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Review

# Old cogs, new tricks: A scaffolding role for connexin43 and a junctional role for sodium channels?

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ABSTRACT

**Cardiac conduction is the process by which electrical excitation is communicated from cell to cell within the heart, triggering synchronous contraction of the myocardium. The role of conduction defects in precipitating life-threatening arrhythmias in various disease states has spurred scientific interest in the phenomenon. While the understanding of conduction has evolved greatly over the last century, the process has largely been thought to occur via movement of charge between cells via gap junctions. However, it has long been hypothesized that electrical coupling between cardiac myocytes could also occur ephaptically, without direct transfer of ions between cells. This review will focus on recent insights into cardiac myocyte intercalated disk ultrastructure and their implications for conduction research, particularly the ephaptic coupling hypothesis.**

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Cardiac conduction is the process by which electrical excitation is communicated from cell to cell within the heart, triggering the synchronous contraction of the myocardium. Since being first demonstrated by Engelmann in 1874, [20] conduction has been the subject of intense scientific inquiry. Interest in the phenomenon stems mainly from the link between aberrant conduction and potentially lethal arrhythmias in a variety of pathologies.

## 1. Historical background

The current understanding of conduction is based largely upon the core conductor model [45]. The roots of this theoretical paradigm can be traced back to the application of continuous cable theory to cardiac conduction by Silvio Weidmann in the 1950s [75]. Subsequent experimental results, while numerous, have largely fit into the framework of this model, which envisions conduction as having two functional components: Membrane excitability and intercellular coupling. Membrane excitability, or the ability of an excitable membrane to depolarize in response to a given stimulus, is thought to be the province of membrane ion channels, particularly voltage-gated sodium channels. Intercellular coupling is seen as occurring via the passive, electrotonic flow of positive charge between cells via low resistance pathways afforded by gap junction (Gj) channels. However, emerging experimental evidence suggests that this view, while perhaps tidy, may not offer a complete and accurate description of cardiac conduction. For a detailed discussion of the electrotonic model of cardiac conduction, the reader is referred to the previous reviews by Spach et al. [58] and Kleber & Rudy [29].

The challenge to the electrotonic model of cardiac conduction comes in the form of ephaptic coupling, a process by which electrical excitation is communicated between cells via an extracellular electric field or ion accumulation/depletion without involvement of Gjs [61,65]. This mechanism, known to occur in other excitable tissues such as the brain, the retina and the uterine myometrium, [28,74,76] has long been hypothesized to play a role in cardiac conduction by Nicholas Sperelakis and others [12,33,40,42,63,64]. However, the lack of direct experimental evidence and a well-defined functional unit, i.e., an ephapse, has meant that the investigation of ephaptic coupling has remained almost exclusively the province of mathematical models. In this article, we will focus emerging evidence for ephaptic coupling in the heart and their theoretical implications – in particular, new functions for Gjs and voltage-gated sodium channels, blurring the boundary between excitability and intercellular coupling.

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## 2. Intercellular coupling: gap junctions and beyond

Over the last century, our understanding of cardiac intercellular coupling has gone through a series of revisions. In the early days of conduction research, the cytoplasms of cardiac myocytes were thought to be contiguous, thus accounting for electrical coupling. However, with the identification of high resistance membrane bounding each myocyte, [56] it was postulated that there had to exist low resistance pathways coupling neighboring myocytes [13]. Using electron microscopy to study the intercalated disk at high resolution, Dewey, Sjostrand and Andersson suggested that it may constitute a connecting surface between myocytes [55]. Subsequently, in the early 1960's, using electron microscopy, Lloyd Barr and colleagues identified 'fused membrane' structures connecting adjacent myocytes, which they dubbed *the nexus* [18]. Around the same time, Van der Kloot and Dane proposed the intercalated disk as the likely site of low resistance electrical contact between myocytes [72]; shortly thereafter, Barr, Dewey and Berger provided direct evidence of the nexus's involvement in conduction [5]. In 1967, Revel and Karnovsky demonstrated the nexus to be membranes separated by a gap rather than fused and coined the term 'gap junctions' [46]. The resistance of Gj was initially considered to be low enough to render coupled myocytes electrically continuous, thus conferring a syncitial nature upon the myocardium. However, experimental studies of action potential propagation at high temporal resolution revealed Gj resistance to be high enough to render cardiac conduction discontinuous at the cellular level [60].

Cardiac Gjs have long been recognized to undergo remodeling in developmental [3,23] and disease scenarios [36,43,57]. In this regard, one key question has been the precise relationship between the degree of Gj uncoupling and the resulting level of conduction slowing. While conduction slowing in response to pharmacological uncoupling has been well characterized, [4,7,15,17,26,30,52] the electrophysiological impact of pathophysiologic Gj remodeling is less clear [2,8]. Experiments in transgenic mice with 50% reduced expression of connexin43 (Cx43), the principal ventricular Gj protein, have yielded mixed results: Some studies reported slower conduction compared to wild-type (WT) littermates [19,24] while others found no difference [6,41,67,68,70,71]. Even more perplexingly, conduction, albeit slowed and susceptible to failure, still occurs in mice with a cardiac-specific conditional knockout of Cx43 resulting in a severe (>80%) loss of Cx43 [14].

All these findings point back to a question first posed by Sperelakis during the 1960's [62]: Can ephaptic coupling sustain cardiac conduction in the absence of Gjs? While initially viewed as an alternative to Gj coupling, more recent *in silico* studies have suggested the possibility of so-called *mixed-mode* coupling involving both mechanisms [31–33,40]. These models envision intercellular coupling as occurring as follows: A depolarized myocyte withdraws sodium ions from the restricted junctional cleft via its intercalated disk-localized $Na_v1.5$ channels (Fig. 1A). The resulting depletion of positive charge from the junctional cleft would render the local extracellular electrical potential more negative. Consequently, the transmembrane potential across the apposed membrane of the neighboring myocyte becomes more positive, causing the activation of $Na_v1.5$ channels (Fig. 1B). Thus electrical activation is communicated from myocyte to myocyte without direct transfer of ions between them (Fig. 1C). Based on this view, the models almost unanimously predict that ephaptic coupling would require that:

(a) the membranes of adjacent myocytes are closely apposed (<10 nm apart) and,

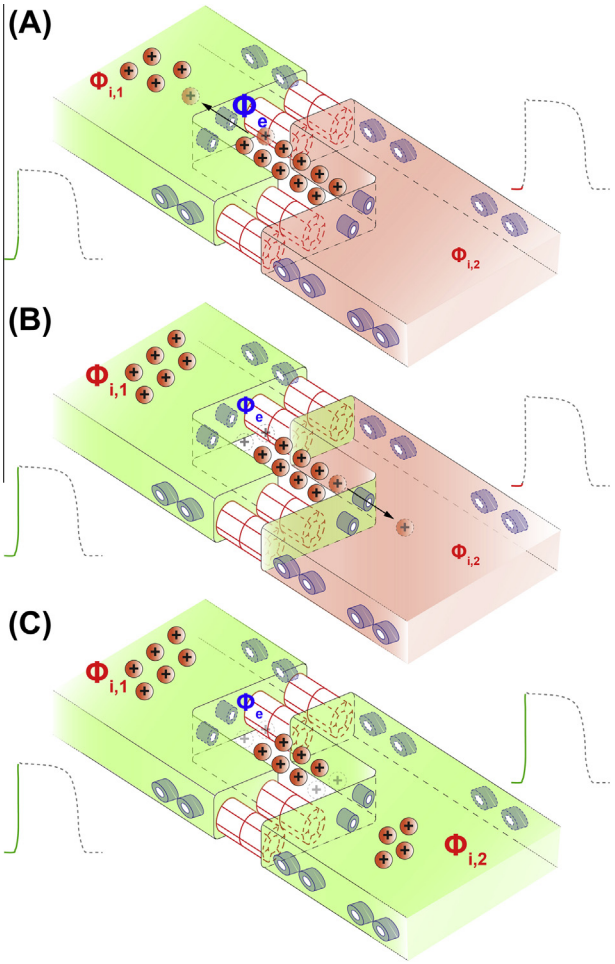


**Fig. 1.** Schematic cartoon illustrating the mechanism of ephaptic coupling. (A) Sodium channels (shown in blue) on the depolarized myocyte's membrane activate, withdrawing positively charged sodium ions ($Na^+$) from the restricted extracellular cleft at the intercalated disk. This raises the intracellular potential ($\Phi_{i,1}$) of the first myocyte. (B) Concomitantly, the depletion of positive charge from the restricted extracellular cleft lowers the local extracellular potential ($\Phi_e$). There is a resultant increase in the transmembrane potential across the membrane of the second myocyte which is defined as the difference between its intracellular potential ($\Phi_{i,2}$) and the extracellular potential ($\Phi_e$). In turn sodium channels located at or near the intercalated disk of the second myocyte activate. (C) Entry of sodium ions into the second myocyte via its sodium channels further depolarize it, triggering an action potential. Thus activation is communicated 'ephaptically' from cell-to-cell without the direct transfer of ions between them.

(b) the closely apposed membranes are rich in cardiac sodium channels ($Na_v1.5$) [12,31–33,40,63,64,66].

## 3. Ion channels at the intercalated disk: functional implications

Recent insights into the ultrastructural organization of ion channels within cardiac myocytes have sparked interest in the ephaptic coupling hypothesis, particularly when interpreted in the context of the aforementioned model predictions. The first evidence that cardiac sodium channels are preferentially localized at the intercalated disks of cardiac myocytes came in 1996, when Dr. Sidney Cohen published immunofluorescence images of rat TTX-resistant sodium channels (rH1) [11]. Since then there has been mounting evidence for the intercalated disk localization of ion channels, long predicted by mathematical models as a requirement for ephaptic coupling [12,31,39,40,61,65,77]. Since then, other studies have recapitulated the preferential localization of