful conditions, the ability of the power supply to provide a steady electron current is impacted. The power supply circuit involves a second order RC filter (the resistance actually formed by diodes). This circuit is shocked by the sudden change in axoplasm potential (Section xxx). The result is a “ringing” in the power supply circuit. As a result, the axoplasm potential is restored by following phase 1 and 2 as shown. Note the second dip in the phase 1 & 2 response just before rejoining the nominal phase 3 response. Under greater stress, the amplitude of the ringing can cause a deeper notch and higher peak in the phase 1 & 2 waveform as indicated by the dotted line.

The stressed condition is frequently described as consisting of a spike (peak of phase 0) and dome (maximum in phase 2) configuration. This nomenclature is strictly colloquial and observation-based. It has no relationship to the underlying mechanisms. It will be shown in Section 20.3.5 that the phase 1 and 2 portion of the waveform has nothing to do with a putative transient outward positive ion current ($I_{to}$) through the axolemma.

The stressed condition is also found in the visual modality where it is uncovered in experiments to define “dark adaptation.” It is also found in man-made amplifiers responding to high amplitude, low frequency staccato conditions. The stressed condition in cardiac tissue has been well documented by Litovsky & Antzelevitch (Section 20.3.2.1). The circuitry of the cardiac tissue will be developed in Section 20.3.5.

Phases 1 and 2 are not normally reported under nominal in-vivo conditions involving antidromic stimulation by an in vivo free running waveform. Clinical texts seldom illustrate
waveforms containing phases 1 and 2. The academic literature more frequently reports the stressed conditions, based on in vitro parametric stimulation of the cell by square wave electrical signals. The pedagogical literature has generally chosen to illustrate the response shown by the dashed line in the top frame of the figure.

It is technically possible for the peak in the phases 1 and 2 waveform to exceed the peak of the phase 0 portion, this would be quite unusual even under stressed conditions in vivo.

The reported waveforms from cardiac tissue are generally acquired with no mechanical load opposing contraction by the muscle tissue. Under load, the waveforms may show a different form.

### 20.3.2.1 Signal transmission within the cardiac system

**Figure 20.3.2-3** is an expansion of a

![Waveform expansion](image)

**Figure 20.3.2-2** Two fundamental waveforms of cardiac tissue. Bottom; the fundamental form of the SA & AV node tissue (with a relatively slow rise for the leading edge). Top; the form of the non-nodal tissue showing a rapid rise followed by a phase 3 repolarization under nominal conditions. Phases 1 & 2 appear under special conditions. See text.

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by other neurons in order to shield the neurons and provide a lower effective capacitance per unit length between their axoplasm and the surrounding fluid matrix (Section xxx).

If the neurons of the Purkinje system do rely upon their nearest neighbors to help reduce their effective capacitance relative to their fluid environment, investigating the performance of these neurons individually in vivo can be expected to result in different values than when ensemble in vitro. Wit et al. encountered this effect in the 1970s.61

The rates of rise of ventricular and atrial muscle are difficult to measure without a digital recording oscilloscope. The leading edge is seldom defined on conventional oscilloscopes set up to capture the complete waveform.

20.3.2.2 Interpreting the papers of Antzelevich & colleagues

Antzelevitch and colleagues have been very active during the two decades around the turn of the 20th Century, culminating in a recent chapter 3 in the specialized text on cardiac arrhythmia by Podrid & Kowey. The material deserves close examination.

To set the stage, Grant & Carboni, also writing in chapter 2 of Podrid & Kowey attempt to describe the current flows in both SA node tissue and atrial and ventricular tissue using the conventional chemical theory of the neuron. They define the shape of the atrial and ventricular tissue waveforms without the prominent notch following the initial peak in the waveform. They do illustrate the shape of the SA node waveform that is that of a relaxation oscillator. They use a variety of current waveforms and current waveform names to illustrate the components of these waveforms. These names appear to be those of the Luo-Rudy model and based on continuum-based mathematical analysis (using numerical integration to solve differential equations). Unfortunately, some of the current components remain unnamed. Linovsky & Antzelevitch (1988) note the profusion of conflicting names for the same currents developed by various mathematical modelers (discussed further below).

Litovsky & Antzelevich present a paper in 1988 that set the foundation for their work to follow.62 While brief, it does suggest they employed the oscilloscope in a DC (direct coupled), rather than an AC (capacitor coupled) mode. While they stress their waveforms were measured transmembrane potentials, they do not suggest they are obtained using “gigaseal” patch clamp techniques. They also describe the fluid content of their micro-electrodes but do not indicate if their properties were fully compensated for in terms of square wave response or potential offset. They also describe their parametric stimulation using a square wave generator with 1-3 msec duration pulses at 2.5 times diastolic threshold intensity.

Figure 20.3.2-4 provides more definition of the waveforms they encountered, with a stimulation time line added for perspective. Any delay between the stimulation and the individual responses is not shown. The form of the waveforms is dependent on the duration between stimulus pulses and Litovsky & Antzelevich used a basic cycle length (BCL) of 500 msec to obtain these waveforms.


They have added additional information to the discussion in a variety of ways. They note that the degree of the notch following the first peak is not only a question of the source of the tissue topographically but also on whether it was taken from the inner or outer wall of the heart at the same location. They also report the depth of the notch appears to depend on the age of the subject and is clearly dependent on the BCL used during stimulation as shown clearly in their figure 2. Their figure 3 shows the depth of the notch (but not other features of the waveform) is susceptible to modification pharmacologically. They employed 4-AP (4-aminopyridine) as the suppressant.

Figure 3 also shows the notch disappears for waveforms collected after very short durations following a waveform containing a significant notch. It also shows the waveforms collected very soon after the initial waveform may achieve an amplitude that exceeds the phase 0 amplitude of the initial waveform. After using two dosages of 4-AP, they noted, "Neither concentration of 4-AP produced any significant change in maximum rate of rise of the action potential upstroke (2 experiments)."

Their Table 1, summarizing the parameters for these waveforms shows a wide standard deviation for samples of 24 in these experiments. In many cases the SD is 8-10% of the mean.

The conclusion is drawn that the notch is not an intrinsic feature of myocytic waveforms but is a developmental feature enhanced by parametric stimulation at widely varying pulse rates. A first order explanation of the myocytic waveform need not account for the notch.

Their figure 2 also brings into question why they use such long BCL's. A BCL of 2000 msec corresponds to a heart rate of only 30 ppm. The significant notches seen at this low rate are much less prominent at 150 ppm, again suggesting the notches are not intrinsic features of the myocytic waveforms.

Figure 20.3.2-5 reproduces their figure 3. It was acquired to study the in-vitro effect of 4-aminopyridine (4-AP) on the performance of cardiac tissue obtained from dogs. The tissue samples were taken from the inner and outer tissue of the same region of the ventricular muscle. The results are described as representative. The experiment consisted of stimulating the tissue for a period of time at a BCL of 2000 msec (panel A) and then introducing the 4-AP as a 1 mM solution. Panel B was recorded thirty minutes after 4-AP administration. The dosage was then raised to 5 mM and panel C was obtained 20 minutes after raising the dosage.
The individual pulses following the initial waveform were acquired by averaging the pulse waveforms from ten pulses introduced at shorter intervals than for the preceding pulses (a premature stimulus). The waveforms in the control group on the left do not show significant phase 1 & 2 activity for BCL's of less than 282 msec (considerably shorter than the 2000 msec (30 ppm) pre-test interval). The waveforms on the right show minimal phase 1 & 2 activity for all pulse intervals.

In the analytical part of their paper, they present Figure 20.3.2-6 analyzing the epicardium data from the above figure. The figure is valuable although it appears to contain some editorial problems. The simplest is the time constant parameter is described as $\tau$ in the data frames by $\tau$ in the caption and text. The text and figure assert that the waveforms associated with the 5 mM 4-AP administration do not exhibit a long time constant component. However, this is erroneous based on inspection of the frame C of the previous figure. The frame B and frame C waveforms are virtually identical. A dashed line has been introduced into frame C of the copy of the figure reproduced here.
Recovery of phase 1 amplitude in epicardium. Each panel shows a semi-logarithmic plot of the difference between phase 1 amplitude of the basic beat (BCL = 2,000 msec) and that of premature beats introduced at progressively longer S1 - S2 intervals (once after every 10th basic response) (see inset). The Δphase 1 amplitude (Δ) is plotted on a logarithmic scale so that an exponential decline provides a straight line. Curves fitted by eye. A: Under control conditions, two exponential components are apparent. The slow component decreases with a time constant (τ) of 500 msec (Δphase 1 amplitude at a diastolic interval of 0 = 13 mv). Subtraction of this component from the measured value yields a second exponential process (β) that decays with a t of 70 msec (amplitude = 18 mV). C: A τ = 570 msec line has been added to the original figure. See text and original caption. From Litovsky & Antzelevitch, 1988.
The figure clearly shows the presence of two distinct processes with time constants of approximately 70 msec and 500-600 msec depending on the stress level. The response is dominated by the longer time constant process for BCL intervals greater than 400 msec (rates less than 150 ppm). Only at BCL intervals shorter than the normal biological range (pulse rates significantly higher than 200 ppm) is the process related to the shorter time constant significant.

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Figure 20.3.2-7 from Antzelevitch (figure 2.3 of chapter 2 in Podrid & Kowey) provides additional information compared to the previous figure credited to Podrid & Kowey (their figure 3.9). Podrid & Kowey noted, “Several studies have shown that the ventricular myocardium is far from homogeneous, as previously thought, and that it is composed of at least three electrophysiologically and functionally distinct cell types: epicardial, mid-myocardial (M) and endocardial.” Note carefully the notch following the initial peak in most of these waveforms. They note, “Major differences in the magnitude of the action potential notch and corresponding differences in transient outward current, Ito, have been described between right and left ventricular epicardium. Similar inter-ventricular differences in Ito have also been described for canine ventricular M cells.” “The M cell is distinguished by the ability of its action potential to prolong disproportionately relative to the action potential of other ventricular myocardial cells in response to a slowing of rate or in response to action potential duration (APD)-prolonging agents.” “These regional differences in Ito have been demonstrated using whole-cell patch clamp techniques in canine, feline, rabbit, rat and human ventricular myocytes.”

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In brief, the duration of the nominal waveforms of cardiac muscle changes as a function of their stimulation rate. At higher rates, the durations shorten. However, the refractory fraction of the pulse-to-pulse duration remains fixed, insuring that tetanus cannot occur in cardiac muscle.

The initial rise of these waveforms is very fast and independent of the BCL. The rate of rise of these waveforms is between 500 and 1000 Volts/sec (much higher than the 2–5 Volts/sec of the nodal cells). This suggests a very robust Activa within the myocytes of the cardiac system. The trough following the peak in the waveform is also essentially independent of the BCL, suggesting that the later portions of the waveforms are controlled by different mechanisms than the initial rise and the trough portions.

They note, “Several studies have shown that the ventricular myocardium is far from homogeneous, as previously thought, and that it is composed of at least three electrophysiologically and functionally distinct cell types: epicardial, mid-myocardial (M) and endocardial.” Note carefully the notch following the initial peak in most of these waveforms. They note, “Major differences in the magnitude of the action potential notch and corresponding differences in transient outward current, Ito, have been described between
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right and left ventricular epicardium. Similar inter-ventricular differences in \( I_{to} \) have also been described for canine ventricular M cells. The M cell is distinguished by the ability of its action potential to prolong disproportionately relative to the action potential of other ventricular myocardial cells in response to a slowing of rate or in response to action potential duration (APD)-prolonging agents. These regional differences in \( I_{to} \) have been demonstrated using whole-cell patch clamp techniques in canine, feline, rabbit, rat and human ventricular myocytes.

These waveforms do not easily associate any individual feature with the zero potential with reference to the extra-neural medium.

Waveforms of the “spike and dome” type are typically abnormal in live mammals of over 40 Kgms.

20.3.2.3 Describing the electrolytic currents flowing within the cardiac myocyte

The electrophysiological literature contains endless and conflicting lists of potential ionic currents flowing through the outer lemma of a myocyte treated as a single compartment. These include many conceptual currents including the recent “funny current” to account for the inadequacies of recent mathematical modeling of waveforms. Amin et al\(^{64}\) assert, “It is also called the funny current (\( I_f \)) because it displays unusual gating properties. \( I_f \) is a mixed \( \text{Na}^+ / \text{K}^+ \) current, which activates slowly upon hyperpolarization and inactivates slowly in a voltage-independent manner (deactivation) upon depolarization. \( I_f \) conducts an inward current during phases 3 and 4 and may underlie slow membrane depolarization in cells with pacemaker activity (i.e., cells with \( I_f \) and little or no \( I_{to} \)).

Wahler has provided a discussion of these currents which can be considered typical (2001, page 201). He notes, identification of the specific current or currents responsible for a specific phase of the various waveforms can be controversial. Following a time-honored practice, he asserts the current \( I_{to} \) (recognized by other investigators) is actually composed of two separate currents (\( I_{to1} \) and \( I_{to2} \)), which are carried through two physically distinct channels. He fails to describe the “exact nature” of the second component.

Mathematical models adopting the continuum-based theory to explain the intermittent waveforms of the neural system rely upon the chemical theory of the neuron. They have required continual modification to account for more precise laboratory measurements, in spite of the fact that no simple positive metallic ions in solution have been shown to pass through the natural lemma of a cell. On the contrary, repeated experiments have failed to show such porosity in both natural and artificial lemma. Use of various equations involving a chemical gradient also suffer from the fact that the uninterrupted biological lemma is an absolute barrier to small alkali and alkali earth ions (thus disrupting the putative gradient). To avoid this problem, it has become common to introduce a dynamically controlled pore into the lemma of the myocyte to support passage of each of the putative ions through the lemma.

As in the case of the Antzelevitch et al. and other papers, it has become common to associate multiple currents, or components of individual ion currents (labeled fast, slow, rectified, “funny,” etc.) with each phase of the cardiac action potential.

20.3.2.4 The waveforms reported in cultured myocytes

Xiao et al. have reported parametrically stimulated cardiac action potentials in their line of

\(^{64}\)Amin, A. Tan, H. & Wilde, A. (2010) Cardiac ion channels in health and disease Heart Rhythm vol 7 pp117–126
HL-5 cells acquired from Claycomb. The reported waveforms were acquired under patch clamp conditions and involved injecting hyperpolarizing currents to maintain a membrane potential around -78 mV before parametrically inducing AP’s with 100 msec square pulses. The waveforms do not show the complexity of those reviewed above and their short duration (<100 msec) are not compatible with the durations commonly associated with viable cardiac myocytes, although the duration may be typical for mice (resting pulse rate, 500-600 ppm). The waveforms in their figures 1, 3 & 7 appear to be the impulse response of a passive axoplasm described as a simple RC circuit. They do assert that the waveforms in figure 1-D were from the spontaneous generation of AP’s by one cell. This cell exhibited a very noisy resting potential indicative of analog amplifier operation. They also note the recurrent appearance of artifacts during the phase 0 portion of their waveforms.

Much of their effort was focused on the so-called “funny current,” If. Their waveforms in figures 4 through 7 do show nonlinear characteristics within their cells and noisy waveforms subject to parametric pulse amplitude. While indicative of nonlinear dynamics, they are not indicative of amplification within the cells (a clear requirement for an active device). The nutritive solution they used did not incorporate glutamate but the “pipette solution for recording Aps and If contained (in mM) 130 K-glutamate, . . . “

Only about 8% of their HL-5 cells showed spontaneous activity. Their discussion appeared to describe more negative than positive results from the study.

20.3.2.5 An alternate view of the electrolytic currents

The Electrolytic Theory of the Neuron takes an entirely different and verifiable approach. It associates all rapid changes in the electrical parameters of the myocyte to the movement of electrons along conduction phospholipid channels in the basic leaflets of the lemma itself.

To apply the Electrolytic Theory to the cardiac myocytes requires establishing several parameters beyond question.

1. While it is commonly asserted that the myocytes exhibit a resting potential near -80 to -90 mV, and a maximum positive potential of +30 mV. These values are generally inconsistent with those found in other neural tissue, particularly with respect to the reversal of polarity at zero millivolts.

2. It is generally asserted that the waveforms of the myocytes exhibit a “threshold” separating one region of operation from another.

A threshold is a clear indication of a driven oscillator. The principle type of oscillator exhibiting a threshold is the relaxation oscillator.

Confirming the precise limiting potentials and the precise shapes of the waveforms of myocytes is not easily. Recent literature seldom cites a source for these measurements.

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Figure 20.3.2-9 illustrates the difference between these two approaches that is applicable to the myocytes and all other tissue incorporating a neural function.

20.3.3 Developing the architecture of the cardiac mini-brain

Researchers exploring the cardiac system appear to have difficulty in describing the cytology of cardiac cells in detail. There appears to be little differentiation between neural and muscle tissue at the histological and cellular levels. While the label, conduction system, implies an electrical system of neural origin, this may not be the original authors intent. Much of the literature associates electrolytic waveforms with cells labeled myocytes, implying muscle tissue.

The waveforms recorded from myocytes exhibit very rapid changes in the potential across the lemma of the cells. These changes exceed the changes in potential associated with the stimulus. Such changes require an active device within the myocyte. The rate of change of the potential across the lemma is typically faster than the change in tension created by the myocyte and typically precede that tension.

The conditions discussed in the above two paragraphs require the presence of an active device independent of the myofibrils and sarcomere within the myocyte. It appears this active device is similar to that found in nominal neural tissue.

The conclusion can be drawn that the cells known as myocytes are in fact hybrid cells exhibiting the electrolytic properties of neurons and the electro-mechanical properties of conventional striate muscle cells. As such, the cardiac myocytes could be named neuro-myo-cytes.

The hybrid myocytes (neuro-myo-cytes) are multi-compartmented cells although imaging the internal lemma using electron-microscopy is difficult. The cells must be infused with a plastic and then cut and oriented so the internal lemma are imaged edge-on. Such imaging is very difficult to accomplish. Usual practice is to define a lemma based on differences in chemical concentration in the regions on its opposing sides.

Careful analysis of the data discussed in previous sections of Section 20.3 support the conclusion that the myocardium consists of hybrid cells (myocytes) combining neural and muscular properties. This assertion will be assumed in the remainder of this section and will be developed in detail in following sections.

The assertion that myocytes can act as both neurons and muscles simultaneously leads to a great simplification within the myocardium. The conduction system expands and contracts in rough synchronism with the muscle tissue, avoiding potential damage to the neurons of the conduction system due to differential and repeated stretching.

If the cardiac myocytes are in fact functional hybrids, their etiology probably differs from the conventional hypothesis (Demir et al., 1999). They exhibit a cellular structure very similar to
the sensory neurons of the auditory modality. If correct, the cardiac myocytes probably can be traced back to stem cells via the neurological branch of the cellular tree. The neurons are known to be adaptations of endothelium. The myocytes of the endocardium are continuous with the endothelium that lines the entire circulatory system.

Based on the above assertions, the architecture of the cardiac system becomes easier to understand. Three situations contribute to the determination that the cardiac system contains its own mini-brain as in the enteric system:

1. The comments by Ter Horst & Zipes relative to the presence of neural tissue (to the extent of 30% by volume) within the cardiac system.
2. The size of the cardiac muscle (significantly larger than 2 mm in major dimensions) calls for the employment of stage 3 signal propagation techniques to save power.
3. The “conduction” data of Lederer showing the presence of stage 3 signal propagation neurons (albeit with an extended pulse duration).

Figure 20.3.3-1 provides an initial proposal for the neural architecture of the cardiac neural sub-system.

![Diagram](image)

**Figure 20.3.3-1** Proposed architecture of the neural sub-system within the cardiac system. The architecture follows that of the primary system. See text.

The system is modeled after the parent neural system. Stage 5 is a limited cognitive stage, responding to autonomic and internal sensory inputs and issuing instructions. Stage 6 is an instruction interpretation stage issuing detailed commands to the neuroaffectors. Stage 7 consists of neuroaffectors (primarily of the paracrine type) that directly impact the myocytes. Signals are projected (rather than conducted or diffused) over long distances by the stage 3 and stage 7 neurons.

Insufficient information has been located as to whether the SA node and AV node are connected at the stage 5 level and/or the stage 6 level.
The stage 6 and stage 7 neurons within the cardiac system employ cardiac action potentials with pulse widths significantly wider, but pulse rates with a significantly lower maximum frequency, than in action potentials of the parent system.

There is no obvious need for a discreet set of stage 3 neurons between the SA node and the stage 7 neuroaffectors of the right atrium since the stage 7 neurons incorporate the signal projection capability normally found in the stage 3 neurons. A similar argument applies to the path between the AV node and the neuroaffectors of the right ventricle.

Based on the recent literature, it is likely the stage 7 neuroaffectors are releasing nitric oxide to control the muscle tissue. Epinephrine remains a significant neuromodulator of stage 7 neurons, and thereby affects the muscles by controlling the release of nitric oxide.

The topology of the neural subsystem, primarily on the surface of the heart, appears to be optimized to insure optimum ejection of fluids from the chambers of the heart. Tension appears to be generated initially at the farthest point of each chamber from its exit port.

### 20.3.3.1 The master oscillators of the SA & AV nodes

Iaizzo & Laske have provided an introduction to the subject of the SA node,

“All myocytes within the heart have the capacity to conduct the cardiac impulse. A population of myocytes is specialized to generate the cardiac impulse and then to conduct it from the atrial to the ventricular chambers. This population has become known as the conduction system.”

“One of the most conspicuous features of sinoatrial nodal cells is that they possess poorly developed contractile apparatus (a common feature to all myocytes specialized for conduction), comprising only about 50% of the intracellular volume. In general, although it typically cannot be seen grossly, the location of the sinoatrial node is on the roof of the right atrium at the approximate junction of the superior vena cava, the right atrial appendage, and the sulcus terminalis. In the adult human, the node is approximately 1 mm below the epicardium, 10–20 mm long, and up to 5-mm thick.”

The cells of the SA node resemble nominal myocytes but are more specialized to meet their quasi-neural responsibilities.

The high reliability required of the master oscillators used within the cardiac mini-brain suggests the character of the oscillator circuits used. This requirement suggests the oscillators are of the same form as the free-running oscillators used elsewhere in the neural system, a free-running relaxation oscillator. Such oscillators will go into single pulse generation mode whenever the circuit attempts to relax completely to its nominal quiescent state. Even under abnormal local physiological conditions, the relaxation oscillator will attempt to perform until they are totally starved of resources.

Relaxation oscillators are of the switching type; they cannot be effectively modeled using continuum-based mathematical techniques. Their modeling using switching techniques is the ultimate in simplicity; two distinct RC time-constant controlled phases controlled by the instantaneous gain of the active element which is in-turn controlled by internal positive feedback.

In the case of the SA node oscillator(s), the proposed circuit is identical to that of the stage 3 action potential generating circuits, but with different time constants in the attack and relaxation portions of the pulse waveform.

The question of whether the SA node involves a syncytium of neurons often surfaces. The
concept of a syncytium should be separated from a circus (discussed below). A single relaxation type oscillator provides very high reliability and all of the required functionality, i.e., it can be driven (controlled) over a limited frequency range by inputs from the autonomic neural system. However, in the absence of such autonomic input, it will oscillate independently. There is no obvious requirement for a syncytium of neurons in the SA node. The use of two or more such neurons operating in series may or may not offer increased reliability depending on their individual characteristics.

Cell culturists were among the first to argue, and have long argued, that cardiac muscle is not a syncytium but a reticulum of joined cells.

Grant & Carboni (page 40) provide an interesting comment related to the potential topology of the SA, and potentially AV, nodes. They assert, “There is a difference in sensitivity of the pacemaker cells to parasympathetic and sympathetic stimulation; increase in activity of either system is accompanied by a shift in the site of origin of the earliest pacemaker activity.” Canale et al. (page 68) cite Boineau et al. (1980) “who found a number of anatomically different point of origin of the pacemaker, each corresponding to a particular range of heart rate.” Such switching action is consistent with the idea of a mini-brain. It is also consistent with the idea of a selection of lead pacemaker through switching rather than through modification of the physical parameters of an oscillator. Finally, it suggests an additional mechanism to assure reliability of the overall timing function. Strogatz has reviewed the work of several other investigators from a theoretical mathematics approach.

Further review of his citations is needed to determine if the author’s employed an appropriate physiological model.

Figure 20.3.3-2 shows the proposed waveforms of the SA node oscillator compared to the nominal measured waveform. The total waveform was created by replicating the waveform of Lederer (page 484) at a pulse rate of 86 ppm. The waveform represents the axoplasm potential of the three chamber Activa within the neuron. Annotation has been added to show the threshold voltage of the neuron as well as its active (analog) operating region. It is not known what the zero voltage point refers to precisely. An excursion of the pulse waveform into positive potential area typically requires the presence of an inductance in the axoplasm (collector) circuit of the neuron. This criteria can be met by an elongated axon (without a Node of Ranvier).

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67 Canale, E. Campbell, G. et al. (1986) Cardiac Muscle. NY: Springer-Verlag pg195

Overlaying the reported waveform is the theoretical waveform for a free-running relaxation oscillator of the same configuration as used as an encoder in stage 3 signal projection circuits (Section 2.3). The nearly straight leading edge of the waveforms is indicative of a constant current source, the Activa collector terminal, charging a fixed capacitance. The rate of voltage change for the leading edge of the reported waveform is 2.37 Volts/sec. This rate of change may be limited by the test equipment employed. The capacitance of a patch clamp probe frequently exceeds that of the axoplasm compartment under investigation. Estimating the total capacitance of the axolemma plus test probe at 20 pF, the required charging current would be about 47.4 micro-micro amperes (47.4 picoamperes). The waveform changes character near its peak. First, the constant charging current begins to decrease as the Activa approaches saturation. In that region, the charging current begins to decrease until it effectively reaches zero at Activa saturation. Then, the oscillator circuit switches to its discharge phase and the pulse voltage begins to decrease according to a different set of conditions. The power supply attempts to restore the axoplasm potential to the sub threshold potential called for by the dendroplasm/podaplasm instantaneous voltage. The impedance of the power supply is characterized as that of a diode. Thus the falling waveform is more linear than expected by an RC circuit. The slowly rising potential in the active (analog) region represents an amplified version of the voltage difference between the dendroplasm and the podaplasm. When this potential reaches the threshold level, the circuit goes into a positive feedback condition and begins the generation of an additional pulse. The re-running process repeats itself.

The time at which the dendroplasm minus podaplasm potential reaches threshold can be altered by analog current inputs to the dendrites. This added signal incrementally raises or lowers the voltage of the slowly rising waveform, thereby reducing or lengthening the time before the waveform reaches threshold and pulse generation begins.

Grant & Carboni have indicated the rate of rise of the voltage during diastolic depolarization (phase 4) is 0.02 to 0.2 volts/sec.

The rising portion of the waveform is typically associated with the “Na+ current”, or a putative current of positive charges entering the axoplasm of the chemical neuron model. In the context of the Electrolytic Theory of the Neuron, this current is actually due to negative charges leaving the axoplasm via the Activa. The independence of the current associated with the rising portion of the waveform is demonstrated by pharmacological experiments that show changes in this current independent of other waveform components.
20.3.3.1.1 The AV node oscillator as a slave

Mechanically, the role of the AV node is to cause contraction in the ventricles after they have been filled with blood by the previous contraction of the atrial chambers.

It is generally recognized that the AV node oscillator operates in delayed synchronism with the SA node, but upon failure of the SA node oscillator, the AV node oscillator will carry on at a slightly lower frequency of pulse generation (typically 50 ppm versus a nominal 70 ppm while the subject is resting). This is a characteristic of driven relaxation oscillators. The AV node oscillator merely has a slightly longer discharge period than the SA node, causing it to operate at a lower rate. It accepts a current pulse into its dendroplasm (emitter) circuit from the SA node oscillator, or a relay neuron providing such a signal, before it normally reaches threshold. This results in an incremental increase in the dendroplasm potential. As a result, the circuit reaches threshold at a shorter interval determined by the SA node under normal conditions. The overall waveforms of the AV node are identical to the SA node shown above. The incremental change in the AV node dendroplasm potential occurs at the root of the ascending pulse waveform.

It remains unresolved whether there is an explicit neural connection between the SA node and the AV node or whether the AV node is stimulated by the mechanical activity of the right atrial muscle (Podrid & Kowey, pg 3).

20.3.3.1.2 Is the SA or AV node oscillator a syncytium

Historically, and based on limited information, the medical community has described each node of electrical activity in the heart as a syncytium, normally without further definition. Because of the high reliability requirement, it is unlikely these nodes contain a syncytium based on multiple oscillators in series. Similarly a “ring oscillator” arrangement is unlikely. Failure of a single neuron in these configurations would lead to disablement of the system. The potential for a node consisting of a set of oscillators operating in parallel, with potential cross excitation, is more likely. Failure of one neuron would then only reduce the effectiveness of a limited portion of the heart.

Boyett et al. have provided a review that suggests a parallel arrangement69. They provide considerable material on the SA node of the rabbit. The material is extensive and deserves close study.

“A characteristic feature of the SA node is much connective tissue, mainly collagen and fibroblasts, although the extent of connective tissue is species-dependent and varies from 50% in the rabbit, guinea-pig and rat to 75–90% in the cat. Another characteristic feature of the SA node under the light microscope is a high density of nuclei; in the monkey, the density in the SA node is approximately double that in the atrial muscle. The cells in the center of the SA node are reported to be 5–10 microns in diameter in the human and dog. In contrast, atrial cells in these species are ~100 microns in length and 15–20 microns in diameter.”

Boyett et al. use typical morphological jargon to describe the SA node cells;

“In the human and dog, in the centre of SA node, there are characteristic ‘P’ cells (or ‘typical nodal’ cells), which are believed to be the leading pacemaker cells. The cells are not only small as reviewed above but also ‘empty’. This is because they contain only a few poorly organised myofilaments (running in all directions and not organised into myofibrils). In the human and dog, typical nodal cells also contain fewer and randomly distributed mitochondria and little sarcoplasmic reticulum. Empty cells are characteristic of the SA node in a number of species.”

Their analysis of SA node operation suffers from the background of these morphologists and

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the chemical theory of the neuron. These difficulties will not be addressed here. They do note;

“The conduction velocity within the SA node is very low (0.03–0.05 m/s compared to that in
the surrounding atrial (~1 m/s).”

They do describe a “leading pacemaker site,” and “tentatively conclude, that the gradient
model represents the most likely organization of the rabbit SA node.” They do not provide a
block diagram of their gradient model. They compare the gradient model and the mosaic
model by showing plots of cell densities across the extent of the morphology of the SA node.
Their figure 1 does locate the leading pacemaker site within 100 microns in the rabbit SA
node. The site is localized based on isochrones (time in ms taken for the action potential to
propagate from the leading pacemaker site to the area shown) developed by probing. The
isochrones extend out over 3-5 millimeters from the leading pacemaker site.

Based on the size of the individual neurons, and localization of the leading pacemaker site, it
can be concluded that the SA node depends on a single pacemaker neuron oscillator
supporting a large group of orthodromic neurons, probably all capable of free-running
oscillation in the absence of stimulation from the leading pacemaker, and possibly
interconnected to insure synchronous operation. This suggests the syncytium of the SA node
is essentially a high reliability driven parallel oscillator arrangement.

Boyett et al. have apparently relied upon a paper by Joyner et al70, that involves totally
synthetic modeling of the relationship between mathematical models of two cells (one SA
node cell and one atrial wall cell). The assumptions in the Joyner paper include linearity of
the “conductance” between cells and adequacy of the models adopted from separate
predecessors. These assumptions are not defendable. Recognition that a synapse (not
addressed in these papers) is an “active diode” explains how a small SA node cell can
“drive” a large atrial cell via its dendritic terminal.

Boyett et al. go on, “According to the gradient model, there is a progressive regional variation in the properties
of SA node cells from the centre to the periphery (their Fig. 9). According to this model, the regional variation
in the roles of i_{Na} and i_{Ca,1} can be explained by a decline in the expression of Na⁺ channels from the periphery to
the centre.” In the language of the Electrolytic Theory of the Neuron, a decline in Na⁺ channels
is actually a decline in the current through the electrostenolytic power supply supporting the
particular neuron. This may be due to a change at the cellular level, or it may be due to a
variation in the porosity of the extra-neural medium.

Verheijck et al. have explored the synchronization of two electrically coupled but physically
isolated rabbit sinoatrial nodal cells71. Their technique depends on the earlier work of Wilders
et al72. Wilders et al. say;

“In this paper, we introduced a technique, model clamp, for coupling an isolated
cardiac cell to a simulated cardiac cell that we implemented with our previously
published detailed model of an isolated rabbit SA node cell.”

The technique is basically one of parametric stimulation at the axoplasm. Verheijck et al.
develop the concept of “mutual entrainment” in some detail but also note a number of
limitations of their modeling. Their discussion supports a syncytium or “democratic”
mechanism of pulse mode-locking among SA node cells but does not discuss or investigate a

Drives a Large Atrium Biophys J vol 50, pp 1157-1164

Coupled Rabbit Sinoatrial Node Cells J Gen Physiol vol 111, pp 95–112

72Wilders, R. Verheijck, E. Kumar, R. et al. (1996) Model clamp and its application to synchronization of rabbit
sinoatrial node cells Am. J. Physiol. 271 (Heart Circ. Physiol. 40): H2168-H2182
potential leading pacemaker site. Their experimental results are compatible with either arrangement.

Their technique involves considerable custom software and assumes a single compartment SA nodal cell. It appears they are using patch clamp techniques to contact the axolemma of the two cells (maximum diastolic potential −60mV and positive peak amplitude −427mV). The cells are interconnected via a PC computer, amplifiers and voltage to current convertors with a sample interval of 130 microseconds. The concept of a dendritic input was not considered in the paper.

Demir et al. have attempted to model the SA node of rabbit. They open with the strong statement,

“DESPITE NEARLY FOUR DECADES of histological, electrophysiological, pharmacological, and biochemical investigation, relatively little is known regarding the ionic mechanisms underlying the effects of vagal stimulation of the mammalian sinoatrial node (SAN) cell. Cholinergic and adrenergic modulation of cardiac pacemaker activity continues to be a topic of considerable interest in cardiac electrophysiology and related mathematical modeling.”

Their work is totally pharmaceutically-based and dependent on the chemical theory of the neuron. They model the typical neural membrane by employing ten(!) parallel branches plus that of the lemma capacitance. They describe their cell as exhibiting this many separate pores in the lemma. The number of free variables in their model allows it to emulate any arbitrary waveform, not just that of the SA node leading pacemaker site. In their discussion, they compare their results with those from eight other mathematical models. Interestingly, they assert their model is based on voltage clamp data from “myocytes,” rather than neural cells. They annotate twelve broad categories of caveats related to their efforts.

Recently, Tsujimae et al. contributed a paper limited to the effect of certain pharmaceuticals on the presumed rectified I, current of a cardiac cell. They provided a brief listing (with citations) of the many models of cardiac tissue.

“There are many mathematical models for cardiac APs, e.g., models for Purkinje fibers (19, 20), rabbit sinoatrial node (4), and ventricular cells of rat (22, 23), guinea pig (35, 40), dog (9, 38), and human (24) and models for atrial cells of dog (15, 25) and human (2, 21). No model is perfect, but each has its use for examination of different properties of cardiac AP behavior, with their respective limitations taken into account. In practical terms, it is important to adjust models according to the purposes for which they will be used.”

The last sentence is of critical importance. These mathematical models are not generic. Science yearns for a generic electrophysiological model of the neurons of the cardiac system that can be used in any situation.

Pullan has provided a book filled with mathematical models of the cardiac system. As discussed, these models are all single purpose models employing 10-20 empirically determined independent variables. While Pullan claims the models they reviewed are biophysically-based, they are in fact mathematical models representing a single non-recurring waveform with no component or mechanism explaining how oscillation is sustained. Most of the models cited by Tsujimae et al. (2007) are less biophysically-based than the biophysical-based mathematical model type reviewed in Pullan (2005).

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20.3.3.2 The distribution of signals via the Purkinje fibers

Figure 20.3.3-3 shows the gross arrangement of the “conduction system” of the heart from Iaizzo & Laske. It shows the great number of Purkinje fibers radiating laterally from the two branches of the Bundle of His, also known as the Purkinje Bundle. As noted by Lederer, the velocity of signal propagation within this system can be as high as 4 m/sec in the conduction bundle extending from the AV node to the bottom of the heart and radiating laterally as the bundles extend back toward the top of the ventricles. Because of the size of the heart in larger mammals, this high propagation velocity, nominally the same as that of the stage 3 neurons supporting the skeletal muscle system, is important. The ventricles empty by contracting from the bottom of each ventricle in a spiral manner. To accommodate this geometry, it is important that neural signals from the AV node reach the bottom of the ventricles quickly, and in correct time relationship with the SA node signals supporting contraction of the much closer atriums. This high velocity also insures the ability of the system to excite virtually all of the myocytes of a given chamber of the heart nearly simultaneously. To accomplish the spiral contraction of the chambers, lateral Purkinje fibers extend nominally perpendicularly from the main bundles on the surface of the muscle of each contracting chamber.

Figure 20.3.3-3 The conduction system of the heart. Normal excitation originates in the sinoatrial (SA) node and then propagates through both atria (intemodal tracts shown as dashed lines). The atrial depolarization spreads to the atrioventricular (AV) node, passes through the bundle of His, and then to the Purkinje fibers that make up the left and right bundle branches; subsequently, all ventricular muscle becomes activated. From Iaizzo & Laske, 2010.

Figure 20.3.3-4 illustrates the distribution of AV node signals to the myocytes of the left and right ventricles. A similar figure can be drawn for the distribution of signals from the SA node.
to the right and left atriums. The cardiac action potentials are initially propagated at high velocity down the bundles of His (alternately named the Purkinje Bundle). The signals are then projected laterally by the Purkinje fibers to multiple groups of myocytes. After transitioning from the conduction system to the main myocyte tissue, the signals are projected from myocyte to myocyte for an indeterminate distance via synapses located within the intercalated disks.

After a review of the literature, it is estimated that a Purkinje fiber may synapse with 10 to 100 myocytes. Following excitation by the oscillatory waveform from the Purkinje fiber, each myocyte synapses with a series of orthodromic myocytes causing a progressive contraction of the overall muscle. This arrangement of the Purkinje fibers and myocytic chains is compatible with the morphological arrangement of the cardiac muscles. De Bakker et al. have shown a similar but simpler depiction of myocyte-to-myocyte signal projection (2010, page 69).

The frame on the right shows the interface at the intercalated disks in greater detail. The synapse is shown in accordance with the Electrolytic Theory of the Neuron. As noted in the caption, the two portions of type 2 lemmata facing each other force all large molecules out of the synaptic junction space. The remaining molecules of water can no longer vibrate with a Brownian motion as expected at biological temperatures. As a result, they assume a liquid crystalline structure. This liquid crystalline water is often encountered (unexpectedly) in cryo-fracture experiments in histology. The resulting structure is the same as found elsewhere in the neural system. It can operate in one of two modes. The synapse can form an Activa which is biased to operate as an active diode passing the electrical current through the synapse loss-free. This operation mimics the synapses found widely in the neural system. Alternately, the structure can be biased to operate as a driven circuit, effectively regenerating the nominal cardiac myocyte waveform. In that case, its operation mimics the operation of Nodes of Ranvier elsewhere in the neural system. The literature does not provide sufficient information to determine which mode is used in actual practice. However, experiments with isolated single myocytes would suggest the cell includes an active component and the input synapse only operates as an active diode.

The mechanism of mechanical coupling between myocytes via the intercalated disks is not well understood. A desmosome is shown conceptually in the figure. However, since these myocytes are connected in series, significant tension can be generated in some intercalated disks. Thus, the actual desmosome structures can be expected to be more pervasive within the disks.
20.3.3.2.1 Electrical specialties of the Purkinje fibers

Canale et al. have noted the very rapid projection velocities of the Purkinje fibers and the fact they are either myelinated (only sparsely) or embedded in glia. These electrically insulated long fibers conform to the loss-free LC fiber of stage 3 neurons and exhibit the same high signal projection velocities as the stage 3 neurons.

They have also noted the very limited amount of sarcomere in these cells, and the associated absence of T-channels extending into the interior of the cardiocytes to serve the sarcomere (page 83). They give these velocities as 1.7 to 1.8 m/sec in peripheral fibers and...
2.1 to 2.5 m/sec in false tendons, compared to 0.4 to 0.9 m/sec in the main cardiocytes of the heart (page 81). Their citations relating to the velocity of propagation being proportional to the diameter of the axon should be disregarded in favor of the discussion in Section xxx of this work.

20.3.3.3 Circus movement or reentry

The study of arrhythmia has led to several interesting phenomena present in the abnormal heart. Antzelevitch & Burashnikov have noted75, “The circuitous propagation of an impulse around an anatomic or functional obstacle leading to reexcitation of the heart is referred to as circus movement reentry.” Multiple forms of this abnormality are documented in their paper.

20.3.4 The cardiac myocyte as a hybrid neuron & muscle

The electrical waveforms of the cardiac muscles differs fundamentally from the waveforms of skeletal muscle. Figure 20.3.4-1 reproduces a figure from a recent text on medical physiology.

It should be noted that Moczydlowski used a figure taken from a work more than 50 years old. Its continued relevance speaks to the speed of advancement in understanding of muscles.

A recent indication of the conceptual level of the recent research is the emergence of a “funny current” to join the ranks of ionic currents and rectified ionic currents, etc.

The figure shows the stimulus, a rectangular current pulse driving a coil exciting the antidromic neuron an undefined distance from the neuro-muscular synapse. The responses were obtained with fluid filled voltage probe of the muscle cell at defined distances from the synapse. Because the stimulus, and presumed neural signal was narrower in time than the initial response, it can be considered an impulse with respect to the response. The initial response shown is precisely that expected of a passive element as defined by the excitation/de-excitation equation of Section xxx. The subsequent responses are also those of a passive element given by the equation if the variable time is replaced by the variable position divided by the velocity of signal travel within the electrolyte of the muscle.

These initial and subsequent responses are drastically different from those associated with cardiac muscle. The responses of the cardiac muscle to stimulation show all of the characteristics of an active device either within the myocyte or associated with the antidromic synapse. Both of these possibilities are realizable within the electrolytic environment of the neuro-muscular environment.

The simplest possibility assumes the cardiac myocyte is an electrically passive component driven by a synapse that has been modified from its normal configuration of an Activa wired as an “active diode.” In this case, the synapse becomes an active driven relaxation oscillator exactly like those associated primarily with Nodes of Ranvier (NoR) within the histological boundary of a single neuron. In this case the driven oscillator would be formed between the axon of a neuron and the input structure of a myocyte (instead of between one axon segment and a second axon segment forming the NoR).

The more complex possibility assumes each cardiac myocyte incorporates an electrolytically active device within its envelope. This active device would be formed like that in a conventional neuron, and would consist of three electrolytically isolated compartments with the lemma between the compartments forming an Activa. Such an Activa would generate waveforms within its axolemma exactly like those reported for myocytes. As a result, the sarcomere would be stimulated by the voltage potential between the axoplasm and the

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surrounding extra cellular matrix. Simultaneously, the axon chamber could form an electrolytic synapse with one or more subsequent myocytes. In this case, the synapse would be wired as an active diode rather than a relaxation oscillator.

Two factors may impede resolving which of the above approaches is actually employed. First, the conclusions of most investigations into cardiac muscle remain conceptual based on the chemical theory of the neuron. Second, it may be difficult to differentiate between these two options due to the very small size of cardiac myocytes. It is difficult to probe the myocyte at different distances from the synapse with antidromic myocytes. It is equally difficult to determine the specific performance of the synapse prior to the myocyte. However, larger synapses in similar topological positions (NoR) have been explored in detail (Section xxx).

Figure 20.3.4-1 Endplate and related potentials orthodromic to the endplate of a frog neuromuscular junction. The endplate was excited by parametric stimulation of the (stage 7) antidromic motor neuron. The stimulation qualifies as an impulse relative to the duration of the initial endplate response. The set of responses follow the performance predicted by the excitation/de-excitation equation precisely when the time function is replaced by a distance function divided by the projection velocity of the signal. See text. From Fatt & Katz, 1951.
20.3.4.1 Experiments to resolve myocyte mechanical operation

Making adequate electrophysiological measurements is very difficult. Richardson & Xiao have offered a guide to establishing adequate conditions for these experiments76. Their figures 16.2 & 16.6 focus on the difficulty of eliminating the artifact that is frequently described as an overshoot in such recordings.

20.3.4.1.1 The fundamentals of actomyosin operation

The text, “Myosin: A Superfamily of molecular motors” indicates how complex the study of myosin has become. A family called myosin II is now focused on myosin as it occurs in cardiac and skeletal muscle. This family is frequently labeled the sarcomeric myosins.

Holmes has reviewed the mechanical aspects of myocyte operation using caricatures and high resolution electron microscope reconstructions77. He relies upon the Lynn-Taylor description of the mechanical cycle of the actomyosin complex78 and cites the more recent work of Geeves & Holmes79 and Coureaux et al80. Reggiani & Bottinelli present more detailed information on the myosin II family81. The Coureaux et al. paper discussed the very detailed molecular structure of a variation of the myosin II family and described aspects of contraction that could not be explained at the time of their work;

“What cannot be deduced from the myosin V rigor structure are details of the myosin state that immediately precedes the weak ADP-binding state (AM.ADPW) in the actin-myosin ATPase cycle”.

“A second missing structural state is that from which phosphate is released when myosin is bound to actin. Actin


After providing considerable additional information concerning the contraction of the sarcomere at the molecular level, Geeves & Holmes appear to concur with the above view written at about the time of their work. As of 2005, there was no complete explanation of the contraction mechanism in muscle. They provided Figure 20.3.4-3 with the caveat, “No crystal structure analogous to species 4 has been defined to date and its exact form is poorly defined.” They attribute the mechanical operation of the myocyte completely to the consumption of ATP. They describe the myosin-products complex (ADP.Pi) in detail. They also note, “Binding to actin causes a crossbridge to change its shape so as to move the actin approximately 10 nm, the ‘powerstroke’ or ‘working stroke.’” Regarding the operation of the Lynn & Taylor loop, they conclude, “However, concrete evidence remains in short supply.”

Sellers & Weisman indicate the actual motion of myosin V proteins has now been recorded using fast atomic force microscopy (AFM). They also note, “Optical-trapping nanometry revealed that a single two-headed molecule of myosin V takes 36 nm steps when moving processively on actin filaments.”

Most of the above studies focus on the role of ADP and ATP in the mechanical operation of actomyosin. They do not discuss the role of the electrolytic aspects of signaling in exciting the actomyosin. They seldom mention Ca++. Sellers & Weisman note with respect to their particular myosin Va, “It is unlikely that calcium is the physiologically relevant regulator of myosin Va in-vivo, however.”

Resolving the great divide in the role of Ca++ between the conventional histologist/cytologist and the proteonomic investigator may be difficult.

20.3.4.1.2 Fundamentals of actomyosin excitation

A. Huxley has addressed the earliest understanding of cardiocyte excitation. He noted, “The idea was originally supported by the observation that an intracellular injection of a solution containing calcium salts could cause contraction (with citations).” He noted three problems with the subsequent assumption that calcium transport across the lemma led to contraction, the diffusion time would exceed the known reaction time, the amount of calcium ion to enter during an action potential was much too small to provide one calcium ion per myosin molecule, and there was direct evidence for a specific inward-conduction mechanism. He concludes, “The picture which is emerging and which seems to be well supported is that the action potential causes some influence to spread inwards along a transverse component of the sarcoplasmic reticulum, probably the middle element of the ‘triads,’ which causes some other components of the reticulum to release calcium ions . . .”

Huxley’s observations were supported by the experiments of Huxley & Taylor (1958). They used a one to two micron micro-pipette to introduce a negative potential within a local region of the muscle fiber of a frog relative to the surrounding medium. It was noted, “no response occurred when the pipette was opposite an A-band, but when it was opposite an I-band, the application of a negative voltage to the pipette commonly caused contraction in that one I-band.” Both papers are worthy of review.

the role of actin as a piezoelectric transducer in the outer hairs of the sensory neurons of hearing supports the likelihood that actomyosin is also a piezoelectric transducer sensitive to a transverse electrical potential. Calcium ions are not known to play a role in the

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transduction by actin in hearing. The actin of the outer hairs is not recycled. In the case of cardiocyte (and general myocyte) contraction, it is possible the role of calcium ions is primarily related to the relaxation of actomyosin following contraction.

20.3.4.2 Experiments to resolve myocyte electrical operation

Several recent specialized texts have included figures showing a significant region of negative impedance in the in-vitro voltage–current characteristic measured in myocytes. Figure 20.3.4-3 summarized these responses. Although it is not clear from what compartment of the myocyte these waveforms were obtained, the appearance of a negative impedance region is clear evidence for the presence of an active device within the cell.

- Xiao et al reported their region of negative impedance in experiments with the HL-5 cultured cells from mice. Figure 4 of their data shows a negative impedance of -1000 ohms/pF. Unfortunately, their reported capacitances varied from 8 to 62 pF. They did not correlate any capacitance with specific I-V curves. Better statistically relevant values are needed.

- Wahler has reported regions of negative impedance in several sets of experiments with chick, guinea pig and rat ventricular myocytes.

- Kohl et al. reported their regions of negative impedance in a collage involving work with recombinant and native cardiac cells. They provided several citations.

White et al. have prepared a well referenced “invited review” on efforts to evaluate a strain of cultured cardiac cells labeled HL-1 from the mouse. They claim the strain has retained the electrical and mechanical properties of cardiac cells “that continuously divide and spontaneously contract while maintaining a differentiated cardiac phenotype.” While listing the studies that have involved the HL-1 strain, they provide little information to support their above claim. One of their key citations is Claycomb et al. of 1998. A second is Sartiani et al. of 2002.

Claycomb et al. describe the establishment of a cardiac myocyte cell line that (i) can be passaged indefinitely in culture, (ii) can be recovered from frozen stocks, (iii) retains a differentiated cardiac myocyte phenotype, and (iv) maintains contractile activity. While their results describe a variety of pharmaceutical applications to the cells, it does not address the contractility of the cells. Its figure 3 shows a uniquely passive response of the “control” cells to parametric electrical stimulation using patch clamp techniques and depolarizing pulses. Their electron micrographs do suggest a reasonably complete regeneration of cardiac muscle cells.

References:


88 Claycomb, W. Lanson, Jr, N. Stallworth, B. Egeland, D. et al. (1998) HL-1 cells: A cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte Proc Natl Acad Sci USA vol. 95, pp. 2979–2984
Sartiani et al. report on Xiao et al. have provided data on experiments with an HL-5 strain of murine atrial cardiomyocytes. They noted, "We should point out that in the present study, single HL-5 cells, not confluent monolayer cultures, were studied using the patch clamp technique." They report on both the parametric response of the cells to impulse excitation and the voltage–current characteristic of those cells. Together, the results provide a clear picture of their character. The impulse responses show the compartment of the cell accessed exhibits a passive character with a time constant of about xxx msec. The negative dynamic impedance recorded in their voltage–current data (on the order of –1000 ohms/picofarad over a range of –50 to –30 mV in figure 4) shows clearly the presence of an Activa within the confines of the isolated cell. They also note a capacitance of 8 to 62 pF for these small non-myelinated cells. Their static voltage–current characteristics for the cells (figures 5 & 6) are suggestive but less clear as to the diode-like impedance of the compartment of the cells accessed. They provide statistically relevant characteristics comparing their HL-5 cells with native adult murine ventricular cells.

Table 2. Characteristics of action potentials in murine HL-5 and adult ventricular cells

<table>
<thead>
<tr>
<th>Myocyte</th>
<th>n</th>
<th>MP, mV</th>
<th>AAP, mV</th>
<th>O5, mV</th>
<th>Vmax, V/s</th>
<th>APD50, ms</th>
<th>APD90, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-5</td>
<td>32</td>
<td>–77±1.3</td>
<td>96.5±3.1</td>
<td>19.2±2.7</td>
<td>20.2±4.2</td>
<td>41.5±3.0</td>
<td>120.0±7.1</td>
</tr>
<tr>
<td>Adult</td>
<td>25</td>
<td>–80±2.3</td>
<td>112.7±2.5*</td>
<td>32.4±2.5*</td>
<td>54.2±4.5†</td>
<td>8.9±0.7†</td>
<td>28.3±2.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of cells. *P<0.05; †P<0.01 vs. corresponding values of HL-5 cells.

Inducible cardiac action potentials were encountered during patch clamp experiments when the resting potential was held to –70 mV or more negative values (figure 2).

Xiao et al indicate the presence of “funny currents,” \( I_f \), although they do not introduce that name. They cite Sartiani et al., 2002. A “funny current” is indicative of a problem in a conceptual model.

It is appropriate to describe cardiac myocytes (or cardiomyocytes) more properly as cardiocytes based on their hybrid operation as both neural and muscle tissue. Incorporation of the identifier myo- gives an inappropriate indication of their form.

It can be concluded the individual HL-5 cells studied by Xiao et al. included an Activa within the isolated cardiocyte.

20.3.5 Modeling the major waveforms of the cardiac system

Many of the past modeling activities have suffered from imprecise knowledge of the test configuration used to acquire specific waveforms. It is critically important to know how the measured waveforms were obtained. Were they obtained under in-vivo operating conditions where stimulation was from antidromic structures, or were they obtained by parametric excitation under (frequently dual probe) patch-clamp conditions. To what extent were the measuring probes compensated to insure a “flat” frequency response (no

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Traditionally, the waveforms of the cardiac system have been modeled following the formalism of Hodgkin & Huxley, and various extensions of the chemical theory of the neuron. The procedure is normally based on continuum-based modeling of fundamental mechanisms with no switching during the duration of the waveform.

Pullan et al. have reviewed at least five of these types of models as of 200590. Like the mathematical models reviewed by Tsujimae et al., these models employ a set of differential equations that have not been solved in closed form. Instead, the investigators have relied upon numerical integration to provide their solutions. Like the models of Tsujimae et al., the models of Pullan et al. begin with a set of initial conditions and generate a non-repetitive waveform. Such models are incapable of emulating an oscillatory condition. Like the models of Tsujimae et al., the models of Pullan et al. become more complex as the level of precision sought increases. Summarizing Pullan et al. (page 78), “The level of complexity inherent in these cellular models has increased significantly over time, starting with only three currents and three gating variables, in the original Hodgkin-Huxley model. The Beeler-Reuter model contains four currents and six gating variables, while 12 currents and seven gating variables are included in the model of DeFrancesco and Noble. The more recent version of the Luo-Rudy model contains eleven currents, four fluxes and 11 gating variables, while the Noble et al. model contains 27 ionic currents, five fluxes, three sub-cellular spaces and tracks nine ion concentrations.”

With the number of variables used in these models, ANY arbitrary waveform can be modeled to ANY desired level of precision.

Oscillatory waveforms are not normally described using continuum-based mathematical modeling.

The continuum-based approach is in a sense fallacious. The variables introduced by Hodgkin & Huxley, h, i, & j are “dimensionless gating variable” used to switch the value of these multipliers between zero and one at arbitrary times, sometimes more than once (Pullan et al., page 93).

Nickerson & Hunter have diagramed all of the models they could locate, separating them into biophysical and simplified models91. Their paper is illuminating as to the piece-meal assembly of various models has been. None of the waveforms resulting from these models can be tracked back to physiological mechanisms (other than the purported flow of positive ions through the putative pores of a membrane. Figure 20.3-5 shows the results of three of the most discussed models. This figure can only lead to consternation among the physiology community. The waveforms either model different underlying mechanisms, or they fail to converge on a common waveform. Their figure 7.4 shows a similar situation for three other models compared to the experimental waveform.
Nickerson & Hunter note in their excellent review;

“We have surveyed the development of cardiac cellular electrophysiology models originating with the Hodgkin and Huxley model and evolving through the early Noble Purkinje fiber remodels and more recent Noble and Rudy ventricular myocyte models.

Beginning with the initial four ODE models from Noble in 1962, models are now being developed with many tens of differential equations requiring hundreds of parameters and integrating multiple aspects of cellular physiology. Such increases in complexity inevitably result in significant barriers to the use of the models by independent scientists or even application of existing models in novel scenarios.

All of the models described in Sect. 7.1 were primarily made available to the scientific community as textual descriptions appearing in scientific journals, i.e., journal articles. These original articles may include: a listing of mathematical equations of which the model consists; various parameter sets and boundary conditions which reproduce the various simulation experiments presented in the article; plots illustrating certain outcomes from the simulation experiments; descriptions of the various numerical methods and algorithms used; descriptions of model changes required for specific outcomes, etc. In order to perform simulation experiments with, or based on, these original models, modelers must first translate the textual description into a computable format. This is traditionally a time-consuming and error-prone process.

As the amount of biophysical detail represented in these cellular models continues to
increase, the technical overhead required to support and develop these models similarly rises. Tracking the provenance of model parameters, for example, is particularly problematic."

The Electrolytic Theory of the Neuron introduces an entirely different framework for describing the waveforms of the neural system. Instead of a single compartment cell, the Theory proposes a multi-compartment cell with the cells electrolytically isolated with an active (transistor-like) electrolytic device formed by the membranes between the compartments. The operation of the cell under this theory reduces the number of differential equations and parameters required to less than two dozen total (compared to tens and hundreds respectively).

Figure 20.3.5-2 compares the electrical characteristics of a myocyte under the chemical theory (as presented by Demir et al. in 1999 and others) and the Electrolytic Theory of the Neuron (as developed in Section xxx of this work). In frame A; the myocyte based on the chemically theory and employing continuum-based mathematical modeling involves the flow of multitudinous metallic ions through pores in the cell wall where the impedance of each of the pores is controlled by undocumented dynamic mechanisms. The figure suggests these mechanisms are conceptually related to PKA (an enzyme protein kinase A) and cAMP (an intracellular second-messenger, cyclic adenylic acid catalyzed by another enzyme). The theory originally assumed the predominant inward-directed conventional current is formed of simple ions of sodium and the predominant outward-directed conventional current is formed of simple ions of potassium. Recent versions have proposed a variety of combinations of currents and an array of rectified, delayed and “funny” currents. A full explanation of this complex figure can be found in Demir et al. A summary description of the individual currents is presented by Grant & Carboni writing in Podrid & Kowey (2000, page 37). They list five inward and seven outward conventional currents. Their description includes several currents labeled with question marks. It does not agree in detail with Demir et al. or with most other investigators. Katz (2011, chapter 14) offers an even longer listing; he lists twelve currents involving different names if not different functions than Grant & Carboni. Katz also defines, in words, multiple currents that contribute to various regions of the nominal waveform that he attributes to Purkinje fibers. He provides a “quaint” explanation of the term rectification (including “true” and “anomalous” forms without defining a diode per se). He does not offer specific citations in support of his presentation.

It appears that, when a new, more precise, or different waveform is acquired, it is compared to the waveform predicted by the set of partial differential equations of an earlier experiment based on continuum-based mathematical modeling. If a residue is found, an attempt is made to explain this residue by introducing another “current” and probably another partial differential equation to account for it. The coefficients in these empirical solutions have no significant theoretical or in-vivo physiological meaning. Failing to solve the differential equations in closed form fails to insure the individual solutions are orthogonal to each other, resulting in terms like “calcium-activated repolarizing potassium current.” Assuming the waveforms of myocytes involve switching circuits avoids these problems.

Frame B presents the alternate configuration of a myocyte according to the Electrolytic Theory. It relies upon the flow of electrons which actually form most electrical currents and flow in the opposite direction to the direction of the conventional current.

The charges involved in conventional current flow was defined erroneously by Benjamin Franklin in the 18th Century. He assumed the charges were positive and flowed from + to −. Scientists have known since the 19th Century, the actual charges were electrons flowing from − to +. No ionic flow is involved in conventional electrical and electrolytic circuits. Even in electroplating in a chemical bath, it is the electrons that move; the electrons approach and neutralize positive ions that then precipitate. Unfortunately, schools still teach elementary electrical theory on the assumption that the “conventional current” flows from + to −.

As reviewed in Section xxx, Hodgkin & Huxley were careful to describe an inward flowing conventional current as flowing from an area of high sodium concentration to
one of low sodium concentration. They used the euphemism, “inward Na⁺ current,” to describe this current. Neither they nor anyone else has ever demonstrated that sodium ions actually flowed through the cell wall. Experiments have repeatedly shown negative results. To alleviate this problem pores were invented. These pores have not been demonstrated successfully to date.

The outward potassium ion current” perpetuated since Hodgkin & Huxley is actually involves the inward flow of electrons through the electrostenolytic power source and the axolemma of the axon compartment (as described below). The “inward sodium ion current” is actually the outward flow of electrons through the type xxx lemma of the poditic chamber (also described below) of the myocyte. The input synapse is made with the dendritic compartment. It is the potential of this compartment relative to the potential of the poditic compartment that controls the electron flow (the actual current) through the Activa. The output synapse is made with the orthodromic myocyte.
Figure 20.3.5-2 Comparing the chemical and electrolytic representations of the myocyte. A; shows a representation under the chemical theory of the single compartment neuron and continuum-based modeling from Demir et al., 1999. B; shows a representation under the multi-compartment electrolytic theory and switching-based modeling. The dashed arrows represent “conventional” current flow. The solid arrows represent the actual flow of electrons in a circuit. No metallic ions related to electrical signaling penetrate the myocyte wall or pores in the myocyte wall. See text.
A neuron or myocyte is a cell formed with multiple internal membranes (lemma) that form electrically isolated compartments.

Look initially at the right half of frame B. All biological cells, including neurons and myocytes, are polarized initially by the electrosynthetic process shown in caricature at upper right. This process is developed in detail in Section xxx. Glutamic acid (glutamate to a pharmacologist) is the primary source of energy. It decomposes into GABA and CO$_2$ on the outside surface of the axolemma with the release of an electron at an energy of about 150 milli electron volts. This electron is injected into a specialized semiconducting region of the axolemma. Multiple electrons pass through the semiconducting cell wall and creates a potential of 150 mV maximum on the capacitor, $C_A$, formed by the insulating portion of the axolemma. The direction of electron travel is indicated by the solid arrow. The direction of the “conventional current,” $I_A$, is indicated by the dashed arrow; this ephemeral current is equivalent to the putative outward potassium ion current of Hodgkin & Huxley.

The axolemma of myocytes, to the extent they can be defined histologically, are not myelinated. Thus, the value of the capacitance $C_A$ is much higher in a myocyte than in a typical stage 3 neuron of similar dimensions. $C_A$ for myocytes are generally given as 50–80 pF, about ten to twenty times that of stage 3 neurons. This capacitance plays a major role in the dynamic performance of the myocytes.

Consider the left portion of frame B. Myocytes, like neurons, are characterized by several electrolytically isolated compartments. These compartments are generally formed by internal lemma utilizing lap joints at the exterior cell wall. The left-most compartment is the dendritic compartment. The next compartment is the poditic compartment, and the right-most is the axon compartment. Each compartment is filled with an electrolyte, a plasma, bearing one of these prefixes. The lemma of these chambers are fundamentally insulating, but have limited regions of specialized lemma generally acting as a diode permitting one-way travel of electrons along the backbone of specialized phospholipids. Each compartment exhibits a potential labeled $V_D$, $V_P$ & $V_A$. A dotted box encompasses the Activa formed by the close extremely close proximity of the two internal lemma. Charge flow through this Activa depolarizes the capacitor, $C_A$, axoplasm. The potential on the capacitor is generally described as the potential of the axoplasm. However, this potential would exist if the axoplasm could be removed without disturbing the charges distributing themselves along the lemma of the compartment in accordance with the laws of electrostatics. The charge flow through the Activa is described by the solid arrow. The conventional current associated with this charge flow is shown by the dashed arrow; this ephemeral current corresponds to the “inward sodium ion flow” of Hodgkin & Huxley.

The charge transfer through the Activa is controlled by the difference in potential between the dendritic and poditic compartments ($V_D - V_P$).

In conventional electrical engineering literature, this potential is described as the difference between the emitter and base ($V_E - V_B$) with the axon potential known as the collector potential ($V_C$).

Using conventional current terminology, the current passing through the Activa is equal to the difference in dendrite to podite potential multiplied by the transconductance, $g_m$, of the Activa. This current charges the capacitor, $C_A$, causing a depolarization with a rate of change given by the product, $(V_D - V_P)g_m/C_A$ in Volts per second or mV/msec.

A resistance, $R_p$, is shown representing a specialized semiconducting portion of the podalemma. A similar resistance, $R_d$, is shown for the dendrolemma. The product of the dendrolemma resistance and the dendrolemma capacitance, $CD$, equals the time constant of the dendrite compartment, $TD$. This time constant plays an important role in the dynamic operation of the Activa in myocytes.

For reference purposes, two electrolytic synapses (frequently described as gap junctions) are
shown. Their utility will be addressed below.

The literature focuses on two distinct waveforms, the one associated with the SA and AV nodes is clearly that of a free-running oscillator that can be synchronized to an antidromic pulse train. The waveform associated with the remainder of the cardiac system, while clearly involving a threshold and a very high rate of initial rise, appears to be a driven saturable amplifier rather than a driven or free-running oscillator.

As noted in Section 20.3.2, the pace of basic research, as separate from applied research, in the interface between the neural and muscular portions of the cardiac system has moved forward at a glacial pace. While a distinct conduction system is typically described, its cells are poorly differentiated from myocytic cells not associated with the conduction system.

There is not a clear distinction between the waveforms associated with the neural system and the muscular system in cardiology. The use of the term myocytes when discussing neural tissue adds to the confusion. The size of the heart in the larger mammals suggests that electromagnetic techniques, similar to those used in stage 3 signal projection neurons, are employed to distribute the cardiac action potentials over distances considerably greater than two millimeters. Thus myocytes with properties similar to stage 3 neurons are to be expected in the conduction system. They should also be expected to faithfully regenerate the cardiac action potential when acting as driven mono-pulse oscillators.

Stage 7 neuroaffector neurons are also expected to employ signal projection techniques and to interface with the muscle tissue. The character of this interface is not well defined; it appears to be primarily paracrine. The agents release by the neuroaffector neurons is also open to question, with recent literature introducing nitric oxide as an important neuroaffector agent in the cardiac system.

A complication arises due to the apparent interaction of individual myocytes via electrical synapses (frequently called gap junctions of width <100 Angstrom). The only recognized synapses represent electrolytic “active diodes” (Section xxx). They are not bilateral impedances as assumed by Joyner et al. and by the Wilders team. These interactions appear designed to enhance the uniformity of muscle stimulation within a region of the heart muscle(s).

Release of chemical agents by the stage 7 neurons result in the stimulation of the myocytes for the duration of the chemicals presence in the paracrine region. Nitric oxide in particular is a free radical known to exhibit a short lifetime. The concentration of the active species in the vicinity of the myocytes is significant in the stimulation of muscle tissue.

Within the cardiac system, multiple types of waveforms, differing significantly from each other and from cardiac action potentials, are encountered. It is suggested these waveforms be described as “mylo-affector potentials” to differentiate them from stage 1 generator potentials and stage 3 & 7 action potentials. These mylo-affector potentials are also affected significantly by a variety of hormonal agents acting as neuro modulators on stage 7 neurons and as mylo-modulators on cardiocytes.

Because of their significant neural activity, it is appropriate to describe cardio myocytes as cardioctyes (reducing their focus on muscular activity alone) and assign their neural function to a new Stage 8.

20.3.5.1 The SA node oscillator and the cardiac action potential

In this and the following sections, the title “cardiac action potential” will be limited to waveforms generated by the SA and AV node oscillators and any stage 3 or stage 7 neurons faithfully reproducing this waveform. The more complex waveforms associated with muscle tissue will be described using different labels.
Section xxx develops the fundamental structure of the multi-compartmented neural cell. Section xxx develops the electrical parameters of such a neural cell. Section xxx develops how such a neuron can oscillate spontaneously, generating a free-running stream of “action potentials.” Such free-running oscillators employ positive internal feedback and are seen to switch operating modes at the peak of each pulse and at the change from negative to positive slope in the inter-pulse interval.

The Electrolytic Theory of the Neuron,

• Recognizes that the inward transfer of positive ionic charges through the axolemma proposed by Hodgkin & Huxley is actually the outward transfer of negative electrons from the axoplasm via an active electrolytic device, the Activa within the neuron.

• Recognizes the putative outward transfer of positive ionic charges through the axolemma is actually the inward transfer of negative electrons from the extra-neural environment into the axolemma via the electrostenolytic power source acting as a “load impedance.”

• Recognizes that the free-running oscillating neuron employs positive feedback in the Activa circuit between the axoplasm and the podaplasm above a specific threshold voltage ($\Delta V_T$) between the dendroplasm and the podaplasm.

• Recognizes the rising potential of the action potential during depolarization is due to an electron current exiting the axoplasm compartment. The rate of potential rise is given by the difference in potential between the dendroplasm and the podaplasm ($\Delta V_d$) multiplied by the transconductance of the Activa, ($g_m$) and divided by the capacitance of the axolemma compartment.

• Recognizes that the transconductance ($g_m$) of the Activa decreases as the axoplasm potential approaches the podaplasm potential. At the point where $g_m$ goes to zero, the Activa enters the cutoff region. The axoplasm circuit switches to the re-polarizing mode.

• Recognizing that during the re-polarization mode, the change in axoplasm potential is due primarily to the electron current entering the axoplasm through the combination of the axolemma membrane and the electrostenolytic power source on the exterior of that lemma.

• Recognizing that after the re-polarizing period, the axoplasm potential is controlled by the balance between the re-polarizing current through the axolemma and the product of $\Delta V \times g_m$. The gradual rise in the axoplasm potential in this region (the linear region of Activa amplification) prior to reaching threshold is due to the time constant of the dendroplasm/podaplasm circuit of the Activa.

Based on the Electrolytic Theory of the Neuron, the total performance of the neuron as a free-running oscillator can be described using less than seven measurable parameters;

• the resistance and capacitance forming the RC time constant of the dendroplasm/podaplasm circuit,
• the transconductance, $g_m$, of the Activa within the neuron,
• the capacitance of the axoplasm compartment,
• the electrostenolytic power supply maximum potential and its internal impedance, and
• the impedance of the podaplasm compartment required to achieve positive feedback above the threshold potential, $\Delta V_T$.

No other parameters or variable are required to describe the operation of the action potential generating neural circuit.

The parameters of the SA and AV node oscillators generating cardiac action potentials (widths typically 150-300 ms) are significantly different than those neurons of the primary system generating conventional action potentials (width 1-2 ms).

[xxx need a theoretical waveform here ]
20.3.5.2 Waveforms subsequent to the cardiac action potentials

[Time scales have been aligned for illustrative purposes (to remove any projection time element) and assume a threshold within the myocyte equivalent to a potential of −40 mV on the AV stimulus waveform.

[The duration of the excitation provided by the AV waveform compared to the duration of the myocyte electrolytic waveform is quite different from that of the stage 7 electrical stimulation of skeletal muscle. This difference must be taken into account when analyzing the operation of the bulk of the cardiac myocytes.

Because of the potentially different operation of the main myocytes when excited either by the nodal waveforms, or the output of a previous myocyte, these operations will be addressed separately.

20.3.5.2.1 AV waveform & main myocyte operation

Figure 20.3.5-3, expanded from Stanfield, is also critical to understanding the operation of the cardiac system92. A myocyte is a transducer of chemical energy into mechanical energy under electrolytic control. As such, it is described as a multi-port device. The critical points to observe are three:

- Blood is being pumped, or other work is being done in connection with the tension shown.
- This work requires the dissipation of chemical energy from that supplied by the mitochondria of the myocyte.
- The work is controlled by the level of stimulation by the neural system.

The temporal relationship between these waveforms and responses raises many questions concerning the neuron-like operation of the myocyte. The detailed shape of the waveforms and responses in the literature also raise questions about how they were obtained.

1. Does the significantly higher rate of rise of the initial myocyte waveform, compared to the rate of the driving AV waveform demonstrate the presence of an Activa within the myocyte?

2. If the myocyte incorporates an Activa, is the electrical waveform applied to the dendroplasm of the myocyte a true copy of the AV membrane potential shown, i.e., does the input synapse of the myocyte operate as an active diode over the relevant range?

3. If the myocyte contains an Activa, is that Activa biased to operate as a driven ‘pulse’ oscillator or as a high gain ‘linear’ amplifier?

4. At what AV membrane potential did the myocyte membrane potential depart from the resting potential baseline?

5. Did the myocyte membrane potential exhibit significant noise during its resting interval?

6. Did the myocyte membrane potential exhibit significant noise during phase 1, 2 or 3 operation?

7. Is the waveform reported for the myocyte the result of a lumped constant network, i.e., is the same waveform measured at the edge of the axoplasm closest to the Activa as at the extreme of the axoplasm leading to the output synapse?

8. In determining the refractory period, what was the shape of the initial electrical stimulus to the myocyte? Was the stimulus parametric or physiological?

9. Is the waveform driving the contraction mechanism of the same shape as the initial axoplasm waveform?

10. Is the overshoot in the myocyte waveform an artifact of the test configuration, i.e., is it present under physiological excitation or only when parametric excitation of the axoplasm is present?

11. If physiologically relevant, under what physiological excitation and operating conditions is the overshoot present?

12. Does the use of a “square” parametric excitation pulse, rather than a stimulus more closely matching the physiological AV pulse impact the operations of the myocyte?

13. Does the shape of the myocyte waveform remain the same under all

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**Figure 20.3.5-3** Electrical and contraction profiles of a myocyte cell in cardiac muscle on a common time scale. Lower waveforms from Stanfield, 2011. Dashed portion of myocyte response added for discussion purposes.
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physiological excitation conditions?

14. Was the myocyte required to perform work during measurement of the tension profile?

15. If the myocyte was performing work during the tension waveform, what was the temporal profile of the work activity?

16. If the AV membrane profile was used to cause contraction within the myocyte, what was the profile of any intermediate activity, such as the release of calcium ions or changes in the molecular configuration of tropomyosin or troponin?

17. If the myocyte waveform was required to initiate a subsequent process within the myocyte, was electrical energy consumed in this process?

Some of these questions can be addressed with confidence based on the material reviewed above.

1. A rate of initial voltage rise in a waveform greater than that of the waveform instigating the rise clearly defines the presence of an active amplification device between the two waveforms, in the absence of a transformer. Within a neuron, this device is known as an Activa. Every neuron or neuron-like cardiac myocyte in an animal biological system includes at least one Activa. The Activa is a three terminal device as opposed to the two-terminal lemma of a cell.

The variable resistors used to describe a lemma under the chemical theory of the neuron are also three-terminal devices with their instantaneous resistive value controlled by an unseen hand.

The Electrolytic Theory of the Neuron completely describes the performance of the Activa and its associated circuit elements in closed mathematical form. No gating variables or arbitrary current profiles are involved.

The increased rate of voltage rise, typically 500-1000 volts/second, can be caused by a high transconductance, $g_m$, within the Activa (when operating as an analog amplifier) or a net feedback coefficient greater than 1.0 in the circuit surrounding the Activa (when operating as a pulse amplifier).

2. Indications are that the dendrite potential applied to the input terminal of the Activa is a true copy of the AV potential stimulating the myocyte for AV potentials exceeding the threshold of the synapse acting as an active diode. This threshold does not imply amplification within the synapse. Measuring the dendrite potential in cells the size of cardiac myocytes in the laboratory is very demanding.

3. The nominal shape of the axoplasm waveform described in the literature for a myocyte, other than one associated with the conduction system, suggests the Activa is biased to operate as a high gain analog amplifier, with potential saturation (area of phases 1 and 2) at the most positive excursion of its dynamic range. The typical resting potential of atrial myocytes is from -80 to -90 mV (Grant & Carboni, 2001). The saturation level is near zero relative to the surrounding extracellular medium but this relationship to zero appears to be coincidental.

4. A precise answer to the question as to the AV membrane potential (of the conduction system myocyte directly stimulating a first cardiac myocyte in a serial chain) at which the cardiac myocyte waveform departs the resting potential is difficult to answer. No experimental report has been found describing two cells meeting this direct association criteria. It is reasonable to expect the AV membrane potential must rise at least five mV (to insure no extraneous interference - noise) but less than 10 mV to stimulate significant action by the cardiac myocyte.

5. Most records in the literature (example, Kuriyama & Csapo, 1961) do not show more noise on the axoplasm waveform while at its resting potential than during dynamic operation. This suggests the Activa, or its antidromic synapse, is in cutoff until external physiological
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stimulation raises its dendrite to podite potential above a critical bias value. This condition can also be achieved parametrically by rapidly changing the axoplasm potential via patch clamp techniques, but such excitation may have other side effects.

6. The appearance of phase 1 and 2 features in a myocyte waveform are relatively rare events, particularly for natural stimulation within the physiological pulse range in-vivo. When phase 1 and/or 2 features are present, they do not exhibit excess noise.

7. The cardiac myocytes are typically quite small relative to the size of the aperture of a patch-clamp probe. As a result, it is likely the patch-clamp measurement represents a lumped-constant measurement of the axoplasm potential, even though there may be finite projection of the potential from its point of origin near the Activa to its impression upon the output synapse and any chemo-mechanical transduction mechanism within the myocyte. The interior of a cardiac myocyte is quite crowded, making it quite possible to record the potential within the sarcoplasmic reticulum rather than that within the axolemma and outside of the sarcoplasmic reticulum.

8. The refractory period is defined in terms of the ability to excite a second electrolytic waveform in a cell following its initial excitation. The sensitivity to excitation depends on the instantaneous dendrite to podite potential compared to the threshold value of that potential. During phase 4, the sensitivity varies continuously as the dendrite to podite potential decays toward its resting potential. Thus, the definition of the refractory period varies with the test stimulant amplitude. It may also vary with the shape of the test stimulus, particularly if it is introduced parametrically into the cell. Physiological stimulation frequently encounters a lead-lag network associated with the dendrite circuit impedances relative to the impedance of the input synapse.

It should be made clear that the refractory period is a characteristic of a relaxation oscillator circuit. It occurs during the relaxation interval, nominally labeled phase 4 in cardio-electrophysiology. It does not occur in an overdriven amplifier circuit such as in ventricular muscle. The caricature in Boron & Boulpaep (page 487) is labeled ventricular muscle. It shows an effective refractory period during phase 1 & 2 that actually describes the interval when the Activa of the muscle tissue is in saturation.

9. At this time, it is not known what the final mechanism is that causes the contraction of the myocyte. Therefore, it is not possible to specifically identify the time profile of the final stimulus to the sarcomere of the myocyte. The stimulus profile provided by the axoplasm potential and the resulting tension profile are the only time functions available. The delay between the beginning of the tension profile and the beginning of the axoplasm profile in Stanfields representation (a measurable delay and a significant different profile) suggest additional chemical activities are probably involved. These activities likely involve calcium ions interacting with tropomyosin and/or troponin and those entities interacting with the actomyosin complexes.

10 thru 13. The question of whether the overshoot associated with phases 0 and 1 is physiological or an artifact is a difficult one. Investigators are not known to have demonstrated their test probe and instrumentation exhibited a flat frequency response before or after their experiments. Absent such a demonstration, it is quite possible the overshoot is an artifact of the test set.

Currently, the absolute potentials of the power supplies and biasing circuits of the myocytes are unknown. As indicated in Section xxx, achieving an absolute axoplasm potential within about 10 mV of zero relative to the surrounding medium is difficult in a conventional neural circuit. The key question is what is the sources of electrical power to the myocytes. Is the principle source the electrostenolytic process involving the glutamate/GABA reaction and generating -154 mV? Is there an alternate source providing a positive potential? The negatively charged glutamic amino acid is uniquely qualified to provide the negative potential key to neural operation. However, a variety of chemicals could provide a positive electrostenolytic potential. No such electrostenolytic potential has been encountered in the neural system.
When an overshoot appears in laboratory results, it is typically the result of parametric stimulus in-vitro, and under unusual pulse rate conditions such as those of Litovsky & Antzelevitch.

Absent better in-vivo data under more physiological conditions and the demonstrated performance of the test equipment, the overshoot will be considered a secondary effect, if not an artifact of the research environment.

14. Most laboratory experiments have focused on the tension generated by the myocyte in response to stimulation. Tension is not as effective measure as work, the energy expended as a function of time. Tension is frequently measured under one of two conditions; isometric contraction, the development of tension without physical movement (no external work is accomplished), and isotonic contraction, the development of tension during mechanical contraction of the length of the cell (external work is the product of the tension and motion as a function of time).

15 thru 17 Analysis of the output waveforms of the cardiocytes to date suggests the neural circuit of the cardiocytes excites the contraction mechanism electrostatically. Therefore, the cardiocyte need not dissipate any power to support this activity. All of the energy consumed in the process of contraction comes from the presence of chemical agents stored within, and apparently recycled by, the cell.

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It is also important to confirm whether these waveforms were acquired using patch clamp techniques, which were only developed in the mid 1970's by Sakmann & Neher. Only patch clamp waveform recording assures the waveforms are not due to the summation of waveforms from multiple adjacent cells.

20.3.5.2.2 Main myocyte to myocyte excitation and operation

Katz has presented a conceptual figure of a main myocyte (cardiocyte) waveform without attribution (page 373) and made several assertions based on it. He uses the term propagated action potential without demonstrating it involves propagation in the sense used in this work. He does not even demonstrate the broader term projection applies. Second, he asserts these waveforms involve a threshold level that when exceeded initiates a regenerative process. The following material will demonstrate the main myocyte waveform is generated by a high transconductance amplifier operating above threshold but does not involve a regenerative process (except under conditions of stress, see Section 20.3.5.6).

20.3.5.3 The proposed cellular topology of myocytes

20.3.5.4 The electrolytic circuit of cardiac muscle

The recorded rates of rise of the waveforms associated with myocytes is very strong evidence of an active amplification device within these cells. This section will assume the presence of an Activa within each myocyte, like that assumed to be present within the SA and AV node cells, and all other neural cells. The circuit will have the configuration illustrated in Figure 20.3.5-4. Frame B is drawn with the synapses in the form of active diodes, rather than three-terminal Activa circuits similar to Nodes of Ranvier. In an alternate view, the cytological structure on the left would become part of the synapse instead of the myocyte. The circuit in frame A would remain the same but would be associated with the revised cytological structure.

The evidence for amplification within a cell from patch clamp investigations on single myocytes isolated in-vitro would suggest the configuration illustrated here is the most likely one.

The circuit shown is similar to that of all other neural tissue with the exception of its output
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circuit. The output circuit exhibits two interfaces between the axolemma of the cell and other elements. The primary interface is with the sarcoplasmic reticulum and/or sarcomere of that cell. This interface supports the contraction of the cell. The second is the conventional synapse with a orthodromic cell, embedded in the intercalated disk. This interface supports the unusual ability of cardiac myocytes to stimulate orthodromic myocytes directly.
The proposed electrolytic circuit of a cardiocyte (myocyte). A; the neural circuit controlling the sarcomere as the chemical energy to mechanical energy (tension) transducer. The role of the sarcoplasmic reticulum remains open to question. B; the histological representation of the same circuit showing an end-on view of the cardiocyte. See text.
It should be noted that, like other Activa within neural cells, the potential of the axoplasm is not determined by Nernst’s Law of chemistry. It is determined by the mechanisms associated with the “transistor effect.” The axoplasm potential under quiescent conditions (the resting potential) is determined by the difference between the dendroplasm and podaplasm potentials, and the electrolytic load formed by the orthodromic synapse (usually negligible), the impedance of the axoplasm power supply (typically dominant) and the impedance of the sarcoplasmic reticulum and/or sarcomere. The quiescent axoplasm potential can be adjusted to any potential below the limit set by the electrostenolytic supply by adjusting the dendrite to podite voltage difference. It is typically adjusted to meet the requirements of the orthodromic mechanisms it impacts. It varies from about –150 mV in pulse circuits such as Nodes of Ranvier, to about –70 mV at the output of most sensory neurons operating in the analog regime.. In the cardiac situation, the nominal quiescent potential is frequently reported as –80 to –90 mV. Such an intermediate value is suggestive of the unique combination of signaling and transduction roles for cardiac myocytes. Sperelakis et al. have provided a more explicit list of resting potentials for various types of cardiac myocytes, but without supporting statistics or citations.

Figure 20.3.5-5 shows the operating characteristic and temporal responses for the proposed neural circuit of the cardiocyte.
The top of the figure shows the operating characteristic with a superimposed load line representing the impedance of the electrostenolytic source. The static curves represent the operating characteristic when minimal current is flowing through the poditic impedance and a poditic source of +52 mV. Under this condition, a single stimulant pulse will cause the axoplasm potential to change from the quiescent value of about –80 to –90 mV to a maximum value of +35 mV as shown in the lower frame of the figure. As the current through this impedance increases, the average potential of the base terminal (poditic terminal of the Activa) becomes more negative and approaches about +12 mV (moving all of the operating curves to the right). Within the normal range of pulse rates, the peak potential of the axoplasm approaches zero volts from its quiescent value of about –80 to –90 mV.

The bottom frame of the figure illustrates the temporal responses expected from the proposed circuit. Under single pulse or low pulse rate stimulation, the output shows a distinctly peaked initial response followed by an exponential rolloff with a time constant determined by the poda impedance. This rolloff reflects the change in the potential of the base terminal of the Activa during a single stimulation pulse. As the pulse rate is increased (into the in-vivo range), the capacitance associated with the poda impedance achieves a steady state potential such that the peak of the waveform never exceeds the nominal zero...
volt potential level. The refractory period shown is due to both the duration of the stimulation and the time constants of the cardiocyte circuit following the termination of stimulation.

At potentials other than the quiescent potential, the axoplasm potential is also impacted by any electrolytic stimulation applied to the dendroplasm and/or podaplasma. The stimulation is particularly important in the case of the myocytes because of its temporal duration. It is also important because of the relatively low rate of rise of the leading edge of the stimulus waveform compared to a stage 3 action potential.

The voltage range of a myocyte waveform is controlled by the maximum and minimum (rail) voltages available to the cell from its electrostenolytic processes, in the absence of significant inductance within the cell’s electrolytic topology.

It is possible that the myocytes use different rail voltages than stages 1 through 7 of the neural system. This may be necessary to satisfy unique requirements associated with activating the contraction mechanism at the molecular level. However, no sources of electrolytic potential, other than the glutamate/GABA reaction have been identified.

There appear to be only limited data from original investigators on the maximum and minimum potentials recorded in myocytes. The test sets used to acquire these waveforms are seldom defined explicitly to the level required to insure any “overshoots” are not inherent to the test set.

The role of the sarcoplasmic reticulum and its store of calcium has not been resolved. Most of the molecular physiologists studying the myocytes do not assign a role to calcium in the excitation of the actomyosin complex. On the other hand, the pharmacologists and medical investigators assign a critical role to the Ca++ ion. This role may be due to its presence in high concentrations and its historical association with currents through the membranes based on the chemical theory of the neuron. If the role of the Ca++ ion is significant, it is likely that its release is under electrolytic control and its complexing with the proteins tropomyosin and troponin leads to the contractions of the actomyosin complex in accordance with the recent models based on Lynn & Taylor.

An alternate proposal is that the electrical potential of the axoplasm controls the contraction of the sarcomere directly through the type 2 lemma shown as a diode in the figure. This approach leaves no direct role for Ca++ in myocyte contraction.

The transduction performed by the sarcomere will be addressed in Section xxx. It is noteworthy that the process is fundamentally different from that associated with skeletal muscle because of the high duty cycle of the stimulus waveforms and the response profile of the contractions. The short-term tension is not proportional to the pulse rate of excitation.

Frame B of the figure leaves the interconnection of the neural portion of the myocyte and the sarcomere open. The sarcomere and sarcoplasmic reticulum are shown immersed in the axoplasm but insulated from it. More research is needed before the precise operation of the sarcomere can be specified and the internal structure of the myocyte detailed completely.

The input and output synapses are shown as active diodes in accordance with the Electrolytic Theory of the Neuron. The electrostenolytic power supply for the axoplasm is shown explicitly. Its maximum voltage corresponds to Vm in frame A. Its impedance network corresponds to Zm in frame A. The “currents” shown correspond to the actual flow of electrons through the various elements of the myocyte, rather than the fictitious currents of positive charges. The dominant role of the two electron currents occur during the phases of the electrolytic waveform indicated. The major current, eA+, during phase zero is due to electrons leaving the capacitor, CA, formed by the axolemma via the Activa and the podite impedance, Rp. This flow results in the depolarization of the axoplasm relative to the extra-myocyte environment. The flow of electrons into the axoplasm and their accumulation along the axolemma surface (forming the capacitance, Cm) during phases one, two and three result in the re-polarization of the axoplasm. This inward electron flow (outward
conventional current flow) may be modulated by electron flow through the Activa (which may be active during these intervals).

Only the resistive component, $R_D$, of the impedance, $Z_{ds}$, and the resistive component, $R_P$, of the podite impedance are shown in frame B. No values for the voltage and capacitive components could be found in the literature. Their values are probably similar to that of other neurons. However, better estimates of their value depend on resolving the specific operating mode of the Activa and its supporting neural circuit elements (Section xxx).

It is proposed that any apparent excursion of the electrolytic potential of myocyte axoplasm into positive values relative to the surrounding extra-myocyte space is due to the test protocol or test set configuration, including the use of a stimulus pulse with a leading edge rate of rise greater than the five volts/sec associated with in-vivo SA and AV node waveforms. Lehmann-Horn et al. have described this problem94;

When fast changes of the membrane potential need to be captured (e.g., action potentials), it is important that the recording system has a high response time to follow the signal (i.e., minimal or adjustable high-pass filtering). Typically, one of the main problems arises from different sources of capacitances within a given experimental recording setup; these are primarily electrode and stray capacitances. The electrode capacitance is best reduced by keeping the level of the bath solution low. Stray capacitances can often be reduced by using amplifiers with a driven shield. If necessary, it is possible to compensate remaining capacitances by adjusting a so-called negative capacitance, which is an integral component of most commercial amplifiers. Yet, it must be noted that this will increase the noise and might produce oscillations that may cause damage to the cell being investigated. Note that if a second electrode is to be used to stimulate the cells of study, this electrode must be shielded from the recording electrode in order to avoid capacitive artifacts in the recorded potentials.

These comments are in line with those of Richardson & Xiao discussed above.

Kuriyama & Csapo have provided examples of myocyte waveforms that vary with the test set and test protocol95. Their investigation was focused on uterine muscle. Similar data is being sought for cardiac muscle. They reference Hodgkin, 1958, and noted, “Special emphasis was put on the ‘overshoot,’ the short-lived reversal of the membrane potential, predicted by the ionic theory.” Figure 20.3.5-6 shows the overshoot conditions they encountered in the waveforms they acquired. After discussing the overshoot, they note, “We have seldom recorded action potentials with overshoot in spite of the successful penetrations of several hundred myometrial cells.” Csapo reproduced this figure with additional labeling in 1973 (Boume, vol II, page 23) showing the overshoots were associated with the “microelectrode technique;” apparently referring to a probe penetrating the myocyte lemma. It may have referred to a two probe technique, since he indicated depolarization of the “membrane potential” was induced electrically by stepwise increasing the injected current.

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Hodgkin generally did not claim a theoretical basis for any of his work. It appears overshoot is more commonly encountered in modern times when less than ideal patch-clamp procedures are used.

The “theoretical basis” is probably contained in the remarks of De Bakker et al. “Depending on the cell type, the transmembrane voltage ranges from –60 to –90 mV.” “A typical ventricular action potential is generated in case a depolarizing current, usually from activated neighboring cardiomyocytes, reduces the membrane potential toward about –60 mV. At this value of the membrane potential, sodium channels open and the membrane potential will move toward the sodium equilibrium value of about +20 mV.”

De Bakker & van Rijen are quoting textbook values based on Nernst’s Equation. Kuriyama & Csapo along with many other investigators have noted a problem with the calculated Nernst values, “A persistent problem in the electrophysiological investigation of smooth muscle is the discrepancy between the theoretical and measured values of the membrane potential.” The same can be said for the relevance of the calculated sodium equilibrium value. Figure 20.3.5-6 illustrates a variety of theoretically possible waveforms from myocytes based on the impedance of the Activa collector circuit (axon circuit in a conventional neuron). The two limiting cases are a rapid initial rise followed by remaining at a high level essentially indefinitely (charging of a pure capacitor), and a rapid initial rise followed by an exponential decay resulting from a finite resistance in parallel with the collector capacitance. [xxx add ]

20.3.5.4.1 The electrolytic circuit of the nodal oscillators

Investigators have acquired a large variety of waveforms from cells in the vicinity of the SA and AV nodes. Lacking a theoretical framework to rely upon, it is very difficult to determine which waveforms are those of the oscillators rather than subsequent signal processing stages.

The oscillators of the SA and AV nodes are very similar to those of stage 3 neurons, both in the shape of their action potentials.
potentials and in their stimulation by the autonomic neural system. It will be shown that these oscillators are also relaxation oscillators. Based on knowledge of how these oscillators operate, it is possible to ascertain the output current capacity of the Activa, the transconductance of the Activa and the load impedance of the electrostenolytic impedance once a precise value is obtained for the axolemma capacitance.
Tseng & Hoffman have presented a variety of canine cardiac action potentials. Figure 20.3.5-8 shows one of their control waveforms from a single ventricular cell obtained by "whole cell" current recording patch clamp techniques. They describe their patch clamp technique as the continuous clamp technique. The current was elicited in response to a step to +30 mV from -60 mV. The probe resistance was 2–3 megohms. They cite their 1987 paper for background on their technique. It contains considerable information as developed in Section 20.3.xxx. While they recorded the current through their patch clamp channel, it is the potential of the axoplasm that is of primary interest here. An auxiliary potential scale has been added to this figure based on an impedance of two megohms for discussion.

The Tseng & Hoffman waveform is an excellent example of a switching type relaxation oscillator by inspection. A dashed straight line is overlaid on the leading edge of the waveform to stress its linearity. Until the region of Activa saturation is reached, the leading edge has a slope of xxx. A dashed exponential curve overlays the trailing (descending) edge. The trailing edge is seen to follow the exponential very closely.

In a relaxation oscillator, the mechanisms forming the two edges are entirely separate. The leading edge is formed predominantly by the removal of electrons from the axoplasm via the Activa during a period when the feedback coefficient exceeds 1.0 and is at its maximum. During this interval, the slope of the line is defined by rate of change in axoplasm potential divided by the capacitance of the axolemma. This slope is also equal to the charge current through the Activa divided by the capacitance, \( C_A \), of the axolemma. The Activa approaches saturation at maximum axoplasm depolarization. The reduced current through the Activa causes the feedback coefficient to collapse and the Activa to turn itself off (vertical line at the peak). The re-polarization of the axoplasm then begins exponentially with a time constant given by the impedance of the electrostenolytic supply and the capacitance, \( C_A \), of the axolemma.

[xxx need to find a good original voltage record of a SA or AV node waveform for human].

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20.3.5.4.2 The electrolytic circuit of the main myocyte

Figure 20.3.5-10 develops the transfer function of the neural circuit of the proposed myocyte, including the nominal transconductance characteristic. The conventional Activa has an operating range (commonly described as its dynamic range) of about 20 mV (Section xxx). If the entire AV node waveform (such as that of Stanfield, [Figure 20.3.xxx ] is transferred to the dendrite of the myocyte (cardiocyte to indicate it is more than a muscle cell), the Activa is over-driven, it will attempt to reproduce a region of the applied waveform compatible with its dynamic range. By setting the lower limit of that dynamic range (known as cutoff) at ~35 mV of the AV node waveform, and the upper limit of the range (known as the saturation level) at ~14 mV, the resulting predicted output waveform will be as reproduced in frame C. Frame C is in excellent agreement with the representation of the membrane potential of the myocyte presented by Stanfield.

The transfer function shown in frame B is linear over the majority of the dynamic range of the Activa, diverging into regions of hard saturation and cutoff as indicated. Within its operating range, the Activa presents an effective voltage amplification factor of 4:1. The effect is to sharpen both the leading and trailing edges of the output relative to the input. The sharpness of the trailing edge may be limited by the ability of the electrostenoletic supply to re-polarize the axoplasm of the cardiocyte due to the capacitive load on the circuit. The dashed line shows the optimum output reproduction of the input limited only by the transfer function.

Saturation tends to flatten the output peak significantly compared to the input waveform. The precise curvature of the transfer characteristic at high depolarization determines the precise shape of the peak.

The shape of the region of peak depolarization may also depend on second order effects found anywhere in the overall circuit of the input synapse and Activa circuits.

This analysis provides a predicted first order output waveform for the main myocyte that depends entirely on measurable quantities and known electrolytic mechanisms. The only effect currently beyond theoretical explanation is the curvature of the transfer characteristic between the linear regime and saturation and the linear regime and cutoff.

The first order output is constrained to zero potential in this analysis. Even this potential
requires a positive electrosenolytic power supply to insure normal operation of the Activa within the cardiocyte. The saturation potential across an Activa in the above circuit is minimally 20 mV, suggesting a positive electrosenolytic supply, $V_p$, of at least +20 mV is a requirement to support this analysis. To accommodate a possible overshoot of the output waveform under stressed conditions to +30 mV, an electrosenolytic supply providing up to +50 mV is needed.

No analysis of potential electrosenolytic sources providing a positive potential to the podaplasm of the Activa has been undertaken.

There are alternate methods of shifting the potentials within biological tissue, relative to the surrounding matrix, but these require further analysis.

It is proposed the main myocytes are over-driven analog amplifiers resulting from too large a signal being impressed upon the dendritic terminal of the Activa. The result is a nearly square pulse appearing at the axoplasm of the myocyte. Figure 20.3.5-10 shows the detailed neural circuit of the proposed myocyte. The initial potential difference between the dendroplasm and the podaplasm results in a potential of -85 mV in the axoplasm. The estimated potential of the podaplasm would be -38 mV. The fine line in the input waveform represents the axoplasm of the antidromic AV node signal. As the potential of this waveform depolarizes, the antidromic synapse begins to conduct and the heavy portion of the waveform is impressed upon the dendroplasm. As the dendroplasm potential becomes more positive than -35 mV, electrons begin to flow through the Activa and the axoplasm begins to depolarize as shown in the waveform on the right. The dynamic range of the Activa is exceeded when the dendroplasm exceeds -14 mV and the Activa goes into saturation. The maximum depolarization of the output circuit is reached at the same time. The output potential remains essentially constant until the dendroplasm again polarizes to more than -14 mV. As the dendroplasm polarizes, the Activa reduces the charge current withdrawn from the axoplasm and expects the axoplasm circuit to repolarize (dashed line). The axoplasm supply, $V_a$, attempts to re-polarize the axoplasm capacitance, $C_a$. If the supply is current limited, the output waveform will follow the solid line along a nominally exponential curve.
The only unusual feature of the circuit is the type 2 lemma of the poditic impedance oriented to make the outside of the cell lemma negative instead of positive and the presence of an electrostenolytic poditic source supporting that condition. Such a requirement is caused by the putative output signal going positive with respect to the extracellular space (at least under conditions of stress). This requirement has not appeared elsewhere in the neural system of animals. The chemistry of such an electrostenolytic source has not been explored.

The fact the output waveform is a clipped version of the input waveform and not a regenerated pulse waveform shows that internal feedback is not an important feature of this circuit (under normal operation, see next section). The poditic impedance is necessarily small relative to the output impedance (the collector impedance) of the Activa.

When the output waveform of the above cardiocyte is impressed on an orthodromic cardiocyte, no additional sharpening of the leading and trailing edges is achieved or required by this circuit. The orthodromic cardiocyte has a limited dynamic range also and the applied waveform over-drives the orthodromic neuron.

It is likely the quiescent potential of the sarcoplasmic reticulum is the same as the quiescent axoplasm. Depolarization of the axoplasm surrounding the sarcoplasmic reticulum is believed to cause the release of additional Ca++ ions into the confines of the sarcomere, thus causing contraction of the sarcomere. The interval of the output waveform can be compared with the intervals of the cardiac cycle in the human heart given by Katz (page 307).

To provide a more precise analysis and representation of this circuit will require obtaining more accurate axoplasm potentials of a specific myocyte and the axoplasm potential of the

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Figure 20.3.5-10 The electrolytic circuit of the main myocyte (cardiocyte) with the input and output waveforms illustrated. See text.
antidromic myocyte exciting it.

20.3.5.4.3 The electrolytic circuit of the Purkinje bundle myocyte

[xxx review Katz pg 387–389 ]

20.3.5.4.4 Role of potassium & other neuro-inhibitors in myocyte waveforms

Tseng et al. (1987) has provided valuable information on the role of potassium in determining the shape of the canine cardiac myocyte waveforms. The cells were from the “deep myocardium.” Figure 20.3.5-11 shows the effect of changing the external concentration of potassium from its nominal value of xxx. Their caption read;

“(A) Effects of [K]o on the steady state I-V relationship of a dog ventricular myocyte. (B) Effects of [K]o on action potential configuration of the same myocyte. [K]o was changed from 4 to 1, 2, 8, and 16 mM. Between exposure to each test solution, the superfusate was switched back to 4 mM [K]o until the resting membrane potential and the action potential configuration had recovered to control values. At 1 mM [K]o, the resting membrane potential was −118 mV and the action potential either had a very long duration or the cell failed to repolarize fully and the membrane potential stayed at −40 to −50 mV. As [K]o was elevated, the resting membrane potential decreased and the action potential duration shortened. At 16 mM [K]o, the action potential was not all-or-none: it was a graded slow response.”

They employed a switched patch clamp technique to gather their data. They described their compensation technique for another part of their experiments using a continuous clamp technique but were less explicit concerning the switched patch clamp experiments. They also described their curve fitting procedures in words but did not show the underlying data points.

They reported “For apparent single cells, the width was 20-33 microns and the length was 110-140 microns.” They gave the capacitance [of the axoplasm] of their cells as 54 to 80 pF depending on the method of calculation.

“Impalements with single microelectrodes could be maintained for >2 h when the cells were held near the resting potential. With holding potentials of −45 mV or more positive and clamp steps beyond the physiological range, the cells deteriorated more rapidly (1-1.5 h).” These numbers are suggestive of the fuel reserves for the electrostenolytic process(es).

“Fig. B shows the action potential configurations at different extracellular K concentrations; these data were obtained from the same cell that provided the data in A.”

The ratio of the axolemma capacitance, C_A, to the collector impedance of the Activa and the impedance of the electrostenolytic power supply is critically important in understanding
the shape of the output waveforms of the cardiocyte. The capacitor introduces a phase shift in the relationship between the input and output circuits of the Activa.

The upper graph clearly shows a significant change in the negative impedance region of the Activa generating these waveforms. Such a change is most closely associated with the poditic terminal impedance or the impedance of the electrostenolytic source supplying the podaplasm (R_P). A change in this impedance has a direct effect on the internal feedback characteristic of the overall circuit.

The Electrolytic Theory of the Neuron provides an explanation for each of the waveforms in B based on the curves in A; a capability that is not shared with the chemical theory. As a result, the data collected by Litovsky & Antzelevitch and Antzelevitch et al. can also be interpreted explicitly.

**Figure 20.3.5-12** replots the Tseng et al. figure in a more standard form. Tseng et al. noted that their compensation method was incomplete. Note also they provide no data points. They indicated they had "similar" data but did not present it. Frame A has been reoriented to allow comparison of the waveforms to the conventional waveforms of an Activa within the cardiocyte. An additional set of axes have been added to support the theoretical waveform expected for the Activa in the absence of any topically applied potassium to the cardiocyte (heavy dashed curve marked 0K). It is assumed their statement, "Also, a low concentration of Ba (0.1 mM) made the steady state I-V relationship almost linear (data not shown)," would be similar to the 0K curve. Lacking data points to indicate otherwise, the waveforms of Tseng et al. have also been modified to suggest the curves all converge on the theoretical waveform rather than extend toward the upper right indefinitely.

With these modifications, the family of waveforms is immediately recognizable as the I-V characteristic of an Activa with a variable level of internal feedback via the poditic impedance, R_P. The result of this feedback is shown explicitly in the response waveforms of frame B. In the absence of any feedback (R_P ~ 0), the expected waveform is shown and labeled control. This is the waveform of an overdriven direct coupled Activa as developed above (Section xxx). The shoulder of the waveform is intentionally shown at other than zero millivolts for purposes of discussion. As the value of R_P is increased (due to the effect of the topical potassium), the output waveform changes progressively until at a potassium concentration of 16K, the feedback factor is at or near 1.0 and the output waveform approximates that of a driven monopulse relaxation oscillator. The same circuit configuration, with high value of R_P, is addressed in Section 9.3.3.

This analysis suggests a re-calibration, or more precise compensation of their test configuration, is needed to move their axes by 0.6 nanoamperes and about 50 mV.

Under this interpretation, the individual waveforms do not cross the displaced voltage axis as suggested by Tseng et al. for their original axes (page 676). Such performance would not be expected under any conditions as this would force the Activa into the cutoff region resulting in the rectification of the potentials at the zero current line.
Under the Electrolytic Theory of the Neuron, the only currents flowing in and out of the axoplasm of the cardiocyte consisted of electrons via the Activa and the axoplasm electrostenolytic supply. Only these electron currents are required to describe the operation of the cardiocytes in closed mathematical form. No alkali or alkali earth ion currents are involved. Phases 0, 1, 2 & 3 of the waveforms are entirely accounted for by conventional electrical circuit analysis (except for any notch that was not encountered in these experiments. The myriad of putative alkali and alkali earth ion currents, such as those documented by Katz (page 377-384), and diagramed on pages 389-390, can be ignored when discussing the output waveforms of the cardiocytes.

Tseng et al. only investigated the role of potassium as a neuro-inhibitor. Many other materials may play a similar role. The waste products of the poditic electrostenolytic process will naturally act as neuro-inhibitors if they are not removed from the region of the cardiocyte promptly.

The inhibition of the poditic electrostenolytic process provides a direct and quantifiable explanation for the interval-duration relationship in cardiac physiology. This relationship describes how the duration of the axolemma potential waveform becomes shorter as the pulse rate increases from its resting value. The relationship is directly related to neuro-inhibition of the poditic electrostenolytic process.

In attempting to understand their data, Tseng et al. noted a significant decrease in the resistance of the axoplasm (about 2:1) with increasing potassium concentration (over their range of 16:1) when measured with their parametric patch clamp technique. They did not specify the potential being maintained by their test set during these changes.

The only feature of the typical cardiocyte output response not accounted for is the brief transient, followed occasionally by a notch, forming phase 1. The time constant of the transient reported by Litovsky & Antzelevitch is 70 ms in dogs. This transient can be generated so many ways in a common podaplasm circuit, and/or due to the presence of the test set. Using a square pulse in the parametric stimulus can contribute to this problem if the probe is not capacitively compensated. The specific source will not be addressed unequivocally until more information is developed. It is most likely due to the stray capacitance between the axoplasm and the dendroplasm. It is possible the source of the transient is an integral part of a more complex mechanism capable of generating a damped sinusoid. Such a damped sinusoid can create a significant notch and dome characteristic.

These analyses lead to two conclusions; each cardiocyte contained an active amplifying device, an Activa, operating in the common or “grounded” podaplasm mode, and the topically applied potassium significantly interfered with the electrostenolytic process connected to the podaplasm. The application of potassium substantially increased the internal impedance, RP, of this process in a completely reversible manner. The means by which potassium interferes with a complex stereochemical process remains undetermined.

These analyses indicate the changes in the electrical waveforms of cardiocytes reported in the literature (noted above) are indications of the stress the cardiocytes are encountering. These stresses are due primarily to an insufficiency of fuel to support the electrostenolytic processes supporting the cardiocytes or to the buildup of waste products in the extracellular fluids of the muscle matrix. The problem appears more significant with regard to the electrostenolytic supply of the podaplasm than that of the axoplasm.

20.3.5.4.5 The podaplasm electrostenolytic power source

The chemistry of the poditic electrostenolytic supply involves capturing an electron from inside the podalemma and ejecting it into the extracellular matrix. The assumed unbalanced reaction could look like:
X + Y + e\(^{-}\) = XY or
X + e\(^{-}\) = Y + Z

While the reactants have not been identified, the reaction probably involves a positively charged amino acid (in contrast to the negatively charged amino acid of the axoplasm supply). If so, there are only three candidates, lysine, arginine and histidine. Lysine and arginine are both capable of participating in a reaction involving a nitrogen in a NH\(^{2}\) or NH\(^{3}\) group, or a reaction involving a more complex group containing one of these as a subgroup. Lysine has been labeled a diamino amino acid. Arginine can be labeled a triamino amino acid.

The energy required for the deamination of the \(\varepsilon\)-amino group of lysine, a saturated aliphatic, has not been located in the literature. In most biological reactions, lysine is transaminated rather than deaminated. Pohlki & Doye have discussed the hydroamination of aliphatics with a double or triple carbon bond\(^{98}\). They and others have noted these reactions are nearly thermodynamically neutral but involve a high activation barrier, hence the need for a catalyst in the inorganic reaction. A stereo-specific receptor on the surface of a type 2 lemma should be an appropriate alternative in the organic environment.

Deamination of cytosine has been reported as 28 kcal/mol by Frederico et al\(^{99}\). However, cytosine is an aromatic amino acid.

The energy of the sought reaction would be between 30 and 50 electron-volts. This is a very low value corresponding to a chemical reaction involving only 700–1100 calories/mole (well below the free energy of formation of ATP at 7000 calories/mole). This value might suggest a rearrangement within an electrostenolytic fuel source rather than a chemical reaction.

There is little information in the literature concerning the extra-cardiocyte matrix from which information about the participants in an electrostenolytic process could be derived. The great majority of the available information is concerned with the metabolism of the work generating processes.

Canale et al. have noted heart cells from neonatal rats grown in monolayer culture take up the essential amino acids provided by the culture medium (page 205). They go on; “Excessive utilization of glutamine, glutamic acid and arginine suggests that these amino acids are involved in intermediary metabolism. Moderate amounts of glycine and proline, and high amounts of alanine are released into the culture medium. Release of these amino acids represents \textit{de novo} synthesis and not protein breakdown.” Their subsequent comments relate to the citric acid cycle.

Glutamic acid and arginine are well known participants in the neural system and glutamine is well represented in the citric acid cycle and similar cycles.

The \(\varepsilon\)-amino group of lysine is known to be reactive. The \(\alpha\)-amino group of amino acids are known to form a Schiff’s base with almost any aldehyde. The \(\alpha\)-amino group of the amino acids are known to be replaceable by oxygen in a transamination. A rearrangement of lysine, arginine or histidine at such low energies is possible. They would likely involve either a hydride shift or alkyl shift, so called 1,2 shifts according to the carbonium ion theory\(^{100}\). These shifts result in the release of a positive charge. This would result in a less saturated arrangement than in amino acids and cause release of the new form by the receptor site.

Current usage replaces carbonium ion theory with the term carbocation theory

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because of a need for more specificity. In present day chemistry a carbocation is any positively charged carbon atom.

The minimum energy required for isomerization, apparently involving breaking a $\pi$-bond, is reported to be $2.64 \times 10^5$ J/mol or xxx.

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Lysine is a diamino amino acid. It is also known as 2,6-diaminohexanoic acid and $\alpha,\varepsilon$-diaminocaproic acid. Can be synthesized by the action of ammonia on $\alpha$-bromoisocaproic acid

Leucine is a mono amino acid. It is also known as 2-amino-4-methylvaleric acid and $\alpha$-aminoisocaproic acid or 2-amino-4-methylpentanoic acid.

These two amino acids are both considered essential amino acids. They are not known to be synthesized by the human body.

The terminal $\text{H}_2\text{N}^+$ group of lysine appears particularly sensitive to separation from the molecule. Such removal would leave an isomer of the amino acid, leucine.

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The dipole potential of lysine is needed. Li et al. have analyzed the dipole potential of the material in water$^{101}$. [Li has a translation problem in that it indicates lysine contains an “alkali amido H$_3$N$^+$” which clearly should read basic amido H$_3$N$^+$]. Lysine immediately complexes with a large number of water molecules. Li et al. selected seven water molecules arbitrarily and discussed their reasoning. The resulting situation may not conform to the desired dipole potential of lysine in a stereochemical relationship with a lemma receptor. Li et al. have focused on the total energy of the complex rather than the net dipole. The level of their computations is intense. Ahluwalia has measured the surface potential of a lysine polymer film$^{102}$. [xxx Ahluwalia continues below]

---

Szent-Gyorgyi has pointed out$^{103}$, “that unsaturated ketones and aldehydes or diketones (like methylglyoxal) and dialdehydes, respectively, can act as electron acceptors with regard to proteins, thus converting these insulators to conductors.” Such a reaction offers interesting possibilities to both modify the podalemma and to provide the required electrostenolytic reaction.

A necessity for using one of these amino acids to support an electrostenolytic process is that receptors exist on the podalemma of the cardocytes involved. There appears to be no research record in this area. Arginine is believed to combine with a receptor on the stage 7 paracrine axolemma as part of the process of releasing nitric oxide. Arginine and lysine are very similar structurally. Both are capable of forming a Schff’s base with the terminal amine group and of participating in a transamination of the $\alpha$-amino group.

Bach, Wachtel & Miller have provided some information on the activation energy of a Schff’s

---


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base formed on the surface of a common lemma constituent, phosphatidylethanolamine. The values they provide appear too high to be compatible with a 30 to 50 mV electrostenolytic source, particularly the values recorded at biological temperatures below 40 °C of 1.25 and 1.34 eV. It would be inefficient to use such a reaction as a source of only 30 to 50 mV.

Alternate reactions to Schiff’s base formation might involve the total release of the terminal amine group of lysine or the replacement of the alpha amine with a doubly-bonded oxygen (to form α-Keto-ε-amino caproic acid, Lehninger, pg 444). Lehninger has also discussed an electron-accepting protein, ferredoxin, that has a standard reduction potential of -0.432 volts at pH 7.0. This is a more convenient but still high potential for use in the electrostenolytic supply of the cardiocyte podaplasma. It also reacts readily with NAD⁺ and NADP⁺ in order to pass along its captured electron and reconstitute itself.

An energy value for the release of NH₃⁺ from lysine has not been found in the literature to date. A trans-cis transition in 2-butene has a minimum energy of 2.6 kcal/mole (experimental) or 1.2 kcal/mole (computed). The computed value is equivalent to 52 mV.

A value of +52 mV (1.2 kcal/mole) can be taken as the likely potential of the poditic electrostenolytic supply based on the experimental record showing a saturation level near +30 mV for the axoplasm potential and the nominal potential drop across the Activa in saturation of about 20 mV.

In the proposed electrostenolytic process, lysine is a positively charged polar molecule while isoleucine is a non-polar (hydrophobic) molecule.

Ahluwalia et al. have provided good data on several modified forms of L-lysine that can be used to extrapolate a dipole potential for lysine alone. The estimated values for a monolayer of 100% L-lysine reflect the minimal reduction in packing factor compared to the more complex constituents.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Area/molecule</th>
<th>Surface potential @ 20 Ang./residue</th>
<th>Surface potential @ 100 Ang./residue</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% cis-azobenzene-L-lysine</td>
<td>42 Ångstrom²</td>
<td>370 mV</td>
<td>205 mV</td>
</tr>
<tr>
<td>57% L-lysine + 43% cis-azobenzene-L-lysine</td>
<td>31</td>
<td>300</td>
<td>75</td>
</tr>
<tr>
<td>Est. 100% L-lysine</td>
<td>28</td>
<td>250</td>
<td>~52</td>
</tr>
</tbody>
</table>

The surface potentials at 20 Ångstrom/residue are peaks in the surface potential. The potentials at 100 Ångstrom/residue reflect a flattening of the film pressure function as the area per residue is increased in a Langmuir apparatus. Their conclusion was that the

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backbone of the molecules was perpendicular to the surface of the water interface.

The estimated dipole potential of L-lysine at 100 Angstrom/residue, 52 mV based on the Langmuir apparatus results, is suggestive of the potential that could be realized by L-lysine acting as a biasing element at a suitable receptor on the appropriate region of type 2 lemma of the overall podalemma. Upon the loss of NH$_3$ through hydrolysis, the remaining non-polar isoleucine exhibits zero dipole potential.

Similar Langmuir apparatus results are described in Section xxx.

---

As a working hypothesis, the following scenario will be presented.

- The principle source of energy for the poditic electrostenolytic process is lysine.
- A region of type 2 lemma is present on the podalemma that provides opposite polarity of charge flow to that associated with the type 2 region of the axolemma.
- A suitable receptor is present on the type 2 surface of the podalemma.
- Lysine is stereo-chemically bound to a receptor on the external surface of the podalemma via its groups associated with its alpha carbon.
- The dipole potential of the lysine causes a nominal potential of +52 mV at the inner surface of the type 2 lemma.
- The lysine becomes polarized by acquiring an additional H$^+$ at its terminal NH$_2$ group.
- The polarized lysine is deaminated by hydrolysis, leaving $\alpha$-aminocapric acid as a residue and removing one electron from the interior of the type 2 podalemma. $\alpha$-aminocapric acid is also known as isoleucine.
- The leucine residue no longer satisfies the required stereochemical conditions and is released by the receptor.
- Another lysine molecule becomes attached to the receptor and the process is repeated.
- A nominal potential of +52 mV is maintained on the inner surface of the type 2 lemma as long as the electrostenolytic process can utilize lysine to form leucine in a timely manner.
- The process exhibits a rising internal impedance if the necessary lysine to leucine conversion rate cannot be maintained.

The energy equation based on this scenario is:

\[
\text{lysine} + \text{H}_2\text{O} + \text{H}^+ \rightarrow \text{isoleucine} + \text{NH}_3\text{OH}
\]

or

\[
\text{NH}_2(\text{CH}_2)_4\text{CH(NH}_2\text{)}\text{COOH} + \text{H}_2\text{O} + \text{H}^+ \rightarrow \text{CH}_3(\text{CH}_2)_3\text{CH(NH}_2\text{)}\text{COOH} + \text{NH}_3\text{OH}
\]

Free energy value of reaction here xxx
20.3.5.5 The electrolytic circuit of a myocyte

Figure 20.3.5-13 is a two-dimensional representation of the complete electrolytic and mechanical arrangement of the cardiac myocyte. The housekeeping portion is generic to any biological cell (Section xxx). The neural and housekeeping portions combined are generic to any neuron (Section xxx). The type 3 membranes between the neural and housekeeping portions of this cell are out of the plane of this figure. The muscle and housekeeping portions combined are generic to any muscle. The unique feature of the cardiac myocyte is the intercalated disk between serial cardiac myocytes.

Figure 20.3.5-13 The proposed fully elaborated electrolytic myocyte. Dashed area at upper left is Purkinje fiber or an antidromic myocyte. Dashed area at lower left is orthodromic myocyte. Butterflies represent the mechanical bonding between sarcomeres (intercalated disks when adjacent synapses on the left are included.) Neural and housekeeping portions are generic to neurons. See text.

A region of type 2 membrane is shown between the axoplasm of the neural portion and the sarcomere of the muscle portion. This region probably acts as an electrolytic diode between these areas. However, the literature does not address the presence or character of this membrane. The figure does not include a sarcoplasmic reticulum at this time because the function of such an element has not been described adequately. If present, the sarcoplasmic reticulum is probably part of the interface between the neural and muscle portions of the myocyte.
Several electrostenolytic sites are shown providing electrical polarization to the various compartment of the myocyte. Several catecholamine sensitive sites are also shown. These allow modulation of the operation of the neural and muscle portions via the paracrine hormone system.

The fact that the intermyocyte matrix is high in sodium ion concentration relative to the interior of the cell, and the potassium concentration is higher on the inside has no bearing on the operation of the myocyte. There is not chemical gradient between these two regions. The pumps used to maintain the virtually static concentration differences are not relative to the active operation of the myocyte.

20.3.5.6 The temporal serape of a cardiocyte

Figure 20.3.5-14 provides an informative illustration of the timing of events associated with a single main cardiocyte. The serape diagram is used in electrical engineering to allow study of the simultaneous waveforms at multiple points in an overall circuit. It can be particularly useful in analyzing the operation of a single complex circuit. The circuit of interest is diagramed on the left and a time sequence of the circuit potentials are shown on the right. The maximum and minimum potentials (the rail voltages) available from the electrostenolytic power supplies are shown as +52 and -154 mV. The voltage supplies have been dual labeled to reflect their histological designations, $V_{PP}$ and $V_{AA}$ for the poditic and axonal supplies; and for their semiconductor designations, $V_{BB}$ and $V_{CC}$ for the Activa base and collector supplies. The impedance of the dendroplasm circuit is assumed to be very low; hence, all dendroplasm waveforms are shown as horizontal lines at this level of detail. The squiggly lines are used to connect the circuit locations to the relative waveforms in the graphic.

This serape is drawn as a first order variant that does not include any capacitive elements that would introduce transients in the waveforms. Each set of potentials representing a specific case are shown connected to the next set by straight lines. A version of a second order serape will be presented in the next section.
In this case five specific states of operation are shown.

- The quiescent state shows the circuit as typically described absent any stimulation; a axoplasm potential of -85 to -90 mV, a podaplasm potential of about +43 mV and a slightly more positive dendroplasm potential at +45 mV. Because the dendroplasm potential is more positive than the podaplasm potential, an electron current is flowing through the Activa within the cardiocyte.

- The long BCL drive state shows the waveforms resulting from the over-driven condition described earlier. It is assumed that this is the n-th pulse waveform, where n is small, in a long series of pulses with a basic cycle length (BCL) in the biological range for a specific species. The dendroplasm potential has been driven farther positive by the stimulus via the synaptic input. As a result, the Activa begins passing a larger electron current and the axoplasm potential falls rapidly (phase 0) until the saturation condition is reached where the voltage between the axoplasm and podaplasm has fallen to about 20 mV. The large current through the Activa generates a significant change in the podaplasm potential due to the finite impedance of the poditic electrostenolytic supply.

- The inter pulse intervals will not be described in detail in this initial description of a serape.

- The intermediate loading state shows the waveforms resulting from the over-driven

Figure 20.3.5-14 First-order temporal serape of a main cardiocyte operating cycle. See text for details related to this figure.
condition for the n-th pulse in a long sequence where n is larger than in the above short BCL situation. Due to the loading of the poditic electrostenoletic supply, the effective poditic impedance has risen to the point where the peak axoplasma potential is reduced marginally and the axoplasma potential shows the effect of a significant feedback factor (still much less than 1.0). Detailing the changes in podiplasm and axoplasma potentials with time requires a larger scale graphic.

• The unity feedback state (F. B. = 1.0) shows a critical condition in the cardiocyte waveforms, usually only encountered in the main cardiocytes, and frequently in a large group of cardiocytes simultaneously. Following an incremental positive excursion of the dendroplasm (due to any cause), electron current through the Activa begins to flow causing a more negative podaplasm potential. As a result, the dendroplasm to podaplasm potential is increase and additional current flows. The result is a rapid increase in Activa current, a rapid positive excursion in axoplasma potential. This condition continues, almost linearly, until the saturation condition is reached, where the axoplasma to podaplasm potential difference is nominally 20 mV. At that point, the current through the Activa cannot increase further, and the podaplasm potential no longer rises. Instead, it begins to relax based on its circuit time constant. This reduces the podaplasm to dendroplasm potential difference and the current through the Activa begins a rapid decrease. The axoplasma potential during phase 3 is now controlled primarily by the flow of electrons into the axoplasma from the axoplasma electrostenoletic supply. As a result, the time constant of the phase 3 waveform is equal to the time constant of the supply impedance (shown here as R) times the axolemma capacitance, C. During much of phase 3, the podaplasm to dendroplasm potential is driven negative. Until this potential approaches its quiescent value, the circuit is said to be in a refractory period. The circuit will not respond to a marginal level of stimulation during this period. After a period shown as “T” in the figure, any extraneous stimulation will cause a repetition of this cycle. The result is known as fibrillation of the cardiocyte, and more generally fibrillation of the muscle containing the cardiocyte if multiple cardiocytes participate.

• The cutoff state is shown for reference. It is more important in stage 3 signal projection neurons and possibly in the pacemakers of the SA and AV nodes. In this condition, the dendroplasm to podaplasm potential is zero or negative, the Activa is in cutoff and no current flows through it. As a result, the axoplasma potential goes to its negative extreme associated with its electrostenoletic supply (typically -150 to -154 mV).

Note, there is no unique relationship between any of the potentials and the extra-cardiocyte potential represented by zero potential on the right scale. The “dome” defined by some investigators, and discussed more in the next section, does not relate to the zero potential of the extra-cardiocyte matrix.

In this figure, the sarcomere are shown as internal to the axolemma but with an independent connection to the extra-cardiocyte matrix. Electrically, they are believed to appear as a capacitive load drawing no power from the neural elements of the cardiocyte.

20.3.5.6.1 The “overshoot” in cardiocyte responses is a relaxation phenomenon

Experimentalists have encountered a variety of modified cardiocyte waveforms associated with the main cardiocytes of contraction (not including the SA and AV node, and Purkinje fiber waveforms). They occur under two general situations: the first output waveform following the first stimulation pulse of a series, output waveforms resulting from stimulation using pulse trains at long intervals.

The first situation usually appears as a short term peak in the leading edge of the waveform followed by the rapid decay to a more sustainable level (defined as stage 1). This has been labeled an overshoot; however, this analysis will show it is not an overshoot. It is the expected transient performance of a first-order RC circuit associated with the podaplasm (an earlier element in the overall cardiocyte circuit).
The second situation usually appears as a rapid descent of the axoplasm potential after reaching the leading edge peak followed by a gradual rise to a sustainable level before the typical phase 3 fall-off occurs. This element of the waveform has been labeled stage 2. The major source of this phase 2 waveform also appears to be associated with the podaplasm electrostenolytic source operating as a second-order RC circuit.

To understand these waveforms, it is necessary to consider the transient events associated with the cardiocyte waveforms. Figure 20.3.5-15 develops the situation using a more complex serape. This serape expands the two electrostenolytic power supplies and introduces other capacitances into the circuit. The voltage scale has been interrupted to provide more detail in the areas of more positive potential.

![Diagram](image)

**Figure 20.3.5-15** Second-order temporal serape of a main cardiocyte operating cycle. All waveforms shown are the first in separate pulse strings. See text for details.

Little is known about the impedance values associated with the electrostenolytic supplies, and the number of potential variations is very large. It is even possible the element shown as $R_p$ includes some positive reactance. In this discussion, the dendrolemma capacitance will be assumed to be zero and the dendroplasm potential will not change during any of the illustrated intervals.

Looking at the quiescent condition, there is an electron current flowing through the cardiocyte as indicated. However, the potentials on the capacitors $C_p$ and $C_{p1}$ ($V_p$ and $V_{p1}$), are close together and near the ideal potential, $V_{pp}$. Upon application of a significant positive potential to the dendroplasm via the synapse, the difference in potential between the dendroplasm and podaplasm is highly positive and significant current begins to flow through the axoplasm/podaplasm circuit ($e^-$). This current significantly and quickly reduces the potential of the podaplasm, $V_{p}(t)$. Because of this sudden change in $V_{p}(t)$, charge begins...
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to flow out of CP via RP into the more positive potential on CP1 with a marginally longer time constant set by the product of RP and CP1. The result is the waveform shown for VP during the first pulse. The net effect of this action is the axoplasm potential, V_A(t) during the first pulse is shown to exhibit a peak followed by an exponential decay caused by changes in the dendroplasm/podaplasm potential, V_D–VP(t). This explanation assumes the transconductance of the cardiocyte Activa remains constant during this time interval, a condition that will not exist if the Activa is actually in saturation. As a result, the actual shape of the axoplasm potential during the first pulse interval depends on how large the stimulation potential is.

If the Activa is in total saturation, the transconductance is zero following a large positive axoplasm excursion, then the question of the compliance of the axoplasm electrostenolytic supply becomes important. The supply can initially satisfy the electron current demanded by the dendroplasm/podaplasm potential via the charge stored in capacitor C_A. However, over the long term, V_A(t) will only be sustainable at a slightly more negative potential determined by the capacitor C_1. V_A(t) will decay with a time constant dominated by R_A*C_A.

In the schematic, the ideal voltage sources and initial impedances are an electrical representation of the chemical electrostenolytic process. The impedance is closely associated with the availability of fuel (either glutamic amino acid or the proposed lysine amino acid). This can be a particular problem in the laboratory if the culture medium does not supply these constituents at the required rate over the life of the experiments. Claycomb's medium and Tyrode's medium do not normally supply these amino acids, even when augmented. One laboratory team did include glutamic acid in their medium but any specific effect was not documented.

The exact shape of the waveform V_C(t) during phases 1 and 2 is totally dependent on the relative values of the elements in the RC networks. If the values lead to a critically damped situation (labeled critically damped state), the differential equation will be second order, and the peak will be more rounded before it descends. V_C(t) will depart phase 0 horizontally before turning down. If the impedance labeled RC_1 contains a positive reactive component, V_C(t) can depart phase 0 with a rapid descent followed by a rise to a sustained level during phase 2 (labeled the 1st pulse with reactance). This wave shape has been described as a notch and bowl by laboratory investigators.

During pulses subsequent to the first pulse in a string, the potentials associated with the cardiocyte do not have time to stabilize at their quiescent or cutoff state. These pulses do not generally exhibit such prominent features as those in the above second-order serape.

The waveforms of Litovsky & Antzelevitch, 1988, are well explained by the discussion and figures above without requiring the passage of any alkali or alkali earth ions through the lemma of the cardiocytes during any time interval.

20.3.5.7 Fibrillation and asystole in the heart

The temporal serape illustrates a serious, and often fatal, heart condition. If the feedback factor of a cardiocyte becomes greater than 1.0, the cardiocyte is subject to free-running oscillation. The oscillations will start any time the dendroplasm of the cell becomes incrementally more positive than the podaplasm. Once started, the oscillation will not stop without intervention. This condition is in addition to the fibrillation usually assumed to be due to improper calcium or potassium balance. It is primarily due to either inadequate supply of fuel to the poditic electrostenolytic process (assumed here to be lysine) of the cardiocyte, or inadequate removal of the waste products (assumed here to be an isomer of isoleucine, α-aminocaproic acid) from the process.

While fibrillation in a local area may not be noticed, the adjacent cardiocytes are subject to the same abnormality. If a large number of cardiocytes goes into fibrillation, the heart muscle has been described as looking like a bowl of worms, since the cardiocytes are oscillating (contracting) asynchronously.
Fibrillation is usually discussed with respect to individual heart chambers. While atrial fibrillation can be tolerated over an extended period, left ventricular fibrillation is life threatening in the very short term. Failure to stop ventricular fibrillation within four minutes is usually fatal in humans.

Hurst et al. have illustrated the random character of the ECG resulting from significant fibrillation leading to ventricular standstill, asystole\textsuperscript{107}.

Electrical shock is the current treatment of choice for severe fibrillation. This cardioversion is obviously of electrical character and attempts to reset the timing of the heart muscle. Any action it has on the calcium or potassium concentrations of the cardiocytes is not obvious.

xxx have provided a paper discussing models of the human heart and pharmaceutical intervention in arrhythmia\textsuperscript{108}.

### 20.3.6 The electrocardiogram, ECG, EKG & vectorcardiography

Stanfield offers a good chapter relating the ECG (frequently EKG in the clinical environment) to the electrical and mechanical actions of the heart muscle. Boron & Boulpaep provides material related more directly to various medical conditions. Figure 20.3.6-1 from Stanfield provides good correlation between the various activities, sounds and ECG signals of the heart.

Ottoson provides more fine grained information concerning the specific signals from various portions of the human heart\textsuperscript{109} (pages 102-106) using free hand caricatures. Note how the signals do not have the form of action potentials at the detailed level. Those waveforms at the atrioventricular node and the ventricular muscle cells exhibit their own particular characteristics.

Ottoson also addresses the modern multi-lead ECG (originally known as the vector electrocardiogram technique\textsuperscript{110}). Benchimol has written from the perspective of the clinician.

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\textsuperscript{107}Hurst, J. et al. (1974) The Heart: Arteries and Veins, 3\textsuperscript{rd} Ed. NY: McGraw-Hill page 552


\textsuperscript{110}Benchimol, A. (1973) Vectorcardiography. Baltimore, MD: Williams & Wilkins
20.3.7 Histology of the diseased heart

Recently, Estes of the Tufts Medical Center in Boston has noted a disease of the athletic heart.
which may contribute to early death. Figure 20.3.7-1 shows three views of the wall of a heart\textsuperscript{111}. The left two frames illustrate the organized cell structure reminiscent of a brick wall (with signals passed along a given course of bricks in accordance with the cardiocyte structure developed in this work. The right frame shows a more granulated structure that may be passing electrical signals from cardiocyte to cardiocyte in a less organized structure.

![Figure 20.3.7-1](image)

**Figure 20.3.7-1** Three images of the heart, one showing granulation. From Beil., 2014.

Beil quoting Maron et al. has noted, “Cardiomyopathy has been consistently demonstrated as the most common cause of exercise-related SCD in young athletes. Data from U.S. autopsy series have reported higher death rates from hypertrophic cardiomyopathy (HCM) in black compared with white athletes (20% vs. 10%, respectively), raising concern that this condition may exhibit a more malignant phenotype in black individuals.”

Chandra et al. have provided a review of this subject matter with an extensive bibliography\textsuperscript{112}.

### 20.4 The Uterine system

The dynamics of the uterine system are unique and suggest significant variations in the neural and muscular aspects of that system compared to other major anatomical systems. Csapo has provided a summary of the operation of this system\textsuperscript{113}.

### 20.5 Referred pain originating in the visceral system

[xxx cite section where material in chapter 24 of Kandel et al. is discussed.]

Ottoson has provided a simple map suggesting most pain arising in the gastrointestinal tract is perceived as occurring along the medial line of the abdomen (page 477). His discussion was more comprehensive.

\textsuperscript{111}Beil, L. (2014) Sudden Death Sci News April 5, pp 22-26

\textsuperscript{112}Chandra, N. Bastiaenen, R. Papadakis, M. Sharma, S. (2013) Sudden Cardiac Death in Young Athletes Practical Challenges and Diagnostic Dilemmas *J Am Coll Cardiol* vol 61(10), pp 1027-1040

\textsuperscript{113}Csapo, A. (1973) The uterus–model experiments and clinical trials In Bourne, G. ed. The Structure and Function of Muscle, 2\textsuperscript{nd} ed. NY: Academic Press Chapter 1
20.5.1 Referred pain involving the cardiac system

Kandel et al. (2000, page 475) provides a first order discussion of referred pain involving the heart. Figure 20.5.1-1 reproduces frame A of that figure. Frame B of that figure provides a speculative explanation for the mechanism involved. It suggests the nociceptors of the cardiac system travel to the CNS via the spinal cord rather than the visceral nerve.

Ottoson provides a somewhat different mapping (page 477).

This author can provide additional personal information. In two bouts of coronary artery blockage involving the coronary arteries, a blockage in the right coronary artery caused a sensation of superficial sunburn to an area extending all the way across the T2/T3 area of the upper chest, a subsequent blockage of the “left artery descending” caused a sensation of superficial sunburn to an area extending all the way across the T3/T4. No unusual sensations related to my arm, shoulder or back were perceived.

Figure 20.5.1-1 nociceptors in the cardiac system can be perceived as occurring elsewhere in the body. Reproduced in Kandel et al., 2000 from Teodori & Galletti, 1962.
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