

## A DYNAMIC POPULATION OF EXCITABLE CELLS: THE TASTE RECEPTOR CELLS

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### INTRODUCTION

Taste receptor cells (TRCs) are specialized epithelial cells that detect chemicals occurring in food and beverages. They are housed in sensory organs – the taste buds – distributed mainly in the oral cavity. TRCs use a vast array of ion channels and receptors for transduction and relay electrical signals to the brain through afferent nerve fibers.

In many mammals, taste buds are either absent or immature at birth (13). This implies that TRCs have to undergo a functional maturation to achieve those membrane properties described in the adults and required for transduction. On the other hand, TRCs are continuously replaced by new cells (9). Thus, TRCs represent a dynamic population of excitable cells that undergoes two basic neurobiological processes: *postnatal development* and *cell turnover*.

We have begun to study the functional maturation of developing TRCs by focusing our attention on their electrophysiological properties. TRCs are heterogeneous as to the expression of ion channels (1) and this poses problems when one wants to study their maturation over time. We have recently developed an electrophysiological-pharmacological approach that allows one to evaluate the pattern of functional heterogeneity for voltage-gated currents in a given cell population and to study how this pattern evolves with time (2, 6).

Here we present data on the so-called Na/OUT cells, a group of taste cells endowed with specific membrane properties (1). These cells, in fact, are characterized by the expression of voltage-gated  $K^+$  and  $Cl^-$  currents ( $I_K$  and  $I_{Cl}$ , respectively), in addition to voltage-gated  $Na^+$  current ( $I_{Na}$ ).  $I_K$  and  $I_{Cl}$  mediate repolarizing currents and, like in neurons, they underlie action potential waveform and firing properties in Na/OUT cells (2). Action potential firing appears to be one important step in the transduction and signalling of sensory information in TRCs (24). By applying the patch-clamp technique to single Na/OUT cells in taste buds isolated from mouse vallate papilla, we addressed the following issue: *what happens to  $I_K$  and  $I_{Cl}$  in Na/OUT cells during postnatal development?* On the basis of our findings, we propose a mechanism of functional maturation occurring during postnatal development that could also take place, in adulthood, during cell turnover.

## METHODS

*Tissue preparation.*

CD-1 mice were used. Vallate taste buds were isolated with an enzymatic-mechanical procedure described previously (1, 2). Isolated buds were plated on the bottom of a chamber that consisted of a standard glass slide onto which a silicon ring (~1–2 mm thick and 15 mm internal diameter) was pressed. The glass slide was precoated with Cell-Tak (~3  $\mu\text{g}/\text{cm}^2$ ; Becton Dickinson) to improve adherence of isolated taste buds to the bottom of the chamber. The chamber was placed onto the stage of an inverted IX70 Olympus microscope and taste buds were viewed with Nomarski optics at 750 $\times$ (2). During the experiments, isolated taste buds were continuously perfused with Tyrode solution (in mM: 140 NaCl, 5 KCl, 2  $\text{CaCl}_2$ , 1  $\text{MgCl}_2$ , 10 HEPES, 10 glucose, 10 Na pyruvate, pH 7.4 with NaOH) by means of a gravity-driven system. Drugs were dissolved in Tyrode solution. All chemicals were from Sigma.

*Patch-clamp recording.*

Membrane currents of single cells in isolated taste buds were studied at room temperature (22–25  $^{\circ}\text{C}$ ) by whole-cell patch-clamp (8), using an Axopatch 1D amplifier (Axon Instruments). Signals were recorded and analyzed using a Pentium computer equipped with Digidata 1320 data acquisition system and pClamp8 software (Axon Instruments). pClamp8 was used to generate voltage-clamp commands and to record the resulting data. Signals were prefiltered at 5 kHz and digitized at 50- $\mu\text{s}$  intervals. Patch pipettes were made from soda lime glass capillaries (Baxter Scientific Products) on a two-stage vertical puller (model PP-830, Narishige). The standard pipette solution contained (in mM): 120 KCl, 1  $\text{CaCl}_2$ , 2  $\text{MgCl}_2$ , 10 HEPES, 11 EGTA, 2 ATP, 0.4 GTP, pH 7.3 with KOH.

## RESULTS

*Electrophysiological heterogeneity of Na/OUT cells during postnatal development*

In adult mice, the relative contribution of  $I_K$  and  $I_{Cl}$  to the whole repolarizing current in Na/OUT cells varied conspicuously from cell to cell. The pattern of this variability was visualized by testing the effect of tetraethylammonium (TEA, a potassium channel blocker (19)) on the whole repolarizing current, and then by evaluating the distribution of the TEA effect among recorded cells. As demonstrated previously (6), this was possible because the current remaining during TEA block was always a chloride current. The analysis of the distribution of TEA effect among Na/OUT cells during postnatal development revealed that: 1) a subset of Na/OUT cells possessed almost exclusively  $I_K$  (TEA block > 90%). We named these cells as “*K cells*”. *K cells* occurred throughout the development and were the most numerous at all ages (> 60% of recorded cells); 2) another subset of Na/OUT cells possessed both  $I_K$  and  $I_{Cl}$  (10% < TEA block < 90%) and were named as “*K+Cl cells*”. These cells, like *K cells*, occurred also throughout the development, but were less numerous than *K cells* (20–30% of the all Na/OUT cells); 3) a third subset of Na/OUT cells was characterized by expressing almost exclusively  $I_{Cl}$  (TEA block less than 10%); for this reason, we called them “*Cl cells*”. *Cl cells* were not present at early developmental stages (PD 2–4) and could be detected among recorded cells starting between PD 4 and PD 8. *Cl cells* were the less numerous among Na/OUT cells (< 10%).

In conclusion, the comparison of the distributions of TEA effect indicated that there was a change in the electrophysiological diversification of Na/OUT cells during postnatal development. Namely, the functional subsets described in the adult animals (*K cells*, *K+Cl cells*, and *Cl cells*) appeared after PD 4. Before then, only two subsets could be identified: *K cells* and *K+Cl cells*.

*Amplitude of  $I_K$  and  $I_{Cl}$  in Na/OUT cells during postnatal development.*

The analysis of the amplitude of  $I_K$  and  $I_{Cl}$  in the three subsets of Na/OUT cells during postnatal development (Fig. 1) revealed that: 1) a gradual increase in the current amplitude occurred in both *K cells* and *K+Cl cells*; 2) the amplitude of  $I_{Cl}$  in *Cl cells* was fairly large since the beginning of the appearance of these cells (PD 8) and did not change during development. On the basis of these observations, we reasoned that the appearance of *Cl cells* endowed with large current could be explained by considering these cells as a later stage in the development of another, pre-existing cell type. Specifically, we hypothesized that some *K+Cl cells* could give rise to *Cl cells*; this was supported by two observations: 1) before PD 8, the only cells expressing  $I_{Cl}$  were *K+Cl cells*, and 2) the relative contribution of  $I_{Cl}$  to the overall repolarizing current in *K+Cl cells* increased during development, concomitantly with the increase in current amplitude.

The average amplitude of potassium currents in *K cells* increased significantly during postnatal development (Fig. 1A). Thus, it was reasonable to conceive that cells endowed with sizeable  $I_K$  in taste buds of adult animals could represent later stages of a cell line expressing only  $I_K$ . Conversely, cells endowed with small  $I_K$  should be considered at the beginning of their functional development. Interestingly, *K cells* at the beginning of their "postnatal development" (that is, with low-amplitude current) could be found also in adult animals. Similar arguments held also for repolarizing currents in *K+Cl cells* (Fig. 1B).

## DISCUSSION

Taste receptor cells undergo two different developmental processes, postnatal development and cell turnover, which bring about the appearance of complex membrane properties in these excitable cells. How the electrophysiological phenotypes are acquired during the maturation processes is poorly understood. We studied the functional maturation of a specific group of taste cells – the Na/OUT cells – during postnatal development. We have provided data that give some insights into the cellular mechanism underlying the development of the functional heterogeneity of these cells. In the adult, Na/OUT cells show three different phenotypes according to the presence of  $I_K$  and  $I_{Cl}$  (Fig. 2): cells expressing  $I_K$  (*K cells*), cells expressing both  $I_K$  and  $I_{Cl}$  (*K+Cl cells*), and cell expressing  $I_{Cl}$  (*Cl cells*). On the contrary, at the early stages of postnatal development (PD 2-4), only *K cells* and *K+Cl cells* can be detected in taste buds. We propose that *K+Cl cells* and *Cl cells* might constitute a functional cell line in which the latter derive from some *K+Cl cells* (Fig. 2). Since *Cl*

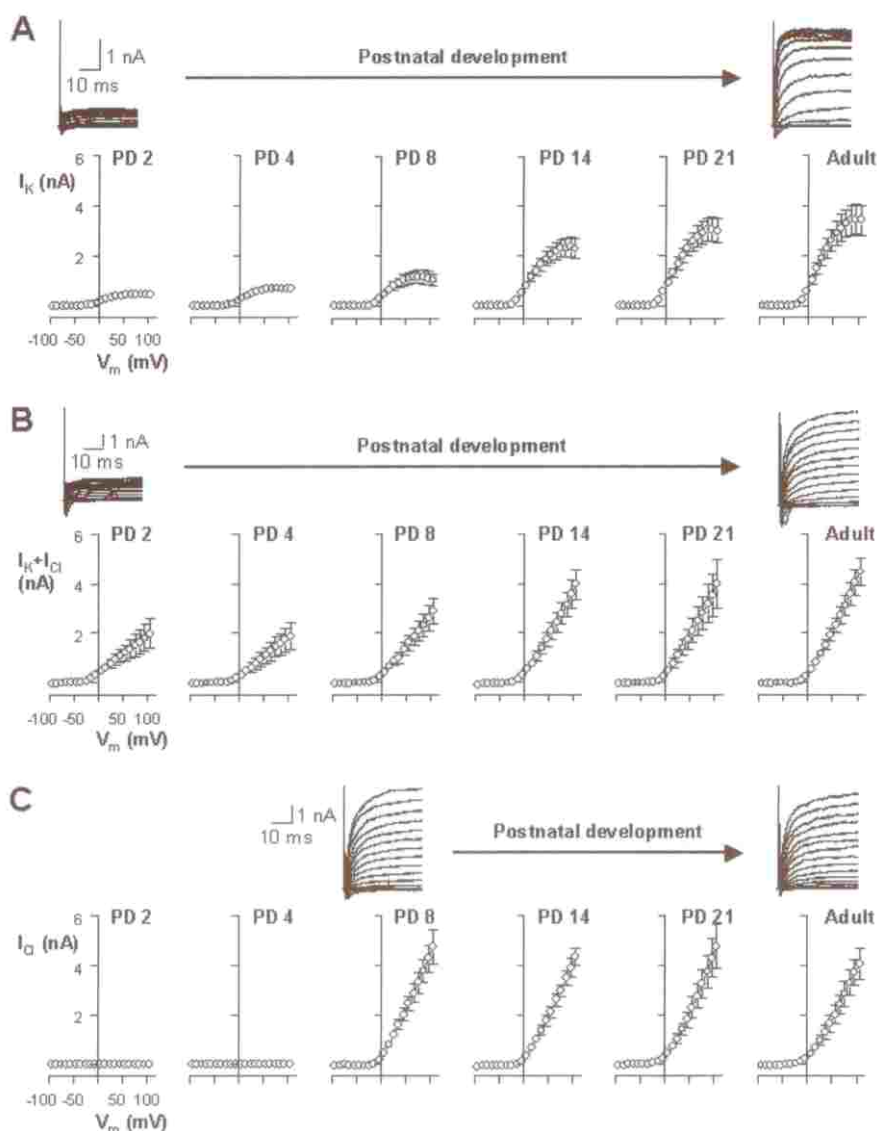


Fig. 1. - Voltage-gated outward currents in the three subsets of Na/OUT cells.

(A: *K* cells; B: *K* + *Cl* cells; C: *Cl* cells) during postnatal development. Membrane currents were elicited by a series of depolarizing pulses from a holding potential of  $-80$  mV. Outward currents (upward deflections in the current records) were carried by potassium ions and/or chloride ions. Downward deflections in the current records; voltage-gated  $\text{Na}^+$  currents. Current-voltage relationships of voltage-gated  $\text{K}^+$  currents ( $I_K$ ),  $\text{K}^+$  and  $\text{Cl}^-$  currents ( $I_K + I_{\text{Cl}}$ ), and  $\text{Cl}^-$ -currents ( $I_{\text{Cl}}$ ) are shown at the bottom. Current amplitude was measured at the end of 40-ms pulses. Each point represents the mean  $\pm$  SEM.  $n$ :  $> 21$  (A),  $> 7$  (B), and  $> 5$  (C). Note that at earlier developmental stages (PD 2-4), cells expressing only  $I_{\text{Cl}}$  were absent. PD, postnatal day;  $V_m$ , membrane potential.

cells do not express functional  $I_K$ , we hypothesize that the disappearance of pre-existing  $\text{K}^+$  currents in some *K*+*Cl* cells may represent the mechanism underlying the



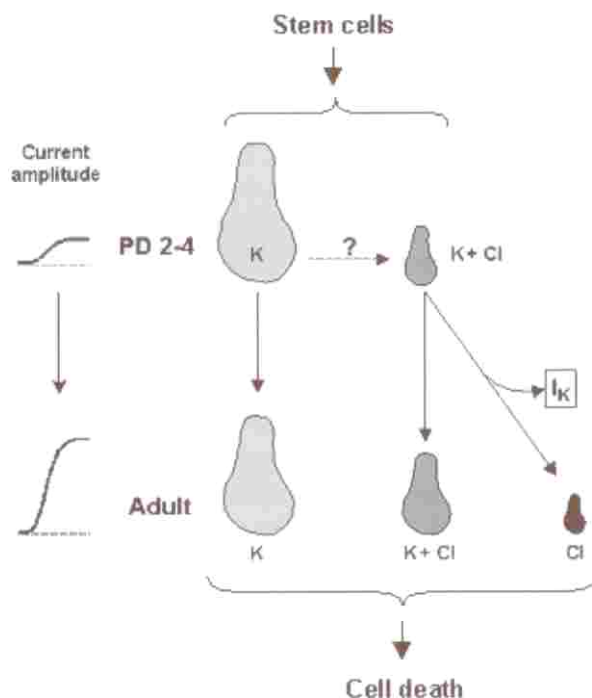


Fig. 2. - Proposed pattern of functional maturation in Na/OUT cells of the mouse vallate papilla.

$K$ , cells expressing only  $I_K$ ;  $K + Cl$ , cells expressing both  $I_K$  and  $I_{Cl}$ ;  $Cl$ , cells expressing only  $I_{Cl}$ . The size of the cells in the cartoon reflects the relative occurrence of each functional subtype among patched cells. In both  $K$  cells and  $K + Cl$  cells, the amplitude of the outward current increases during development. On the contrary,  $Cl$  cells displayed always very large current independently of the age. A possible lineage relationship between  $K$  cells and  $K + Cl$  cells at early developmental stages is indicated by the dashed arrow.

appearance of  $Cl$  cells (Fig. 2).  $K$  cells, on the other hand, could represent a separate functional line.

In developing neurons and sensory cells, voltage-gated ion channels undergo remarkable rearrangements, including changes in density in the plasma membrane (15, 20, 25) as well as transient expression followed by their disappearance at later developmental stages (16, 17). Our data suggest that similar general processes may take place also during the functional maturation of taste Na/OUT cells. In particular, our working model hypothesizes that in some  $K+Cl$  cells,  $I_K$  should be gradually turned off to allow the formation of  $Cl$  cells (Fig. 2). It is likely that this process occurs throughout the development and also, once the adult phenotypes are acquired, during cell turnover in mature taste buds. The functional importance of  $I_K$  in the lineage of  $Cl$  cells is unclear.  $I_K$  affects profoundly the firing properties of excitable cells, including taste cells (2). It is well established that firing activity plays a crucial role in synaptogenesis and in the refinement of synaptic connections (4, 21). Thus, it is tempting to speculate that the presence of potassium currents in a cell line that eventually will lose such currents may be important for the development of functional connectivity inside the taste buds with the nerve endings. At this regard, we recall that we studied Na/OUT cells, that is, spiking cells (2). Alternatively, potassium currents might be necessary for cell maturation. It is well known that differentiation and ion fluxes are strictly interrelated during development of excitable cells (22, 23).

In taste cells of adult mammals,  $I_K$  is modulated by several extracellular signals, including gustatory stimuli (3, 5, 7, 18), leptin (14), and cholecystikinin (12). Findings

by the Herness' group with rat taste cells indicate that adrenergic signalling enhances  $I_{Cl}$  (10) while inhibits  $I_K$  (11). Caffeine, a bitter-tasting stimulus, inhibits  $I_K$  but has no effect on  $I_{Cl}$  (26). According to our data, we should expect a change in the "picture" of peripheral taste modulation as the functional maturation of taste cells proceeds during postnatal development. This, of course, will have obvious repercussion on the operation of taste buds as input devices for controlling food selection and intake.

In adult rodents, taste cells turnover in about 10-14 days (9). An obvious question is then: do the changes of  $I_K$  and  $I_{Cl}$  during postnatal development reflect a mechanism that operates also in adult animals? The observation that the lowest values of the outward currents recorded in both *K cells* and in *K+Cl cells* did not change during postnatal development suggested the appearance of new, immature taste cells throughout all ages and during cell turnover. In addition, once established during development, the electrophysiological heterogeneity is maintained in the adult. Thus, postnatal development and cell turnover are likely strictly related. It is tempting to speculate that changes in the functional expression of  $I_K$  and  $I_{Cl}$  in developing cells might represent the process that will take place during turnover in adult mammals.

#### SUMMARY

Taste receptor cells (TRCs) represent an unique opportunity to study a dynamic population of excitable cells that undergoes two basic neurobiological processes: *postnatal development* and *cell turnover*. We have begun to investigate the functional properties of TRCs and how they mature over time by applying the patch-clamp technique to single cell in taste buds isolated from mouse vallate papilla during postnatal development. We have focussed our attention on a well-defined functional group of taste cells, called Na/OUT cells, and on their voltage-gated  $K^+$ , and  $Cl^-$  currents ( $I_K$  and  $I_{Cl}$ , respectively). As in neurons,  $I_K$  and  $I_{Cl}$  underlie action potential waveform and firing properties in these cells. By analyzing the relative occurrence of  $I_K$  and  $I_{Cl}$  among cells, we found that in adult mice three different electrophysiological phenotypes of Na/OUT cells could be detected: cells with only  $I_K$  (*K cells*); cells with both  $I_K$  and  $I_{Cl}$  (*K+Cl cells*); and cells with  $I_{Cl}$  (*Cl cells*). On the contrary, at early developmental stages (2-4 postnatal day, PD) there were no *Cl cells*, which appeared at PD 8. The analysis of the changes in current amplitude (which continuously increased in developing cells) during postnatal development suggested that *Cl cells* and *K+Cl cells* likely represented a single functional line different from *K cells*. In addition, electrophysiological data were consistent with the interpretation that *Cl cells* derived from some *K+Cl cells* by suppression of  $I_K$ . The dynamics of the expression of  $I_K$  and  $I_{Cl}$  during postnatal development likely reflects a mechanism that could also operate during turnover.

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