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Estimating and Eliminating Junctional Current in Coupled Cell Populations by Leak Subtraction. A Computational Study

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Abstract. The quantitative characterization of ion channel properties in pancreatic β-cells under typical patch clamp conditions can be questioned because of the unreconciled differences in experimental conditions and observed behavior between microelectrode recordings of membrane potential in intact islets of Langerhans and patch recordings of single cells. Complex bursting is reliably observed in islets but not in isolated cells under patch clamp conditions. E. Rojas et al. (*J. Membrane Biol.* **143:**65–77, 1995) have attempted to circumvent these incompatibilities by measuring currents in β-cells in intact islets by voltage-clamping with intracellular microelectrodes (150–250 MΩ tip resistance). The major potential pitfall is that β-cells within the islet are electrically coupled, and contaminating coupling currents must be subtracted from current measurements, just as linear leak currents are typically subtracted. To characterize the conditions under which such coupling current subtraction is valid, we have conducted a computational study of a model islet. Assuming that the impaled cell is well clamped, we calculate the native and coupling components of the observed current. Our simulations illustrate that coupling can be reliably subtracted when neighbor cells' potentials are constant or vary only slowly (e.g., during their silent phases) but not when they vary rapidly (e.g., during their active phases). We also show how to estimate coupling conductances in the intact islet from measurements of coupling currents.

Key words: Pancreatic beta-cell — Islet of Langerhans — Voltage clamp — Gap junctions — Electrotonic coupling

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Introduction

A persistent difficulty in studying the electrophysiological properties of pancreatic β-cells has been reconciling microelectrode recordings of membrane potential in intact islets of Langerhans with patch clamp recordings of ion channels in single cells. Complex bursting patterns are reliably observed in islets [2], but in isolated cells under the same conditions as patch clamp experiments, most reports show continuous spike activity (e.g., [6, 15]). Hence, the correspondence of the characterizations of individual channels to the bursting phenomenon observed in cells within islets may be questioned. Several major differences in the conditions under which the two experiments are performed may account for the disparity. Recordings of membrane potential are made with intracellular microelectrodes (tip resistance typically 150–250 MΩ) impaled in cells in the intact islet, typically at 37°C using HCO_3^-/CO_2 buffer to control pH. Patch and voltage clamp recordings, on the other hand, are usually done on isolated β-cells at room temperature using HEPES buffer. It would not be surprising if one or more of these conditions alters ion channel function, resulting in regular bursting in cells in the intact islet but not in individual cells at patch clamp conditions. In fact, bursting in the intact islet reverts to constant spiking as temperature is decreased to room temperature, similar to single cell behavior under similar conditions (C.L. Stokes et al., *in preparation*). In addition, the functional characteristics (e.g., open and closed probabilities, conductance and time constants) of various channels in several cell types change with temperature [3, 7]. In β-cells, the buffer selected to control pH changes K-ATP, but not K-Ca, channel behavior [4].

To address these issues, Rojas et al. [14] carried out voltage clamp experiments on individual cells within intact islets to characterize the ion channels under condi-

tions in which bursting indisputably occurs. In doing so, an additional difficulty was introduced. The β-cells are electrically coupled to neighboring cells through gap junctions, and one must separate native membrane currents in the clamped cell from coupling currents originating in neighboring cells. Rojas et al. [14] have proposed that the standard leak subtraction protocol [1] will do this. Here, we test this proposition with a computational islet of Langerhans. We subject it to the same manipulations as the real experimental islet. We assume the impaled cell is well clamped and take advantage of our knowledge of the underlying currents in the model to test how well the simulated experimental protocol extracts the membrane channel currents and removes the unwanted coupling component. For the model, we demonstrate that the leak subtraction protocol works well when neighboring cells are in the silent phase of their bursts, but poorly during the active, spiking phase because of rapidly changing coupling currents. In addition, we demonstrate that the total gap junctional conductance coupling the clamped cell to neighbors can be estimated by recording the total current during voltage-clamping.

Materials and Methods

Modeling

The model islet is a 5 × 5 × 5 cube of cells coupled by gap junctions [17, 19]. One cell in the cube, generally the center cell, is considered to be impaled by a microelectrode through which the cell's membrane potential can be clamped to a specified voltage. The membrane potential of each cell is described by the model of Smolen and Keizer [18] because this model has the most detailed fitting of voltage clamp currents, especially Ca^{2+} currents. The details of the model and even the underlying theory of what slow process drives bursting, however, are not important for our purposes. The model produces bursts of electrical activity (Fig. 1A) for suitable values of a putative glucose-dependent parameter by means of the following channel currents: the delayed-rectifier K^+ current (I_{K-V}); the K-ATP current ($I_{K\text{-}ATP}$); the two high-threshold voltage-activated Ca^{2+} currents identified by Satin and Cook [16] and a glucose-activated, voltage-independent Ca^{2+} current ([13]; combined in our equations as I_{Ca}). We ignore complications such as the voltage drop across the series resistance of the electrode. Under zero-current clamp conditions, the membrane potential in the impaled cell, V_m, varies in time according to the differential equation.

$$C_m \frac{dV_m}{dt} = -I_{ion} - I_{coup} - I_{elec}, \tag{1}$$

where I_{ion} is the sum of the native membrane currents,

$$I_{ion} = I_{Ca} + I_{K-V} + I_{K-ATP}, \tag{2}$$

I_{coup} is the sum of the coupling currents from immediate neighbors, j,

$$I_{coup} = \sum_j g_{c,j}(V_m - V_j), \tag{3}$$

I_{elec} is the current passed through the electrode, C_m is membrane capacitance, $g_{c,j}$ is coupling conductance between the clamped cell and the jth neighbor, and t is time. There are seven other auxiliary variables not listed above. Five represent activation and inactivation gating of the ionic currents as in the Hodgkin-Huxley equations. The remaining two are intracellular Ca^{2+} and ADP concentrations. The model postulates that voltage-dependent variations in Ca^{2+} slowly modulate ATP production and give rise to bursts of action potentials separated by silent periods. ATP feeds back on to membrane potential through the ATP-sensitive K^+ channel, whose conductance, $g_{K\text{-}ATP}$, is assumed to decrease with ATP and increase with ADP. The rate of ATP production is assumed to depend also on glucose concentration. Details may be found in [18] with corrections listed here in the Appendix. Calculations were carried out on a Cray YMP computer. With parameters representing a stimulatory concentration of glucose and under current clamp with $I_{elec} = 0$, the model islet bursts (Fig. 1A).

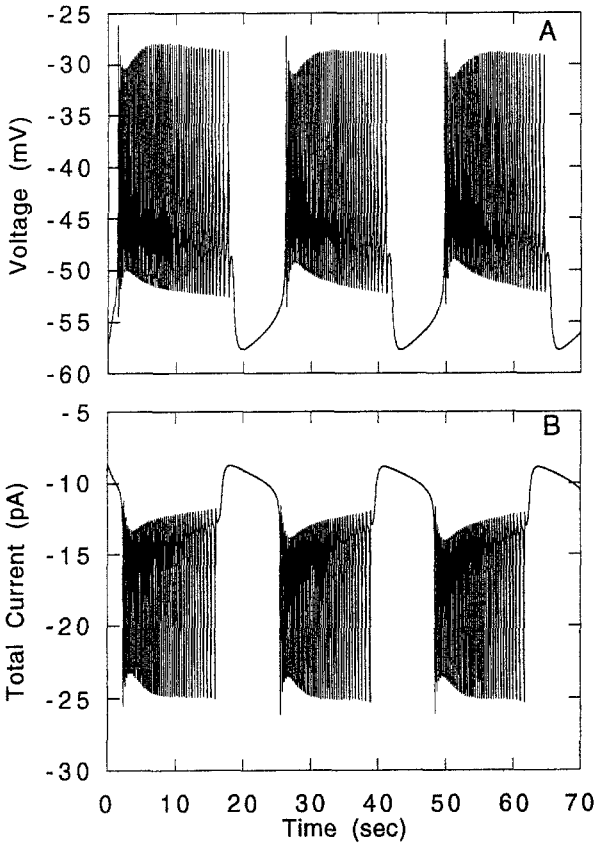

Fig. 1. (*A*) Computer simulation of a bursting β-cell in a cluster of 125 cells (a 5 × 5 × 5 cube) with g_c = 100 pS, using Eqs. (1–3). (*B*) Total cell current measured in the center cell when it is clamped at $V_m = -70$ mV and the rest of the cluster is bursting.

Under voltage clamp conditions, the potential of the clamped cell, V_m, is held constant, so $dV_m/dt = 0$, and

$$I_{elec} = I_{ion}(V_m) + I_{coup}(V_m). \tag{4}$$

I_{elec} is now equivalent to the measured current in a whole-cell voltage clamp experiment, and is the sum of the native membrane currents, evaluated at the clamping potential, and the invading coupling currents from the neighboring cells. The neighbor cells are still free to burst, but are perturbed to an unknown extent by the coupling current.

As in the experiments we are modeling [14], we apply the P –

P/N protocol for leak subtraction [1]. The purpose is to remove the linear component of the total current, purportedly representing any voltage-independent "leak" current, under conditions where currents of interest are inactive. The residue represents currents through the channels other than the leak.

In their voltage clamp experiments in the intact islet, Rojas et al. [14] have implemented this protocol to remove such linear currents, which presumably include coupling currents. The protocol is to precede the depolarizing test voltage pulse of size P by N (typically four) hyperpolarizing prepulses of size P/N, a modification of the scheme of Armstrong and Bezanilla [1] (Fig. 2*A*). The experimental observable, the leak-subtracted current I^{sub}, is the sum of the current increments during the test- and prepulses relative to the holding current:

$$I^{sub} = I^t - I^h + \sum_{j=1,N_1} (I^{p,j} - I^h), \tag{5}$$

where I^h, $I^{p,j}$, and I^t are the recorded values of I_{elec} at the holding, prepulse, and test potentials, respectively. (Superscripts refer to elements of the subtraction protocol; subscripts identify cells and current components.) In general, any current measured at the prepulse voltages will be subtracted from the current measured during the test pulse. For a current to be linear, and hence subtracted by the *P-P/N* protocol, it must have both a voltage-independent conductance and an ohmic driving force. That is, it must be representable in the form $I_{leak} = g_l(V_m - V_l)$ where g_l is the leak conductance. For such a current, $I^{sub} = 0$ (*see* Discussion). For example, Fig. 2*B* illustrates the effect of leak subtraction on $I_{K\text{-}ATP} = g_{K\text{-}ATP}(V_m - V_K)$, which is very nearly linear in the model. (It is not perfectly linear because ADP concentration changes slightly during the pulses.)

For nonlinear, voltage-dependent channels, the currents during prepulses are typically small since prepulse potentials are hyperpolarizing and nonactivating. This is illustrated in Fig. 2*C*, which shows the effect of leak subtraction on $I_{K\text{-}V}$, a typical nonlinear voltage-dependent current.

The coupling current is not in general linear, even if the coupling conductance g_c is voltage independent, because the response of the neighboring cells is uncontrolled. Some current injected into the clamped cell will travel through gap junctions and change the voltage of the neighbor cells. Clamping a cell to a voltage negative to its neighbor will hyperpolarize the neighbor, while clamping positive to the neighbor will depolarize it. This effect increases with g_c. If the potential of the neighbor does not vary linearly with the clamp potential, coupling current will not be linear, and leak subtraction will not work correctly. In addition, the clamp potential is rapidly varied between V^h_m, V^p_m, and V^t_m according to the protocol, producing transient alterations in the neighbors' potentials that further complicate subtraction efforts.

Our task then is to determine under what conditions leak subtraction allows a good approximation of native membrane currents when coupling currents are present ($g_c > 0$), as in the intact islet experiments of Rojas et al. [14]. When we consider the effects of subtraction on currents in coupled cells, we will take as the standard of comparison the current obtained after leak subtraction in the absence of coupling ($g_c = 0$), which corresponds to voltage clamp of an isolated cell.

Results

What Leak Subtraction Does to the Coupling Current

We first examine the effect of leak subtraction on the coupling current. Figure 3 shows the total coupling cur-

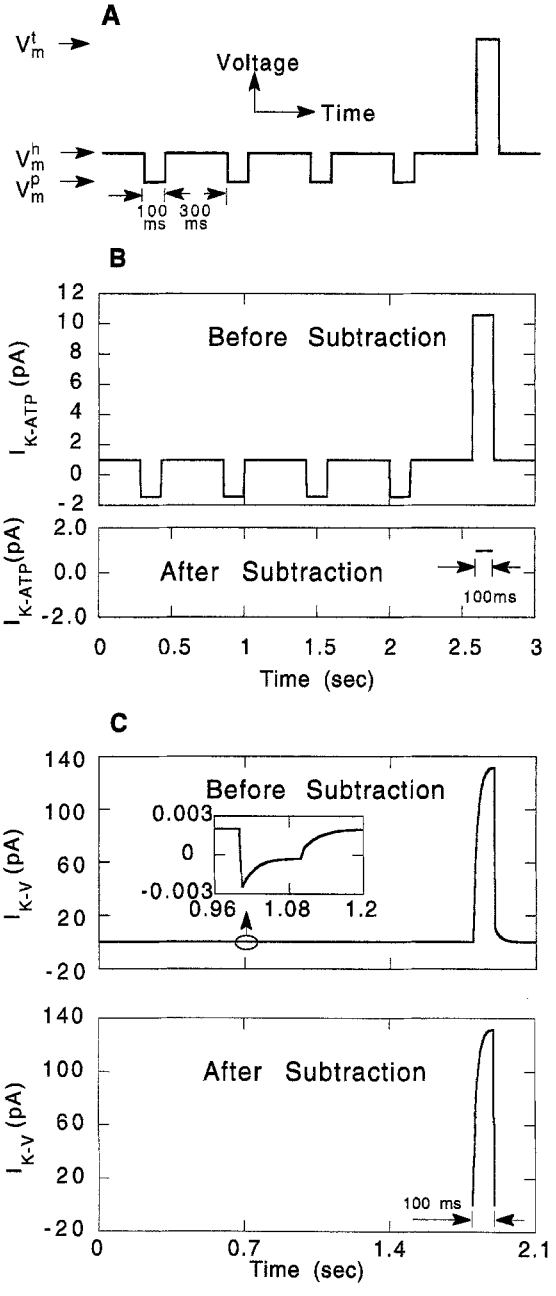

Fig. 2. Leak subtraction protocol using the *P-P/N* scheme. $V^h_m = -70$ mV, $V^t_m = -20$ mV, $g_c = 100$ pS in parts *B* and *C*. (*A*) The voltage clamp protocol using leak subtraction. N prepulses of magnitude $-P/N$ precede the test pulse of magnitude p. All test and prepulses are 100 msec. $V^h_m = -70$ mV throughout. We used four prepulses ($N = 4$) as is typical in experiments. (*B*) Response of a linear current ($I_{K\text{-}ATP}$) to voltage-clamping and leak subtraction. The top curve is the total current as it responds to prepulses and a test pulse. The lower curve is the current left after applying leak subtraction by adding the current during the four prepulses to the current during the test pulse. This linear, steady-state current is eliminated by the protocol. (In contrast to the experiments, there is no capacity transient because we neglect the series electrode resistance, and, hence, V_m steps instantaneously to the command potential.) (*C*) Response of a nonlinear current ($I_{K\text{-}V}$) to voltage-clamping and leak subtraction. The top and bottom curves are the same as in *B*. This nonlinear current is little reduced by the subtraction protocol.

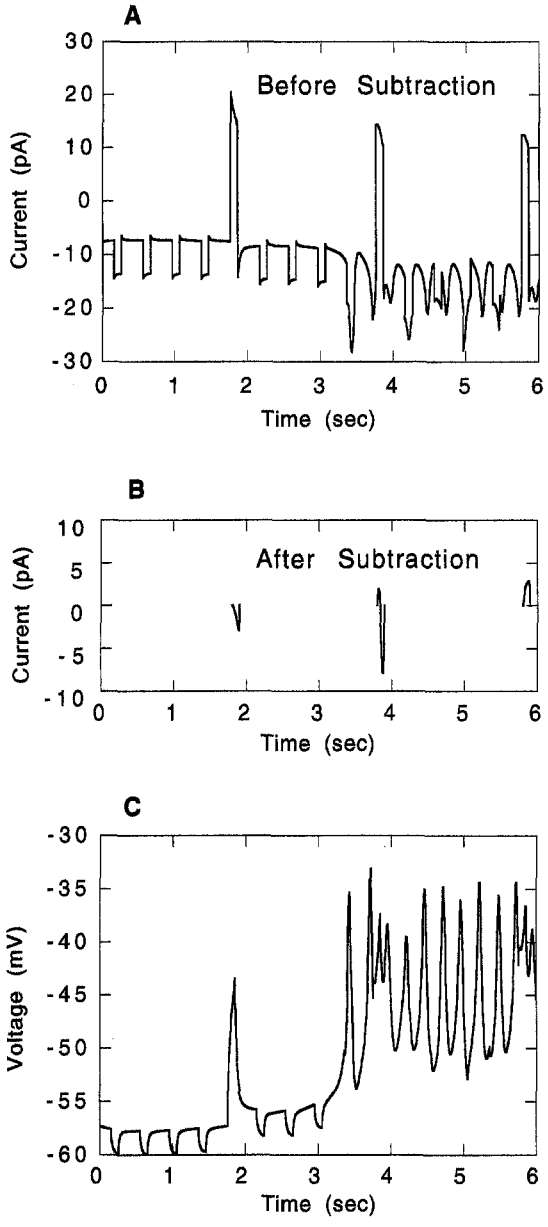

Fig. 3. Effect of leak subtraction on the coupling component of current. $V_m^h = -70$ mV, $g_c = 100$ pS, and $V_m^t = -20$ mV. (*A*) Total current and (*B*) leak-subtracted current during the middle and end of the neighbors' silent phase and beginning of the neighbors' active phase, and (*C*) the corresponding membrane potential of the neighbor, V_n.

rent and what remains after leak subtraction at various points in time. When the neighboring cells are in the early or middle part of the silent phase, their voltages vary in a nearly linear manner, and hence the subtraction reduces the coupling current amplitude by a factor of about 10 (left portion, Fig. 3*A*, *B*). As the neighbors approach the active phase, however, the coupling current fluctuates erratically and develops rapid, large amplitude transients during both the prepulses and test pulse (middle and right portions, Fig. 3*A*, *B*). The results of the subtraction are unpredictable during these times, with the coupling currents sometimes exaggerated (middle portion, Fig. 3*A*, *B*) and sometimes canceling nicely (right portion, Fig. 3*A*, *B*). Hence, the subtraction protocol cannot be relied upon to correctly remove coupling currents during these periods.

The membrane potential of a neighbor cell is shown in Fig. 3*C*. During the first set of pulses, well within the silent phase, the neighbor exhibits attenuated, nearly passive, responses to the transmitted steps from the clamped cell. It is more depolarized during the holding periods, and undergoes smaller relative deflections during the test- and prepulses. Thus, the coupling current of the clamped cell (Fig. 3*A*) is inward during the holding- and prepulses, but outward during the test pulses. As the silent phase ends, the voltage of the neighbor ramps up and exceeds the threshold for spiking. (This is primarily because the threshold has been lowered by a slow decrease in $g_{K\text{-}ATP}$, which is not shown.)

We conclude that during the silent phase the behavior of I_{coup} is intermediate between that of $I_{K\text{-}ATP}$ and $I_{K\text{-}V}$; it is not perfectly linear, but it is not strongly nonlinear. During the active phase, rapid transients render the subtraction protocol ineffective.

Current-Voltage Relationships

Despite all of the potential problems, the foregoing suggests that the leak-subtraction method will work reasonably well if one uses only data taken when the unclamped cells are in the silent phase. Figure 4 shows the total currents (I_{elec}) *vs.* time at a family of test potentials, V_m^t, with $g_c = 100$ pS, before and after leak subtraction. The currents are reduced slightly in magnitude by subtraction. This is primarily due to removal of coupling current, which is outward during the test pulse because the neighbor cells do not depolarize as much as the clamped cell. Note that the transient inward current component, clearly visible after subtraction, is masked by the coupling current before subtraction.

Figure 5 summarizes peak total current *vs.* membrane potential before and after leak subtraction for several values of g_c. The unsubtracted *I-V* curves for $g_c > 0$ are shifted upward relative to the curve for $g_c = 0$, which corresponds to an isolated cell. The upward shift increases with g_c and again reflects the hyperpolarizing effect of the coupling current.

After subtraction, the coupling current is much reduced. Note, however, that the subtracted *I-V* curves show a small, but systematic, *downward* shift, reflecting the residual coupling current which is inward. *Compare* with the leftmost portions of Fig. 3*A*, *B*. The subtraction protocol overcorrects because the response of the neighboring cells to the steps in V_m is nonlinear. A more precise mathematical analysis is given in the Discussion and in Fig. 7.

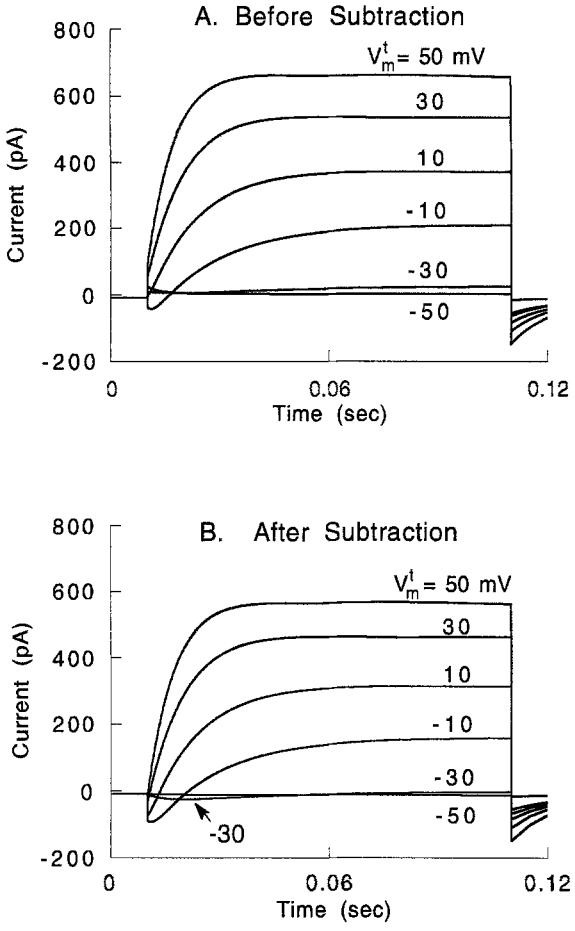

Fig. 4. A family of currents I_{elec} (Eq. 4) *vs.* time (*A*) before and (*B*) after leak subtraction. $g_c = 100$; V_m^t ranges from −60 to +50 mV. Here and hereafter, currents are calculated using data taken only when neighbors are in their silent phases.

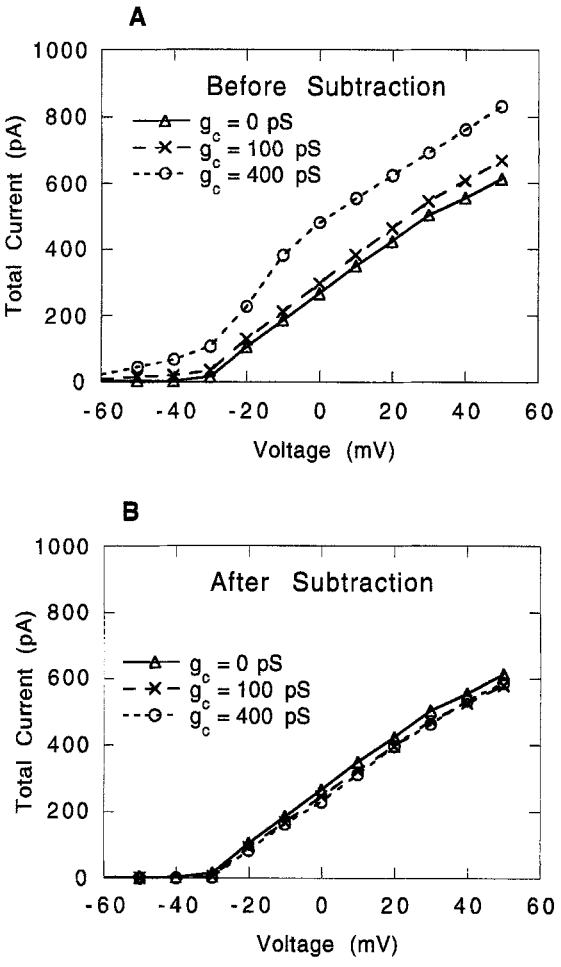

Fig. 5. Current-voltage relationship for the peak total cell current I_{elec} (Eq. 4) for $g_c = 0$, 100, and 400 pS conductance. When $g_c = 0$, the given *I-V* curve is equivalent to that from voltage-clamping a single, isolated cell. Curves for $g_c = 100$ correspond to Fig. 4. (*A*) Before subtraction, coupling current is outward and increases with g_c. (*B*) After subtraction, the residue of coupling current is small but inward.

As g_c increases, the error due to the residual coupling current increases, and leak subtraction becomes less effective. However, even with $g_c = 400$ pS, where the unsubtracted coupling current is about one-third as large as the native current, the protocol works fairly well. The mathematical analysis below shows why the error increases with g_c.

We can also generate *I-V* curves for individual currents. For example, I_{Ca}-*V* curves can be obtained by setting all K^+ conductances to zero; this simulates addition of TEA. Alternatively, one can compute leak-subtracted, peak total current curves as in Fig. 5*B* with normal and zero extracellular Ca^{2+}, and subtract one from the other. The results of the latter procedure are displayed in Fig. 6. We obtain reasonable, though downward shifted, I_{Ca}-*V* curves, even with $g_c = 400$ pS (Fig. 6) and even though the inward current component was barely visible prior to leak subtraction in the total current records (Fig. 4*A*). At large values of V_m^t where the error is large and the native current is small (e.g., $V_m^t = +50$ mV), the relative error is large. The mathematical analysis shows why the residual error increases with V_m^t.

Estimation of Coupling Conductance

The variation of the junctional current between the neighbors' silent and active phases (Fig. 1*B*) can be combined with voltage clamp recordings (Fig. 1*A*) to estimate the total gap junctional conductance between the clamped cell and its neighbors. At any time, the total electrical current in the clamped cell is equal to the native membrane currents and the coupling currents such that we can write,

$$I_{elec}^{AP} = I_{ion}(V_m) + \sum_j g_{c,j}(V_m - V_{n,j}^{AP}) \qquad (6)$$

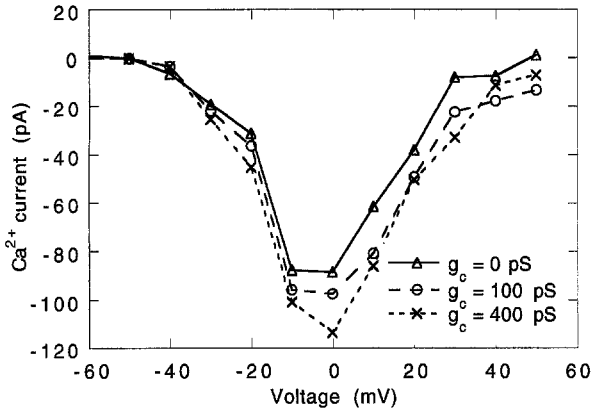

Fig. 6. Current-voltage relationship for the peak, leak-subtracted, voltage-dependent calcium current for several values of coupling conductance. When $g_c = 0$, the given *I-V* curve is equivalent to that from voltage-clamping a single, isolated cell. The calcium current was obtained by a method similar to that used experimentally: Total cell current was measured during normal simulations with extracellular Ca^{2+} present (Fig. 5) and without extracellular Ca^{2+}. The difference gives the displayed curves.

$$I_{\text{elec}}^{SP} = I_{\text{ion}}(V_m) + \sum_j g_{c,j}(V_m - V_{n,j}^{SP}) \tag{7}$$

where *AP* and *SP* represent values measured when the neighbors are in their active and silent phases, respectively.

In an experimental situation one would not know $V_{n,j}^{AP}$ or $V_{n,j}^{SP}$, the potential of the neighbors in the active or silent phases. However, one could switch to current clamp, allow the clamped cell to resume bursting, and take V^{AP} and V^{SP} of the impaled cell under current clamp as estimates of $V_{n,j}^{AP}$ and $V_{n,j}^{SP}$. For this to be valid, we must assume that the $V_{n,j}$ are not perturbed very much by the current injected into the impaled cell when it is under voltage clamp. An alternative sufficient condition is that $V_{n,j}^{AP}$ and $V_{n,j}^{SP}$ are perturbed about the same amount. Simulations with the model show the latter condition to hold within about 7% when $g_c = 100$ pS, and within about 15% when $g_c = 400$ pS. Eddlestone et al. [5] showed that cells were approximately synchronized, so we can take all the V_j to be equal. With these simplifications we can subtract Eq. (7) from Eq. (6) to obtain

$$I_{\text{elec}}^{AP} - I_{\text{elec}}^{SP} = \left(\sum_j g_{c,j}\right)(V^{AP} - V^{SP}) \tag{8}$$

which can be solved for $\Sigma_j\, g_{c,j}$, the total junctional conductance of the clamped cell with its neighbors. In Fig. 1 average values are $I_{\text{elec}}^{AP} = -19$ pA, $I_{\text{elec}}^{SP} = -9$ pA, $V^{AP} = -42$ mV, and $V^{SP} = -59$ mV. This gives an estimate for $\Sigma_j\, g_{c,j}$ of 588 pS, close to the true value of 600 pS (six junctions of 100 pS each). The error is worse for larger coupling, but even at $g_c = 400$ pS, the error is < 25%.

Discussion

Summary

We have explored with simulations the possibility of voltage-clamping a cell within an electrically coupled tissue, with specific application to the pancreatic β-cell in the intact islet of Langerhans. A key experimental difficulty in using intact islets not faced with isolated cells is that the former is electrically coupled to neighboring cells through gap junctions. Current may flow between the clamped cell and its neighbors through the gap junctions, depending on the relative magnitudes of their membrane potentials. Hence, measured current represents not only native membrane currents through ion channels but also the current through the gap junctions. Rojas et al. [14] have used the *P-P/N* protocol to subtract the gap junctional currents as if they are part of a linear leak current. Simulations with parameters appropriate to the pancreatic islet confirm that voltage-clamping a cell in a coupled ensemble using this subtraction protocol is a reasonable procedure if done with care. Mathematical analysis of a simplified case below characterizes more generally when spurious results will be obtained.

The islet simulations show that coupling leads to relatively small errors when measurements are made during neighboring cells' silent phases and leak subtraction is used to remove coupling currents. Recording during neighbors' active phases gives meaningless results, however, because the rapid oscillation of the neighbors' potentials causes the coupling currents to vary rapidly. Rojas et al. [14] showed that it is experimentally feasible to make voltage clamp measurements during the silent phase because the neighbors' active phase is apparent by the invasion of large inward action currents in the current record when at the holding potential (Figs. 1*B*, 3*A*).

We have shown that oscillations of the neighbors can be converted from a contaminant to a source of useful information. By clamping to a fixed potential (Fig. 1*B*), measurements of invading burst currents can be used to estimate gap-junctional conductance between the clamped cell and its neighbors (Eq. 8). This type of data is unique. Until now, measurements of gap junctional conductance have been made only in cell pairs that survive dissociation of an islet [10, 12]. We do not know whether these are representative of conductance *in situ.* Perez-Armendariz et al. [12] found that 65% of cell pairs were electrically connected, and that the conductance was highly variable from pair to pair, ranging from 25–600 pS, with a mean of 215 pS. Meda et al. [10] found that only 20% of pairs were connected. Computer simulations suggest that the former value is adequate to mediate synchrony of bursting in islets, while the latter is not [19]. Measurements in islets could help resolve this

critical issue. If one could impale several cells in an islet sequentially, one could assess the variation in coupling strength from cell to cell. Note that our procedure gives the total coupling conductance of the clamped cell to all its neighbors rather than to individual neighbors.

Furthermore, our simulations suggest that one could study the kinetics of the glucose-stimulated increase in gap junctional coupling in an islet. Eddlestone et al. [5] have demonstrated that g_c increases with glucose, and that the increase is time dependent, as judged from the delay of a few minutes for bursts to synchronize after adding glucose. Our simulations suggest that by measuring the increase in magnitude of the invading burst currents when clamping at a fixed potential, one could follow the rate of increase of glucose-stimulated gap junctional coupling.

Similarly, one could study the rate and magnitude of the effects of other substances on gap junctional coupling, such as the long-chain alcohols [9]. The method of Eq. (8), would give an estimate of the effect on g_c and subtracting the coupling current would show whether the native currents are also significantly affected.

Our results depend on some specific features of the "tissue preparation." First, there are electrically "silent" periods during which currents can be measured; during this time the unclamped cells are far below threshold and have a nearly linear response. Second, the islet is a discrete cluster of small cells which are not spatially extended like neurons, and hence each cell may be considered iso-potential. The discreteness contributes somewhat to isolating the cells electrically from each other. For example, reduced coupling in discrete systems can cause failure of action potential propagation; in continuous systems, such as large single neurons, the propagation speed is merely reduced [8].

Our assumption that all the gap junctional connections (as well as all the cells) in a cluster are identical, however, should not limit the applicability of the results. While all cell properties and gap junctions in real islets are not likely to be identical [12], we expect that varying coupling conductances randomly (as in [19]) would give qualitatively similar results.

Mathematical Analysis of Leak Subtraction

Certain results on the leak subtraction protocol can be found analytically. It is easy to show that leak subtraction of a linear current results in a zero net current, as stated previously in Modeling under Materials and Methods. To simplify, we assume that currents in each of the N prepulse steps are the same, so that $I^{p,j} = I^p$ in Eq. (5). This gives for the subtracted current,

$$I^{\text{sub}} = I^t - I^h + N(I^p - I^h). \tag{9}$$

For a linear current component, $I_{\text{leak}} = g_l(V_m - V_l)$, $I^t = g_l(V^t_m - V_l)$; $I^h = g_l(V^h_m - V_l)$; $I^p = g_l(V^p_m - V_l)$, where $V^t_m = V^h_m + P$; $V^p_m = V^h_m - P/N$. Substituting these expressions for I^t, I^h, and I^p in Eq. (9), we find that $I^{\text{sub}}_{\text{leak}} = 0$; the leak is completely subtracted as expected for a linear current.

Now apply leak subtraction to the coupling current component. For simplicity, consider the case of two cells, one clamped to V_m and one neighbor with unknown potential V_n. Then, $I_{\text{coup}} = g_c(V_m - V_n)$. If V_n were constant, them I_{coup} would be formally equivalent to a linear leak and would be completely subtracted with the *P-P/N* protocol, as just shown above. In general V_n varies in an unknown way, but a condition for complete subtraction of this component can be derived as follows:

Let $V_n = V^h_n$, V^p_n, or V^t_n, respectively, when $V_m = V^h_m$, V^p_m, or V^t_m. Substituting $I^t = g_c(V^t_m - V^t_n)$; $I^h = g_c(V^h_m - V^h_n)$; $I^p = g_c(V^p_m - V^p_n)$ in Eq. (9), we find

$$I^{\text{sub}}_{\text{coup}} = -g_c[(V^t_n - V^h_n) - N(V^h_n - V^p_n)].$$

Assuming $g_c \neq 0$, $I^{\text{sub}}_{\text{coup}} = 0$ if and only if $(V^t_n - V^h_n) = N(V^h_n - V^p_n)$. This means that the coupling component is completely subtracted only if the deflections from baseline of the neighbor are in the same proportion as those of the clamped cell.

In general this will not hold. For the cell pair, a qualitative picture of how nonlinear the response of V_n will be to changes in V_m can be estimated as follows. The equation for V_n is

$$C_m \frac{dV_n}{dt} = -I_{ion}(V_n, g_{\text{K-ATP}}) - g_c(V_n - V_m). \tag{10}$$

The parameter $g_{\text{K-ATP}}$ sets the overall level of excitability of the cell in the Smolen-Keizer [18] model (for a neuron a similar role could be played by a neurotransmitter or bias current). The steady-state response of the neighbor is implicitly defined by

$$V_m = V_n + I_{\text{ion}}(V_n, g_{\text{K-ATP}})/g_c. \tag{11}$$

An immediate consequence is that V_n approaches V_m for very large g_c; in this limit the neighbor responds perfectly linearly because the pair is iso-potential. The measured current is then double that of an isolated cell because the two cells respond like one cell with double the membrane area.

The response for several values of g_c and $g_{\text{K-ATP}}$ is plotted in Fig. 7. First, consider the case of relatively weak coupling, $g_c = 10$ pS. When $g_{\text{K-ATP}} = 250$ pS (corresponding roughly to the beginning of the silent phase) and $V^t_m < -20$ mV, V_n stays on the bottom branch of the S-shaped curve and the response is nearly linear. Because the bottom portion of the curve bends upwards, however, $V^t_n - V^h_n > N(V^h_n - V^p_n)$ and $I^{\text{sub}}_{\text{coup}} < 0$; that is why the leak-subtracted *I-V* curves in Fig. 5*B* are shifted downward. The curvature reflects the nonlinear re-

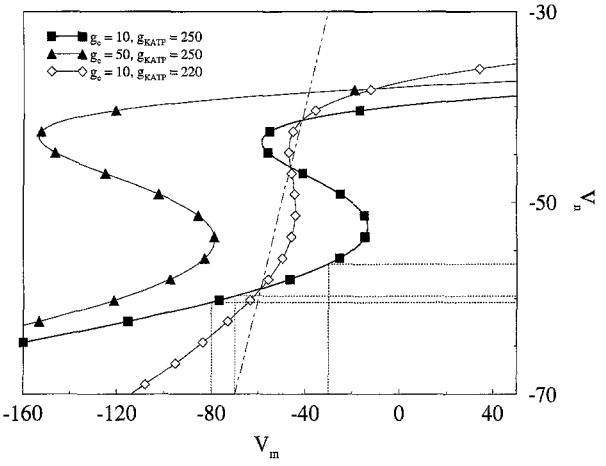

Fig. 7. The curves show for a pair of cells the response of the neighbor's potential, V_n to changes in the potential of the clamped cell, V_m using Eq. (11). The dotted lines represent V_m^p, V_m^h, and V_m^t and the corresponding V_n^p, V_n^h and V_n^t (*see text*). For relatively small coupling, g_c, large K^+ conductance, $g_{K\text{-}ATP}$, and modest V_m^t, the response is nearly linear (squares). Larger values of V_m^t to the right of the knee of the S-curve stimulate the neighbor to spike. If g_c is increased (diamonds) or $g_{K\text{-}ATP}$ is decreased (triangles) the spike threshold shifts to the left.

sponse of the neighbor to changes in V_m. Stepping up V_m steps up V_n, which opens additional channels and further depolarizes the neighbor. The curvature increases toward the right knee of the S-curve, so the error increases with V_m^t. If V_m^t is increased past the knee, V_n^t will exceed threshold, and the neighbor will begin to spike. I_{coup}^{sub} cannot be accurately predicted from the graph then, but will likely be large and varying in time. Similarly, if g_c is increased, the S-curve will deform towards the straight, dotted-dashed line $V_n = V_m$, and it will be easier to exceed threshold. I_{coup}^{sub} will also increase, as in Fig. 5*B*. Finally, if g_c = 10 pS, but $g_{K\text{-}ATP}$ is decreased to 220 pS (corresponding roughly to the end of the silent phase), the S-curve shifts to the left, and the neighbor will be able to spike even when the clamped cell is at the holding potential, V_m^h.

The results discussed above and illustrated in Fig. 7 should be widely applicable because they do not depend strongly on particular features of our β-cell model, but are typical of many excitable cell models. However, this simple example neglects the effects of distal neighbors for which the effects of clamping are attenuated, moderating the response of the near neighbors. Therefore, the values of g_c must be scaled up for large clusters, and one cannot draw quantitative conclusions for the large-cluster simulations. Nonetheless, our analysis of cell pairs qualitatively accounts for the phenomena seen. The analysis suggests that the key factor that permits approximate subtraction of the coupling current is the suppression of excitability by a large K^+ conductance. This occurs naturally in pancreatic islets as a result of the slow cyclic oscillations (bursting). Müller and Lux [11] modeled point-clamp in neurons with axons and also found that large outward currents help isolate the clamped region from the rest of the neuron.

In summary, our computational models have shown that a standard, leak subtraction protocol will also subtract coupling currents during voltage-clamping in electrically coupled tissues, but works reasonably only during time periods in which the neighbor cells' potentials are varying slowly compared to the voltage clamp protocol. In the bursting islet, subtraction of coupling currents works while the neighbors are silent, but not during their active phase. We have calculated analytically the error in the subtracted current for a simplified two-cell case, and the result will be similar for a larger cluster of coupled cells. Finally, we have shown how coupling currents in the intact islet can be estimated from a combination of voltage- and current-clamping using microelectrodes in the intact islet.

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Appendix

Calculations were done with the model of Smolen and Keizer [18], using parameters corresponding to their Fig. 6. Corrected equations only are listed below with original equation numbers on the left. **(Eq. 6).** There should be a + between the two exponential terms in the denominator:

$$\tau_J(V) = \frac{T_J}{\left\{ \exp\left[\frac{(V - V_J)}{2S_J^-}\right] + \exp\left[\frac{-(V - V_J)}{2S_J}\right] \right\}} + T_J^* \tag{A1}$$

(Table, p. 13) Parameters K_1 and K_2 should have units of mM. τ_m should be 1.28 msec.
(Eq. 19) There should be a − in front of S_I:

$$I_\infty(V) = \frac{1}{\left(1 + \exp\left[\frac{(V_I - V)}{-S_I}\right]\right)} \tag{A2}$$

(Eq. 20) There should be a + between the two exponential terms in the denominator:

$$\tau_n(V) = \frac{T_N}{\lambda\left\{ \exp\left[\frac{(V - V_K)}{65}\right] + \exp\left[\frac{-(V - V_K)}{65}\right] \right\}} \tag{A3}$$

(Eq. 27) Replace k by k_a:

$$\frac{d[\mathrm{ADP}]}{dt} = -k_a \exp\left\{ R\left[1 - \frac{[\mathrm{Ca}]}{R_1}\right] \right\} [\mathrm{ADP}] + k_a[\mathrm{ATP}] \tag{A4}$$