Identification of Electrophysiologically Distinct Cell Subpopulations in *Necturus* Taste Buds

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**ABSTRACT** We used the patch clamp technique to record from taste cells in thin transverse slices of lingual epithelium from *Necturus maculosus*. In this preparation, the epithelial polarity and the cellular organization of the taste buds, as well as the interrelationships among cells within the taste bud, were preserved. Whole-cell recording, combined with cell identification using Lucifer yellow, allowed us to identify distinct subpopulations of taste cells based on their electrophysiological properties. Receptor cells could be divided in two groups: one group was characterized by the presence of voltage-gated Na⁺, K⁺, and Ca²⁺ currents; the other group was characterized by the presence of K⁺ currents only. Therefore, receptor cells in the first group would be expected to be capable of generating action potentials, whereas receptor cells in the second group would not. Basal taste cells could also be divided into two different groups. Some basal cells possessed voltage-gated Na⁺, K⁺, and Ca²⁺ conductances, whereas other basal cells only had K⁺ conductance. In addition to single taste cells, we were able to identify electrically coupled taste cells. We monitored cell-cell coupling by measuring membrane capacitance and by observing Lucifer yellow dye coupling. Electrical coupling in pairs of dye-coupled taste receptor cells was strong, as indicated by experiments with the uncoupling agent 1-octanol. Electrically coupled receptor cells possessed voltage-gated currents, including Na⁺ and K⁺ currents. The electrophysiological differentiation among taste cells presumably is related to functional diversifications, such as different chemosensitivities.

**INTRODUCTION**

Taste receptor cells have been shown to be coupled via chemical and electrical synapses, raising the possibility that some degree of signal processing occurs in taste buds (Roper, 1992). Very little is known about the extent and functional significance of synaptic interactions in taste buds, especially electrical coupling. West and Bernard (1978) indicated that some cells in taste buds were electrically and dye coupled. Subsequent studies have extended and refined these initial reports. It is now well...
established that subsets of taste receptor cells are coupled in groups of approximately
two or three when examined with Lucifer yellow (Teeter, 1985; Yang and Roper,
1987; Sata and Sato, 1989). The physiological properties of these cells and how these
properties compare with those from adjacent, noncoupled taste cells have not been
elucidated.

This report is the first in a series on the functional characteristics of electrically
coupled and noncoupled taste cells in a new, semi-intact preparation of lingual
epithelium from *Necturus maculosus*. Taste bud cells are large in this species (Farbman
and Yonkers, 1971) and thus are amenable to detailed electrophysiological investiga-
tions, including patch recording. To date, patch clamp recording has been used to
study the electrical properties of isolated taste cells in *Necturus*, as well as other
species (*Ambystoma*: Sugimoto and Teeter, 1990; catfish: Teeter, Brand, and
Kumazawa, 1990; frog: Avenet and Lindemann, 1987a, 1988; Avenet, Hofmann, and
Lindeman, 1988; Miyamoto, Okada, and Sato, 1988; mouse: Spielman, Mody,
Brand, Whitney, MacDonald, and Salter, 1989; *Necturus*: Kinnamon, Cummings, and
Roper, 1988a; Kinnamon, Dionne, and Beam, 1988b; Kinnamon and Roper, 1988a;
Cummings and Kinnamon, 1992; rat: Akabas, Dodd, and Al-Awqati, 1988, 1990;
Herness, 1989; Behe, DeSimone, Avenet, and Lindemann, 1990). Recently, however,
it has been shown that patch clamp recordings can be successfully obtained in thin
slices of nervous tissue without the use of proteolytic enzymes and cell isolation
(Blanton, Lo Turco, and Kriegstein, 1989; Edwards, Konnerth, Sakmann, and
Takahashi, 1989; reviewed in Konnerth, 1990). This has introduced the possibility of
using patch recording of taste buds in slices of lingual epithelium to study synaptic
connections, particularly electrical synapses, with voltage clamp techniques.

Here we show that a slice preparation of *Necturus* lingual epithelium, used
previously for intracellular recording studies (Bigiani and Roper, 1991a; Ewald and
Roper, 1992a), is suitable for patch recording techniques without any enzymatic
treatment. In this preparation the epithelial polarity and the cellular organization of
the taste bud, as well as the interrelationships among cells within the taste bud, are
preserved. Therefore, taste cells can be studied electrophysiologically in a cellular
environment that more closely resembles that in vivo. We have identified coupled
cells and monitored electrical coupling with a single patch recording electrode by
measuring membrane capacitance (e.g., Santos-Sacchi, 1991). This report focuses on
the voltage-dependent ionic currents in electrically coupled and noncoupled taste
cells in *Necturus* in the lingual slice preparation. The findings show that there are
distinct subpopulations of cells within the taste bud that can be distinguished on the
basis of their electrophysiological properties.

Preliminary reports of part of this work have been presented in abstract form
(Bigiani and Roper, 1991b, c; Bigiani, Avenet, and Roper, 1992).

**MATERIALS AND METHODS**

**Tissue Preparation and Optical Setup**

Mudpuppies (*Necturus maculosus*) were obtained from commercial suppliers and maintained at
4–10°C in fresh water aquaria. They were fed minnows weekly.

The procedure to obtain lingual slices has been previously described (Bigiani and Roper,
Briefly, mudpuppies were anesthetized in ice water and rapidly decapitated. The tongue was removed and placed in cold (4–5°C) amphibian physiological solution (APS; see below). The lingual epithelium was gently freed by blunt dissection. The lingual tissue, with the epithelial surface oriented upward, was carefully fixed to a block of firm supporting material, such as carrot, with cyanoacrylic glue. Transverse slices (~200 μm thick) of the lingual epithelium were cut with a vibrating razor blade (a modified ladies’ Remington electric shaver mounted on a micromanipulator, as suggested to us by A. R. Cinelli and J. S. Kauer) while the tissue was kept moist with ice-chilled APS.

Slices of lingual tissue were scanned with a compound microscope at 50x. Sections containing taste buds were selected and pinned out in a shallow recording chamber (~1 ml vol) containing APS. The chamber was placed onto the stage of a fixed-stage upright Zeiss microscope equipped with a 40X water immersion objective (Nikon CF, working distance = 2.0 mm). When viewed this way (i.e., at 500x), entire taste buds with their cellular organization were clearly visible in the preparation (Fig. 1A).

During the experiments, the tissue was continuously perfused with APS (flow rate: ~3–5 ml/min) by means of a gravity-driven system.

Solutions

Our standard bathing solution (APS) consisted of 112 mM NaCl, 2 mM KCl, 8 mM CaCl₂, and 5 mM HEPES (buffered to pH 7.2 with NaOH). This solution has been used previously in electrophysiological experiments with Necturus taste cells to obtain stable and reliable intracellular and patch microelectrode recordings (Kinnamon and Roper, 1987; Kinnamon et al., 1988a, b; Kinnamon and Roper, 1988a, b; Roper and McBride, 1989; Bigiani and Roper, 1991a; McBride and Roper, 1991; Cummings and Kinnamon, 1992; Ewald and Roper, 1992a). 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 BAPTA). This solution has been used previously for patch clamp recordings from isolated Necturus taste cells (Kinnamon et al., 1988b; Kinnamon and Roper, 1988a; Cummings and Kinnamon, 1992). The osmolality of bathing and pipette solutions (248 ±4% mmol/kg) was checked before experiments. All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Gigaseal Whole-Cell Recording Technique

We obtained patch clamp recordings on taste cells in tissue slices using the whole-cell configuration of the patch clamp technique (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981).

Patch pipettes were fabricated from soda lime glass capillaries (microhematocrit tubes; Baxter Scientific Products, McGaw Park, IL) using a two-stage vertical micropipette puller (model PB-7; Narishige, Tokyo, Japan). When filled with the intracellular solution the patch pipette resistance was typically 2–10 MΩ.

Whole-cell currents were measured at room temperature (20–22°C) using an Axopatch-1A amplifier (Axon Instruments, Inc., Foster City, CA). Signals were prefiltered at 5 kHz and digitally recorded at 50-μs intervals with a Macintosh IIx computer equipped with a MacADIOS II data acquisition board (GWI Inc., Somerville, MA). Signals were displayed on the computer screen using SuperScope software (GWI Inc.). Leakage and capacitive currents were not subtracted from records unless otherwise indicated.
Procedure to Patch Clamp Taste Cells in Slices of Lingual Epithelium

To form high resistance (\(G \Omega\)) seals, the patch pipette must have an unimpeded access to the cell membrane (Hamill et al., 1981). Recent publications have described techniques to obtain \(G \Omega\) seals onto neurons in tissue slices without using proteolytic enzymes (Blanton et al., 1989; Edwards et al., 1989). These techniques, involving mechanical cleaning of the slice surface to expose the membrane of neurons or direct insertion of the pipette through the tissue to patch on neurons under the ependymal layer, are not applicable to slices of lingual epithelium. Lingual epithelium appears to be a more compact, tougher tissue compared with brain tissue. It was necessary to select slices in which taste buds were quite superficial and thus already partially exposed on the surface of the tissue. If necessary, we used a patch pipette, broken to a tip diameter of 20–50 \(\mu \text{m}\), to remove any debris by suction. Patch pipettes were manipulated under visual control at 500×.

After contacting the cell membrane, a high-resistance seal (> 2 \(G \Omega\)) was usually obtained by removing the positive pressure to the patch pipette or by applying a gentle suction to the patch pipette. Cells were left to stabilize for 1–3 min before collecting data. Stable whole-cell recordings could sometimes be maintained for up to 2 h while the tissue slice was continuously perfused with APS.

Cell Membrane Capacitance and Cell Input Resistance Measurement

Cell membrane capacitance and cell input resistance were calculated using a subroutine written by us. A similar procedure has also been used by Santos-Sacchi (1991) to study electrically coupled Hensen cells. Briefly, leakage and capacitive currents flowing through the membrane were measured when a 20-mV hyperpolarizing voltage pulse was applied to the patch pipette from the holding potential of \(-80\ \text{mV}\); the cell membrane capacitance was calculated by integrating the capacitive transient at the onset of the voltage pulse and dividing it by the amplitude of the voltage step. Cell input resistance was estimated by dividing the voltage step by the amplitude of the leakage current.

In our experimental recording chamber, stray capacitance resulting from immersion of the pipette in the bath solution could not be completely compensated electronically with the patch clamp amplifier. Consequently, membrane capacitance would be overestimated. Therefore, we evaluated the uncompensated stray capacitance while the recording electrode was still in the cell-attached configuration before breaking through the plasma membrane and subtracted this value from the capacitance obtained in the whole-cell configuration: \(C_{\text{cell}} = C_{\text{whole-cell}} - C_{\text{cell-attached}}\). Although it was possible to reduce stray capacitance by coating the pipette shank with Sylgard (Edwards et al., 1989) and by using electronic compensation, we found the above procedure more convenient and more reliable.

The access resistance 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 the whole-cell configuration; values typically ranged from 5 to 15 \(\text{M}\Omega\). Series resistance was not compensated during the recordings.

Identification of Cell Types by Dye Injection

We added Lucifer yellow CH (dipotassium salt; Sigma Chemical Co.) to the patch pipette solution (2 mg/ml; Edwards et al., 1989) so that cells would be filled during whole-cell recording and could then be identified at the end of experiments. This way, we could correlate the electrophysiological characterization with morphological identification of the cell under study. In this article we present data obtained only from cells that could be morphologically identified with confidence after being filled with Lucifer yellow dye. Incompletely filled cells, or ambiguously labeled cells were not included in the database. Lucifer yellow did not appear to
interfere with the physiological properties we investigated. Characteristics of voltage-dependent currents that we obtained were similar to those measured in the absence of the dye.

RESULTS

Slice Preparation and Cell Types

*Necturus* taste buds are ovoid structures 100–150 μm long and 90–120 μm wide, and containing ~100 taste cells (Farbman and Yonkers, 1971; Cummings, Delay, and Roper, 1987; Delay and Roper, 1988). By using thin sections of lingual epithelium, we have been able to obtain a preparation in which taste buds retain their cellular organization and taste cells can be approached by patch pipettes under direct visual control (Fig. 1 A). Since taste buds in mudpuppy are distributed over the entire surface of the tongue, sectioning the lingual epithelium at 150–200 μm thickness often yielded a high percentage of slices that contained taste buds in optimal condition for patch clamping, that is, with taste cells partially exposed on the slice.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Photomicrographs of living taste buds in slices of *Necturus* lingual epithelium. (A) Bright field observation of slices allows one to visualize whole taste buds and to resolve single taste cells. Patch pipettes can be positioned accurately under direct visual control. (B) Injecting the fluorescent dye, Lucifer yellow, allows one to identify taste cells in the slice after recording their electrophysiological properties. Arrowhead, taste pore. Calibration = 50 μm.
surface. Lingual slices lasted several hours in APS, and could be stored overnight at 5°C without any evidence of alteration.

Two main classes of cells occur in mudpuppy taste buds: receptor cells and basal cells (Farbman and Yonkers, 1971; Cummings et al., 1987; Delay and Roper, 1988, 1989; Taylor, Delay, and Roper, 1992). Receptor cells (dark cells and light cells) are elongated cells with processes extending from the base of the taste bud to the taste pore, whereas basal cells (stem cells and Merkel-like cells; Delay, Taylor, and Roper, 1993) are ovoid cells situated near the base of the taste bud. With careful observation it was possible to distinguish cellular outlines of different cells (see, for example, Fig. 1 A). However, it was not possible to distinguish cell types unambiguously in the living isolated slice preparations using bright field optics. We overcame this problem by filling cells with Lucifer yellow CH during patch clamp recording so that the shape and the extent of the cell studied could be examined at the end of the experiment with epifluorescence optics (Fig. 1 B). In addition to distinguishing receptor cells from basal cells, Lucifer yellow dye filling allowed us to identify coupled cells (e.g., Yang and Roper, 1987). Lucifer yellow loading proved to be very rapid. The dye diffused particularly well in the narrow processes of taste cells (Fig. 1 B).

We were able to distinguish four main categories of Lucifer yellow-filled taste cells according to their morphology and organization: receptor cells (single, elongated cells extending from the base toward the top of the taste bud); dye-coupled receptor cells (two adjacent, dye-filled receptor cells); basal cells (spherical cells lying at the base of the taste bud, without apical process); dye-coupled basal cells (two adjacent, dye-filled basal cells). It has not been possible to distinguish dark from light receptor cells (e.g., Delay and Roper, 1988), or stem from Merkel-like basal cells (e.g., Taylor et al., 1992; Delay et al., 1993). These distinctions were beyond the resolution of our experimental methods.

Whole-Cell Recording

Using the patch clamp technique, we were able to voltage clamp taste cells in slices of Necturus lingual epithelium and characterize their voltage-activated membrane currents. Membrane currents were elicited by voltage steps from a holding potential of $-80 \text{ mV}$, chosen as our standard reference potential so that membrane currents from different taste cells could be compared. Cell membrane capacitance and input resistance were also measured at a reference holding potential of $-80 \text{ mV}$. These parameters were evaluated immediately after the whole-cell recording configuration was established and recordings had stabilized. The presence of the dye Lucifer yellow in the patch pipette solution had no obvious effect on electrophysiological properties of taste cells, as indicated by preliminary experiments where Lucifer yellow was omitted from the patch pipette. All the recordings were performed by patching onto cell membranes located in the basal half of the taste buds, where most cell bodies are found (Farbman and Yonkers, 1971; Delay and Roper, 1988). Stable recordings were

1 We did not test whether chemical stimuli evoked receptor potentials in taste cells in this study. Consequently, we cannot state definitively that all elongated cells are receptor cells. On the other hand, it is highly likely that a fraction of these cells are receptor cells since elongated cells possess synapses and reach the taste pore (Cummings et al., 1987; Delay and Roper, 1988).
FIGURE 2. Whole-cell recording from a taste receptor cell in a lingual tissue slice. (A) Transient inward and sustained outward currents elicited by a series of depolarizing pulses between -70 and +50 mV, in 10-mV increments, from a holding potential of -80 mV. Pulse duration was 60 ms in this and in all following figures unless otherwise noted. Shown here are the first 50 ms. Capacitative and leakage currents were not subtracted from the records in this and in the following figures unless specifically noted. Bath perfusion: APS. (B) Current–voltage relationships for the records shown in A. Currents were measured at the peak for the transient inward current, and by averaging the final 5 ms of the trace for the outward current. (C) Whole-cell currents from the same cell after 3 min perfusion with 1 μM TTX. The transient inward current is blocked completely. Voltage protocol as in A. (D) Whole-cell currents from the same cell after 2 min perfusion with 10 mM TEA. The outward current is strongly reduced but not totally abolished. TEA reached its maximum effect in <60 s in the lingual slice preparation. Prolonged exposure to TEA did not completely eliminate the outward current. Note that the transient inward current reappeared in the recording because TTX was being washed out. Voltage protocol as in A. Same scale bars for A, C, and D.
obtained from 178 cells. In 75 of these it was possible to characterize their physiological properties and to identify cell type unambiguously with Lucifer yellow. The following data were collected from these 75 taste cells.

**Group I receptor cells.** Most taste bud cells (56 of 75) identified after recording were receptor cells (Fig. 1 B). In response to a series of depolarizing voltage steps from a holding potential of −80 mV, the majority of these cells (group 1, n = 40 of 56) displayed a transient inward current followed by a sustained outward current in regular APS (Fig. 2 A). The transient inward current activated at membrane potentials above −50 mV (Fig. 2 B). The inward current was blocked reversibly by 1 µM tetrodotoxin (TTX) in the bath, indicating that it was a Na⁺ current (Fig. 2 C). Outward currents activated at membrane potentials above −20 mV did not inactivate and increased almost linearly with depolarization (Fig. 2 B; see also Fig. 5 B). These currents were blocked reversibly by bath perfusion with 10 mM tetraethylammonium bromide (TEA), indicating that they were K⁺ currents (Fig. 2 D). All these properties.

**Figure 3.** Calcium currents and current–voltage relationship from taste receptor cell in the tissue slice perfused with 1 µM TTX, 10 mM TEA, and 20 mM BaCl₂. (A) Inward calcium (carried by Ba²⁺) currents elicited by a series of depolarizing pulses between −40 and +15 mV, in 5-mV increments, from a holding potential of −80 mV. Recordings were obtained after 8 min of bath application of the test solution. (B) Current–voltage relationship for the cell in A. Peak amplitudes of barium currents are plotted.
of the outward currents are typical of the delayed rectifier K⁺ current described in isolated *Necturus* taste cells (Kinnamon and Roper, 1988a). After correcting for linear leakage currents, the peak amplitude of Na⁺ currents obtained in one series of recordings was 3,397 ± 1,520 pA (or 57 ± 25 pA/pF when normalized for membrane capacitance; means ± SD; n = 17). The equivalent values for the delayed rectifying K⁺ currents, measured at +30 mV, were 1,369 ± 540 pA (or 23 ± 8 pA/pF; n = 17).

In addition to transient inward and sustained outward currents, this category of receptor cells (group 1) also possessed Ca²⁺ currents. Ca²⁺ currents were unambiguously shown by blocking Na⁺ and K⁺ currents with 1 μM TTX and 10 mM TEA, respectively, and by replacing Ca²⁺ with Ba²⁺ (e.g., Kinnamon and Roper, 1988a).
Fig. 3 shows typical recordings of \( \text{Ba}^{2+} \) currents during perfusion with TTX, TEA, and \( \text{BaCl}_2 \). All receptor cells in group 1 that were tested with this bathing solution \((n = 12)\) showed \( \text{Ba}^{2+} \) currents, although the amplitude of \( \text{Ba}^{2+} \) currents varied from cell to cell (mean \( \pm \) SD: 572 \( \pm \) 337 pA; or 9 \( \pm \) 5 pA/pF when normalized for cell capacitance; \( n = 10 \)).

**Group 2 receptor cells.** A second, smaller group of identified taste receptor cells (group 2, \( n = 14 \) of 56) showed distinctly different membrane current properties.

Whole-cell currents in APS consisted of an outward current without any transient inward currents (Fig. 4A). Perfusion with 10 mM TEA reduced the outward currents, indicating that they were \( \text{K}^+ \) currents, without unmasking any inward currents (Fig. 4D). Like the delayed rectifier \( \text{K}^+ \) currents described in group 1 receptor cells, outward \( \text{K}^+ \) currents in group 2 receptor cells activated at membrane potentials above \(-20 \text{ mV}\) (e.g., Fig. 4B). However, unlike the delayed rectifier currents of group 1 cells, \( \text{K}^+ \) currents in group 2 cells showed inactivation. Inactivation was more
evident with longer depolarizing pulses (e.g., 300 ms, Fig. 5 A). Inactivation of these currents was voltage dependent, as typifies A currents in molluscan neurons or Ambystoma taste receptor cells (Connor and Stevens, 1971; Sugimoto and Teeter, 1990). However, unlike A currents in these other cells, the inactivation of outward K+ currents in group 2 taste receptor cells was not totally removed by holding the cell membrane at potentials more depolarized than -80 mV (Fig. 6). The amplitudes of the inactivating K+ currents in these taste cells were significantly smaller than the delayed rectifier K+ currents in the other category of receptor cells (cf. Fig. 5), even when normalized for cell size (Table I).

In addition to the absence of a fast transient inward current, group 2 receptor cells did not possess any detectable Ca2+ currents, even when Ba2+ was substituted for Ca2+ (n = 6 cells). Fig. 7 compares recordings obtained from the two types of receptor cells in the presence of TTX, TEA, and Ba2+, showing unambiguous Ba2+ currents in the group 1 cell (Fig. 7 B) and their absence in the group 2 cell (Fig. 7 A).
Although Ca\textsuperscript{2+} currents in many cells are known to decline during whole-cell recording (Bean, 1992), the lack of Ba\textsuperscript{2+} currents in some mudpuppy taste receptor cells could not be explained by such rundown. For example, rundown of Ba\textsuperscript{2+} currents in group 1 receptor cells was observed only over a course of some tens of minutes. Second, in group 2 receptor cells, Ba\textsuperscript{2+} currents could not be elicited at any time after establishing the whole-cell configuration. Last, although the series resistance of the patch pipette can affect the magnitude of currents through voltage-gated channels (Marty and Neher, 1983), series resistance values were in the same range during recordings from both kinds of receptor cells.

The mean values of membrane capacitance, input resistance, and voltage-dependent currents of group 1 and 2 receptor cells are summarized in Table 1. The difference in input resistance between group 1 and group 2 receptor cells is due in part to the larger size (pF) of group 1 cells, but is also due to the presence of inward currents that were activated by stepping the membrane to more negative potentials from the holding potential of -80 mV. These inward currents were greatly reduced when the cells were bathed in TEA (10 mM) and BaCl\textsubscript{2} (20 mM), indicating that they were carried by potassium ions. These properties resemble those described for inward rectifier K\textsuperscript{+} currents in other tissues (Hille, 1992), including isolated Necturus receptor cells (Kinnamon and Roper, 1988a). Group 2 receptor cells, i.e., those without Na\textsuperscript{+} currents, did not show any detectable inward rectification.

**Electrically and dye-coupled receptor cells.** By using the fluorescent dye Lucifer yellow in the pipette solution, it was possible to reveal dye coupling between some receptor cells. We observed dye coupling between eight pairs of adjacent cells. Fig. 8 C shows an example of dye-coupled receptor cells. The membrane currents we
recorded from all coupled cells \((n = 8\) pairs\) were similar to those recorded from the group 1 taste receptor cells, above. That is, coupled receptor cells possessed a transient inward \(Na^+\) current, followed by a sustained outward \(K^+\) current (Fig. 8A). Thresholds for \(Na^+\) current activation ranged from \(-40\) to \(-30\) mV, and the peak amplitude was reached between \(-20\) and \(0\) mV (see, for example, Fig. 8B). Outward \(K^+\) currents did not inactivate.

Santos-Sacchi (1991) showed that membrane currents recorded from electrically coupled Hensen cells in the organ of Corti reflected the summation of voltage-dependent currents from the ensemble of coupled cells. We investigated this possibility in taste buds by measuring the \(Na^+\) and \(K^+\) currents recorded from coupled taste cells. The mean peak amplitude of \(Na^+\) currents was \(3,266 \pm 955\) pA (mean \(\pm\) SD; \(n = 7\)). The mean amplitude of \(K^+\) current (measured at \(+30\) mV) was \(1,327 \pm 655\) pA \((n = 7)\). These values did not differ significantly from comparable values from single receptor cells in group 1 \((P > 0.8\) for \(Na^+\) currents; \(P > 0.8\) for \(K^+\) currents\). Therefore, it seems likely that the main contribution to current measurements was derived from the membrane of the patched cell. However, a careful inspection of the current traces recorded from coupled cells occasionally revealed the presence of small deflections that occurred with a relatively constant latency and that were superimposed on the sustained outward current (Fig. 9A and B). The latency decreased when larger depolarizing voltage pulses were applied through the patch pipette (Fig. 9A and B). These observations suggest that the deflections represented excitation of the adjacent, coupled cell. This was not investigated in detail because of the negligible contribution of these transients to the whole current.

The electrophysiological properties we measured for coupled receptor cells were relatively uniform. Thus, based on considerations of probabilities alone, it is likely

| Passive membrane properties | Cell capacitance | Input resistance | Inward currents | Outward current |
|-----------------------------|------------------|------------------|----------------|----------------|
|                             | \(pF\)           | \(G_\Omega\)      | \(pA/pF\)      | \(pA/pF\)      | \(pA/pF\)      | Properties          |
| Group 1                     | 59 \(\pm 18\)\(^1\) | 1.0 \(\pm 0.7\)\(^1\) | 57 \(\pm 25\) | 9 \(\pm 5\)  | 23 \(\pm 8\)\(^2\) | Noninactivating    |
| \((51)\)                    | \((36)\)         | \((17)\)         | \((10)\)       | \((17)\)      |                  |
| Group 2                     | 43 \(\pm 11\)\(^1\) | 2.7 \(\pm 2.5\)\(^1\) | 0              | 0             | 12 \(\pm 7\)\(^1\) | Inactivating       |
| \((13)\)                    | \((13)\)         | \((14)\)         | \((6)\)        | \((12)\)      |                  |

Values are expressed as means \(\pm\) SD (number of cells in parentheses). Cell capacitance and input resistance were measured at a holding potential of \(-80\) mV as described in Materials and Methods. Voltage-dependent membrane currents were elicited by depolarizing the cells from the holding potential. Inward current amplitude was measured at the peak of the current trace. Outward current was measured at the end of a 60-ms voltage pulse to \(+50\) mV. Leakage current was subtracted from the membrane currents.

\(^1\)Significantly different \((P < 0.005)\).

\(^2\)Currents through Ca\(^{2+}\) channels were measured by using Ba\(^{2+}\) as the main charge carrier.
that both cells in a pair of coupled cells have identical properties. The mean values of passive membrane parameters for dye-coupled receptor cells (pairs) are summarized in Table II. On average, these cells showed a membrane capacitance significantly higher than the capacitance measured from noncoupled receptor cells, whereas the opposite can be noted with regard to the values of input resistance. These differences are understandable given that the capacitance includes a contribution from the
second, coupled cell, and more current is required to hold a pair of coupled cells at any given potential.

One important aspect of measuring the membrane capacitance and input resistance from cells in the slice preparation is that these parameters can serve as an effective on-line monitor of electrical coupling between cells in living tissue. For example, it should be possible to measure changes in capacitance and input resistance during application of agents known to block gap junctions and thus to decouple electrically coupled cells (Santos-Sacchi, 1991). Fig. 9 C demonstrates the effect of 1 mM 1-octanol (a gap junction blocker: Johnston, Simon, and Ramon, 1980; Spray and Bennett, 1985) on what were subsequently identified as dye-coupled receptor cells. 1-Octanol caused a decrease in membrane capacitance and an increase in the input resistance.
Uncoupling agents like 1-octanol are known to affect membrane channels other than gap junctions (Swenson and Narahashi, 1980; Hirche, 1985). We were able to confirm this in our experiments. 1-Octanol blocked the voltage-gated Na⁺ and K⁺ currents in coupled as well as in noncoupled taste cells (data not shown). However, 1-octanol had no effect on the membrane capacitance or the input resistance on taste cells that were subsequently identified with Lucifer yellow as single receptor cells (data not shown; cf. Bigiani and Roper, 1991c).

In two pairs of coupled receptors, the effects of 1-octanol reached a plateau and were stable over tens of seconds (e.g., Fig. 9 C). In these cells we measured a reduction in cell capacitance of 43 and 41%, respectively. If we use the mean reduction from these experiments (42%) to predict what the effects of 1-octanol might have been on all six pairs of coupled cells shown in Table II, we obtain capacitance values of ~55–65 pF. This matches well the capacitance measured for group 1 taste receptor cells.

We have been able to identify dye-coupled receptor cells only in experiments performed from late winter (~January) to summer (~July). During this period, the yield was 8 coupled cells out of 38 successful, stable recordings from receptor cells with complete Lucifer yellow identification, that is, ~21%. In contrast, at other times of the year (October–December) all receptor cells identified with Lucifer yellow were noncoupled (n = 26 stable recordings). This observation suggests that electrical coupling between taste receptor cells might present seasonal variations, at least in Necturus.

It is interesting to note that during uncoupling, the reduction in membrane capacitance seemed to precede the increase in input resistance. For example, in Fig. 9 C the half-time for the capacitance change was 44 s, whereas for input resistance change it was 110 s, and capacitance decreased to single cell values before resistance change was complete. This result suggests that membrane capacitance is a more sensitive indicator of the state of coupling than the input resistance, a conclusion also reached by Santos-Sacchi (1991).

### TABLE II

| Passive membrane properties | Inward currents | Outward current |
|-----------------------------|-----------------|-----------------|
| Cell capacitance (pF)       | Na⁺ (pA)        | K⁺ (pA)         |
| 102 ± 19                    | 3,266 ± 955     | 1,327 ± 655     |
| Input resistance (GΩ)       | Ba⁺⁺ (pA)       | Properties      |
| 0.4 ± 0.4                   | Not measured    | Noninactivating |

Values are expressed as the mean ± SD (number of cells in parentheses). Cell capacitance and input resistance were measured at a holding potential of ~80 mV as described in Materials and Methods. Voltage-dependent membrane currents were elicited by depolarizing the cells from the holding potential. Na⁺ currents were measured at their peak. K⁺ was measured at the end of a 60-ms voltage pulse to +30 mV. Leakage current was subtracted from the membrane currents. Na⁺ and K⁺ currents were not normalized to cell capacitance as in Table I since cell capacitance (column 1) includes a contribution from the electrically coupled adjacent cell.

*Currents through Ca²⁺ channels were measured by using Ba²⁺ as the main charge carrier.
Group 1 basal cells. After Lucifer yellow loading, basal cells could be identified in proximity to or at the base of the taste bud. These cells were ovoid and lacked apical processes that reached to the taste pore (Fig. 10 C). Whole-cell recordings revealed the presence of two electrophysiologically distinct types of basal cells in Necturus taste buds. In response to a series of depolarizing voltage steps from a holding potential of −80 mV, some basal cells (group 1, n = 5 of 9) showed a transient inward current followed by a sustained outward current in APS (Fig. 10 A), similar to those observed in group 1 receptor cells (cf. Fig. 2 A). TTX (1 μM) and TEA (10 mM) reversibly blocked the inward and outward currents, respectively, indicating that they were carried by Na⁺ and K⁺ ions. After subtracting linear leakage currents from the traces, the peak amplitude of Na⁺ currents was 3,142 ± 1,412 pA (mean ± SD; n = 5) and the amplitude of K⁺ current elicited by a voltage step to +30 mV was 833 ± 618 pA (n = 5). These values did not differ significantly from those found for group 1 receptor cells (P > 0.7 for Na⁺ currents; P > 0.07 for K⁺ currents). This group of basal cells also possessed Ba²⁺ currents (Fig. 10, D and E).

Group 2 basal cells. The second group of basal cells (group 2, n = 4 of 9) did not present any transient inward currents but only a TEA-sensitive outward current. This K⁺ current showed inactivation (Fig. 11 A), although less marked than the inactivation of K⁺ currents in group 2 receptor cells (cf. Fig. 5 A). Ba²⁺ currents were also absent in group 2 basal cells, as revealed by treating the tissue with our standard solution for unmasking currents through Ca²⁺ channels (Fig. 11, B–D).

Membrane capacitance and input resistance values of group 1 and 2 basal cells are summarized in Table III. Basal cells having Na⁺ currents (group 1 basal cells) on average had a higher membrane capacitance and a lower input resistance than basal cells lacking Na⁺ currents (group 2 basal cells). These differences were statistically significant (P < 0.02 for membrane capacitance; P < 0.045 for input resistance).

Dye-coupled basal cells. In our survey, we recorded membrane properties from one pair of dye-coupled basal cells. The membrane currents in this pair closely resembled those in group 2 basal cells. That is, a TEA-sensitive, outward current was recorded from the pair. This current showed inactivation over a period of 300 ms. There was no evidence for any inward Na⁺ or Ca²⁺ currents. Table III includes the values of membrane capacitance and input resistance for this pair of coupled basal cells.

Although dye-coupled non-taste epithelial cells have been described in Necturus lingual epithelium (Yang and Roper, 1987), the pair of cells we recorded were clearly within a taste bud and we assume, therefore, that they were basal taste cells. Furthermore, non-taste epithelial cells do not possess any voltage-activated currents (Kinnamon and Roper, 1988a; our unpublished observations). Due to the fact that we identified only one pair of coupled basal cells, it was not possible to study the properties of this type of cell in any detailed manner. It is possible that this recording represented basal cells undergoing mitosis.

Taste cells with intermediate properties. Table IV summarizes the membrane properties of the different types of taste cells that we have studied. Lucifer yellow staining combined with electrophysiological recordings has allowed us to identify
Figure 10. Whole-cell recordings from basal taste cells in lingual tissue slice. (A) Currents elicited in a group 1 basal cell by a series of depolarizing pulses between -70 and +60 mV, in 10-mV increments, from a holding potential of -80 mV. Bathing medium: APS. (B) Current-voltage relationships for the cell in A, measured as in Fig. 8 B. (C) Identification of the cell type with Lucifer yellow shows that the cell was a basal cell. Dashed line outlines the full extent of the intact taste bud in this slice. Arrow, taste pore. Calibration = 50 μm. (D) Currents elicited in another group 1 basal cell by a series of depolarizing pulses between -40 and +40 mV, in 10-mV increments, from a holding potential of -80 mV. The bathing medium contained 1 μM TTX, 10 mM TEA, and 20 mM BaCl₂ to reveal Ca²⁺ currents carried by Ba²⁺. (E) Current-voltage relationship for the cell in D. Peak amplitudes of barium currents are plotted.
FIGURE 11. Whole-cell recordings from a group 2 basal taste cell. (A) Currents elicited by a series of depolarizing pulses between −70 and +50 mV, in 10-mV increments, from a holding potential of −80 mV. Pulse duration was 300 ms. Bathing medium, APS. Note the absence of inward currents and the slight inactivation of the outward current. (B–D) Properties of the whole-cell currents and the current–voltage relationship from the same basal taste cell as shown in A. (B) Currents elicited in APS by a series of depolarizing pulses between −70 and +50 mV, in 10-mV increments, from a holding potential of −80 mV. Pulse duration: 60 ms. (C) Whole-cell currents from the same cell after perfusing 5 min with 10 mM TEA and 20 mM BaCl₂. The outward current is markedly reduced but not totally blocked. Note that the treatment with the test solution did not unmask any inward calcium (barium) currents. (D) Current–voltage relationship for the cell in A–D. Outward current amplitude (obtained by averaging the final 5 ms of the traces shown in B and C) as a function of membrane voltage ($V_m$) before (APS) and during addition of 10 mM TEA and 20 mM BaCl₂ to the bath.
distinctly different subtypes of taste cells. 96% of tested cells (72 of 75) could be unambiguously assigned to one of those categories. We found only three cells (two receptor cells, one basal cell) with membrane properties “intermediate” to those showed by the well-established subtypes. This suggests that grouping taste cells according to their electrophysiological properties is not merely an arbitrary division along a continuum.

**DISCUSSION**

In this study we have demonstrated the feasibility of patch clamp recording on taste cells maintained in situ by using transverse slices of *Necturus* lingual epithelium and have begun to determine the characteristics of electrically coupled and noncoupled taste cells. Our data have revealed several groups of physiologically distinct taste cells. Table IV summarizes these groups.

**Electrophysiological Subtypes: Receptor Cells**

Cells that were identified as receptor cells could be divided into two groups: one group was characterized by voltage-gated Na⁺, noninactivating K⁺, and Ca²⁺ currents. This group of receptor cells could be expected to be excitable and generate action potentials when depolarized, based on the presence of their inward Na⁺ and Ca²⁺ currents. Indeed, it has been shown that some taste receptor cells in *Necturus* generate action potentials when depolarized (Roper, 1983; Avenet and Lindemann, 1983).

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**TABLE III**

Comparison of Passive Membrane Properties and Voltage-dependent Inward and Outward Currents in Two Types of Basal Taste Cells and Electrically Coupled Basal Cells in *Necturus* Lingual Slices

| Passive membrane properties | Inward currents | Outward current |
|----------------------------|----------------|-----------------|
|                            | Na⁺ | Ba²⁺* | K⁺ | Properties        |
|                            | *pF | GΩ  | pA/pF | pA/pF | pA/pF |
| Group 1                    | 42 ± 5 | 0.8 ± 0.3 | 55 ± 11 | 5 | 11 ± 2 | Noninactivating |
| (3)                        |     | (3) |     | (1) | (3) |
| Group 2                    | 18 ± 9 | 3.4 ± 2.4 | 0 | 0 | 21 ± 9 | Inactivating |
| (3)                        |     | (3) |     | (3) | (3) |
| Dye coupled                | 27 | 3.4 | 0 | 0 | 351 pA | Inactivating |
| (1)                        |     | (1) |     | (1) | (1) |

Values are expressed as means ± SD (number of cells in parentheses). Data were collected and analyzed as in Table I.

*Currents through Ca²⁺ channels were measured by using Ba²⁺ as the main charge carrier.
*Statistically different (P < 0.02).
*Not statistically different (P > 0.140).
*K⁺ current was not normalized to cell capacitance as for non-coupled cells because cell capacitance here includes a contribution from the adjacent cell.
The other group of receptor cells was characterized by inactivating K+ currents only. These cells would not be expected to be excitable.

The presence of two distinct receptor populations in *Necturus* is consistent with findings in rat taste buds, where two populations of taste cells have been identified, one with a K+ current only and the other with both K+ and Na+ currents (Akabas et al., 1990; Behe et al., 1990). The existence of receptor cell subpopulations in *Necturus* taste buds may be explained in several ways. For example, electrophysiological differences may represent functionally distinct cellular subgroups, such as chemosensory cells vs. sustentacular cells. The existence of sustentacular cells in taste buds has been postulated by some researchers on morphological criteria (see, for example, Farbman, 1980). Others, however, on the basis of studies on cell turnover and the widespread occurrence of synapses on all classes of taste receptor cells, have concluded that sustentacular cells do not exist (for review, see Roper, 1989). Alternatively, the two groups may represent chemosensory cells that respond to different categories of taste stimuli such as sour, bitter, or salty (*Necturus* do not appear to respond to sweet stimuli: McPheeters and Roper, 1985). In this respect, it is interesting to note that, to date, studies on the chemical sensitivity of mudpuppy taste receptors have selectively investigated cells that possess voltage-dependent Na+ currents (Kinnamon et al., 1988b) or action potentials (Avenet and Lindemann, 1987b; Kinnamon and Roper, 1988b; Bigiani and Roper, 1991a), i.e., equivalent to group 1 receptor cells reported in this paper. The chemosensitivity of receptor cells possessing outward currents only (group 2 receptor cells) has not yet been systematically explored. One might speculate that the electrophysiologically distinct subpopulations correspond to dark and light taste cells (Murray, 1973). In the mudpuppy, dark cells represent the majority (~70%) of receptor cells in taste buds, whereas light cells represent a minority (~30%) (Farbman and Yonkers, 1971; Cummings et al., 1987; Delay and Roper, 1988). In our study, the ratio of group 1 to group 2 receptor cells was 3:1, closely matching the ratio of dark to light cells reported in *Necturus*.

| Table IV |
|---|
| **Summary of the Electrophysiologically Distinct Groups of Cells in Necturus Taste Buds** |

| Category   | Electrophysiology | C_{cell} | R_{m} | I_{Na} | I_{K} |
|------------|-------------------|----------|------|--------|------|
| Receptor   |                   |          |      |        |      |
| Group 1    |                   | 59       | 1.0  | +      | +    |
| Group 2    |                   | 43       | 2.7  | -      | -    |
| Coupled    |                   | 102      | 0.4  | +      | NM   |
| Basal      |                   |          |      |        |      |
| Group 1    |                   | 42       | 0.8  | +      | +    |
| Group 2    |                   | 18       | 3.4  | -      | -    |
| Coupled    |                   | 27       | 3.4  | -      | +    |

Data are abstracted and summarized from Tables I–III. For clarity, sample sizes and SD have been omitted in this table. These data are provided in the previous tables.

NM, not measured.
However, recent data suggest that dark cells and light cells alike have inward \( \text{Na}^+ \) currents in *Necturus* taste buds (McPheeters, Barber, Kinnamon, and Kinnamon, 1991). Lastly, the presence of a subset of receptor cells that lacks inward \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) currents (group 2 receptor cells) may correspond with developmentally immature receptor cells, as suggested by recent findings (Mackay-Sim, Delay, Kinnamon, and Roper, 1991). Until recordings are correlated with cell identification at the electron microscopic level, it will not be possible to identify the cell morphotype or state of maturation with confidence and the above interpretations remain speculation.

Although work in progress on isolated receptor cells has suggested that there are some electrophysiological differences among taste receptor cells in *Necturus* (McPheeters et al., 1991; Mackay-Sim et al., 1991), the presence of a group of taste receptor cells that possess only an inactivating \( \text{K}^+ \) current has not yet been described. Mackay-Sim et al. (1991) found that receptor cells that were believed to be immature (developing) in *Necturus* possessed voltage-dependent \( \text{K}^+ \) currents only. However, they did not note any inactivation of these currents, possibly because of the relatively brief (20–40 ms) test pulses used in that study or possibly due to changes caused by their cell isolation procedures.

Inactivating \( \text{K}^+ \) currents such as those we reported here have been first described in molluscan neurons and termed \( I_A \) by Connor and Stevens (1971). Thereafter, currents with properties similar to those of molluscan \( I_A \) have been described in a variety of cells, including *Ambystoma* and rat taste cells (Behe et al., 1990; Sugimoto and Teeter, 1990; for review, see Rudy, 1988). Variations in the steady-state inactivation of \( A \) currents (complete near \(-40 \text{ mV}\) in typical \( I_A \)) and in activation threshold (between \(-60 \) and \(-50 \text{ mV}\) in typical \( I_A \)) have also been reported (see, for example, Tables 3 and 4 in Rudy, 1988). Although the transient \( \text{K}^+ \) currents we observed in *Necturus* taste receptor cells did not seem to match the properties of typical \( A \) currents (for example, inactivation was not complete at \(-40 \text{ mV}\); the activation threshold was between \(-20 \) and \(+10 \text{ mV}\)), we have considered these responses to be an \( A \)-like current or the summation of an ensemble of channels, some having \( A \) properties and some having delayed rectifier properties, since they resemble transient \( \text{K}^+ \) currents described in other tissues (frog skeletal muscle: Nakajima, Iwasaki, and Obata, 1962; Adrian, Chandler, and Hodgkin, 1970; frog myelinated nerve: Schwarz and Vogel, 1971).

The lack of detectable \( \text{Ca}^{2+} \) currents in a subset of taste receptor cells (group 2 receptor cells) may suggest that these cells are not directly involved in chemical synaptic transmission, at least as classically described, i.e., with \( \text{Ca} \)-dependent transmitter release. However, studies have shown that in a subpopulation of rat taste receptor cells, denatonium (a bitter-tasting compound) induces \( \text{Ca}^{2+} \) release from intracellular stores (Akabas et al., 1988). This raises the possibility that synaptic transmission in taste cells could occur without activating voltage-dependent \( \text{Ca}^{2+} \) channels. Alternatively, cells lacking voltage-gated \( \text{Ca}^{2+} \) conductance could represent a class of cells that are not directly involved in chemosensory transduction, such as supporting or sustentacular cells (see above).
Electrophysiological differences among *Necturus* basal cells were mentioned by Kinnamon and Roper (1988a) in their study on isolated taste cells. Our results on tissue slices confirm and extend their findings by showing the existence of two distinct subpopulations of identified basal cells: one group displaying voltage-gated Na\(^+\), K\(^+\), and Ca\(^{2+}\) currents (group 1 basal cells) and the other one showing only a slightly inactivating K\(^+\) current (group 2 basal cells). This result indicates that some basal cells have the capacity to generate action potentials. It may be significant that the magnitude of Na\(^+\) currents in group 1 basal cells was similar to that in group 1 receptor cells. The presence of a voltage-activated Ca\(^{2+}\) conductance in group 1 basal cells indicates that some basal cells have the potential to be involved in synaptic transmission (as classically described).

The electrophysiological differentiation among basal cells, like that in receptor cells, presumably is related to a functional diversification. Recent studies have described the presence of two types of basal taste cells in *Necturus* taste buds: a stem basal cell and a Merkel-like basal cell (Delay and Roper, 1989; Taylor et al., 1992; Delay, R. J., R. Taylor, and S. D. Roper, manuscript submitted for publication). Stem basal cells are undifferentiated cells that undergo cellular divisions and are thought to generate the other cellular types in the taste bud. In contrast, Merkel-like basal cells are fully differentiated cells that are postulated to take part in synaptic interactions in the taste bud. Merkel-like basal cells contain serotonin and may release this monoamine as a neuromodulator of receptor cell function (Ewald and Roper, 1992b; Roper and Ewald, 1992; Taylor et al., 1992; Delay, R. J., R. Taylor, and S. D. Roper, manuscript submitted for publication). The functional characteristics of isolated stem basal cells and Merkel-like basal cells are currently under investigation (Mackay-Sim et al., 1991; Delay, Kinnamon, and Roper, 1992). Findings to date suggest that our group 1 basal cells correspond to Merkel-like cells, whereas group 2 basal cells correspond to stem cells. Again, detailed correlations of functional properties in the slice preparation with tracers visible at the electron microscopic level are needed to validate our conclusions.

**Electrical Coupling**

A number of investigators have shown dye-coupling between taste cells in different vertebrate species, including *Necturus* (catfish: Teeter, 1985; *Necturus*: West and Bernard, 1978; Yang and Roper, 1987; frog: Sata and Sato, 1989). Dye-coupling implies the existence of low-resistance current pathways between cells (electrical coupling). By using the slice preparation of mudpuppy lingual epithelium, we have been able to voltage clamp coupled cells in taste buds and to characterize their electrophysiological properties. Our data indicate that coupled receptor cells possess membrane conductances similar to those shown by the majority of noncoupled receptor cells (i.e., group 1 receptor cells), including fast inward Na\(^+\) current and delayed rectifier K\(^+\) current. The occurrence of voltage-activated currents in coupled cells is consistent with previous findings showing that these cells generate action potentials (Yang and Roper, 1987). No dye coupling was observed among taste receptor cells that possessed only inactivating K\(^+\) currents. Furthermore, no dye
coupling was observed between receptor and basal cells, consistent with previous reports.

Pairs of dye-coupled taste receptor cells in our experiments, as well as those of Yang and Roper (1987), usually appeared to be equally stained, suggesting that electrical coupling was strong. This was supported by measurements of the cell membrane capacitance. Dye-coupled cells had a much higher membrane capacitance than that obtained from noncoupled receptor cells (Tables I and II). Furthermore, bath application of the uncoupling agent 1-octanol produced a ~40% reduction in cell membrane capacitance from coupled cells.

Santos-Sacchi (1991) found that in tightly coupled supporting cells from the organ of Corti, membrane currents recorded with a patch electrode onto one cell represented the summation of voltage-gated currents from the ensemble of coupled cells. We were also able to detect voltage-dependent currents from the adjacent coupled cell, but these currents contributed only a negligible fraction of the total current. The reason for this small contribution from the coupled cell could be due to the inability to voltage clamp two large, elongated, electrically coupled receptor cells through (presumed) gap junctions (i.e., poor space clamp), and due to the resistance of such gap junctions to current flow.

In our study, 8 of 48 (~17%) taste receptor cells were coupled to other receptor cells. This finding is in good agreement with the incidence of dye-coupled receptor cells (20%) reported by Yang and Roper (1987). However, we noticed a seasonal variation in the incidence of dye coupling not reported previously. A possible explanation of the absence of coupling between receptor cells during October–December is that some receptor cells were only weakly electrically coupled during this period, and therefore were not detected by Lucifer yellow or by capacitance measurements. Recent studies have shown that biocytin and Neurobiotin may reveal more extensive coupling than the fluorescent dye, Lucifer yellow (Vaney, 1991). Experiments conducted with these intracellular tracers might provide further information on the full extent of coupling in Necturus taste buds, especially during October–December. Morphological studies have shown seasonal changes in gap junctions in different vertebrate tissues. Seasonal variations in endocrine function are believed to underlie these differences (Gregory and Tweedle, 1983; Pelletier, 1988). There are major biological changes that occur from season to season in Necturus, including mating, feeding, egg laying, and estivation (e.g., Pfingsten and White, 1989). It seems reasonable to speculate that electrical coupling in Necturus taste buds, as in other vertebrate tissues, could be under “humoral” control. In this respect, it is worth noting that other functions of taste buds, such as sensitivity to acid and salt stimuli, are strongly affected by hormones (Okada, Miyamoto, and Sato, 1990, 1991; Gilbertson, Roper, and Kinnamon, 1992). The functional significance of electrical coupling between some taste receptor cells remains to be elucidated.

**Lingual Slice Preparation vs. Taste Cell Isolation**

Despite the several advantages of the slice preparation (see below), it should be noted that there are certain limitations. Voltage clamp recordings from large, irregularly shaped cells, such as Necturus receptor cells, inevitably introduce the problem of inadequate space clamping. This is particularly true in slices where the cellular
structure and plasma membrane specializations remain intact and therefore are more complex than in isolated cells where cells tend to round up with time. Furthermore, cells can be electrically coupled, therefore introducing other factors affecting space clamp, such as intercellular junctional resistance and the larger cytoplasmic volumes. Lastly, although taste cells in the lingual slice are relatively accessible for patch clamp recording, we have noted that it is easier to obtain seals from cleanly isolated taste cells. In some respects, it is possible to obtain certain data quicker on isolated cells, with the important caveats outlined below.

Nonetheless, the lingual slice preparation presents important advantages over isolated taste cells, namely: (a) no enzymatic treatments are used; (b) the polarity and cellular organization of taste buds are maintained; (c) synaptic interrelationships among cells within the taste bud are preserved; and (d) once a seal is obtained, long-duration stable recordings can be obtained during isolation procedure (Akabas et al., 1990), and even entire taste buds can be disconnected from the surrounding tissue (Behe et al., 1990), those methods still use enzymatic treatments and this may compromise certain physiological properties. For example, trypsin is known to damage amiloride-sensitive Na⁺ channels in some tissues (Garty and Edelman, 1983). Indeed, Akabas et al. (1990) considered the possibility that these channels were disrupted by trypsin during the dissociation procedure to explain their lack in rat taste receptor cells. We believe that the lingual slice preparation is an important new addition to the battery of techniques being used to study peripheral taste mechanisms.

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REFERENCES

Adrian, R. H., W. K. Chandler, and A. L. Hodgkin. 1970. Voltage clamp experiments in striated muscle fibers. Journal of Physiology. 208:607-644.

Akabas, M. H., J. Dodd, and Q. Al-Awqati. 1988. A bitter substance induces a rise in intracellular calcium in a subpopulation of rat taste cells. Science. 242:1047-1050.

Akabas, M. H., J. Dodd, and Q. Al-Awqati. 1990. Identification of electrophysiologically distinct subpopulations of rat taste cells. Journal of Membrane Biology. 114:71-78.

Avenet, P., F. Hofmann, and B. Lindemann. 1988. Transduction in taste receptor cells requires cAMP-dependent protein kinase. Nature. 331:351-354.

Avenet, P., and B. Lindemann. 1987a. Patch-clamp study of isolated taste receptor cells of the frog. Journal of Membrane Biology. 97:225-240.

Avenet, P., and B. Lindemann. 1987b. Action potentials in epithelial taste receptor cells induced by mucosal calcium. Journal of Membrane Biology. 95:265-269.

Avenet, P., and B. Lindemann. 1988. Amiloride-blockable sodium currents in isolated taste receptor cells. Journal of Membrane Biology. 105:245-255.

Bean, B. P. 1992. Whole-cell recording of calcium channel currents. Methods in Enzymology. 207:181-193.
Behe, P., J. A. DeSimone, P. Avenet, and B. Lindemann. 1990. Membrane currents in taste cells of the rat fungiform papilla. *Journal of General Physiology.* 96:1061–1084.

Bigiani, A., P. Avenet, and S. D. Roper. 1992. Electrical coupling between cells in the taste buds of *Necturus maculosus.* *Pflügers Archiv.* 420:R157. (Abstr.)

Bigiani, A. R., and S. D. Roper. 1991a. Mediation of responses to calcium in taste cells by modulation of a potassium conductance. *Science.* 252:126–128.

Bigiani, A., and S. Roper. 1991b. Patch clamp recordings from cells in intact taste buds in thin lingual slices. *Chemical Senses.* 16:501–502. (Abstr.)

Bigiani, A., and S. Roper. 1991c. Membrane capacitance measures electrical coupling between taste cells. *Society for Neuroscience Abstracts.* 17:1216. (Abstr.)

Blanton, M. G., J. J. Lo Turco, and A. R. Kriegstein. 1989. Whole cell recording from neurons in slices of reptilian and mammalian cerebral cortex. *Journal of Neuroscience Methods.* 30:203–210.

Connor, J. A., and C. F. Stevens. 1971. Voltage clamp studies of a transient outward membrane current in gastropod neural somata. *Journal of Physiology.* 213:21–30.

Cummings, T. A., R. J. Delay, and S. D. Roper. 1987. Ultrastructure of apical specializations of taste cells in the mudpuppy, *Necturus maculosus.* *Journal of Comparative Neurology.* 261:604–615.

Cummings, T. A., and S. C. Kinnamon. 1992. Apical K⁺ channels in *Necturus* taste cells. *Journal of General Physiology.* 99:591–613.

Delay, R. J., S. G. Kinnamon, and S. D. Roper. 1992. Membrane properties and transmitter sensitivity of Merkel-like basal cells and taste cells in *Necturus* taste buds. *Chemical Senses.* 17:610. (Abstr.)

Delay, R. J., and S. D. Roper. 1988. Ultrastructure of taste cells and synapses in the mudpuppy *Necturus maculosus.* *Journal of Comparative Neurology.* 277:268–280.

Delay, R. J., and S. D. Roper. 1989. Cell lineage in the mudpuppy, *Necturus maculosus.* *Chemical Senses.* 14:694. (Abstr.)

Delay, R. J., R. Taylor, and S. D. Roper. 1993. Merkel-like basal cells in *Necturus* taste buds contain serotonin. *Journal of Comparative Neurology.* In press.

Edwards, F. A., A. Konnerth, B. Sakmann, and T. Takahashi. 1989. A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. *Pflügers Archiv.* 414:600–612.

Ewald, D. A., and S. D. Roper. 1992a. Intercellular signaling in *Necturus* taste buds: chemical excitation of receptor cells elicits responses in basal cells. *Journal of Neurophysiology.* 67:1316–1324.

Ewald, D. A., and S. D. Roper. 1992b. Basal cells modulate receptor cell function in *Necturus* taste buds by a serotoninergic mechanism. *Society for Neuroscience Abstracts.* 18:596. (Abstr.)

Farbman, A. I. 1980. Renewal of taste bud cells in rat circumvallate papillae. *Cell and Tissue Kinetics.* 13:349–357.

Farbman, A. I., and J. D. Yonkers. 1971. Fine structure of the taste bud in the mud puppy, *Necturus maculosus.* *American Journal of Anatomy.* 131:353–370.

Garty, H., and I. S. Edelman. 1983. Amiloride-sensitive trypsinization of apical sodium channels. Analysis of hormonal regulation of sodium transport in toad bladder. *Journal of General Physiology.* 81:785–803.

Gilbertson, T. A., S. D. Roper, and S. C. Kinnamon. 1992. Effects of acid stimuli on isolated hamster taste cells. *Society for Neuroscience Abstracts.* 18:844. (Abstr.)

Gregory, W. A., and C. D. Tweedle. 1983. Maturation of the goldfish preoptic area: ultrastructural correlates of position and maturation of neurosecretory cells. *Neuroscience.* 10:425–448.

Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv.* 391:85–100.
Herness, M. S. 1989. A dissociation procedure for mammalian taste cells. Neuroscience Letters. 106:60–64.

Hille, B. 1992. Ionic Channels of Excitable Membranes. Sinauer Associates, Inc., Sunderland, MA. 607 pp.

Hirche, G. 1985. Blocking and modifying actions of octanol on Na channels in frog myelinated nerve. Pflügers Archiv. 405:180–187.

Johnston, M. F., S. A. Simon, and F. Ramon. 1980. Interaction of anaesthetics with electrical synapses. Nature. 286:498–500.

Kinnamon, S. C., T. A. Cummings, and S. D. Roper. 1988a. Isolation of single taste cells from lingual epithelium. Chemical Senses. 13:355–366.

Kinnamon, S. C., V. E. Dionne, and K. G. Beam. 1988. Apical localization of K+ channels in taste cells provides the basis for sour taste transduction. Proceedings of the National Academy of Sciences, USA. 85:7023–7027.

Kinnamon, S. C., and S. D. Roper. 1987. Passive and active membrane properties of mudpuppy taste receptor cells. Journal of Physiology. 383:601–614.

Kinnamon, S. C., and S. D. Roper. 1988a. Membrane properties of isolated mudpuppy taste cells. Journal of General Physiology. 91:351–371.

Kinnamon, S. C., and S. D. Roper. 1988b. Evidence for a role of voltage-sensitive apical K+ channels in sour and salt taste transduction. Chemical Senses. 13:115–121.

Konnerth, A. 1990. Patch-clamping in slices of mammalian CNS. Trends in Neurosciences. 13:321–323.

Mackay-Sim, A., R. Delay, S. Kinnamon, and S. D. Roper. 1991. Voltage-dependent currents in developing and mature taste cells in mudpuppy (Necturus maculosus). Society for Neuroscience Abstracts. 17:230. (Abstr.)

Marty, A., and E. Neher. 1983. Tight-seal whole cell recording. In Single-Channel Recording. B. Sakmann and E. Neher, editors. Plenum Publishing Corp., New York. 107–122.

McBride, D. W., Jr., and S. D. Roper. 1991. Ca2+-dependent chloride conductance in Necturus taste cells. Journal of Membrane Biology. 124:85–93.

McPheaters, M., A. J. Barber, J. C. Kinnamon, and S. C. Kinnamon. 1991. Electrophysiological properties of light and dark cells isolated from mudpuppy taste buds. Society for Neuroscience Abstracts. 17:118. (Abstr.)

McPheaters, M., and S. D. Roper. 1985. Amiloride does not block taste transduction in the mudpuppy, Necturus maculosus. Chemical Senses. 10:341–352.

Miyamoto, T., Y. Okada, and T. Sato. 1988. Membrane properties of isolated frog taste cells: three types of responsivity to electrical stimulation. Brain Research. 449:369–372.

Murray, R. G. 1973. The ultrastructure of taste buds. In The Ultrastructure of Sensory Organs. I. Friedmann, editor. Elsevier Science Publishing Co., Inc., New York. 1–81.

Nakajima, S., S. Iwasaki, and K. Obata. 1962. Delayed rectification and anomalous rectification in frog’s skeletal muscle membrane. Journal of General Physiology. 46:97–115.

Okada, Y., T. Miyamoto, and T. Sato. 1990. Aldosterone increases gustatory neural response to NaCl in frog. Comparative Biochemistry and Physiology. 97A:535–536.

Okada, Y., T. Miyamoto, and T. Sato. 1991. Vasopressin increases gustatory neural responses elicited by NaCl and HCl. Comparative Biochemistry and Physiology. 100A:693–696.

Pelletier, R.-M. 1988. Cyclic modulation of sertoli cell junction complexes in a seasonal breeder: the mink (Mustela vison). American Journal of Anatomy. 183:68–102.

Pfingsten, R. A., and A. M. White. 1989. Necturus maculosus (Rafinesque): mudpuppy. In Salamanders of Ohio. R. A. Pfingsten and F. L. Downs, editors. College of Biological Sciences, The Ohio State University, Columbus, OH. 72–78.

Roper, S. D. 1983. Regenerative impulses in taste cells. Science. 220:1311–1312.
Roper, S. D. 1989. The cell biology of vertebrate taste receptors. *Annual Review of Neuroscience.* 12:329–353.

Roper, S. D. 1992. The microphysiology of peripheral taste organs. *Journal of Neuroscience.* 12:1127–1134.

Roper, S. D., and D. A. Ewald. 1992. Peripheral events in taste transduction. In *Sensory Transduction.* D. P. Corey and S. D. Roper, editors. The Rockefeller University Press, New York. 282–289.

Roper, S. D., and D. W. McBride, Jr. 1989. Distribution of ion channels on taste cells and its relationship to chemosensory transduction. *Journal of Membrane Biology.* 109:29–39.

Rudy, B. 1988. Diversity and ubiquity of K channels. *Neuroscience.* 25:729–749.

Santos-Sacchi, J. 1991. Isolated supporting cells from the organ of Corti: some whole cell electrical characteristics and estimates of gap junctional conductance. *Hearing Research.* 52:89–98.

Sata, O., and T. Sato. 1989. Dye-coupling among cells in taste disk of frog. *Chemical Senses.* 14:316. (Abstr.)

Schwarz, J. R., and W. Vogel. 1971. Potassium inactivation in single myelinated nerve fibers of *Xenopus laevis.* *Pflügers Archiv.* 330:61–73.

Spielman, A. I., I. Mody, J. G. Brand, G. Whitney, J. F. MacDonald, and M. W. Salter. 1989. A method for isolating and patch-clamping single mammalian taste receptor cells. *Brain Research.* 503:326–329.

Spray, D. C., and M. V. L. Bennett. 1985. Physiology and pharmacology of gap junctions. *Annual Review of Physiology.* 47:281–303.

Sugimoto, K., and J. H. Teeter. 1990. Voltage-dependent ionic currents in taste receptor cells of the larval tiger salamander. *Journal of General Physiology.* 96:809–834.

Swenson, R. P., and T. Narahashi. 1980. Block of sodium conductance by n-octanol in crayfish giant axons. *Biochimica et Biophysica Acta.* 603:228–236.

Taylor, R., R. Delay, and S. Roper. 1992. Are basal cells in taste buds identical to cutaneous Merkel cells? *Chemical Senses.* 17:706. (Abstr.)

Teeter, J. 1985. Dye-coupling in catfish taste buds. In Proceedings of the 19th Japanese Symposium on Taste and Smell. S. Kimura and I. Shimada, editors. Asahi University Press, Hozumi-cho, Gifu, Japan. 29–33.

Teeter, J. H., J. G. Brand, and T. Kumazawa. 1990. A stimulus-activated conductance in isolated taste epithelial membranes. *Biophysical Journal.* 58:253–259.

Vaney, D. I. 1991. Many diverse types of retinal neurons show tracer coupling when injected with biocytin or Neurobiotin. *Neuroscience Letters.* 125:187–190.

West, C. H. K., and R. A. Bernard. 1978. Intracellular characteristics and responses of taste bud and lingual cells of the mudpuppy. *Journal of General Physiology.* 72:305–326.

Yang, J., and S. D. Roper. 1987. Dye-coupling in taste buds in the mudpuppy, *Necturus maculosus.* *Journal of Neuroscience.* 7:3561–3565.