

Estimation of the Junctional Resistance between Electrically Coupled Receptor Cells in *Necturus* Taste Buds

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ABSTRACT Junctional resistance between coupled receptor cells in *Necturus* taste buds was estimated by modeling the results from single patch pipette voltage clamp studies on lingual slices. The membrane capacitance and input resistance of coupled taste receptor cells were measured to monitor electrical coupling and the results compared with those calculated by a simple model of electrically coupled taste cells. Coupled receptor cells were modeled by two identical receptor cells connected via a junctional resistance. On average, the junctional resistance was $\sim 200\text{--}300\text{ M}\Omega$. This was consistent with the electrophysiological recordings. A junctional resistance of $200\text{--}300\text{ M}\Omega$ is close to the threshold for Lucifer yellow dye-coupling detection ($\sim 500\text{ M}\Omega$). Therefore, the true extent of coupling in taste buds might be somewhat greater than that predicted from Lucifer yellow dye coupling. Due to the high input resistance of single taste receptor cells ($>1\text{ G}\Omega$), a junctional resistance of $200\text{--}300\text{ M}\Omega$ assures a substantial electrical communication between coupled taste cells, suggesting that the electrical activity of coupled cells might be synchronized.

INTRODUCTION

Cell-to-cell communication through low resistance pathways (electrical or electrotonic coupling) has been observed between excitable and nonexcitable cells alike in a variety of tissues, including many epithelia (Bennett and Spray, 1985). Several physiological roles have been attributed to these junctions according to the tissue in which they occur, such as metabolic synchronization (liver, pancreas), synchronization of electrical activity (heart), and modulation of receptive fields (retina) (Bennett and Spray, 1985; Sheridan and Atkinson, 1985; Dermietzel and Spray, 1993). In taste buds also, both electrophysiological and morphological observations have demonstrated the existence of electrical coupling between taste cells. By using two intracellular microelectrodes in current clamp mode, West and Bernard (1978) showed electrotonic spread and dye coupling between neighboring cells in *Necturus* taste buds. The interpretation of these results, however, could be ques-

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tioned due to the cell damage and possible current leakage inevitably produced by intracellular microelectrode impalements. Nonetheless, subsequent dye-coupling observations in taste buds from fish and amphibians have confirmed the presence of coupling between taste cells. Intracellular injections of the fluorescent dye Lucifer yellow have shown that taste receptor cells are coupled in groups of two or three cells (catfish: Teeter, 1985; *Necturus*: Yang and Roper, 1987; frog: Sata, Okada, Miyamoto, and Sato, 1992). In rat vallate taste buds, the existence of electrical coupling has been inferred from the observation of gap junctions between some taste cells (Akisaka and Oda, 1978).

Despite these studies, the details and functional role(s) of electrical coupling in taste buds are still uncertain. This lack of information is mainly due to technical difficulties in probing the properties of the intercellular communications between coupled taste cells. Taste cells are relatively small and inaccessible. Recently, we have begun to obtain data on the physiology and pharmacology of electrical coupling between taste receptor cells by applying the patch clamp technique to *Necturus* taste buds in lingual slices where taste cells are more exposed and detailed experimentation is possible. By measuring cell membrane capacitance and cell input resistance to monitor coupling, we have shown that electrical coupling between taste receptor cells is very sensitive to changes in intracellular pH as well as to the application of the alcohol, 1-octanol (Bigiani and Roper, 1993, 1994).

In this study, we have estimated the junctional resistance between *Necturus* taste receptor cells. Junctional resistance is a quantitative indicator of the state of coupling between cells and allows one to assess the extent to which coupled cells can exchange ions and molecules. Therefore, measuring junctional resistance can provide some insight on the possible functional significance of cell-to-cell coupling in taste buds. Junctional resistance is usually evaluated with dual voltage clamp techniques (Spray, Harris, and Bennett, 1982; Kolb, 1992). However, this approach is feasible only if coupled cells can be isolated in culture (e.g., Lasater and Dowling, 1985) or identified in situ (e.g., Bodmer, Verselis, Levitan, and Spray, 1988). To date, neither of these experimental conditions has been achieved with coupled taste receptor cells. Thus, we have used an analytical approach consisting of modeling the results of membrane capacitance and input resistance measurements obtained with a single patch pipette voltage clamp technique (e.g., Santos-Sacchi, 1991). We have used the slice preparation of *Necturus* lingual epithelium because cellular organization and interrelationships among cells within taste buds are maintained (Bigiani and Roper, 1993; Bigiani, Ewald, and Roper, 1995). Our analysis indicates that, on average, coupled cells have a relatively high junctional resistance of $\sim 200\text{--}300\text{ M}\Omega$. However, because the input resistance of taste cells is $> 1\text{ G}\Omega$, the estimated coupling coefficient for two receptor cells is > 0.8 , suggesting that electrical synchronization might occur between coupled cells in taste buds.

MATERIALS AND METHODS

Tissue Preparation

Mudpuppies (*Necturus maculosus*) were obtained from commercial suppliers and maintained at $4\text{--}10^\circ\text{C}$ in fresh water aquaria. They were fed minnows weekly.

The procedure to prepare lingual slices has been previously described (Bigiani and Roper, 1993; Bigiani et al., 1995). Tissue sections containing taste buds were selected and pinned out in a shallow recording chamber (~1-ml vol) filled with amphibian physiological saline (APS; see below). The chamber was placed onto the stage of a fixed-stage upright Zeiss microscope equipped with 40× water immersion objective (Nikon CF, working distance = 2.0 mm). During the experiments, the tissue was continuously superfused with APS (flow rate: ~3–5 ml/min) by means of a gravity-driven system.

Solutions

Our standard bathing solution (amphibian physiological solution, APS) consisted of 112 mM NaCl, 2 mM KCl, 8 mM CaCl₂, and 5 mM HEPES (buffered to pH 7.2 with NaOH). Elevated calcium concentration was used to stabilize the recordings and maintain the integrity of the tissue preparation. Drugs were dissolved in standard or modified APS solution and applied to the bath using a gravity perfusion system.

The patch pipette solution was as follows: 100 mM K gluconate, 10 mM NaCl, 10 mM HEPES (buffered to pH 7.3 with KOH), 2 mM MgCl₂, and 10⁻⁵ mM free Ca²⁺ [buffered with 1 mM 1,2-bis(*o*-aminophenoxy)ethane-*N,N,N',N'*-tetracetic acid (BAPTA)]. We added Lucifer yellow CH (dipotassium salt) to the patch pipette solution (2 mg/ml; Edwards, Konnerth, Sakmann, and Takahashi, 1989) so that cells would be filled during whole-cell recording and could subsequently be identified at the end of experiments (cf Bigiani and Roper, 1993). All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Recording Technique and Cell Coupling Monitoring

We obtained patch clamp recordings on taste cells in tissue slices as described previously (Bigiani and Roper, 1993). Patch pipettes were fabricated from soda lime glass capillaries (microhematocrit tubes, Baxter, Scientific Products, McGaw Park, IL) using a two-stage vertical micropipette puller (PB-7, Narishige, Tokyo, Japan). Patch electrode resistances ranged from 2 to 7 MΩ. Whole-cell recordings (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981) were performed at room temperature (20–22°C) using an Axopatch-1A amplifier (Axon Instruments, Inc., Sunnyvale, CA). Signals were prefiltered at 5 kHz and digitally recorded at 50-μs intervals with a Macintosh computer equipped with a MacADIOS II data acquisition board (GWI Inc., Cambridge, MA). Signals were displayed on the computer screen and saved on disk by using SuperScope software (GWI Inc., Cambridge, MA). The access resistance (R_a) of the patch pipette tip was estimated by dividing the amplitude of the voltage steps by the peak of the capacitive transients (from which stray capacitance had been subtracted) in whole-cell configuration; values ranged from 4 to 20 MΩ. Access resistance was not compensated during the recordings.

The state of coupling between taste receptor cells was monitored by measuring the cell membrane capacitance and the cell input resistance (Santos-Sacchi, 1991; Bigiani and Roper, 1993, 1994). These membrane parameters were calculated using a subroutine written by us consisting in the following steps: leakage and capacitive currents flowing through the membrane were measured when voltage steps (–20 mV; 65 ms) were applied to the patch pipette from a holding potential of –80 mV; the cell membrane capacitance was calculated by integrating the capacitive transient (obtained by subtracting the leakage current from the whole-cell current) at the onset of the voltage pulse and dividing it by the amplitude of the voltage step. The duration of the voltage steps allowed the membrane capacitance of coupled cells to be charged completely in our experimental conditions. Cell input resistance was estimated by dividing the voltage step by the amplitude of the leakage current evaluated at the end of the voltage step. The same subroutine was used also to evaluate membrane capacitance and input resistance of single (noncoupled) taste receptor cells.

Stray capacitance resulting from immersion of the pipette in the bath solution was subtracted from the recordings as described previously (Bigiani and Roper, 1993).

RESULTS

Electrical coupling between two or more cells can be monitored with a single patch pipette by measuring the membrane capacitance and input resistance of the coupled cells and comparing this with values for noncoupled cells (Santos-Sacchi, 1991; Bigiani and Roper, 1993, 1994). These parameters give information not only on membrane capacitance and resistance of the cells forming the coupled set, but also on the junctional resistance connecting them. We have modeled coupled taste receptor cells to obtain the junctional resistance from capacitance and input resistance measurements.

Modeling Coupled Cells

Electrically coupled taste bud cells can be described by a minimal equivalent circuit comprised solely of resistive and capacitive elements (Fig. 1 *A*). In this circuit, R_a represents the access resistance of the pipette tip; R_M and C_M the membrane resistance and capacitance, respectively, of each cell (for simplicity, we assume that both cells have the same membrane parameters); R_j the junctional resistance of the pathway connecting the coupled cells. Stray capacitance is not shown because it is subtracted from the recordings. Seal resistance (R_s) is also omitted because $R_s \gg R_a$ in our experiments (on average, R_s was $\sim 7 \text{ G}\Omega$, and R_a was $\sim 10 \text{ M}\Omega$). This model assumes that the circuit parameters are independent of voltage (cf., Lindau and Neher, 1988). Because voltage-gated conductances are present in *Necturus* taste cells (Kinnamon and Roper, 1988; Bigiani and Roper, 1993), the voltage commands were chosen to avoid activating voltage-gated ion currents. We found it convenient to use -20 mV voltage pulses applied to the cell membrane from a holding potential of -80 mV .

When a voltage command step is applied to such a network, the recorded current response consists of two components (Fig. 1 *B*, trace *APS*): a transient current representing the charging of the capacitors (capacitive current), and a steady current representing the current flowing through the resistors after the capacitors have been charged (leakage current). By using standard methods of mathematical analysis applied to electrical circuits, it is possible to solve the equation to obtain the current response. The approach is the following: first, the network is solved in the frequency domain to obtain the current response as a function of the complex variable $s = j\omega$ [that is, $I(s)$], where j is the imaginary unit and ω is the angular frequency; then, the current response in the time domain [$I(t)$] is obtained by calculating the inverse Laplace transform of $I(s)$. The equation for the current response is the following:

$$I(t) = \frac{\Delta V}{b_2 R_a} \left\{ \left[\frac{b_2(a + a_1) - a_2(a + b_1)}{a - b} \right] e^{at} + \left[\frac{a_2(b + b_1) - b_2(b + a_1)}{a - b} \right] e^{bt} \right\} + \frac{a_2 \Delta V}{b_2 R_a} \quad (1)$$

where ΔV is the voltage step; a_1 , a_2 , b_1 , b_2 , a , and b are parameters the values of which are expressed by the following equations:

$$a_1 = \frac{2R_j + 2R_M}{R_M C_M R_j} \quad (2)$$

$$a_2 = \frac{R_j + 2R_M}{R_M^2 C_M^2 R_j} \quad (3)$$

$$b_1 = \frac{2R_a R_j + 2R_a R_M + R_j R_M}{R_M C_M R_a R_j} \quad (4)$$

$$b_2 = \frac{R_a R_j + R_M (2R_a + R_j + R_M)}{R_M^2 C_M^2 R_a R_j} \quad (5)$$

$$a, b = \frac{-b_1 \pm \sqrt{b_1^2 - 4b_2}}{2} \quad (6)$$

The first addend of Eq. 1 is a two exponential expression representing the transient component of the current response (capacitive current; a and b are negative values; $a = 1/\tau_1$, and $b = 1/\tau_2$, where τ_1 and τ_2 are the time constants), and the second addend of Eq. 1 is the steady component of the current response (leakage current). Fig. 1 *C* shows a log-plot of the current responses reported in Fig. 1 *B* so that the exponential components of the response can be appreciated.

The computer-assisted procedure used to evaluate the membrane capacitance of coupled cells during patch clamp recordings consisted in subtracting the steady state component from the total current response elicited by a voltage step to obtain the transient component of the current; then to integrate the transient current to obtain the electrical charge (Q) transferred to the membranes during the voltage step (ΔV); and finally to calculate C from $Q/\Delta V$. With the analytical approach, these steps yield the following equation for cell membrane capacitance (C_{pair}):

$$C_{\text{pair}} = \frac{1}{b_2 R_a} \left\{ \left[\frac{a_2 (a + b_1) - b_2 (a + a_1)}{a (a - b)} \right] + \left[\frac{b_2 (b + a_1) - a_2 (b + b_1)}{b (a - b)} \right] \right\} \quad (7)$$

By replacing each parameter in the Eq. 7 with the corresponding value (Eqs. 2–6), we obtain the expression for the membrane capacitance of coupled cells:

$$C_{\text{pair}} = \frac{R_M R_j^2 + 2R_M^2 R_j + 2R_M^3}{(R_a + R_M)^2 R_j^2 + 2R_M (R_a + R_M) (2R_a + R_M) R_j + R_M^2 (2R_a + R_M)^2} R_M C_M \quad (8)$$

The equation for the input resistance of coupled cells (R_{pair}), which is the total resistance of the network shown in Fig. 1 *A*, was obtained by dividing the voltage step

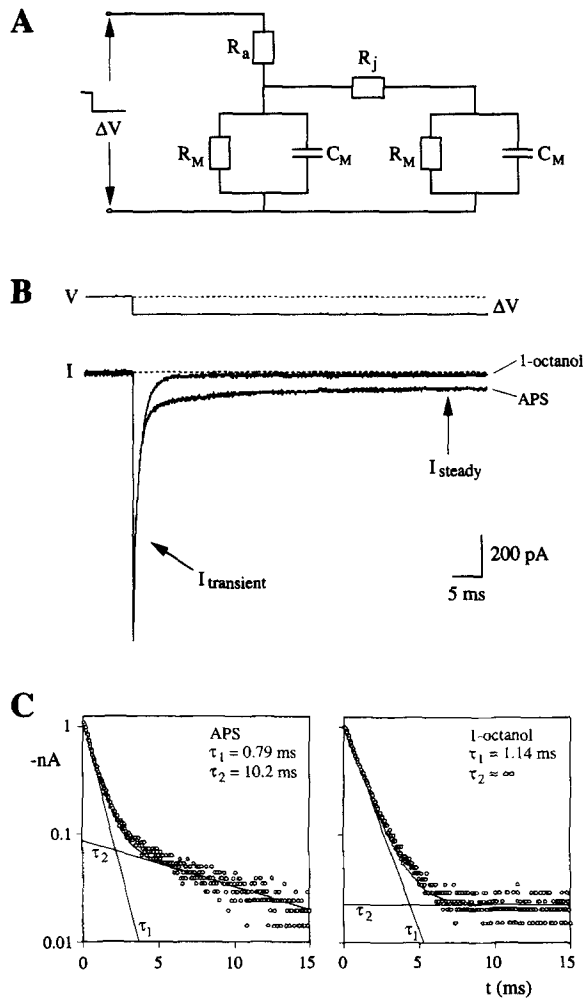


FIGURE 1. (A) Minimal equivalent circuit of two cells electrically coupled and of the tight-seal whole-cell configuration. The cell membrane of each cell is modeled as a parallel combination of the membrane capacitance (C_M) and membrane resistance (R_M). Membrane potential is ignored. R_a represents the resistance of the patch pipette. R_j represents the junctional resistance between the cells. (B) Typical transient and steady components of the whole-cell currents (I) elicited by a voltage step (ΔV) applied to an electrically coupled taste receptor cell. Note the presence of a slow phase in the relaxation of the current when the cell was bathed in APS (trace APS). This phase disappeared after applying 1 mM 1-octanol, a known blocker of electrical coupling (trace 1-octanol). Note also the reduction in the steady current during 1-octanol application. $V_h = -80$ mV; ΔV , -20 mV. (C) Log-plots of the current traces shown in B. The first 15 ms of the responses are shown here and the ordinate has been inverted (note, $-nA$) for clarity. In APS (left), two major exponential components can be observed.

The first component reflects the membrane charging of the patched cell, and the second component of the adjacent, coupled cell. Time constants were derived from the slopes of straight lines, fitted by computer. After uncoupling with 1-octanol (right), only one dominant exponential component remains, consistent with a large increase in τ_2 (i.e., $R_j \rightarrow \infty$). The curved lines plot the double exponential equations derived using the values for τ_1 and τ_2 given in the insets. After 1-octanol treatment, τ_1 is somewhat larger than when the cell was bathed with APS (1.14 vs 0.79 ms), presumably reflecting an increase in R_{in} of the patched cell. This is consistent with the effects of 1-octanol to reduce other ionic conductances in addition to blocking gap junctional conductance (see Fig. 3). Using the analysis based on comparing time constants (B. Lindemann, personal communication), described in footnote 1, R_j for this cell would be 216 M Ω ($R_a = 18$ M Ω).

(ΔV) by the leakage current (the second addend of the Eq. 1, and by replacing a_2 and b_2 with the corresponding values Eqs. 3 and 5):

$$R_{pair} = \frac{(R_a + R_M) R_j + R_M (2R_a + R_M)}{R_j + 2R_M} \quad (9)$$

R_M in Eqs. 8 and 9 is not readily available from the recordings. Therefore, it must be written as a function of experimentally obtained parameters, such as cell input resistance. The membrane resistance of a single cell can be expressed as:

$$R_M = \Delta V / I_{ss} - R_a \quad (10)$$

where ΔV is the voltage step, I_{ss} the steady state current elicited by the voltage step, and R_a the access resistance of the patch pipette tip (cf. Lindau and Neher, 1988). By definition, the term $\Delta V / I_{ss}$ represents the input resistance of the cell (R_{in}). Therefore:

$$R_M = R_{in} - R_a \quad (11)$$

Both R_{in} and R_a can be readily measured from the recordings. By replacing ($R_{in} - R_a$) for R_M in the Eqs. 8 and 9, we obtain the final expression for the membrane capacitance and input resistance of coupled cells :

$$C_{\text{pair}} = \frac{(R_{in} - R_a) R_j^2 + 2(R_{in} - R_a)^2 R_j + 2(R_{in} - R_a)^3}{R_{in}^2 R_j^2 + 2R_{in}(R_{in}^2 - R_a^2) R_j + (R_{in} - R_a)^2 (R_{in} + R_a)^2} (R_{in} - R_a) C_M \quad (12)$$

$$R_{\text{pair}} = \frac{R_{in} R_j + R_{in}^2 - R_a^2}{R_j + 2(R_{in} - R_a)} \quad (13)$$

Both these two equations allow one to evaluate the junctional resistance (R_j) between coupled cells from parameters that can be obtained experimentally.¹

Coupled Cells and Single Cells in Lingual Slices

The Eqs. 12 and 13 can be applied to real coupled cells only if membrane capacitance (C_M) and input resistance (R_{in}) of both cells in the pair are known. Although uncoupling the cells with agent such as 1-octanol or Na acetate yielded recordings from one cell (Bigiani and Roper, 1993, 1994), the adjacent cell was not readily available for measurements by using the single electrode patch clamp technique. Moreover, uncoupling treatments can alter the plasma membrane properties of the cells (see below). Therefore, we tested whether cells obtained from uncoupling coupled cells had the same membrane properties of single (noncoupled) cells. If so, then membrane properties measured from single cells could be used in Eqs. 12 and 13.

In *Necturus* taste buds, three main populations of receptor cells can be identified according to their morphological organization and electrophysiological properties

¹As indicated by Eq. 1, two time constants ($\tau_1 = 1/a$ and $\tau_2 = 1/b$) govern the circuit behavior in response to a voltage step. A simplified expression for R_j can be calculated from τ_1 and τ_2 (suggested by B. Lindemann, personal communication) if $R_a \ll R_m$, $R_a \ll R_j$, which is often the case in taste cells. In such circumstances, $\tau_1 \approx R_a C_m$ and $\tau_2 \approx (R_a + R_j) C_m$, and therefore, $R_j = R_a [(\tau_2/\tau_1) - 1]$.

(Bigiani and Roper, 1993): (a) single, noncoupled taste cells with voltage-gated Na and delayed-rectifier K currents (called group 1 cells); (b) single cells with A-like K currents only (called group 2 cells); (c) electrically coupled cells with voltage-gated Na and delayed rectifier K currents (called group 3 cells). Because both group 1 cells and coupled cells (group 3) possess the same set of voltage-gated ionic conductances in their plasma membrane, it is conceivable that they might also share other electrophysiological membrane properties. To test this possibility, we measured the membrane capacitance and the input resistance from group 3 coupled receptor cells and group 1 receptor cells in *Necturus* lingual slices. Further, we studied how these membrane parameters changed during application of known uncoupling agents. Lucifer yellow-filled pipettes allowed us to distinguish single (group 1) cells from coupled (group 3) ones after the whole-cell voltage clamp recording (cf. Bigiani and Roper, 1993). In APS, group 3 coupled receptor cells (pairs) had a membrane capacitance of 104.0 ± 11.9 pF (mean \pm SEM; $n = 5$) and an input resistance of 0.78 ± 0.42 G Ω . Single receptor cells (group 1) had a membrane capacitance of 63.4 ± 8.0 pF and an input resistance of 1.44 ± 0.32 G Ω ($n = 6$). These data were consistent with those reported previously (Bigiani and Roper, 1993, 1994).

For uncoupling agents we used 1-octanol (Johnston, Simon, and Ramón, 1980; Spray and Bennett, 1985) and Na acetate (Spray, Harris, and Bennett, 1981). Previous studies have shown that these chemicals effectively uncoupled receptor cells in *Necturus* taste buds (Bigiani and Roper, 1993, 1994). During uncoupling experiments, membrane capacitance and input resistance were monitored continuously before and during perfusion of 1-octanol or Na acetate, and final data points were measured after the effects of these agents had reached a steady state (cf., Bigiani and Roper, 1993, 1994). In Fig. 1 *B*, current transients obtained before (trace APS) and after 1-octanol (trace 1-octanol) are superimposed. Fig. 1 *C* provides a log-plot for the current responses shown in Fig. 1 *B*. The transient component of the responses clearly changes from a two-exponential time course to a single-exponential one, reflecting the decoupling. For coupled cells, a decrease ($39.4 \pm 7.3\%$, mean \pm SEM; $n = 5$) in membrane capacitance was observed during uncoupling treatments, whereas no significant differences could be detected for single (group 1) cells. The results of these experiments are summarized in Fig. 2.

Data from both 1-octanol (1 mM) and Na acetate (112 mM, replacing NaCl in APS) experiments were combined in this analysis because both these agents had the same effect on the membrane capacitance (Bigiani and Roper, 1993, 1994). Note that uncoupling agents reduced membrane capacitance of coupled cells to single cell values (t test: $P > 0.8$). The input resistance of coupled cells is a function of gap junction channels and of nonjunctional ion channels (Spray et al., 1981). Because 1-octanol and Na acetate could have different effects on nonjunctional ion channels, we compared data obtained by using the same uncoupling agent. Fig. 3 shows the results of experiments where 1-octanol (1 mM) was used.

As expected, this alcohol caused an increase in the input resistance of coupled cells as a consequence of closing junctional channels (cf., Fig. 1 *B*). However, 1-octanol also produced an increase in the input resistance in single cells, suggesting that the alcohol affected nonjunctional channels as well. The input resistances of cou-

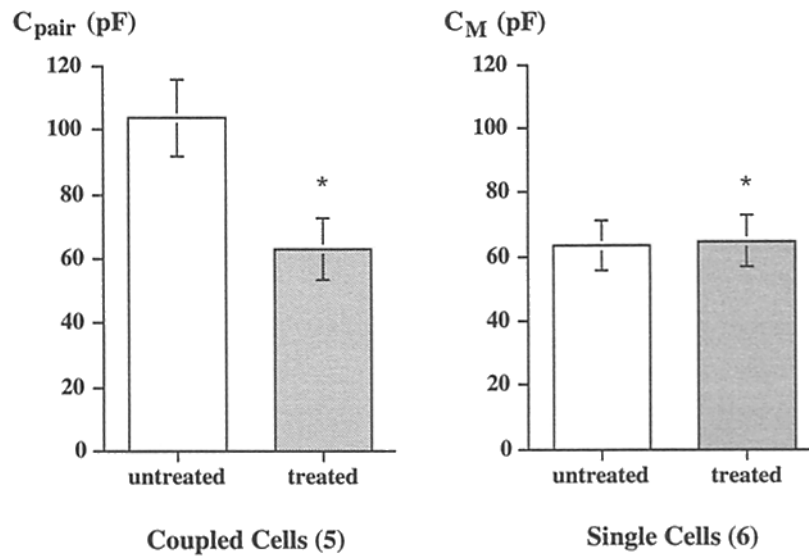


FIGURE 2. Effect of uncoupling agents (1-octanol, 1 mM, and Na acetate, 112 mM replacing NaCl) on membrane capacitance measured from coupled receptor cells (C_{pair}) and single (group 1) receptor cells (C_M) in *Necturus* taste buds. Bars represent mean values (\pm SEM). Number of cells is in parentheses. * Not statistically different ($P > 0.8$).

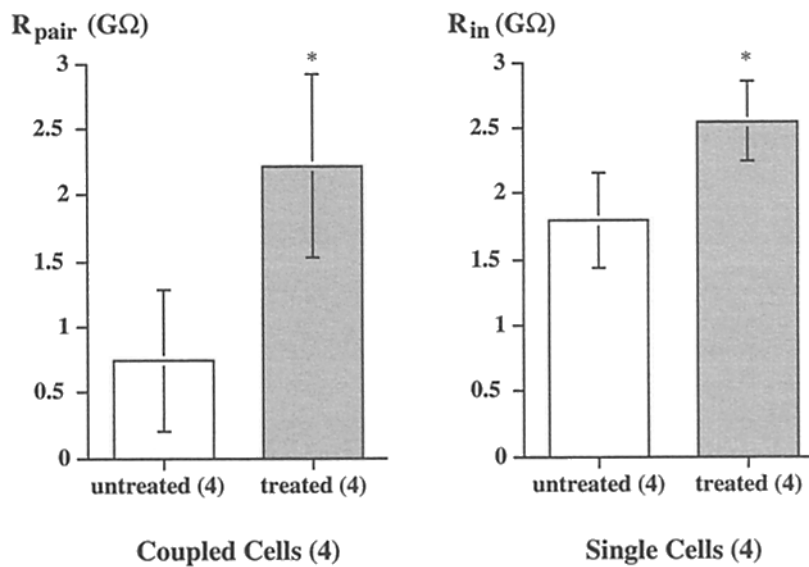


FIGURE 3. Effect of 1-octanol (1 mM) on the input resistance measured from coupled receptor cells (R_{pair}) and single (group 1) receptor cells (R_{in}) in *Necturus* taste buds. Bars represent mean values (\pm SEM). Number of cells is in parentheses. During the application of the uncoupling agents, measurements were taken after changes had reached a steady state. *Not statistically different ($P > 0.6$).

pled versus single cells did not differ significantly (t test: $P > 0.6$) after treatment with 1-octanol (mean values: $2.22 \text{ G}\Omega$ for coupled cells, and $2.55 \text{ G}\Omega$ for single cells).

Because application of uncoupling agents to group 3 coupled cells and to group 1 single cells resulted, on average, in the same membrane properties, it is reason-

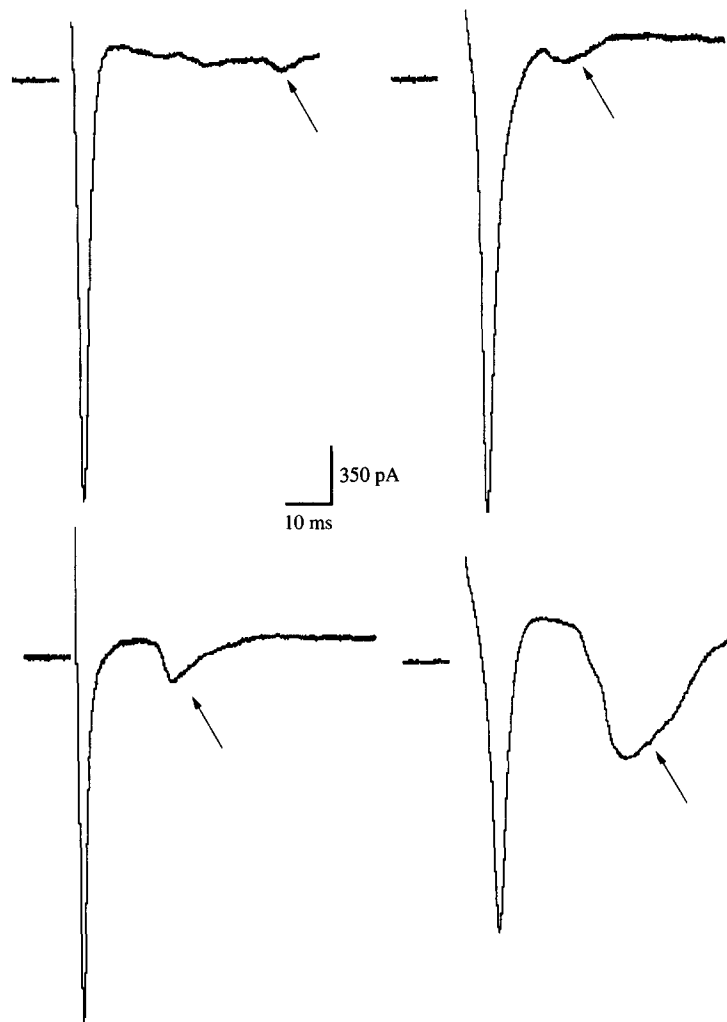


FIGURE 4. Whole-cell currents recorded from four different coupled taste receptor cells. Currents were elicited by stepping the membrane potential from -80 mV (holding potential) to -20 mV . Two transient inward currents are present, a large early one and a smaller, later one (*arrows*). Based on their amplitudes and delays with respect to the onset of the voltage step, it is likely that the first inward current is elicited in the cell under the patch electrode, whereas the second transient (*arrows*) derives from excitation in the adjacent cell. Note that in each record, the second, transient current is always smaller than the first one. Moreover, the second transient current shows a considerable variation from cell to cell, presumably reflecting differences in the junctional resistances among preparations.

able to consider each cell in a coupled set (group 3) as a group 1 receptor cell. That is, each cell in a set of coupled receptor cells has similar membrane properties (excluding junctional channels) as group 1 receptor cells. This now allows us to solve Eqs. 12 and 13.

Junctional Resistance

Based on the electrophysiological results described above, we have assumed that a pair of coupled receptor cells is formed by two group 1 receptor cells connected through a junctional resistance. In addition, both cells of the pair were assumed to have the same membrane capacitance and input resistance, corresponding to the average values measured from group 1 receptor cells in our experiments. If we use the average values for C_{pair} and R_{pair} measured in coupled taste receptor cells in APS (104.0 pF and 0.78 G Ω , respectively), the average values for C_M and R_{in} measured in single cells in APS (63.4 pF and 1.44 G Ω , respectively), and assign to $R_a = 0.012$ G Ω (the average value measured in our experimental conditions), we obtain a junctional resistance of 289 M Ω from Eq. 12 and 233 M Ω from Eq. 13. The average input resistance for single taste receptor cells was 1.44 G Ω . Thus, the junctional resistance was ~ 5 – 7 times lower than the input resistance of a single receptor cell.

The finding that taste receptor cells are coupled by a 200–300 M Ω junction now explains a curious finding that was occasionally observed when recording from coupled cells. Namely, when the membrane voltage was depolarized from -80 mV to -20 mV or above, small transient inward currents were sometimes superimposed on the traces, following the expected large inward (TTX-sensitive) Na current. Fig. 4 illustrates these transient currents. The traces in Fig. 4 show whole-cell currents recorded from different coupled receptor cells when the membrane voltage was stepped from -80 mV (holding potential) to -20 mV.

Two transient inward currents (both TTX sensitive; data not shown) are present in the records: the first large one represents the excitation of the patched cell. The second, smaller transient current represents the excitation of the adjacent, coupled cell (cf., Bigiani and Roper, 1993). The magnitude of this latter current is quite variable, presumably reflecting variations in junctional resistance and cell size in different experiments. It is worth noting that these currents closely resembled ones recorded from single taste cells with a nystatin-perforated patch method (Béhé, DeSimone, Avenet, and Lindemann, 1990): progressive permeabilization of the membrane patch with nystatin channels can be monitored by inward currents, the amplitude of which progressively increases as the access resistance of the pipette tip decreases (cf. Fig. 3 C in Béhé et al., 1990). In other excitable tissues, where the junctional resistance between coupled cells is lower, e.g., 1 M Ω , voltage-dependent ionic currents from adjacent cells do not show such a detectable delay and the recorded whole-cell currents are the sum of the currents from both coupled cells (e.g., Santos-Sacchi, 1991).

Delayed transient inward currents like those shown in Fig. 4 were never observed in single (type 1, noncoupled) taste receptor cells (Fig. 5).

According to this analysis, it is now possible to determine the changes in R_j that occur under different conditions, such as when the taste bud is exposed to agents

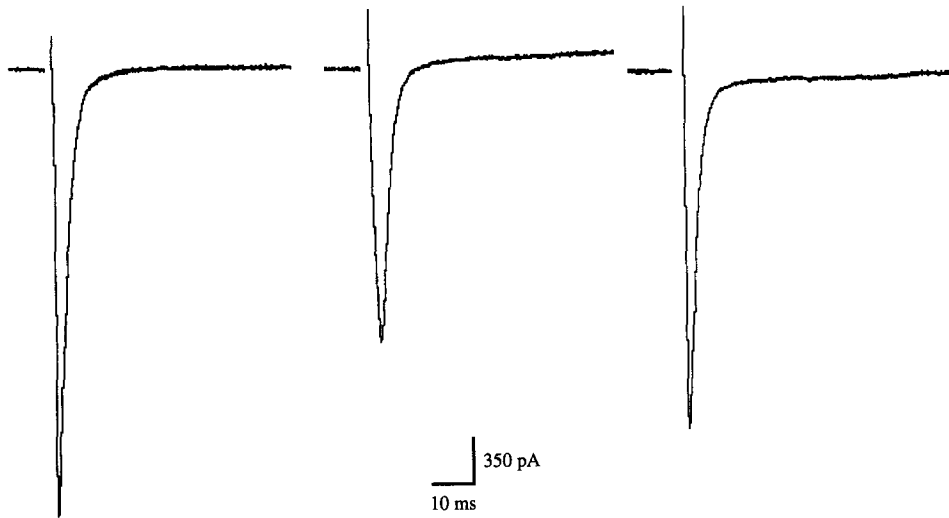


FIGURE 5. Whole-cell currents recorded from three different single taste receptor cells elicited by stepping the membrane potential from -80 mV (holding potential) to -20 mV. Only one transient inward current is present in these records, as expected from excitation of a single cell.

that affect electrical coupling. For example, an extreme case is when cells are bathed in 1-octanol. Fig. 6 shows the time course of decoupling (that is, $\uparrow R_j$) during bath application of 1 mM 1-octanol.

The Eqs. 12 and 13 also provide information about the variations of C_{pair} and R_{pair} as a function of R_j . Fig. 7 shows the results of a computer simulation where the

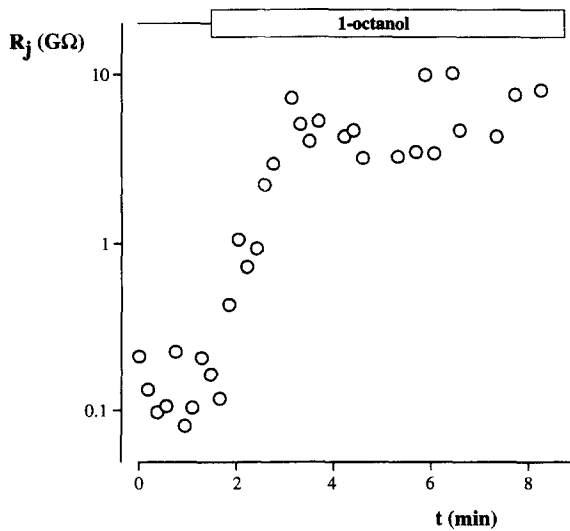


FIGURE 6. Increase in junctional resistance (R_j) when a pair of taste cells is decoupled by applying 1 mM 1-octanol. R_j was calculated according to Eq. 12 by monitoring the membrane capacitance of the coupled cells (C_{pair}). For this calculation, we have assumed that the two cells have the same membrane capacitance ($C_M \approx 70$ pF, that is, the steady state value obtained after 1-octanol application). For R_{in} in Eq. 12, we used the average value of R_{in} measured in single cells, that is, 1.44 G Ω . The pipette access resistance (R_{a}) in this experiment was ≈ 15 M Ω . Before 1-octanol application, R_j was ≈ 0.149 G Ω , and increased to ≈ 7 G Ω during uncoupling.

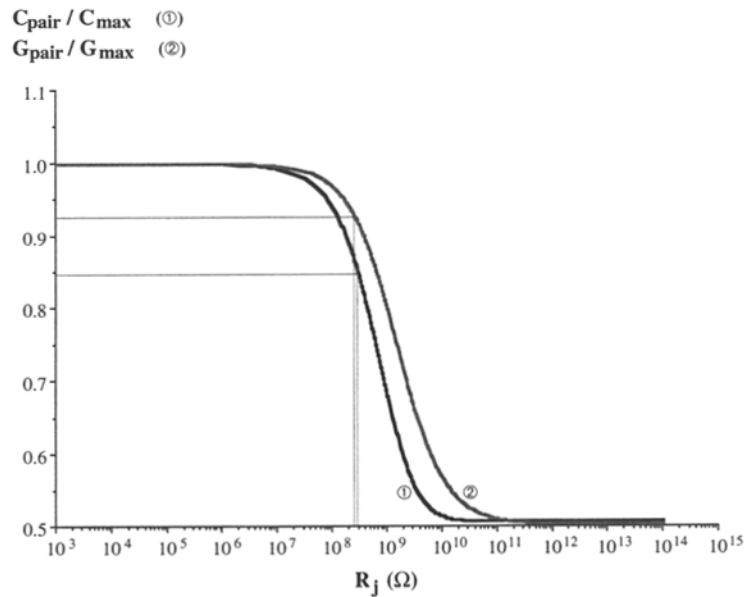


FIGURE 7. Voltage step analysis of the two coupled cell model. Normalized cell capacitance ($C_{\text{pair}}/C_{\text{max}}$; C_{max} = maximum value for C_{pair} obtained from Eq. 12 for $R_j = 0$) and normalized input conductance $G_{\text{pair}}/G_{\text{max}}$; $G_{\text{pair}} = 1/R_{\text{pair}}$; G_{max} = maximum value for G_{pair} obtained from Eq. 13 for $R_j = 0$) are plotted as functions of the junctional resistance (R_j). Graphs were obtained from Eqs. 12 and 13. In these equations, values for C_M and R_{in} are from single receptor cell data (63.4 pF and 1.44 G Ω , respectively), and pipette access resistance is 0.012 G Ω , the average value in our recordings. The conductance plot is shifted to the right of the capacitance plot, indicating that membrane capacitance is more sensitive to increases in R_j . Note also that the normalized membrane capacitance and input conductance of coupled taste receptor cells obtained from our experimental data (0.848 and 0.931, respectively; *horizontal lines*) both yield a junctional resistance between 200 and 300 M Ω (*vertical lines*).

junctional resistance was varied over twelve orders of magnitude, and the normalized membrane capacitance ($C_{\text{pair}}/C_{\text{max}}$) and normalized input conductance ($G_{\text{pair}}/G_{\text{max}}$) of coupled cells were plotted. In this analysis, we used the input conductance of coupled cells (defined as $1/R_{\text{pair}}$) instead of the input resistance. In addition, normalized values were used to allow a ready comparison of the plots.

It is interesting to note that the graph for the normalized input conductance is shifted to the right of the graph for the normalized membrane capacitance. For example, the midpoint in the capacitance plot is reached for $R_j \approx 0.6$ G Ω , whereas in the input conductance plot for $R_j \approx 1.5$ G Ω . Therefore, membrane capacitance represents a more sensitive parameter than input conductance (input resistance) in monitoring coupling conditions (cf. Santos-Sacchi, 1991; Bigiani and Roper, 1993, 1994). That is, changes in junctional resistance will first be reflected by changes in total capacitance before changes in total conductance are recorded. This is confirmed by experiments with 1-octanol or Na acetate, where variations in

measured membrane capacitance from coupled cells always preceded detectable variations in input resistance (see Santos-Sacchi, 1991; Bigiani and Roper, 1993, 1994).

DISCUSSION

Junctional Resistance between Coupled Taste Receptor Cells

In this study, we have been able to estimate the junctional resistance between coupled taste receptor cells in *Necturus* lingual slices by modeling the results from single patch pipette recordings. The data underscore the potential importance of certain basic physiological mechanisms in taste, namely cell-cell communication in the peripheral sensory organs and how this functional coupling might be modulated during taste transduction. The model was based on the following assumptions: (a) coupled taste receptor cells are comprised of two group 1 receptor cells (Bigiani and Roper, 1993) electrically coupled through a junctional resistance; (b) both cells in the coupled set have the same membrane properties, specifically the same membrane capacitance and resistance. The results of our electrophysiological experiments on both coupled (group 3) cells and group 1 (single) cells support the validity of the first assumption. Treating coupled receptor cells with uncoupling agents yielded uncoupled cells with membrane capacitance and input resistance values indistinguishable from those of single (group 1) receptor cells. Therefore, group 1 receptor cells and group 3 (coupled) receptor cells can be considered as belonging to a single population of taste cells as far as certain membrane properties are concerned. This conclusion is in agreement with earlier results, showing that coupled receptor cells and group 1 cells express the same set of voltage-gated ion channels (Bigiani and Roper, 1993; see also Yang and Roper, 1987). Although variations in membrane capacitance and input resistance are expected to occur between the two cells in a coupled pair, by using the average values for these membrane parameters measured from group 1 receptor cells, we fulfilled the second assumption of the model. In addition, dye-coupling studies have shown that taste cells in coupled pairs share similar size and shape (Teeter, 1985; Yang and Roper, 1987; Sata et al., 1992; Bigiani and Roper, 1993). The voltage step analysis of the two coupled cell model (Fig. 1) allowed us to obtain expressions for the membrane capacitance (C_{pair}) and the input resistance (R_{pair}) of the doublet as a function of junctional resistance (R_j), membrane capacitance (C_M), and input resistance (R_{in}) of single cells (Eqs. 12 and 13). Therefore, junctional resistance could be calculated by substituting these membrane parameters with the average values obtained from our recordings. We estimated a junctional resistance value of ~ 200 – $300 \text{ M}\Omega$. Because this value has been obtained by substituting the mean values of the relevant parameters, it should be considered as an estimation of the “average” junctional resistance between coupled receptor cells in taste buds in our experiments. Actual values of junctional resistance for specific pairs of coupled cells could not be evaluated in this study. As suggested by the recordings shown in Fig. 4, junctional resistance is expected to vary from pair to pair.

In our experiments, as well as in other studies (Teeter, 1985; Yang and Roper, 1987; Sata et al., 1992; Bigiani and Roper, 1993) dye-coupled cells always appeared

equally filled with the fluorescent dye, Lucifer yellow. This observation might suggest a strong cell-to-cell coupling. On the contrary, junctional resistance between taste cells in the *Necturus* was quite high. Lucifer yellow dye coupling is generally undetectable if the junctional conductance is below ~ 2 nS (Dermietzel and Spray, 1993), which corresponds to a junctional resistance > 500 M Ω . A value of 200–300 M Ω for the junctional resistance in our experiments is quite close to the threshold for Lucifer yellow detection. It is then possible that coupling between taste receptor cells might be more extensive than predicted on the basis of Lucifer yellow dye coupling observations. Coupling with very high junctional resistance could be still present in *Necturus* taste buds even during October to December period, when few, if any, dye-coupled cells are detected (Bigiani and Roper, 1993). Experiments with new intercellular tracers, such as Neurobiotin (which is at least 30% smaller than Lucifer yellow; e.g., Vaney, 1991), may reveal more extensive coupling among taste bud cells.

High junctional resistance is not a peculiarity of coupled taste receptor cells. Coupled cells from other tissues also present junctional resistances of the same order of magnitude or even higher (Table I).

It is interesting to note that the state of coupling (as inferred from junctional resistance value) between coupled taste receptor cells is very similar to that found in the retina between coupled rod cells (Table I).

Single-channel conductances of gap junctions vary in different tissues, ranging from 50 to 150 pS for various connexins (Bennett, Barrio, Bargiello, Spray, Hertzberg, and Sáez, 1991; Dermietzel and Spray, 1993). Although no data are available on the conductance of single gap junction channels as well as on the type of connexin(s) present in *Necturus* taste cells, our results suggest that the number of gap junctional channels should be ~ 30 – 50 , assuming a single-channel conductance of 100 pS. If a channel occupies ~ 100 nm² of the cell surface (Bennett et al., 1991), then 30–50 channels tightly packed should constitute a single plaque of only ~ 60 – 80 nm in diameter. This might explain the difficulty in identifying arrays of gap junction particles in electron microscopic observations, even though as many as 30% of taste cells in a taste bud may be coupled (e.g., Yang and Roper, 1987; Bigiani and Roper, 1993). In rat taste buds, where gap junctions have been reported, they formed maculae of irregular shapes in which particles were not evenly distributed nor tightly packed (Akisaka and Oda, 1978). The diameters of these zones were ~ 100 – 200 nm. This value is in approximate agreement with our estimation.

Possible Physiological Role(s) of Cell Coupling in Taste Buds

Electrical coupling occurs both in embryonic and adult tissues (Bennett and Spray, 1985). Because taste bud cells turn over (Farbman, 1980; Delay, Kinnamon, and Roper, 1986; Delay and Roper, 1989), it might be possible that coupling between taste cells plays some role in their development, for example by allowing the exchange of developmentally important information (Bennett and Spray, 1985). However, experimental observations are not consistent with this view. Coupled taste receptor cells are elongated cells that reach the taste pore, and possess voltage-activated ion channels such as TTX-sensitive Na and delayed rectifier K channels. In *Necturus*, these properties are not expressed by either the mitotically active taste

bud stem cells, which are thought to generate all the other taste cells (Delay and Roper, 1989; Delay, Mackay-Sim, and Roper, 1994) or by the developmentally immature receptor cells (Mackay-Sim, Delay, Kinnamon, and Roper, 1991). However, epithelial stem cells in the adjacent nontaste stratum basale in lingual epithelium from *Necturus* are coupled (Yang and Roper, 1987). In addition, coupled taste bud cells respond to chemical stimulation (West and Bernard, 1978; Sata et al., 1992). Furthermore, in immature tissues such as developing blastomeres, the junctional resistance is two orders of magnitude lower than in taste buds (Table I). Therefore,

TABLE I
Junctional Resistance (R_j) between Coupled Cells from Different Tissues in order of Increasing R_j , and the Effects of Physiological Modulators on this Resistance

Coupled cells	R_j , $M\Omega$	Modulator
Crayfish neurons ¹	0.05	
Guinea-pig Hensen cells ²	1	
Guinea-pig ventricular cells ³	~2	
Rat ventricular cells ⁴	1.7	
<i>Aplysia</i> L14 neurons in situ ⁵	2.5	
Axolotl blastomeres ⁶	5	
<i>Lymnaea</i> peptidergic neurons ⁷	31	Serotonin ($\uparrow R_j$)
<i>Aplysia</i> L14 neurons in culture ⁵	~33	
White perch horizontal cells ⁸	37	Dopamine ($\uparrow R_j$)
Rat astrocytes ⁹	~77	Noradrenaline ($\uparrow R_j$)
Catfish horizontal cells ¹⁰	100	Dopamine ($\uparrow R_j$)
<i>Necturus</i> taste receptor cells	200–300	
<i>Ambystoma</i> rod-rod ¹¹	300	
Rat glomus cells ¹²	833.6	Dopamine ($\uparrow R_j$); ACh ($\uparrow R_j$)
Rat hepatocytes ¹³	1000	Glucagon ($\downarrow R_j$)
<i>Ambystoma</i> rod-cone ¹¹	5000	

1, Johnston et al., 1980; 2, Santos-Sacchi, 1991; 3, Kameyama, 1983; 4, Weingart, 1986; 5, Bodmer et al., 1988; 6, Spray et al., 1981; 7, Wildering and Janse, 1992; 8, Lasater and Dowling, 1985; 9, Dermietzel, Hertzberg, Kessler, and Spray, 1991; 10, DeVries and Schwartz, 1989; 11, Attwell, Wilson, and Wu, 1984; 12, Monti-Bloch, Abudara, and Eyzaguirre, 1992, 1993; 13, Sáez, Spray, Nairn, Hertzberg, Greengard, and Bennett, 1986.

it is likely that electrical coupling in taste buds might be a feature of differentiated taste cells, that is, of mature receptor cells.

In excitable cells, electrical coupling may serve to synchronize activity or to relay signals rapidly from pre- to postsynaptic elements (for review, see Dermietzel and Spray, 1993). Taste receptor cells, including coupled ones, can generate action potentials if electrically or chemically stimulated (Roper, 1983; Kashiwayanagi, Miyake, and Kurihara, 1983; Avenet and Lindemann, 1987a, 1991; Yang and Roper, 1987; Sugimoto and Teeter, 1990; Gilbertson, Avenet, Kinnamon, and Roper, 1992). The relatively high junctional resistance between taste cells might suggest

that cell-to-cell communication is weak. However, the nonjunctional resistance of coupled cells plays an important role in the electrical communication. For single receptor cells, the input resistance easily exceeds 1 G Ω (1.44 G Ω in our experiments at a membrane voltage of -80 mV). The coupling coefficient, k , for two receptor cells connected through a junctional resistance of 200–300 M Ω would thus be 0.83–0.88.² Therefore, it is possible that some degree of synchronization should occur between active coupled receptor cells. For example, an action potential of 100 mV in one cell would produce (ignoring RC delays and decreases in R_M due to voltage-dependent conductance increases) a depolarization of ~ 80 –90 mV in the adjacent coupled cell, enough to exceed the threshold of action potential activation (e.g., Roper, 1983; Avenet and Lindemann, 1987a). That this actually occurs is shown by Fig. 4. Integration of subthreshold activity, such as receptor potentials, could also be mediated by electrical junctions between taste cells. In this case, the localization of junctional channels would be important in determining the degree of coupling. Because membrane responses to chemical stimuli are expected to occur at the apical membrane of taste receptor cells (Kinnamon and Cummings, 1992), electrical junctions situated near the apical tips would be more effective for signal integration than coupling near the basal ends of taste cells. Electron microscope studies should provide valuable information about this possibility.

It is worth noting that by using simultaneous intracellular recordings from two coupled receptor cells in *Necturus* taste buds, West and Bernard (1978) estimated an average coupling coefficient, k , of ~ 0.1 . This result may seem in disagreement with our calculation for the coupling coefficient (i.e., >0.8). However, if one considers the low input resistance West and Bernard measured for single cells (~ 24 M Ω), most likely caused by cell damage during microelectrode impalements, then a junctional resistance of 200–300 M Ω would yield $k \approx 0.1$, i.e., the value they reported. The same argument holds if the input resistance of one or both cells was to be reduced by physiological mechanisms (e.g., shunting during receptor potentials or neuromodulatory inputs) rather than by cell damage during microelectrode impalements. That is, functional coupling between taste cells will be reduced by any mechanism that decreases membrane resistance, R_M . Conversely, increasing R_M will enhance functional cell–cell coupling. This could have implications for signal processing during taste transduction since receptor potentials can be associated with increases or decreases in input resistance, depending on the specific chemical stimulus (e.g., Bigiani and Roper, 1991).

Another role that could be played by electrical coupling between taste receptor cells, even if the junctional resistance is high, is second-messenger exchange. The second messenger Ca²⁺, cyclic 3',5'-adenosine monophosphate (cAMP) and inositol 1,4,5-triphosphate (IP₃) all diffuse between coupled cells in other tissues (Lawrence, Beers, and Gilula, 1978; Sáez, Connor, Spray, and Bennett, 1989). Second messengers are implicated during taste transduction (Avenet and Lindemann, 1987b; Akabas, Dodd, and Al-Awqati, 1988; Tonosaki and Funakoshi, 1988; Avenet,

²The coupling coefficient, k , is expressed by $k = G_j / (G_j + G_{nj})$, where G_j is the junctional conductance ($G_j = 1/R_j$), and G_{nj} is the nonjunctional conductance of a single cell [$G_{nj} = 1/R_{nj} = 1/(R_{in} - R_a)$] (Dermietzel and Spray, 1993).

Hofmann, and Lindemann, 1988; Hwang, Verma, Bredt, and Snyder, 1990). Thus, it is possible that levels of these second messengers may be coregulated in coupled taste receptor cells. If this is the case, groups of coupled receptor cells could behave as a transduction unit.

Several studies indicate that the degree of coupling is not a static phenomenon, but is subject to modulation by numerous factors, including pH and neurotransmitters (Table I). Serotonin and dopamine, known modulators of electrical coupling in different tissues (Table I), are present in specific subsets of taste cells (Fujimoto, Ueda, and Kagawa, 1987; Delay, Taylor, and Roper, 1993; Kim and Roper, 1995; Kim and Roper, submitted for publication). It is thus tempting to speculate that electrical coupling between taste receptor cells could be regulated by these neurotransmitters. As shown by Fig. 7, the state of coupling in our experimental conditions is set in the steep region of the curve. Therefore, coupling would be readily modulated. Small changes in junctional resistance would produce large variations in cell-to-cell communication. Further investigations are needed to explore this possibility.

Square Wave and AC Analyses of Complex Circuits

The method we used to analyze electrically coupled taste cells, strictly speaking, is more appropriate for simple circuits such as a parallel RC circuit (e.g., Lindau and Neher, 1988). More complex circuits, such as the one shown in Fig. 1 A, are more accurately studied by using AC rather than square wave voltage steps (e.g., Moore and Christensen, 1985). This is because considerably more information about membrane impedance (and therefore, membrane electrical components) can be obtained by analyzing responses to sinusoidal currents over a wide range of frequencies (AC impedance analysis; Jack, Noble, and Tsien, 1983). The junctional resistance, R_j , and the membrane capacitances in Fig. 1 A will make current flow through the entire circuit dependent on the frequency of excitation. That is, the "effective" capacitance of the entire circuit will vary with the frequency of the applied current, in addition to the values of the resistive components, R_a , R_j , and R_M . For example, with high-frequency signals, the capacitance of the adjacent, coupled cell will not be fully charged. Square waveforms such as voltage steps contain components of all frequencies. Therefore, in theory the circuit of Fig. 1 A should require a more extensive mathematical description for evaluating circuit parameters than the simpler expression we used in this study (Eq. 8). Nonetheless, our approach, although not suitable for a more detailed characterization of coupled taste cells, yields a fairly good approximation of R_j . This is shown by the following observations: (a) the membrane capacitance for coupled cells was consistently larger than that for single cells (Fig. 2), indicating that during voltage steps of the duration we used, the membrane capacitance of adjacent, coupled cells is at least partially charged; and (b) values for R_j obtained from the two independent calculations, Eqs. 12 and 13, are very similar.

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