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Receptor Dissociation Constants and the Information Entropy of Membranes Coding Ligand Concentration

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Abstract

The binding of ligands to receptor proteins embedded in cell membranes drives cellular responses that involve either second messenger cascades or directly gated ion channels. It is known that a single class of receptor proteins expresses ~98% of its graded response to ligand concentrations over four orders of magnitude, where the response is measured by the equilibrium proportion of bound ligand–receptor complexes. This four-decadic concentration range is centered on a logarithmic scale around log$K$, where K is the dissociation constant defined by the ratio of ligand–receptor unbinding (k_-) to binding (k_+) rates. Remarkably, this four-decadic concentration range is intrinsic to all homogeneous ligand–receptor (or, equivalently, enzyme–substrate) systems. Thus, adapting the sensitivity of cell membranes to narrower or wider ranges of ligand concentrations, respectively, requires multivalent receptors or heterogeneous populations of receptors. Here we use a normalized Shannon–Weaver measure of information entropy to represent the efficiency of coding over given concentrations for membranes containing a population of univalent receptors with a specified distribution of dissociation constants, or a homogeneous population of strongly cooperative multivalent receptors. Assuming a specified level of resolution in the response of cellular or neural systems downstream from the membrane that 'read' the ligand concentration 'code', we calculate the range of concentrations over which the coding efficiency of the membrane itself is maximized. Our results can be used to hypothesize the number of receptor types associated with the membranes of particular cells. For example, from data in the literature, we conclude that the response of most general olfactory sensory neurons can be explained in terms of a homogeneous population of receptor proteins, while the response of pheromone sensory neurons is satisfactorily explained by the presence of two types of membrane receptor protein with pheromone-binding dissociation constants that have values at least one to two orders of magnitude apart.

Introduction

The ability of cell membranes to code chemical concentration, as a way of controlling cellular processes, is central to all cellular, physiological and sensory systems—in short, to all of life. At their most basic level, endocrine, paracrine, synaptic and chemosensory processes involve messenger ligands (hormones, chemical mediators, neurotransmitters, odorants, flavorants, etc.) binding to specific protein receptors protruding from the surface of cell membranes (Lauffenburger and Lindeman, 1993). If L is the density of ligands, A the density of unbound receptors and B the density of bound receptors, then the ligand–receptor association and disassociation process is represented by the iconograph $L + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$, where k_1 and k_{-1} are the association and dissociation rates, respectively. The ratio K, which is equal to k_{-1}/k_1, is referred to as the dissociation constant for the reaction.

If we are able to manipulate genes to obtain ligand–receptor binding processes with specified dissociation constants, then we can pose the following design question: 'What should the dissociation constants be of one or more types of receptors in a cell membrane in order to obtain the most efficient coding across a membrane with respect to a specified range of ligand concentrations?' The answer depends on what we mean by 'most efficient' coding, which will be clarified below. Also, the question is posed independent of any intracellular processes that may subsequently operate to enhance coding (e.g. second messenger systems), or other mechanisms such as 'spare receptor capacity' (Cleland and Linster, 1999), which enhances the sensitivity of membranes to low ligand concentrations.

A simple example of a ligand code is that some cellular function is 'turned on' (e.g. firing of a neuron, cell division or synthesis of a particular product) if the concentration of ligand outside a cell exceeds a specified threshold. A more

sophisticated example of a ligand code is that a cell exhibits a graded response to ligand concentration (e.g. a graded rate of firing in a neuron or a graded rate at which a cell synthesizes a product). On–off responses are translations of a two-state ligand code: concentration above or below a threshold. Graded responses are translations of an m-state ligand code ($m > 2$), where m can also be referred to as the resolution of the code. This resolution, which corresponds to categorizing the proportion of bound receptors into m discrete intervals, cannot be infinite because of the presence of noise in the system. Noise arises from several sources, including stochastic variations in the ligand concentration outside the cell and the finite size of ligand and receptor populations (i.e. sampling effects during ligand–receptor interactions), and introduces errors that limit the distinguishability of states.

Classical information theory, as developed primarily by Shannon and Weaver (Shannon, 1948; Shannon and Weaver, 1949; Ingels, 1971), provides a mathematical tool for evaluating how well a channel transmits information coded by a set of discretized states. It is the most appropriate mathematical theory available for analyzing the information capacity and transmission properties of a channel. We have, thus, used this theory to evaluate the information-processing qualities of receptor-embedded cell membranes with respect to transmitting information regarding which one of m possible discretized ligand concentration levels exists outside a cell over a period of time sufficiently long to consider our system at equilibrium [but see Getz and Abers (Getz and Abers, 1997)]. This is certainly not the first time Shannon–Weaver information theory has been applied to biological systems (Kueppers, 1990; Chapeau-Blondeau and Raguin, 1997; Panzeri *et al.*, 1999), but it is the first time it has been used to obtain an objective measure and analysis of 'efficient' coding by cell membranes of ligand concentration.

The converse of the 'design' question posed above, couched in the context of information theory, is the following 'existence' question: 'If a membrane expresses receptors of different types characterized by a set of dissociation constants, then what is its information capacity?' Surprisingly, neither this question nor the above 'design' question has been asked, although both the dynamic and equilibrium aspects of reversible and irreversible ligand–receptor binding processes (or, equivalently, substrate–enzyme interaction processes) have been investigated intensively in several contexts, including the control of neuronal (Rospars *et al.*, 1996; Kaissling, 1998; Getz, 1999) and, even, ecological processes (Keating and Quinn, 1998).

Considerable attention has been paid to Michaelis–Menten substrate–enzyme cascades (Brooks and Storey, 1992; Sakamoto, 1994) to multivalent binding processes where more than one ligand or substrate molecule binds to the same receptor or enzyme (e.g. the dynamics of oxygen and hemoglobin where up to four oxygen molecules bind to one hemoglobin molecule), and to situations where different substrates or ligands compete for the same enzyme or receptor (e.g. neurotransmitter antagonists such as curare or a-bungarotoxin which bind to acetylcholine receptors). Less attention has been paid in the literature to situations where the same ligand binds to more than one type of receptor molecule on the surface of a common membrane; although this problem has been of interest in the context of odor coding (Ennis, 1991; Malaka *et al.*, 1995). The issue of whether receptor neurons express more than one receptor protein in their membranes has become more important with the discovery that ~1000 genes or 1% of the genome of mice and rats code for olfactory receptor proteins (Mombaerts, 1999). Information theory can be used to shed some light on this question, as will become apparent in later sections of this paper.

Considerations of receptor valency and heterogeneity are critical for understanding the graded responses of cell membranes across biologically meaningful ranges of ligand concentrations. From an equilibrium analysis it is known that, irrespective of the particular ligand or receptors involved, a homogeneous population of univalent receptors expresses ~98% of its graded response to ligand concentrations (i.e. from 1 to 99% of receptors are bound) over four orders of magnitude in ligand concentration (Rospars *et al.*, 1996). Multivalent or heterogeneous ligand–receptor systems, however, have more complex characteristics in terms of the range of concentrations over which they respond. These characteristics, as is evident from this study, are relatively easy to understand and can be used to hypothesize the likely number of receptor types expressed in the afferents of olfactory sensory neurons. This is currently an important question because it is not known whether one or more receptor proteins are expressed in olfactory receptor cells. For example, if there are no intracellular mechanisms to amplify strongly the effects of ligand binding when much less than 1% of receptors are bound, our analysis suggests that pheromone sensory neurons in some insects probably express more than one receptor protein. Similarly, our analysis suggests that the response range of generalist olfactory sensory neurons in insects is compatible with these neurons expressing no more than one type of receptor protein.

In this presentation, we first review the equilibrium curves for the proportion of receptors in a membrane bound to ligands as a function both of ligand concentration and of the value of the dissociation constants for homogeneous, heterogeneous and multivalent receptor populations. We then explore the concept of coding resolution in the context of 'just discernable differences' in ligand concentration and define the informational transfer properties of the membrane using the Shannon–Weaver entropy measure (Shannon, 1948; Ingels, 1971). We use this measure to define a concept of membrane 'coding efficiency'. We numerically calculate the coding efficiency of membranes with regard to ligand concentrations uniformly distributed over a region of

concentration space defined by a 'range parameter', while the heterogeneity of the values of the dissociation constants of the membrane receptors is characterized in terms of a 'receptor heterogeneity parameter'.

After presenting a suite of results on how coding range, heterogeneity of dissociation constants among receptors, and coding resolution affect coding efficiency, we theoretically bound the coding efficiency of finite resolution systems using an infinite resolution analog to measure the entropy of coding efficiency. Specifically, we find the size of the range over which a theoretical membrane, with an entropy-maximizing linear response, codes the same amount of information as a homogeneous receptor membrane over an infinite range of concentrations. Our presentation concludes with a discussion of the implications of our theory for biological systems. This includes a discussion of data currently available (Kaissling, 1987) on the response of pheromonal receptor cells in the antennae of the male moth, *Antherea polyphemus*, to sex pheromones produced by females conspecifics and an assessment of the size of the range over which human T-cells can efficiently code interleukin-2 concentrations (Smith, 1988).

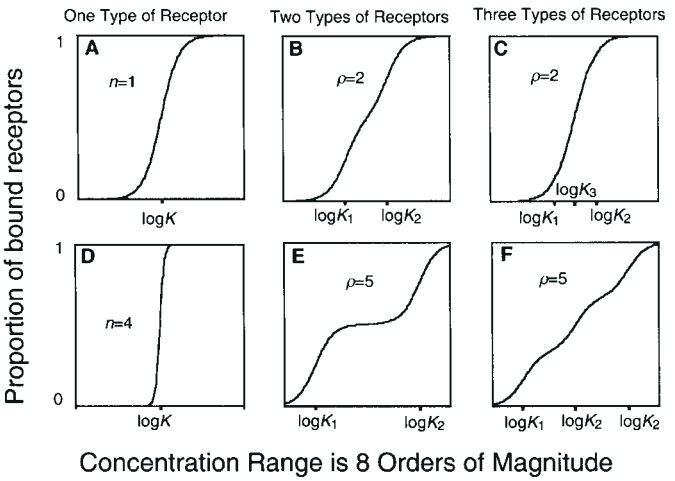

Figure 1 The proportion of bound receptors is plotted here as a function of the logarithm of the ligand concentration over eight orders of magnitude [equation (1), (2) or (3), depending on the case]. The parameter $\rho = \log(K_2/K_1)$ is a measure of receptor heterogeneity in systems with two types of receptor or symmetric systems with three or more types of receptor. The integer n is the valency of the receptor (Hill's constant) in a highly cooperative systems [equation (3)]. When not specified, the default values for ρ and n are $\rho = 0$ (i.e. the system is homogeneous, as in panels **A** and **D**) and $n = 1$ (the receptors are univalent, as in panels **A**, **B**, **C**, **E** and **F**).

Models and analysis

Equilibrium response curves

In our analysis, we focus on the equilibrium proportion, $y(x)$, of receptors (or enzymes) rather than the actual number that have ligands (or substrates) bound to them as a function of x, the decadic logarithmic concentration of ligands at density L (i.e. $x = \log L$). We consider situations where a signal is coded by the concentration of a particular ligand and the membrane transducing this signal expresses one type or several different types of receptors. The equilibrium curve for the case of a homogeneous, univalent ligand–receptor binding process is the well-known S-shaped or logistic curve (Figure 1A)

$$y(x) = \frac{10^x}{K + 10^x} \tag{1}$$

The 'half-saturation' constant, K, which in this context is known as the equilibrium dissociation constant (in the context of enzyme–substrate binding theory, K is known as the Michaelis–Menten constant), is the ratio of the rate at which ligands disassociate and associate with the receptors [e.g. see equation 2.13 in Lauffenburger and Lindermann (Lauffenburger and Lindermann, 1993)], taking into account the logarithmic transformation in x and the proportional, rather than absolute, value of y). In the case of membranes with r types of receptors able to bind to a ligand at density L, if the relative density of these receptors is given by the proportions ρ_i, $i = 1, \ldots, r$, then the equilibrium response curve (1) generalizes, as discussed by Malaka *et al.* (Malaka *et al.*, 1995), to (recall that $L = 10^x$)

$$y(x) = \sum_{i=1}^{r} \rho_i \left(\frac{10^x}{K + 10^x} \right) \tag{2}$$

where K_i is the equilibrium dissociation constant for ligands binding to receptors of the i^{th} type. Our purpose is to consider first systems with one type of receptor and then systems with two or three different types of receptor, all of which respond to the ligand in question. In the case of multiple receptors, to keep the analysis simple, we make the assumption that the different receptor types occur at the same frequencies. We note that unequal frequencies will give results that are intermediate between a system in which the frequencies are equal and a system with fewer types of receptors also with frequencies that are equal. For example, results obtained for a system that has two types of receptors in proportions 3/4 and 1/4 is intermediate between a system in which all receptors are of the first type and a system in which the proportions of the two types are 1/2.

Finally, for highly cooperative n-valent receptor-binding processes (i.e. one in which almost all receptors are either unbound or have n ligands bound to them, with an insignificant proportion of receptors having 1, 2, . . ., $n - 2$ or $n - 1$ ligands bound to them), the equilibrium proportion of bound receptors, as discussed by Brown and Rothery (Brown and Rothery, 1993), can be approximated by the Hill equation:

$$y(x) = \frac{10^{nx}}{K^n + 10^{nx}} \tag{3}$$

Of course, equations (2) and (3) can be combined to generalize to heterogeneous, multivalent systems. But this complexity is not considered here because, as will become apparent below, cooperative multivalency and heterogeneity have opposite effects on the entropy of information in coding ligand concentration over identified concentration ranges.

Information and resolution

The amount of information that can be associated with the equilibrial proportion of bound receptor–ligand complexes in the membrane of a cell depends in practice on several different factors that limit the resolution of a chemical coding system. First, the signals contain noise: ligand concentrations may fluctuate during the production of signals downstream from the membrane, but noise is also added during the transmission process due to turbulence in the transmission medium (e.g. air or water). Second, stochasticity arises due to sampling: the number of receptor and ligand molecules is finite with given probabilities for ligand–receptor encounters (i.e. a ligand–receptor encounter has the elements of a Bernoulli process, but numbers are generally large enough so that this factor is relatively unimportant compared, say, with the first factor). Third, the system is stochastic because distributions are involved: in reality, equations (1)–(3) are the means of stochastic processes in which ligands and receptors encounter one another at rates that are means of distributions with variance dependent, for example, on the variance of ligand velocity distributions. Fourth, computational properties of the system downstream from the membrane determine how large the change in the equilibrium proportion of bound receptor complexes needs to be to get a 'just discernible' difference.

The existence of a just discernible difference implies that even without noise, the resolution of the system is finite. If the intent of the signal is to evoke changes in a single cell over a short period of time then we might expect the resolution of the system to be relatively low. On the other hand, if the 'intent' of the signal is to guide an organism to an odor source, then networks of neurons are involved in the computation and we might expect the resolution of the system to be somewhat higher. It has been argued, for example, that humans appear to have the ability to distinguish about 30 levels of concentration of odors (Wilson and Bossert, 1963).

We analyze coding efficiency in the context of Shannon–Weaver theory (Shannon, 1948; Ingels, 1971). The analysis depends not only on the desired ligand coding range ('design question') or the range of ligand concentrations that can be expected under natural conditions ('existence question'), but, as discussed above, on the resolution of the decoding

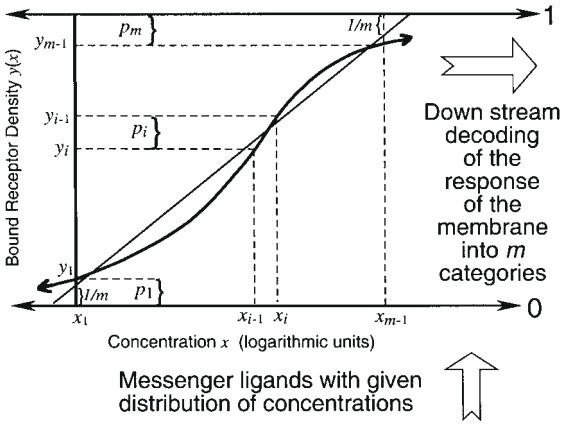

Figure 2 We regard a membrane as a coding channel: the ligand concentration is the signal, the proportion of bound receptors (solid curve) is the transfer function specified by equation (1), and the resolution m depends on systems downstream from the membrane (e.g. cell organelles or brain neuropil) that decode the proportion of bound receptors at equilibrium into m categories, thereby producing a graded response of the systems to ligand concentration outside the cell. The straight line is the linear relationship $y(x)$ specified by equation (7) and using the notation [see equation (5)] $y_i = y(x_i)$. This line is positioned so that $p_1 = y_1 = 1/m$ and $p_m = 1 - y_{m-1} = 1/m$.

process downstream from the membrane (design and existence questions) (Figure 2). Although we have to select, *a priori*, a level of resolution, we explore the properties of systems over a range of resolutions sufficiently broad to provide us with a comprehensive view of the effects of resolution on coding. As a limiting case, we also consider what happens if resolution could become infinite, even though noise ensures that the resolution of real systems can never be infinite. This limit leads to a continuous formulation of the problem, which we then analyze to obtain a benchmark on the efficiency of homogeneous univalent ligand–receptor coding represented by transfer function (1). Finally, we note that in systems with relatively high noise levels, the resolution of coding can only be high if the signal is sampled over a long period of time or over many different cells reading the same signal.

Discrete systems

Suppose we are interested in how efficient a particular system is at coding concentrations into m categories over the interval $x \in (-\infty,\infty)$, where $x = \log L$ is a measure of ligand concentration L. Assume, as depicted in Figure 2, that a minimum and maximum detectable level, x_1 and x_{m-1}, respectively, exist in the sense that all concentrations below the minimum level are coded as category 1 and all concentrations above the maximum level are coded as category m. Consider a partition of the interval $[x_1,x_{m-1}]$ into $(m-2)$ equal subintervals to obtain the m-subintervals $X_1 = (-\infty,x_1)$, $X_1 = [x_{i-1},x_i]$, $i = 2, \ldots, m-1$, and $X_m = [x_{m-1},\infty]$, where obviously

$$x_i = x_1 + (i-1)\frac{(x_{m-1} - x_1)}{m-2}, \quad i = 1,\ldots,m-1 \tag{4}$$

For each of the m–1 values x_i, i = 1, . . ., m – 1, as illustrated in Figure 2, we can use our transformation equation (1) or (2), whichever case applies, to calculate corresponding values

$$y_i = y(x_i), \quad i = 1, \ldots, m-1. \tag{5}$$

If we now define y_0 = 0 and y_m = 1, we can generate a partition of the ordinal axis defined by $Y_1 = [0,y(x_1)]$, $Y_i = y(x_{i-1}),y(x_i)]$, i = 2, . . ., m – 1, and $Y_m = [y(x_{m-1})]$, 1. The length of each of these intervals Y_i is equal to (Figure 2)

$$p_i = y_i - y_{i-1} \tag{6}$$

In general, the values p_i are not all equal. In the context of our minimum and maximum points x_1 and x_{m-1}, the intervals Y_i will only be equal for a linear function defined by

$$y(x) = \frac{(m-2)x + x_{m-1} - (m-1)x_1}{m(x_{m-1} - x_1)} \tag{7}$$

for $x = x_1$ and $x = x_{m-1}$ this function respectively yields y_1 = 1/m and y_{m-1} = (m – 1)/m (Figure 2), as well as producing the required m equal values p_1 = 1/m, i = 1, . . ., m. According to the theory developed below, for a system of resolution m > 2, this linear response function is the most efficient way to code concentration on the partition $\{X_1, \ldots, X_m\}$ of the interval $x \in (-\infty,\infty)$, obtained as described above.

In real systems, noise and imprecision in components downstream from the membrane imply that a concentration x close to a node point x_i will sometimes be assigned to category Y_i [because $y(x_i)$ is its upper bound] and sometimes to category Y_{i+1} [because $y(x_i)$ is its lower bound]. If this is done roughly with equal probability for x in a small neighborhood of x_i, then the values p_i defined in equation (5) will hardly be affected.

With these definitions, we use the Shannon–Weaver measure

$$I_m = -\sum_{i=1}^{m} p_i \log_2 p_i \tag{8}$$

to define the information entropy of the m-resolution membrane coding system formulated above. This measure has the maximum value $I_m = \log_2 m$ when all the probabilities are equal (i.e. p_i = 1/m, i = 1, . . ., m). This is why the linear transfer function (7) has greater entropy than an S-shaped transfer function (1) centered over the concentration range for which function (7) is defined. To enable us to compare the coding efficiency of various dose–response (transfer) functions $y(x)$ for different levels of resolution m, we normalize the entropy value by dividing equation (8) by $\log_2 m$. Thus we define the efficiency of coding of a system of resolution m to be

$$\text{coding efficiency} = \frac{I_m}{\log_2 m} \tag{9}$$

Parameters of analysis

The coding efficiency calculated from equations (8) and (9) for a membrane transfer function defined by equation (1) depends, through equations (5) and (6), on the locations of the values x_i, i = 1, . . ., m – 1 that define the partition $\{X_1, \ldots, X_m\}$ of the concentration space. If the points x_i, i = 1, . . ., m – 1, are all smaller than log$K$ by at least two decadic orders of magnitude then it follows from equations (1), (5) and (6) that $p_i \approx 0$, i = 1, . . ., m – 1 and $p_m \approx 1$. Similarly, if the points x_i, i = 1, . . ., m – 1, are all larger than log$K$ by at least two decadic orders of magnitude then it follows from equations (1), (5) and (6) that $p_i \approx 1$ and $p_i \approx 0$ i = 2, . . ., m. In both cases $I_m \approx 0$, so coding efficiency is close to zero. The maximum value for information entropy for a specific partition $\{X_1, \ldots, X_m\}$ is attained for a membrane characterized by transfer function (1) when the partition $\{X_1, \ldots, X_m\}$ is located symmetrically with respect to the transfer function (1) [i.e. symmetrically around the half-saturation value, log$K$, which yields $y(\log K) = 0.5$], as illustrated in Figure 3. Thus, recalling that $x = \log L$, for membranes with a single type of receptor we maximize coding efficiency for even values of m when $x_{m/2} = \log K$ and for odd values of m when

$$\frac{x_{(m-1)/2} + x_{(m+1)/2}}{2} = \log K$$

We illustrate the drop-off in coding efficiency with deviations from symmetry for the case m = 3 by defining the variable

$$z = \log K - \frac{(x_1 + x_2)}{2}$$

to represent the degree to which the construction of the partition $\{X_1, X_2, X_3\}$, as discussed in the sentence containing equation (4), deviates from symmetry [for example, at z = 0, log$K$ = (x_1 + x_2)/2, which implies that on a logarithmic scale, the value of K is midway between x_1 and x_2]. We then calculate the efficiency of coding using equations (1), (5), (6), (8) and (9) for values of the parameter z ranging from –4 to +4. At z = 0, the coding efficiency given by equation (9) has the value 0.933 (Figure 3A), but drops off rapidly as z decreases below 1/3 or increases above 1/3 (Figure 3A). Thus, if we are interested in characterizing only the most efficient coding by homogeneous receptor

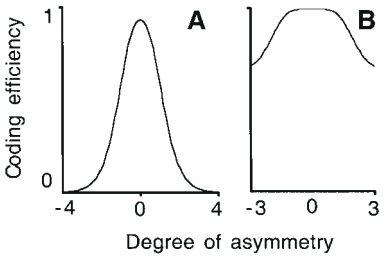

Figure 3 The coding efficiency of a membrane with resolution $m = 3$ receptors is plotted in terms of an asymmetry variable z for the following cases: **(A)** a homogeneous univalent receptor membrane with fixed range parameter $\Delta_X = 1$ and asymmetry variable $z = \log K - (x_1 + x_2)/2$ (see text for details) and **(B)** a membrane with three types of univalent receptor, $\log K_1 = -3$, $\log K_2 = 3$, and the dissociation constant of the third receptor is the asymmetry variable $z = \log K_3$ (i.e. symmetry is given by the value $z = 0$). The range parameter in this case is $\Delta_X = 3$.

membrane systems that have a specified resolution m over a given range of ligand concentrations, then, by letting x_1 and x_{m-1} represent the lower and upper values of the range, respectively, we need only consider the system that is symmetrically located on this range, and use the range parameter

$$\Delta_X = x_{m-1} - x_m$$

to characterize the width of the range. Taking this view, we can plot the coding efficiency of such symmetrically located systems for different resolutions m and for different values of the range parameter Δ_X. Implicit in this view is the expectation that the particular values of the dissociation constants of the receptors in cell membranes have either been designed or evolved to maximize coding efficiency, subject to constraints on the resolution m of the system and on the heterogeneity of receptor types in the membrane. A more general analysis that plots coding efficiency for nonsymmetric systems, as we have done in Figure 3A for the case $m = 3$ and $\Delta_X = 1$, adds little to our understanding of how range affects coding efficiency for systems of different resolution. Note that it will become apparent once we present our results that, for relatively narrow ranges of concentrations, the most efficient coding is not always at the highest level of resolution. In fact, the results presented below indicate that ranges of concentration of 1 to 1.5 orders of magnitude are most efficiently coded by systems with resolutions of around $m = 3$–6 (Figure 4A).

In the two-receptor case, because we are dealing with logarithms, coding efficiency is maximized by locating the partition $\{X_1, \ldots, X_m\}$ symmetrically around the geometric mean of the logarithms of the associated dissociation constants $\log K_1$ and $\log K_2$ (we will demonstrate this numerically below in the context of membranes with three types of receptor). Designating these constants so that $K_1 < K_2$, we define the receptor heterogeneity parameter,

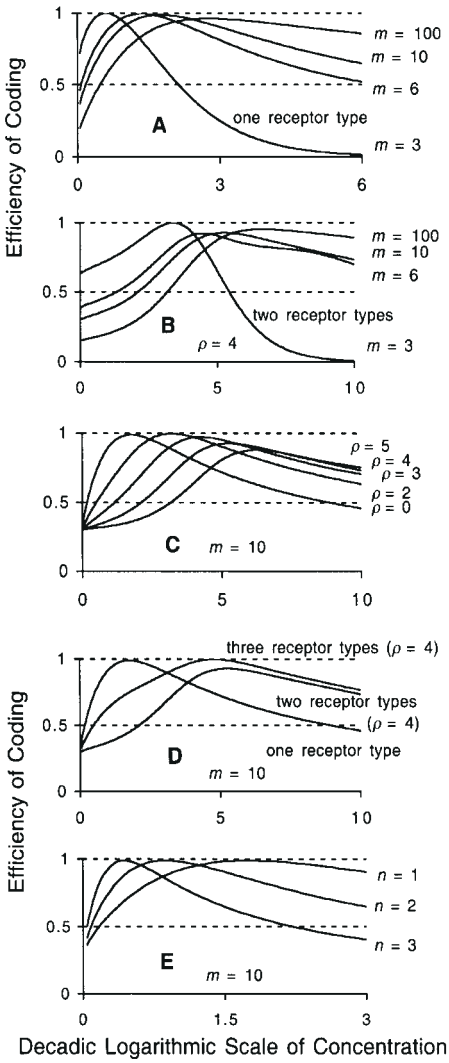

Figure 4 The coding efficiency of selected systems is plotted in terms of decadic range $\Delta_X = x_{m-1} - x_1$ (see Figure 2) for the following cases: **(A)** homogeneous univalent receptor membranes symmetrically located over the defined range of ligand concentrations, **(B)** two-receptor-type membranes with dissociation constants four orders of magnitude apart [i.e. $\rho = \log(K_2/K_1) = 4$] symmetrically located over the defined range of ligand concentrations, **(C)** symmetrically located two-receptor-type membrane systems with high resolution ($m = 10$), **(D)** two- and three-receptor-type membranes systems symmetrically located over the defined range of ligand concentrations that have a decoding resolution of $m = 10$ and dissociation constants four orders of magnitude apart ($\rho = 4$) and **(E)** monovalent ($n = 1$) [equation (1)] and highly cooperative divalent [$n = 2$ in equation (3)] and tetravalent [$n = 4$ in equation (3)] receptor systems with a decoding resolution of $m = 10$ symmetrically located over the defined range of ligand concentrations.

$$\rho = \log(K_2/K_1)$$

ρ is a measure of heterogeneity because at $\rho = 0$ the two-receptor-type system is equivalent to a one-receptor-type system with dissociation constant $K = K_1 = K_2$.

For systems with three types of receptor, consider the

placement of a third receptor type intermediate between two others (i.e. consider the value of a dissociation constant K_3, satisfying $K < K_3 < K_2$). For the same reason that maximum coding efficiency is obtained under symmetrical conditions in the homogeneous-receptor case (see Figure 3A), in the case of three types of receptors maximum coding efficiency also requires symmetry (Figure 3B). This amounts to requiring K_3 to be the geometric mean of K_1 and K_2 [$K_3 = \sqrt{(K_1K_2)}$] (see Figure 1) and requiring the three subpopulations to occur with equal frequency. Thus, in the context of exploring maximum coding efficiency in three-receptor systems that are constrained to have resolution m, we confine ourselves only to these symmetrical situations that can be characterized by the two parameters introduced above: the range parameter Δ_X and the heterogeneity parameter ρ.

In similar vein, using symmetry constraints, we can analyze systems with four, five, six or more types of receptors, in the context of the two parameters Δ_X and ρ. Thus, for example, if we have five types of receptor, a symmetry assumption implies that $K_1 < K_4 < K_3 < K_5 < K_2$, with $K_3 = \sqrt{(K_1K_2)}$, $K_4 = \sqrt{(K_1K_3)} = \sqrt{\{K_1[\sqrt{(K_1K_2)}]\}} = {K_1}^{3/4}{K_2}^{1/4}$ and, similarly, $K_5 = {K_1}^{3/4}{K_2}^{1/4}$. By analogy, symmetry conditions for four receptors imply that $K_1 < K_3 < K_4 < K_2$, with $K_3 = {K_1}^{1/3}{K_2}^{2/3}$ and $K_4 = {K_1}^{2/3}{K_2}^{1/3}$.

The more types of receptors a membrane has, the greater is the range of concentrations over which efficient coding exists (see Figure 1F), although it will become obvious from our simulations that systems with several receptor types do not code smaller ranges very efficiently, unless the heterogeneity parameter ρ is comparatively small.

Results

Finite resolution simulations

Univalent receptor membranes with resolution $m = 2$ are purely 0 and 1 classifiers. Their performance depends on where the classification point, or threshold value, $x_1 = x_{m-1}$ is located with respect to the placement of the transfer function (1) on the concentration axis. If, for example, $x_1 = x_{m-1} = K$, where K is the dissociation constant, then the partition is $X_1 = (-\infty,\ln K)$, $X_2 = (\ln K,\infty)$ and $p_1 = 0.5$, $p_2 = 0.5$, and $(1/\log_2 2)I_2 = 1$. Other partitions will lead to coding with lower efficiencies.

Systems with resolution $m = 3$ are thus the lowest resolution systems that have a free parameter Δ_X with corresponding information entropy measure $I_3(\Delta_X)$ [i.e. the value of expression (9) depends on the value of Δ_X] that can be analyzed to find the value of Δ_X that maximizes $I_3(\Delta_X)$. In this section we analyze the coding properties of discrete channels with the following resolutions: low ($m = 3$), moderate ($m = 6$), high ($m = 10$) and very high ($m = 100$). These levels of resolution span what we might reasonably expect to see in real systems [recall that humans appear to have the ability to distinguish about 30 levels of concentration of odors (Wilson and Bossert, 1963)].

For homogeneous univalent receptor membranes symmetrically located over the defined range of ligand concentrations, our results indicate for the four levels of resolution (Figure 4A) that the maximum efficiency $I_3(\Delta_X)/\log_2 3 = 1$ is achieved when the resolution m is 3 and the range parameter Δ_X is 0.6 (equal to a four-fold change in concentration L). With increasing resolution m, the maximum coding efficiency drops slightly (Figure 4A), but the size of the interval for which coding efficiency is high increases until, for m=100, efficiencies of ~95% occur over a coding interval of width $\Delta_X \approx 3$ [when m=100, maximum coding efficiency is $I_{100}(\Delta_X)/\log_2 100 = 0.96$ at $\Delta_X = 2.75$ (Figure 4)]. For a very high resolution system, a coding efficiency of 90% can be achieved over five orders of magnitude and of 80% over eight orders of magnitude (not shown in Figure 4). Although values to three decimal places cannot be read directly from Figure 4, for a single receptor system with moderate resolution ($m = 6$) we obtained a maximum coding efficiency of $I_6(\Delta_X)/\log_2 6 = 0.996$ at $\Delta_X = 1.35$ (this represents a 22- to 23-fold change in concentration). Thus it is clear from Figure 4A that coding efficiency is critically dependent on the overall resolution of the system. These results suggest that coding efficiencies of >90% for concentration ranges of five or more orders of magnitude require membranes with more than one type of receptor (Figure 4B and C).

In Figure 4B we plot the coding efficiency of two-receptor-type membranes with dissociation constants four orders of magnitude apart [i.e. $\rho = \log(K_2/K_1) = 4$] symmetrically located over the defined range of ligand concentrations. In this case (Figure 4B), very high resolution systems (e.g. $m = 100$) have membranes with a coding efficiency of >90% for concentration ranges of 5.0–9.5 orders of magnitude, while low resolution systems ($m = 3$) only have >90% coding efficiency for concentrations ranges from 2.4 to 4.2 orders of magnitude. When $m = 3$ (Figure 4B), coding rapidly loses efficiency for concentration ranges 4.5 orders of magnitude. For symmetrically located two-receptor-type membrane systems with high resolution ($m = 10$) (Figure 4C), membranes for which $\rho = 3$ have a greater maximal efficiency (the maximum is $I_{10}/\log_2 1.00$, correct to two decimal points, at Δ_X) than either membranes for which $\rho = 2$ (the maximum is $I_{10}/\log_2 10 = 0.99$ at $\Delta_X = 2.2$) or $\rho = 4$ (the maximum is $I_{10}/\log_2 10 = 0.93$ at $\Delta_X = 5.3$). Further, the maximum coding efficiency begins to decline strongly as the value of ρ increases. Two-receptor-type membranes for which ρ=4 provide reasonable levels of coding efficiency (>80%) at high levels of resolution ($m = 10$) over ranges of 3.7–8.5 orders of magnitude (Figure 4B or C).

High-quality coding (>90% efficiency) cannot be achieved with any two-receptor system for ranges of seven orders of magnitude or greater (Figure 4B and C). It does not help to increase the distance between the half-saturation response levels of two receptors (for example, when $\rho = 5$, the coding efficiency is always <90%) because a flat spot in the response

curve develops around the mid-point $\log K_3 = \frac{1}{2}\log K_1 K_2$ (see Figure 1E).

The only way to improve coding efficiency over ranges of concentration greater than seven orders of magnitude is to add a third receptor type to the system to smooth out the flat spot in the middle of the range. In Figure 4D, for example, we compare two- and three-receptor-type membranes systems symmetrically located over the defined range of ligand concentrations that have a decoding resolution of $m = 10$ and dissociation constants four orders of magnitude apart (ρ=4); we see that coding efficiency in the three-receptor-type membrane moderately improves coding over ranges of six to 10 orders of magnitude and strongly improves coding over ranges of one to three orders of magnitude compared with two-receptor-type membrane systems. In Figure 4D we also see, however, that homogeneous (one) receptor systems are still best over smaller ranges.

Coding over large concentration ranges, however, may not be our only concern. High-resolution coding over small ranges of concentrations may be required in systems where signals occur within a relatively narrow range of concentrations (e.g. one order of magnitude or less). Plots of coding efficiency of information for membranes with highly cooperative symmetrically located multivalent-type receptors in systems with a decoding resolution of $m = 10$ (Figure 4E) indicate that divalency ($n = 2$) improves the coding efficiency of monovalency ($n = 1$) over a 0.5 decadic range from $I_{10}(0.5)/\log_2 10 = 0.7$ to $I_{10}(0.5)/\log_2 10 = 0.95$. Further, tetravalency ($n = 4$) (see Figure 1D) achieves its maximum coding efficiency $I_{10}(\Delta_X)\log_2 10 = 0.98$ at $\Delta_X = 0.41$ (i.e. a six-fold range of concentrations). Thus coding over small ranges (one or two decadal orders of magnitude) is not efficient in homogeneous univalent single-receptor-type systems, and even less so in heterogeneous-receptor systems. High efficiencies over small ranges require some other type of mechanism such as highly cooperative multivalent receptors (Figure 4E) for which the transfer function is expressed by equation (3).

Infinite resolution bound

Discrete channels can, in theory, be made continuous by letting $m \to \infty$ [e.g. see Ingels (Ingels, 1971), Chapter 4.5], and by replacing differences with derivatives and sums with integrals. The information capacity of the channel, however, becomes infinite. A finite relative measure of information can be defined over any interval $x \in [x_l, x_u]$ in terms of the derivative $y'(x)$ of the dose–response function $y(x)$ by the integral

$$I(x_l, x_u) = -\int_{x_l}^{x_u} y'(x)\ln y(x)\,\mathrm{d}x \qquad (10)$$

(Ingels, 1971) where, for the convenience of avoiding scaling constants in calculations, we express this entropy measure in terms of a natural rather than the binary logarithmic scale associated with the classical Shannon–Weaver measure. Although a channel with infinite resolution is not realizable in practice, as already mentioned, it provides a standard for comparing systems with finite resolution. The focus of our analysis here is to compare, in the limit as the resolution becomes infinite, the information transfer properties of a homogeneous univalent receptor membrane [i.e. transfer function (1)], with the information transfer properties of the linear transfer function (7). The motivation for this comparison is that the linear transfer function (7) maximimizes information entropy for signals occurring over the associated range of concentrations (its linearity over this range implies all p_i in Shannon–Weaver measure (8) are equal, thereby maximizing expression (9) (Ingels, 1971).

In the limit as $m \to \infty$, defining $x_l = x_1$ and $x_u = x_{m-1}$, transfer function (7) approaches the ramp function

$$y(x) = \begin{cases} 0 & \text{for} \quad x \le x_l \\ (x - x_l)/(x_u - x_l) & \text{for} \quad x_l < x < x_u \\ 1 & \text{for} \quad x \ge x_u \end{cases} \qquad (11)$$

which is the cumulative distribution function of a rectangular distribution on $[x_l, x_u]$. If the derivative of (11) is inserted in equation (10), the integral yields

$$I(x_l, x_u) = \ln(x_l - x_u)$$

Note, this entropy measure itself becomes infinite if we let $x_l \to -\infty$ or $x_u \to +\infty$.

On the other hand, when $y(x)$ is given by the logistic transfer function (1), it is easily shown that integration of expression (10) yields $I(-\infty,\infty) = 2$. Thus, the information entropy of homogeneous univalent receptor membranes has the same information entropy as ramp function (11) defined over a coding range determined by solving $\ln(x_l - x_u) = 2$. On transforming to a decadic logarithmic scale, this implies that $x_l - x_u = e^2/\ln 10 \approx 3.2$. In short, the information entropy of the logistic transfer function (1) over the log-concentration range $(-\infty,\infty)$ is equivalent to the information entropy of a ramp function over a concentration range of 3.2 orders of magnitude.

Discussion

The intrinsic characteristics of the mass action principle of substrate–enzyme or ligand–receptor binding imply that efficient coding of signals, presented at constant levels and assuming that equilibrium conditions prevail, is restricted to one or two orders of magnitude for low resolution systems and four orders of magnitude for very high resolution systems. In fact, when the resolution is infinite, the information entropy of a homogeneous univalent membrane is equivalent to maximum entropy that it is possible to attain over 3.2 orders of magnitude. The only degree of freedom

of homogeneous univalent membranes is the location of the center of the transfer function, log$K$, which must be symmetrically placed to maximize the coding efficiency of membranes over any finite range of ligand concentration. Efficient coding over concentrations larger that four orders of magnitude can be achieved by introducing an appropriate distribution of receptor types, with two types being efficient over six or seven orders of magnitude, three over seven to nine, and, although we limited our numerical studies to three types of receptor, its obvious that increased efficiency can be obtained with more types if the range is greater than 10 orders of magnitude. On the other hand, efficient coding over concentrations less than two orders of magnitude requires other mechanisms such as a membrane containing a population of highly cooperative multivalent receptors. Our analysis, and thus our comments, are confined purely to the information transduction properties of the membranes themselves. Various cellular mechanisms associated, for example, with second messenger systems could amplify signals at very low concentrations to increase the coding performance of a cell as a whole.

In the context of the response of olfactory receptor neurons, our results imply that for neurons exhibiting a graded response over more than four orders of magnitude, additional mechanisms are required for efficient coding. A simple mechanism, as demonstrated here, is that such neurons could be expressing at least two types of receptor proteins in their dendritic membranes. The best data we are aware of relating the response of olfactory receptor neurons to odorant concentration are from studies in insects (Kaissling, 1987; Fujimura *et al.*, 1991). Data presented by Fujimura *et al.* (Fujimura *et al.*, 1991) on the response of cockroach olfactory neurons to stimulation by a number of different odorants, including *n*-alcohols, terpenes, aromatic compounds, acids and acetates, indicate a response range of at least two to three orders of magnitude, but most plots do not show the full range of responses because either the thresholds or saturation points are not apparent. The data of Fujimura *et al.* do indicate that neurons sensitive to terpineol have response ranges >3. No ranges of four orders of magnitude or greater, however, are indicated in their data.

Kaissling (1987), on the other hand, presents data that indicate that pheromonal receptor neurons in the moth *A. polyphemus* are sensitive to at least five to six orders of magnitude of the odorant *E*-6,*Z*-11-hexadecadienyl actetate (see his Figure 29). According to our analysis, a membrane responding to this broad a range of concentrations can easily be explained by a membrane expressing two types of receptor with dissociation constants K_1 and K_2 at least one to two orders of magnitude apart (see Figure 1A–C). If, in reality, the membrane of *A. polyphemus* pheromonal sensory cell has only one type of *E*-6,*Z*-11-hexadecadienyl actetate receptor, then some other type of mechanism is needed to broaden the response of the membrane at low levels of resolution, such as second messenger amplification systems that work preferentially at low odorant concentration levels or spare receptor capacity for detecting odors at low concentrations (Cleland and Linster, 1999).

By contrast, if the membrane of *A. polyphemus* pheromonal sensory cell has two or more types of *E*-6,*Z*-11-hexadecadienyl actetate receptors with different dissociation constants, each receptor type can still remain highly specific for this odorant because specificity and sensitivity, though correlated to some extent, are not completely congruent. Specificity relies primarily on matching complex geometries in the shape of the ligand and receptor molecules while sensitivity depends on forces of attraction between the two, which are dependent partly on geometry and partly on the distribution of electrical charges.

The results reported in Figure 4B and C can be used to estimate the range over which human T-cells can efficiently code interleukin-2. In a review, Smith (Smith, 1988) reports that high-affinity interleukin-2 receptors in T-cell membranes have a dissociation constant K of 7×10^{-10} M while low-affinity receptors in the same T-cell membranes have a dissociation constant of 3×10^{-8} M. Assuming equal distributions of high and low affinity receptors in T-cell membranes, these values yield a heterogeneity index

$$\rho = \ln\left(\frac{3\times10^{-10}}{7\times10^{-10}}\right) = 1.63$$

This value suggests from Figure 4C that for moderate resolution systems (m = 10), coding is >80% efficient over two to six orders of magnitude. Isolated cells, however, might well have lower levels of resolution, in which case the fact that these cells have two types of receptor ensures that they can code at 80% efficiencies over at least two to three orders of magnitude rather than the single order of magnitude seen in Figure 4A for cells with only one type of receptor (i.e. $\rho = 0$).

More precise assessments of the coding properties of real systems require that we take into account additional details that are not included in the analysis presented here. However, the general principles of our equilibrium analysis hold unless the system strongly violates the assumptions of the model (e.g. if coding is fast compared with the association rate constant so that the initial transients are important, or if receptors themselves have multiple states controlled by factors other than ligand binding).

In summary, we have demonstrated how the Shannon–Weaver information measure can be used to obtain a configuration of receptor types and affinity constants that provide efficient coding by membranes over *a priori* specified distributions of messenger ligand concentrations and system resolution. For example, in a high-resolution system (m = 10), the coding efficiency of a membrane will be maximized by a homogeneous population of multivalent receptors for uniformly distributed sets of signals over ligand concentration ranges smaller than one order of

magnitude, by a homogeneous population of univalent receptors for concentration ranges between one and three orders of magnitude and by a heterogeneous population of univalent receptors with optimally selected dissociation constants for concentration ranges greater than three orders of magnitude. Further, the coding efficiency of membranes over small ranges generally improves with decreasing levels of resolution and over large ranges generally improves with increasing levels of resolution (the specifics are determined by the actual size of the range, level of resolution, and valency and heterogeneity of the receptors).

The analysis presented here implicitly assumes that signals follow rectangular distributions over the finite concentration intervals considered in the analysis. In real systems, a complete analysis of the coding efficiency of specific membrane should account for the fact that the distribution of signals is unlikely to be rectangular. Such signal distributions are easily incorporated by multiplying the proportions p_i in the Shannon–Weaver measure (8) by a factor reflecting the degree to which signals arising in the corresponding subinterval X_i differ from a rectangular distribution.

From an evolutionary perspective, we might expect the number of receptor types and their valency with respect to a particular ligand to reflect the natural range of concentrations over which biologically meaningful signals occur. The complexity of the machinery decoding the signal should evolve to reflect the amount of information contained in the original signal, while mechanisms that reduce noise should be favored by natural selection so that the appropriate coding efficiency is achieved for the system as a whole. From a design perspective, the analysis presented provides a method for calculating the number of receptor types and corresponding dissociation constants needed to obtain efficient coding over specified ranges of ligand concentration.

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