J Biosci (2022)47:14
DOI: 10.1007/s12038-021-00251-6

Review

Do oscillations in pancreatic islets require pacemaker cells?

BRADFORD E PEERCY[1] and ARTHUR S SHERMAN[2]*

[1]*Department of Mathematics and Statistics, University of Maryland, Baltimore County, Baltimore, Maryland 21250, USA*

[2]*Laboratory of Biological Modeling, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, USA*

**Corresponding author (Email, arthurs@niddk.nih.gov)*

MS received 21 October 2021; accepted 22 December 2021

The pancreatic islets of Langerhans are biomedically important because they are home to the beta cells that secrete insulin and are hence important for understanding diabetes. They are also an important case study for the mechanisms of bursting oscillations and how these oscillations emerge from the electrical coupling of highly heterogeneous cells. Early work has pointed to a voting/democratic paradigm, where the islet properties are a nonlinear average of the cell properties, with no 'conductor leading the orchestra'. Recent experimental work has uncovered new facets of this heterogeneity, and has identified small world networks dominated by a small subset of cells with a high degree of functional connectivity, assessed via correlations of calcium oscillations. It has also been suggested that these connectivity hubs act as pacemakers necessary for islet oscillations. We reviewed modeling studies that have confirmed the existence of small worldness, and we did not find evidence for obligatory pacemakers. We conclude that democracy rather than oligarchy remains the most likely organizing principle of the islets.

Keywords. Bursting; calcium; diabetes; emergent behavior; gap junctions; networks

1. Introduction

The pancreatic islets of Langerhans have long been of interest because they play a central role in diabetes and are a paradigmatic example of an emergent network. The beta cells of the islets are the only cells in the body that secrete insulin, and dysfunction in this process is a key step in the pathogenesis of diabetes (Ha and Sherman 2020). The islets (numbering hundreds in rodents, and hundreds of thousands in humans) generate oscillations in the circulating insulin with a period of about 5 min, which are important for the efficacy of insulin in regulating blood glucose levels, accomplished by suppressing the release of stored glucose from the liver and the uptake of glucose by muscles and adipose tissue (Laurenti *et al.* 2021; Satin *et al.* 2015). Each islet, however, is already a functional unit capable of generating 5 min oscillations *in vitro*, in addition to oscillations that are as much as an order of magnitude faster (with periods of tens of seconds) and compound oscillations in which fast oscillations occur superimposed on slow ones (Bertram *et al.* 2007).

As in other excitable cells, such as neurons, the oscillations arise from an even more fundamental substrate, with action potentials or spikes on a still faster timescale (<1 s), which are organized into bursts with repeated active (spiking) and silent phases to produce oscillations on the timescales of seconds or minutes.

Bursting activity is illustrated in figure 1 for a simplified model of a single cell (Sherman 1996) in the tradition of Chay and Keizer (1983), which can be thought of as representing the synchronized behavior of an islet. In this class of models, inhibition, represented

This article is part of the Topical Collection: Emergent dynamics of biological networks.

Published online: 20 February 2022

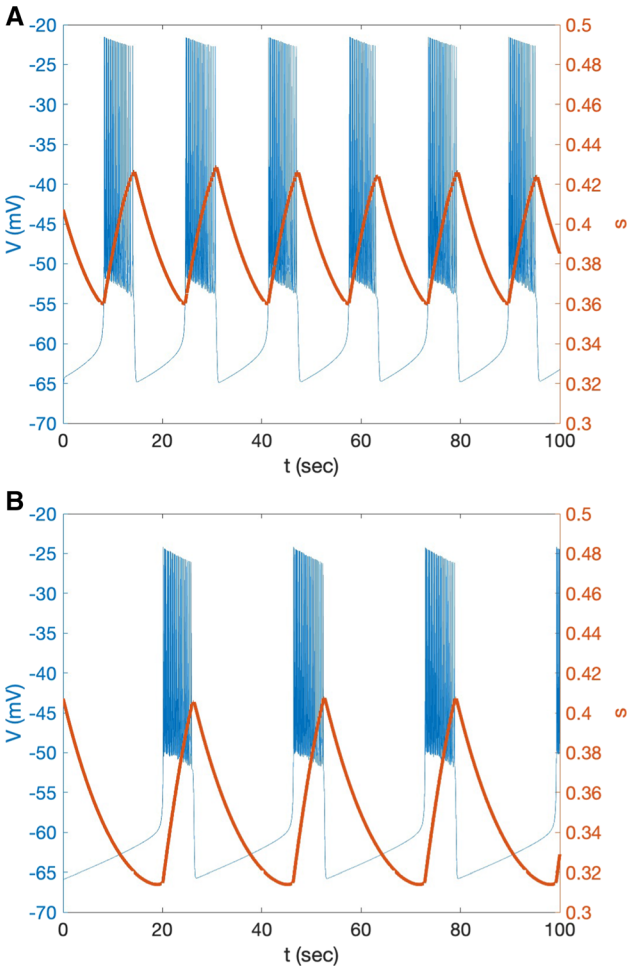

Figure 1. Simulation of a simplified model of a single beta cell, showing bursting, periodic alternation of membrane potential (V, blue) between active (spiking) and silent states, governed by the slow rise and fall of an inhibitory slow variable (*s*, red). Inhibition is the open fraction of a potassium channel from the rise of cytosolic calcium opening K(Ca) channels or the rise of ADP opening K(ATP) channels. The cell in panel (**A**) is more excitable than the one in panel (**B**), exhibiting higher burst frequency and duty cycle. If electrically coupled, cell A could act as a pacemaker entraining cell B, but in the absence of cell A, cell B would still oscillate at a lower frequency.

here by the variable *s*, slowly builds up during the active phase until it reaches an upper threshold level that shuts down the spiking. It then recovers during the silent phase until it reaches a lower threshold that allows the spiking to resume. For the case of fast oscillations illustrated in figure 1, one could think of *s* as representing cytosolic calcium, which opens calcium-dependent potassium [K(Ca)] channels. Panel A of figure 1 represents a highly excitable cell, with a high duty cycle (the fraction of time spent in the active phase), while panel B of figure 1 represents a less excitable cell with a low duty cycle. The degree of excitability could depend on the rate of glucose metabolism, regulated by the rate-limiting enzyme that initiates glycolysis, glucokinase (GK), which determines the ATP/ADP ratio and hence the fraction of open ATP-dependent potassium [K(ATP)] channels. Alternatively, it could depend on the number of K(ATP) channels in the cell. We will see below that both these play roles in the heterogeneity of beta-cell activity in the islets. For slow oscillations, *s* might represent ADP, which opens K(ATP) channels; this process is much slower than calcium accumulation because it depends on the rate of glucose metabolism and ATP production. See Bertram and Sherman (2004) for more on how fast and slow inhibitions work together.

The grouping of spikes into bursts permits the generation of repeated, prolonged rises in intracellular calcium, which are required to drive the exocytosis of insulin-containing vesicles, in a process that shares many features with the release of neurotransmitters at neuronal synapses (Chen *et al.* 2008). We will focus here on mouse islets, which are the most studied biophysically and the most modeled.

The issue of emergent oscillations has recently been posed in a novel and provocative form by experimental advances, allowing a broader view of heterogeneity in character and behavior of beta cells in the islets. A study of fast oscillations in mouse islets (Johnston *et al.* 2016) identified a small subset of beta cells as 'hubs' (the most highly connected cells) of a small world network, where the strength of connectivity was defined by the correlation of calcium dynamics in pairs of cells. Small world networks had previously been identified in the islets (Stožer *et al.* 2013), but Johnston *et al.* further found that if hub cells were electrically silenced by photo-activation of a genetically added inhibitory ion channel, the synchronized bursting oscillations in the entire islet were abolished or greatly reduced. This led to the hypothesis that the hub cells acted as pacemakers that were required for coherent oscillations of the islet.

A follow-up study in zebrafish islets by the same group (Salem *et al.* 2019) found distinct but similar results. A subset of cells was identified, and called 'leader cells' or 'first responders' because they were the first to become activated when glucose was raised above the threshold level for oscillations. Removal of leader cells by photo-ablation (distinct from the silencing studied by Johnston *et al.* 2016) delayed the onset and diminished the amplitude of the subsequent whole-islet oscillations.

These two studies inspired a vigorous discussion about whether hubs really exist and whether hubs and leaders are the same cells. Our aim here is to summarize that discussion and describe modeling studies addressing them as well as related questions. In order to put the discussion in context, we will start with earlier works on emergent oscillations in the islets, which is somewhat at variance with the new hub hypothesis.

2. Early studies of emergent behavior in islets

The issue of emergent behavior arose early on (in the 1980s) in the form of two questions: (1) Are the oscillations driven by specialized pacemaker cells, analogous to the heart, and (2) Is each beta cell an oscillator or do oscillations only occur when many cells (hundreds to thousands per islet) work in concert? The analogy to the heart was encouraged by the finding that the beta cells are coupled by gap junctions, similar to cardiac myocytes, as well as by a number of autocrine and paracrine factors secreted within the islets. The question of emergent behavior was raised by experimental observations that single beta cells isolated from the islets showed only irregular spiking and not bursting (Atwater *et al.* 1983; Rorsman and Trube 1986; Kinard *et al.* 1999).

Both questions were settled, or so it seemed, by a series of modeling studies exploring the hypotheses that bursting in isolated beta cells was prevented by either noise, stemming from stochastic opening and closing of small numbers of channels (Chay and Kang 1988; Sherman *et al.* 1988), or heterogeneity, variation in parameters across cells (Smolen *et al.* 1993). Coupling through gap junctions permitted regular bursting to occur by averaging out the stochastic fluctuations and/or the parameter heterogeneity. Furthermore, no special pacemaker cells were needed: the cell parameters were chosen from normal distributions and synchronized by voting with their transmembrane potential to determine the level of concerted activity by the population. The most active ones act as pacemakers in the trivial sense that they kick off each active phase, but less active cells can take over if the most active are deleted, as long as the overall level of excitability of the population is sufficient.

Aside from the existence of oscillations, the model of Smolen *et al.* (1993) made a non-obvious prediction that coupling sharpens the glucose response curve compared to a heterogeneous uncoupled population of model bursting beta cells with variation in the rate of glucose-dependent ATP synthesis. The collective output is thus not merely a linear summation of the activity of the individual cells but is transformed nonlinearly.

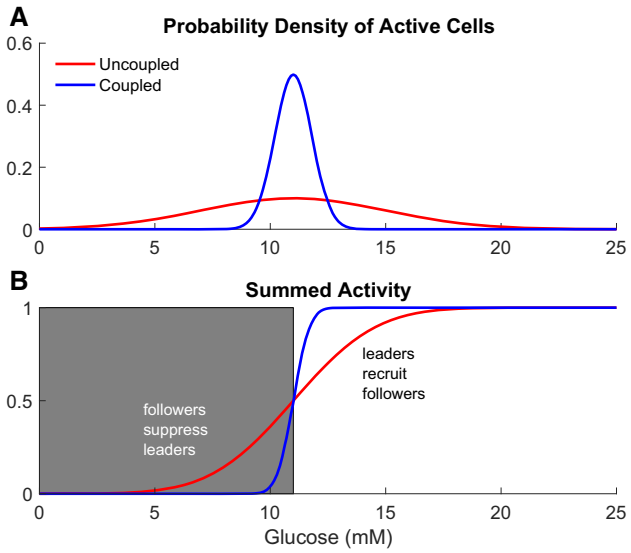

Figure 2. Schematic of effects of coupling on the probability density of cell activation thresholds (**A**) and the corresponding glucose dose response curves (**B**). Without coupling, the threshold distribution is broad and the dose response curve is shallow (red curves). With coupling, the threshold distribution is narrow and the dose response curve is steep (blue curves).

This is illustrated schematically in figure 2. Panel A of figure 2 shows the probability distribution of activation thresholds for uncoupled islets (red) and coupled islets (blue), and panel B of figure 2 shows the corresponding summed activity, which constitutes the glucose dose response curve. When the islet is uncoupled, the distribution is very broad, as observed, for example in Scarl *et al.* (2019), and the dose response curve is shallow. When the islet is coupled and glucose is low, the minority of more active cells are suppressed by hyperpolarizing current from the majority of less active cells. When glucose is high, the more active cells are in the majority and are able to recruit the less active cells. The combined effect is to narrow the distribution of activation thresholds and steepen the dose response curve. Note that even with strong coupling, the schematic indicates some dispersion of activation thresholds, as observed by Stožer *et al.* (2021).

Such steepening of the otherwise shallow response was indirectly observed experimentally by Speier *et al.* (2007), who compared the activity in normal islets to ones with the gap junction protein Cx36 knocked out. This was shown more explicitly by Dwulet *et al.* (2019), who found in simulations similar threshold sharpening due to coupling in Ca^{2+} elevation as a function of the level of expression of glucokinase; glucokinase as the rate-limiting enzyme at the head of

the glycolytic pathway is a surrogate for glucose concentration. The prediction of sharpening was confirmed by blocking glucokinase using mannoheptulose in islets where Cx36 was either intact or knocked out.

They also made the astute observation that the steep dose response curve mediated by electrical coupling makes islets robust to the loss of cells or glucokinase expression, which corresponds to a severe genetic form of diabetes found in neonates (Nguyen *et al.* 2014; Dwulet *et al.* 2019). This robustness comes with a trade-off: once a threshold level of impairment is reached, secretion falls catastrophically. Fortunately, this defect is rare, and the authors pointed out that even then, catastrophe can be averted (in principle) by reducing the gap junctional coupling, which makes the dose response curve more shallow.

3. Identifying novel functional subpopulations in islets

With improvements in optical imaging and other experimental techniques, evidence has begun to emerge that islets may contain subpopulations of cells with specialized roles in islet behavior and properties, such as secretion, proliferation, and degrees of maturity and differentiation, including trans-differentiation between beta cells and alpha cells (van der Meulen *et al.* 2017; Benninger and Hodson 2018; Benninger and Kravets 2021; Joglekar *et al.* 2021). In the interest of brevity and thematic coherence, we will limit our discussion to the consequences of functional measures of heterogeneity and connectivity for oscillations of membrane potential and calcium.

3.1 *Functional connectivity*

The first shot across the bow was a study of functional connectivity, defined by pair-wise correlations between activities, usually of calcium, of cells. Cells that were correlated above a threshold were defined to be connected (Stožer *et al.* 2013). This was a departure from previous attempts to measure connectivity by assessing gap junctional conductance, which limited analysis to pairs of cells and required simultaneous measurements with two electrodes (Eddlestone *et al.* 1984; Perez-Armendariz *et al.* 1991). The use of calcium imaging allowed many more cells to be visualized simultaneously. Note that imaging has mostly been limited to confocal planes, which may be relevant for interpreting the results, as we discuss briefly below.

The study of Stožer *et al.* (2013) resulted in three main findings. The first was a quantification of long-range functional coupling. This is possible in a population coupled only by nearest-neighbor connections because activity transmitted from one cell to another can trigger a regenerative wave that propagates to more remote cells. Second, they studied time-dependent changes in connectivity. The network showed higher connectivity (synchronization) at high glucose where cells were oscillating than at low glucose, which was below the threshold for oscillations. This likely reflects the dependence of correlation on the waves of activity traversing the islet, although it is possible that glucose metabolism also increases gap junctional conductance. Third, and most striking, they found that the network had small world properties, with a power law distribution of cumulative degrees in the graph. This was assessed by a small world network measure defined by a ratio of two ratios: (1) average number of connections between active cells or nodes to the average number of connections to another randomized network with the same number of nodes and connections over (2) global pathway efficiency (inversely related to the average shortest path length between active cells) to the efficiency of a randomized network.

Cappon and Pedersen (2016) carried out simulations of the islets on a three-dimensional hexagonal lattice, similar to the one shown in figure 3. They showed that long-range functional connections can arise from excitation waves without assuming any long-range structural connections, such as those found, for example, in neuronal networks. They also showed that the small world behavior observed in the islets could be

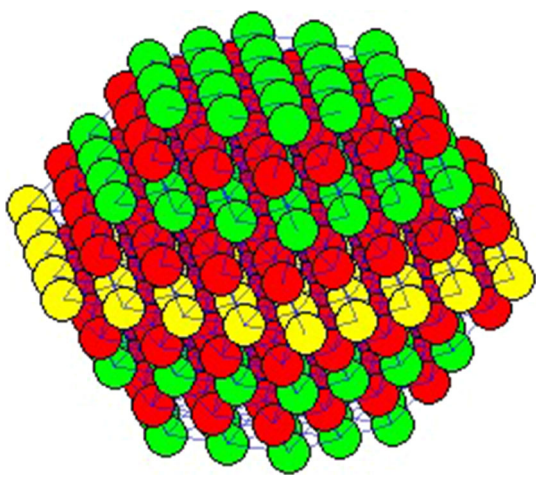

Figure 3. An islet modeled as a hexagonal lattice, similar to those used in the simulation studies described in the text. Pictured is an islet with 323 cells (edge size 5).

recreated in the model provided an appropriate degree of heterogeneity of cell properties and coupling strength was assumed.

Hogan and Peercy (2021) similarly found that it was easy to construct model islets with small world connectivity. Small worldness is not guaranteed—it depends, for example, on having coupling that is not so strong that all cells would be functionally connected—but it exists over a wide range of parameter values.

Taken together, these studies show that a normal or similar distribution of cell properties and coupling is sufficient to account for the new observations of long-range functional connectivity and small worldness, in addition to older modeling results showing that special pacemaker cells were not needed. However, they left open the question of whether these properties have any significance for how islets fulfill their physiological roles of regulating whole-body glucose homeostasis.

3.2 *The hub-follower hypothesis*

As mentioned in the introduction, Johnston *et al.* (2016) addressed this question of physiological relevance by identifying hub cells in mouse islets based on high functional connectivity. When the hubs (or possibly small regions around the hubs) were silenced using optogenetics, the islet was desynchronized, the overall level of activity was reduced, and functional connectivity, quantified as the proportion of correlated links, was also reduced. The silencing was reversible—oscillations resumed once the inhibitory stimulus was removed. Silencing non-hubs did not lead to desynchronization, and so they were dubbed 'followers'. The hubs were reported to have higher expression of glucokinase, which determines how sensitive the cells are to glucose and how active they are at a given glucose level. Relevance to the pathogenesis of diabetes was suggested by the finding that the hub cells were especially vulnerable to inflammation and metabolic stress.

The second paper in this series (Salem *et al.* 2019) extended the findings to zebrafish islets, which offer the advantage that calcium oscillations can be visualized *in vivo* during a larval stage when the animal is transparent. However, the zebrafish preparation has a number of differences that make comparisons with mouse islets uncertain. The oscillations observed in zebrafish islets were very noisy and irregular, possibly owing to their small size (only 30 beta cells vs. hundreds in typical mouse islets). Functional connectivity was assessed and shown to increase when glucose was raised, as in mice, but silencing was not attempted. In lieu of that, the study focused on the first responders—the cells that responded earliest to a step change in glucose. As we shall see later, these are not necessarily the same cells that are first to become active with each burst. Photo-ablation of identified first responders delayed and reduced the amplitude of succeeding responses, but did not eliminate them. An *in vivo* mouse preparation was simulated by transplanting mouse islets into the anterior chamber of the eye of a mouse; these islets exhibited clear, synchronized oscillations very similar to those seen *in vitro*. Again, functional connectivity was confirmed to increase with glucose, but neither silencing nor photo-ablation was attempted. The strongest connection to the prior *in vitro* study from this group (Johnston *et al.* 2016) was the finding that the first cells to fire during each burst were the most connected. Similar findings were obtained with human islets transplanted into mouse eyes.

These papers elicited some pushback from electrophysiologists (Satin *et al.* 2020), who pointed out that the islets lack the specialized structures needed to isolate pacemakers electrically from follower cells, in contrast to the heart, in which pacemakers reside in the sino-atrial node and communicate through paranodal cells to cells in the atrium or in the atrio-ventricular node and communicate through long, thin Purkinje fibers to cells in the ventricles. When individual cells within islets are voltage clamped, the islets are not silenced even when large hyperpolarizing voltage commands are applied. Rather, the clamped cell exhibits oscillating currents propagated from their neighboring cells, whose membrane potential evidently continues to oscillate. In one early study (Cook *et al.* 1981), it was possible to electrically switch a burst off or on, but the electrodes and the currents that needed to be applied were enormous compared to the current that a putative hub could deliver to its neighbors. It was further argued that hubs that are critical for coherent oscillations but also especially vulnerable to stress would be unlikely to survive natural selection and produce islets that, except for relatively rare cases of diabetes, are capable of life-long, robust performance. We add that studies of decoupled islets, e.g. Benninger *et al.* (2011), and isolated beta cells, e.g. Scarl *et al.* (2019), have shown that most beta cells are intrinsically oscillatory, but in the absence of gap junctional coupling, the oscillations are irregular and may occur outside the normal range of glucose values; coupling cures these defects but is not required for oscillations. The hub proponents (Rutter *et al.* 2020) responded that electrophysiological techniques could only sample one cell at a time and would be unlikely to find a hub if such cells were rare. Both commentaries suggested that

if gap junctional coupling is not well-suited to the subdivision of cells into leaders and followers, perhaps other forms of coupling, such as diffusible paracrine factors or neurotransmitters, could carry out this role.

The hub studies represented a marked departure from the prior modeling work cited above, suggesting that, despite considerable heterogeneity, a small subset of pacemaker cells is not required for oscillations. They also constitute somewhat of a Delphic oracle as to whether the hubs are obligatory pacemakers, without which oscillations would not occur, or just cells with disproportionate influence. Put in another way, is the islet an oligarchy, governed by a small, unique population of specialized cells, or a democracy in which some cells are more influential than others? Moreover, are the cells that can silence the islet the ones with high functional connectivity? Finally, are the cells that are first to become active when glucose is elevated (first responders) also the cells that lead off each burst (leaders or pacemakers)? The experimental papers inspired a series of modeling studies designed to answer these questions, and those will be our main focus in the remainder of this review. There are a number of other studies of coupled dynamics in islets with interesting things to say about heterogeneity and its possible functional role that we will not discuss because they do not directly address those questions (see, for example, Loppini and Chiodo 2019; Stožer *et al.* 2019, 2021; Scialla *et al.* 2021).

4. Models of hub properties

The first modeling study to address some of the above questions (Lei *et al.* 2018) created an islet with heterogeneous coupling and beta cell properties selected to mimic those in the experiment of Johnston *et al.* (2016). Hub cells were predefined as cells with higher glucose sensitivity and higher gap junctional coupling conductance. Silencing 1–4% of the most active cells was effective at silencing the islet, similar to the experimental results of Johnston *et al.* (2016). However, silencing non-hub cells was only slightly less effective at silencing the islet (1–10%) at threshold levels of glucose (6–7 mM). Both were less effective if glucose was raised slightly (6.5–7.5 mM). The parameter set that the authors considered to be in best agreement with the data assumed that 90% of the non-hub cells were below the threshold for oscillations, as determined by the Hopf bifurcation for isolated cells. Thus, silencing by a small fraction of highly active cells, but not other cells, required the assumption that almost all of the cells were below threshold. This suggests that oscillations could have been restored by raising glucose slightly, rendering the putative hubs no longer indispensable. Another important point is that Lei *et al.* (2018) acknowledged that they were unable to reproduce a power law distribution of functional connectivity suggested in Johnston *et al.*, and therefore did not identify hub and non-hub cells in this way. Thus, the models we have considered so far have shown either small world connectivity (Cappon and Pedersen 2016) or cells that can silence the islet (Lei *et al.* 2018) but not both.

Hogan and Peercy (2021) carried out another modeling study to systematically explore the conditions for small worldness and cells that can silence the islet. Similar to previous studies, they constructed islets with heterogeneous coupling as well as cellular parameters that affect intrinsic electrical excitability. Rather than predetermine which cells were hub cells, they attempted to achieve emergence of a power law distribution of functional connectivity, as defined by calcium correlation, such that silencing those highly connected cells—defining hub cells by degree of connection links, as in Johnston *et al.* (2016)—could silence the islet. While well-synchronized islets with small world properties (as Cappon and Pedersen were able to find) and power law distributions of functional connections could be generated, the individual hub cells were unable to control islet behavior. Moreover, they found a trade-off between small worldness and coupling strength, and hence synchrony; it was essentially impossible to find islets that had both clear power law connectivity and a high degree of synchrony (figure 4).

Hogan and Peercy therefore abandoned the search for small world islets and turned to exhaustive testing

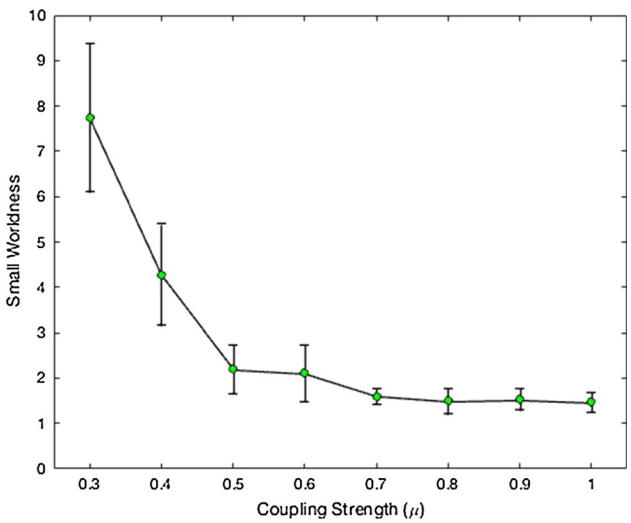

Figure 4. Trade-off of small worldness vs. coupling strength. Mean values and standard deviations from 10 simulations of islets like those in figure 3, but with 1483 cells (edge size 8).

of parameter combinations to search for cells that could control islet activity, as assessed by silencing. These cells were termed 'switch cells', and in fact, two distinct classes of switch cells were found. One, called 'initiators', were cells that always began the wave of excitation, while the other, called 'percolators', were cells that were required to continue excitation waves initiated by other cells. In neither case were the switch cells associated with the most highly functionally connected cells as defined by calcium trace correlation. Furthermore, switch cells were found to exist in islets near the threshold for excitation. Thus, similar to the case studied by Lei *et al.* (2018), being a switch cell was a conditional property, and cells that were required for synchronized activity at a given level of glucose were dispensable at higher levels of glucose. Thus, if switch cells are removed, oscillations may still be possible, but with a slightly right-shifted glucose dose response curve. These simulations, like those of Lei *et al.* (2018) do not support the hub-follower model proposed in Johnston *et al.* (2016). In particular, the properties of functional connectivity hubs and excitation leaders were distinct in these models.

Dwulet *et al.* (2021) also studied conditions under which a small population of cells could have disproportionate control over islet oscillations. Because Johnston *et al.* (2016) had found that hubs (in the sense of connectivity) had higher glucokinase expression, the parameter representing it was normally distributed across the model islet. Silencing the most active cells was able to silence the islet. In the most favorable case, when the distribution was skewed to give more weight to the most active cells, silencing 10% was sufficient. This was considered good agreement with the experiments on the assumption that only a 2D slice of the islet was visualized, so more cells may have been hit by the laser than were seen. However, removing the high GK cells did not prevent the remaining cells from oscillating; the islet duty cycle was just slightly reduced. This was true in spite of the fact that the high GK cells also had higher functional connectivity by virtue of their higher activity (not due to more or stronger gap junctions). Note that removing cells is not the same as silencing them by activating a hyperpolarizing current—the silencing propagates hyperpolarization to neighboring cells, removal does not.

In a further search for cells that could act as pacemakers, Dwulet *et al.* (2021) simulated the effect of removing the cells with the highest frequency or the cells that were earliest to fire during each burst. Both interventions had only minor effects on islet frequency. Similar differences were noted by Hogan and Peercy (2021), who found that it was more challenging to find switch cells when isolating or removing cells rather than silencing them with hyperpolarization; this was especially true when coupling was stronger.

In summary, the properties of being able to silence islets vs. driving oscillations were distinct in the model, in contrast to experimental data (Johnston *et al.* 2016). More broadly, these simulation results suggest that gap junction-coupled islets, composed of cells with a broad range of properties, are very robust to loss of small subpopulations of cells. This holds even when the cells removed have features (high activity, high functional connectivity, high frequency, and early phase) that nominally make them good candidates to act as pacemakers.

The final study we discuss in detail (Kravets *et al.* 2020) has appeared only on a preprint server, but we include it because the work has been presented at several major meetings and has entered into the conversation in the field. Experiments and modeling were combined to examine the properties of first responders, the cells that activate first when glucose is raised above the threshold for oscillations. One finding was that first responders were not indispensable: if a first-responder was ablated experimentally, another took over, and the onset of steady-state oscillations was only slightly delayed, especially in larger islets. This suggests that the larger effects of ablating first responders in small zebrafish islets (Salem *et al.* 2019) are not typical. First responders were also distinct from the cells that led off each burst. Notably, first responders did not have higher metabolic sensitivity to glucose, as they did not differ from later responders in NAD(P)H, a standard marker of glucose response; instead they had lower K(ATP) conductance. This differs from the hub cells identified in the study by Johnston *et al.* (2016) and were found to have higher expression of glucokinase, which would be expected to manifest as higher NAD(P)H. As discussed above, both lower K(ATP) conductance and higher GK would enhance excitability, but they are biochemically distinct, which challenges the identification of the two cell types in the study by Salem *et al.* (2019). Finally, first responders did not have higher functional connectivity than later responders.

5. Conclusions

We have reviewed recent progress in imaging calcium oscillations in pancreatic islets, which has revealed details heretofore unavailable and led to greater

appreciation for the wide variability of properties among beta cells. It has also led to a provocative new hypothesis that synchronized islet oscillations are dependent on a small subset of cells, termed hubs, with the highest degree of functional connectivity in a small world network (Johnston *et al.* 2016; Salem *et al.* 2019).

The modeling studies we have reviewed used different network architectures and intrinsic mechanisms for oscillations but provide a common core of conclusions. The first conclusion is that small world networks of functional links can arise in a natural way from a population with a plausible distribution (normal or skewed normal) of intrinsic properties and coupling parameters (Cappon and Pedersen 2016; Hogan and Peercy 2021). However, high functional connectivity per se does not confer on cells the ability to act as obligatory pacemakers or to desynchronize islets when they are silenced.

Second, heterogeneous networks naturally give rise to leaders that act as first responders or as wave initiators, but the models suggest that these are not the same cells and nor are they hubs in the sense terms of connectivity (Kravets *et al.* 2020; Dwulet *et al.* 2021). Rather, they appear to be the cells that are more excitable, although this is conditioned by local coupling and the properties of the cells in the neighborhood. The ability to lead may actually be facilitated by being more *weakly* coupled than average, as this helps them avoid being inhibited by the less active cells in the network. This is a fundamental feature of gap junction-coupled networks: coupling is symmetrical, and so depolarizing current delivered by a leader is balanced by hyperpolarizing current delivered by a follower.

Third, leaders are not indispensable. When first responders are removed, latency to first activity is prolonged but activity is not prevented (Kravets *et al.* 2020). When wave initiators are removed, oscillation frequency is slightly reduced but oscillations persist. Leaders are then first among equals, and the next rank of cells can take over in their absence. Leadership may also rotate if cell properties drift over time. These are reassuring features, as they make islets more robust to cell loss. The original hub concept, in contrast, would seem to make islets more vulnerable to disruption.

Fourth, silencing cells is not the same as removing them (Dwulet *et al.* 2021; Hogan and Peercy 2021). Silencing propagates a hyperpolarizing current to neighboring cells, whereas removal does not (Satin *et al.* 2020). Silencing a sufficient fraction of the most active cells (5–10%) can desynchronize or silence islets (Lei *et al.* 2018; Dwulet *et al.* 2021). Hogan and Peercy (2021) found that it was possible for silencing a single cell to silence an islet, but whether this happens to a significant degree in real islets remains to be determined. However, and most important, removing the cells capable of silencing the islet does not prevent the rest of the cells from oscillating (Dwulet *et al.* 2021).

Put in another way, leaders can only lead if the followers are themselves close to threshold, which means that in their absence the rising tide of depolarization would still trigger an active phase, just somewhat delayed. To paraphrase the 18th century British historian Sir Edward Gibbon on education (*https://www.brainyquote.com/quotes/edward_gibbon_389013*), pacemakers in a gap junction-coupled network lack efficacy except in those cases where they are almost superfluous. In addition to the limitations of gap junction networks already mentioned, this follows from the particular dynamics of beta-cell electrical activity, an aspect that has not previously been noted in this debate. As shown in figure 1, each burst is triggered when inhibition, say, K(Ca) or K(ATP) channel conductance, slowly falls below the threshold. The most active cells will generally reach this threshold first, but in their absence, other slightly less active cells would reach the threshold a little later. For example, the more active cell depicted in panel A reaches the threshold when s falls to about 0.36, while the less active cell shown in panel B reaches threshold when s falls to about 0.32.

A similar point of view, particularly with regard to the need to carefully distinguish hubs, first responders and wave initiators can be found in Benninger and Kravets (2021), plus extensive additional commentary on the biochemical bases of heterogeneity in beta cells and other cell types. Note, however, that we use the term pacemaker in a more restrictive sense to mean cells that are required for oscillations to occur, rather than cells with only a disproportionate influence on oscillations or the first to become active with each burst. We consider disproportionately influential cells and wave leaders uncontroversial but are doubtful about obligatory pacemakers.

The failure of models based on gap junctions to agree fully with the experimental data on hubs has led to the suggestion that some other form of coupling, such as paracrine interactions or neurotransmitters, may be involved (Rutter *et al.* 2020; Satin *et al.* 2020). We are not aware of any data showing that blocking paracrine or neurotransmitter receptors prevents synchronized oscillations in calcium. However, there are many candidates, and it may be necessary to block all simultaneously to rule this out.

Moreover, experimental observations of coherent oscillations in clusters consisting only of beta cells argue against a need for factors arising in non-beta cells or neurons. Clusters of MIN6 cells connected by Cx36 gap junctions can oscillate, which shows that such exogenous factors are not necessary (Calabrese *et al.* 2003). MIN6 cells are a pale imitation of primary beta cells with regard to oscillations, and so confirmation using pseudo-islets composed only of beta cells would be desirable. Even if confirmed in a better preparation, these results would leave the (remote) possibility that beta-cell/beta-cell interactions mediated by diffusible factors could still play a role. However, clusters of MIN6 cells lacking gap junctions show only asynchronous, irregular activity, which shows that diffusible factors are not sufficient (Calabrese *et al.* 2003; Ravier *et al.* 2005; Benninger *et al.* 2011). There is evidence that incretins enhance the strength of gap junctional coupling but there is no clear evidence that they act as coupling factors themselves (Hodson *et al.* 2013).

There has been little modeling in islets of coupling by diffusible factors, which has been suggested as an alternative to gap junctions. The one study we are aware of is by Stokes and Rinzel (1993), who considered diffusion of extracellular potassium, which is released by spiking cells and depolarizes their neighbors. They found that this mechanism was sufficient to synchronize beta cells in the absence of gap junctions and in the presence of some degree of heterogeneity.

Further theoretical exploration of the possibilities for coupling by diffusible factors and by exogenous neural input is warranted. In addition, models should consider the effects of human vs. mouse architecture: in human islets there are fewer homotypic beta-cell to beta-cell contacts and more heterotypic beta-cell to alpha-cell and delta-cell contacts. This would likely lead to quantitative differences but also possibly to different conclusions about the need for obligatory pacemaker cells. However, this would not change the conclusions about observations in mouse islets from simulations using mouse architecture.

We are left with a body of theoretical work that to date can confirm some important aspects of the recent experimental work on hubs while disagreeing with others. The models in particular do not support the hypothesis that coherent islet oscillations critically depend on a small set of pacemaker cells, and indicate that this is better studied experimentally by ablating the cells rather than silencing them. We have also presented some theoretical reasons why the pacemaker paradigm is unlikely to work in a gap junction-coupled network, especially one driven by the kind of bursting dynamics possessed by beta cells. In the absence of a general theory for pacemaker systems, we cannot rule out that some model, perhaps along the lines suggested in the preceding paragraph, perhaps something else that has not yet been thought of, would overturn this conclusion. We hope this discussion will be of use in bringing theory and experiment into better alignment in the near future.

Acknowledgements

We thank Janita Hogan for previous work on islet modeling, including construction of figures 3 and 4.

Funding

AS was supported by the Intramural Research Program of the National Institute of Diabetes and Digestive and Kidney Diseases (NIH), USA.

References

Atwater I, Rosario L and Rojas E 1983 Properties of the Ca-activated K+ channel in pancreatic beta-cells. *Cell Calcium* **4** 451–461

Benninger RKP and Hodson DJ 2018 New understanding of β-cell heterogeneity and in situ islet function. *Diabetes* **67** 537–547

Benninger RKP and Kravets V 2021 The physiological role of beta-cell heterogeneity in pancreatic islet function. *Nat. Rev. Endocrinol.* *https://doi.org/10.1038/s41574-021-00568-0*

Benninger RKP, Head WS, Zhang M, Satin LS and Piston DW 2011 Gap junctions and other mechanisms of cell-cell communication regulate basal insulin secretion in the pancreatic islet. *J. Physiol.* **589** 5453–5466

Bertram R and Sherman A 2004 A calcium-based phantom bursting model for pancreatic islets. *Bull. Math. Biol.* **66** 1313–1344

Bertram R, Sherman A and Satin LS 2007 Metabolic and electrical oscillations: partners in controlling pulsatile insulin secretion. *Am. J. Physiol. Endocrinol. Metab.* **293** E890–E900

Calabrese A, Zhang M, Serre-Beinier V, *et al.* 2003 Connexin 36 controls synchronization of Ca^{2+} oscillations and insulin secretion in MIN6 cells. *Diabetes* **52** 417–424

Cappon G and Pedersen MG 2016 Heterogeneity and nearest-neighbor coupling can explain small-worldness and wave properties in pancreatic islets. *Chaos* **26** 053103

Chay TR and Kang HS 1988 Role of single-channel stochastic noise on bursting clusters of pancreatic beta-cells. *Biophys. J.* **54** 427–435

Chay TR and Keizer J 1983 Minimal model for membrane oscillations in the pancreatic beta-cell. *Biophys. J.* **42** 181–190

Chen YD, Wang S and Sherman A 2008 Identifying the targets of the amplifying pathway for insulin secretion in pancreatic beta-cells by kinetic modeling of granule exocytosis. *Biophys. J.* **95** 2226–2241

Cook DL, Porte D and Crill WE 1981 Voltage dependence of rhythmic plateau potentials of pancreatic islet cells. *Am. J. Physiol.* **240** E290–E296

Dwulet JM, Ludin NWF, Piscopio RA, *et al.* 2019 How heterogeneity in glucokinase and gap-junction coupling determines the islet [Ca^{2+}] response. *Biophys. J.* **117** 2188–2203

Dwulet JM, Briggs JK and Benninger RKP 2021 Small subpopulations of β-cells do not drive islet oscillatory [Ca^{2+}] dynamics via gap junction communication. *PLOS Comput. Biol.* **17** e1008948

Eddlestone GT, Goncalves A, Bangham JA and Rojas E 1984 Electrical coupling between cells in islets of Langerhans from mouse. *J. Membr. Biol.* **77** 1–14

Ha J and Sherman A 2020 Type 2 diabetes: one disease, many pathways. *Am. J. Physiol. Endocrinol. Metab.* **319** E410–E426

Hodson DJ, Mitchell RK, Bellomo EA, *et al.* 2013 Lipotoxicity disrupts incretin-regulated human β cell connectivity. *J. Clin. Invest.* **123** 4182–4194

Hogan JP and Peercy BE 2021 Flipping the switch on the hub cell: Islet desynchronization through cell silencing. *PLoS ONE* **16** e0248974

Joglekar MV, Dong CX, Wong WKM, Dalgaard LT and Hardikar AA 2021 A bird's eye view of the dynamics of pancreatic β-cell heterogeneity. *Acta Physiol.* **233** e13664

Johnston NR, Mitchell RK, Haythorne E, *et al.* 2016 Beta cell hubs dictate pancreatic islet responses to glucose. *Cell Metab.* **24** 389-401

Kinard TA, de Vries G, Sherman A and Satin LS 1999 Modulation of the bursting properties of single mouse pancreatic beta-cells by artificial conductances. *Biophys. J.* **76** 1423–1435

Kravets V, Dwulet JM, Schleicher WE, *et al.* 2020 Functional architecture of the pancreatic islets: First responder cells drive the first-phase [Ca^{2+}] response. *Biophysics* bioRxiv https://doi.org/10.1101/2020.12.22.424082

Laurenti MC, Matveyenko A and Vella A 2021 Measurement of pulsatile insulin secretion: rationale and methodology. *Metabolites* **11** 409

Lei C-L, Kellard JA, Hara M, *et al.* 2018 Beta-cell hubs maintain Ca^{2+} oscillations in human and mouse islet simulations. *Islets* **10** 151–167

Loppini A and Chiodo L 2019 Biophysical modeling of β-cells networks: Realistic architectures and heterogeneity effects. *Biophys. Chem.* **254** 106247

Nguyen LM, Pozzoli M, Hraha TH and Benninger RK 2014 Decreasing cx36 gap junction coupling compensates for overactive KATP channels to restore insulin secretion and prevent hyperglycemia in a mouse model of neonatal diabetes. *Diabetes* **63** 1685–1697

Perez-Armendariz M, Roy C, Spray DC and Bennett MV 1991 Biophysical properties of gap junctions between freshly dispersed pairs of mouse pancreatic beta cells. *Biophys. J.* **59** 76–92

Ravier MA, Güldenagel M, Charollais A, *et al.* 2005 Loss of connexin36 channels alters beta-cell coupling, islet synchronization of glucose-induced Ca^{2+} and insulin oscillations, and basal insulin release. *Diabetes* **54** 1798–1807

Rorsman P and Trube G 1986 Calcium and delayed potassium currents in mouse pancreatic beta-cells under voltage-clamp conditions. *J. Physiol.* **374** 531–550

Rutter GA, Ninov N, Salem V and Hodson DJ 2020 Comment on Satin et al. 'Take Me To Your Leader': An Electrophysiological Appraisal of the Role of Hub Cells in Pancreatic Islets. Diabetes 2020;69:830-836. *Diabetes* **69** e10–e11

Salem V, Silva LD, Suba K, *et al.* 2019 Leader β-cells coordinate Ca^{2+} dynamics across pancreatic islets in vivo. *Nat. Metab.* **1** 615–629

Satin LS, Butler PC, Ha J and Sherman AS 2015 Pulsatile insulin secretion, impaired glucose tolerance and type 2 diabetes. *Mol. Aspects Med.* **42** 61–77

Satin LS, Zhang Q and Rorsman P 2020 'Take me to your leader': an electrophysiological appraisal of the role of hub cells in pancreatic islets. *Diabetes* **69** 830–836

Scarl RT, Corbin KL, Vann NW, *et al.* 2019 Intact pancreatic islets and dispersed beta-cells both generate intracellular calcium oscillations but differ in their responsiveness to glucose. *Cell Calcium* **83** 102081

Scialla S, Loppini A, Patriarca M and Heinsalu E 2021 Hubs, diversity, and synchronization in FitzHugh-Nagumo oscillator networks: Resonance effects and biophysical implications. *Phys. Rev. E* **103** 052211

Sherman A 1996 Contributions of modeling to understanding stimulus-secretion coupling in pancreatic beta-cells. *Am. J. Physiol.* **271** E362–E372

Sherman A, Rinzel J and Keizer J 1988 Emergence of organized bursting in clusters of pancreatic beta-cells by channel sharing. *Biophys. J.* **54** 411–425

Smolen P, Rinzel J and Sherman A 1993 Why pancreatic islets burst but single beta cells do not. The heterogeneity hypothesis. *Biophys. J.* **64** 1668–1680

Speier S, Gjinovci A, Charollais A, Meda P and Rupnik M 2007 Cx36-mediated coupling reduces beta-cell heterogeneity, confines the stimulating glucose concentration range, and affects insulin release kinetics. *Diabetes* **56** 1078–1086

Stokes CL and Rinzel J 1993 Diffusion of extracellular K+ can synchronize bursting oscillations in a model islet of Langerhans. *Biophys. J.* **65** 597–607

Stožer A, Gosak M, Dolenšek J, *et al.* 2013 Functional connectivity in islets of Langerhans from mouse pancreas tissue slices. *PLoS Comput. Bio.* **9** e1002923

Stožer A, Markovič R, Dolenšek J, *et al.* 2019 Heterogeneity and delayed activation as hallmarks of self-organization and criticality in excitable tissue. *Front. Physiol.* **10** 869

Stožer A, Skelin Klemen M, Gosak M, *et al.* 2021 Glucose-dependent activation, activity, and deactivation of beta cell networks in acute mouse pancreas tissue slices. *Am. J. Physiol. Endocrinol. Metab.* **321** E305–E323

van der Meulen T, Mawla AM, *et al.* 2017 Virgin beta cells persist throughout life at a neogenic niche within pancreatic islets. *Cell Metab.* **25** 911–926.e916

Corresponding editor: Mohit Kumar Jolly