Effect of the Glial Envelope on Extracellular K\(^+\) Diffusion in Olfactory Glomeruli

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Goriely, Anita R., Timothy W. Secomb, and Leslie P. Tolbert. Effect of the glial envelope on extracellular K\(^+\) diffusion in olfactory glomeruli. J Neurophysiol 87: 1712–1722, 2002; 10.1152/jn.00569.2001. In many species, including vertebrates and invertebrates, first-order olfactory neuropils are organized into spherical glomeruli, partially enveloped by glial borders. The effect of this characteristic organization on olfactory information processing is poorly understood. The extracellular concentration of potassium ions ([K\(^+\)]) must rise around olfactory receptor axons in specific glomeruli following odor-induced activation. To explore the time course and magnitude of K\(^+\) accumulation and possible effects of such accumulation on neural activity within and among glomeruli, we developed a theoretical model to simulate the diffusion of K\(^+\) in extracellular spaces of the glomeruli of the moth Manduca sexta. K\(^+\) released by activated axons was assumed to diffuse through the extracellular spaces in glomeruli and the glial borders that surround them. The time-dependent diffusion equations were solved in spherical coordinates using a finite-difference method. The results indicate that the glial envelope forms a significant barrier to the spread of K\(^+\) between neighboring glomeruli, thus reducing the likelihood of cross-talk between glomeruli, and may cause elevation of extracellular [K\(^+\)] to levels that influence neural activity within the activated glomerulus for many seconds. Such effects could enhance olfactory discrimination and sensitivity, respectively.

INTRODUCTION

In olfactory systems of most animal species that have been studied, first-order synaptic neuropils are arranged in spherical structures known as glomeruli. Within each glomerulus, thousands of receptor axons synapse with the dendrites of brain neurons in a fine, dense meshwork (Hildebrand and Shepherd 1997). The receptor axons terminating in each glomerulus respond to specific features of the molecules that make up an odor (Bozza and Kauer 1998; Hildebrand and Shepherd 1997). In fact, the axons of receptor neurons that express a particular olfactory receptor protein have been shown to terminate in just one to three specific glomeruli on each side of the brain, in mammals (Mombaerts et al. 1996) and in insects (Gao et al. 2000; Vosshall et al. 2000). Therefore individual glomeruli are thought to be the sites of processing of information about particular molecular features of odorants, and activation of many axons terminating in one glomerulus may occur simultaneously, possibly without direct stimulation of neighboring glomeruli.

In many species, olfactory glomeruli are surrounded by an incomplete layer of glial cell processes (Pinching and Powell 1971; Raisman 1985; Valverde and Lopez-Mascaráque 1991; Willey 1973). This glial envelope, relative to mammals and other species, is especially prominent in the moth Manduca sexta, where, in addition, glial processes are mostly excluded from the glomeruli (Oland et al. 1999; Tolbert and Hildebrand 1981). The glial cells enveloping the glomeruli are known to play an essential role in glomerulus development in Manduca (Oland and Tolbert 1987; Oland et al. 1995), but the effects of the glial envelope on mature information processing in this or any other olfactory system are not known. One possible role for the glial envelope rests on the finding by many investigators (e.g., Dietzel et al. 1989; Frankenheimer and Hodgkin 1956; Jendelova and Sykova 1991) that potassium ions (K\(^+\)) accumulate around active axons. In the present study, our goal was to examine the hypothesis that the glial envelope in Manduca significantly limits the spread of extracellular K\(^+\) released by receptor axons during activation of a glomerulus. Since increased levels of extracellular K\(^+\) can influence the excitability of neurons (Demir et al. 1998; Khayari et al. 1988b; Korn et al. 1978; McCormick and Contreras 2001; Nicholson 1995), we explore whether the role of the glial envelope as a barrier to diffusion is sufficient to influence the processing of olfactory information by limiting cross-talk between glomeruli and by affecting activity levels within active glomeruli (Khayari et al. 1988a). We tested our hypothesis by developing a mathematical model of diffusion of K\(^+\) within the glomerulus and across the glial envelope, and used experimentally derived values of biological parameters for our simulations. Our model predicts that extracellular K\(^+\) concentrations may rise significantly and remain high in activated glomeruli for several seconds after odor stimulation, thus providing a substrate for sustained ephaptic effects that outlast the stimulus.

METHODS

For the model, glomeruli were assumed to be spherical. The glial envelope was assumed to be a continuous structure covering the glomerulus except for a circular mouth region (Fig. 1). Receptor axons that innervate a glomerulus were assumed to extend to the midplane of the sphere because they are known to dominate roughly the outer half of each glomerulus, while dendrites of antennal-lobe neurons dominate the inner halves (Sun et al. 1993).
Diffusion of $K^+$ in the extracellular spaces was described by the following unsteady diffusion equation for porous media

$$\frac{\partial C}{\partial t} D^* \nabla^2 C \phi + q$$

(1)

where $C = \left[ K^+ \right]_{\text{out}}$ is the concentration in the extracellular space, $\phi$ is the porosity, i.e., the volume fraction of the extracellular space, $D^*$ is the effective diffusivity, which depends on the structure of the porous material, and $q$ represents the amount of solute released into the interstitial space per unit tissue volume per unit time. Neuronal and, for the glial border, glial $K^+$ uptake also could be incorporated into $q$, but were neglected here for reasons addressed in the discussion.

**Morphological measurements**

To determine the diameters of glomeruli and the size of the open mouth of the glial envelopes at the bases of the glomeruli, the glial envelopes surrounding 41 glomeruli in histological cross-sections through 4 antennal (olfactory) lobes were traced using a camera lucida microscope attachment. The histological sections, available from a previous study (Oland and Tolbert 1987), were from brains of two female *Manduca* taken near the end of metamorphic adult development (stage 16) (see Oland and Tolbert 1987; Tolbert et al. 1983), when glomeruli have reached their adult size. The brains had been fixed in 2.5% glutaraldehyde/0.5% paraformaldehyde, osmicated en bloc, dehydrated through a graded series of ethanols, embedded in Epon/Araldite, sectioned at 1 $\mu$m, and stained with toluidine blue. All glomeruli that, on examination of adjacent sections, appeared to be sectioned through their widest point and at an angle that gave the full width of the mouth were traced. The mean diameter of the glomeruli was 65 $\pm$ 14 $\mu$m (mean $\pm$ SD), and the mean width of the mouth openings was 25 $\pm$ 12 $\mu$m, giving an angle of the mouth opening of approximately $\pi/4$.

**Porosity and effective diffusivity of glomeruli**

To predict the diffusive spread of $K^+$, estimates were needed of the porosity $\phi$ and the effective diffusivity, $D^*$. The porosity could not be reliably estimated from the electron micrographs because the fixation procedure is expected to cause shrinkage of the extracellular regions, so a typical value of $\phi = 0.2$ was assumed, based on published values (Gardner-Medwin 1986; Latour et al. 1994; McBain et al. 1990; Nicholson and Phillips 1981). Direct measurements of $D^*$ are not available in this tissue. According to previous studies of diffusion in neural tissues (Nicholson and Phillips 1981), $D^*$ is given by

$$D^* = D_0 / \lambda^2$$

(2)

where $D_0$ is the diffusivity of the solute in water and $\lambda$ is the tortuosity of the medium. The tortuosity is related to the increase in path length for diffusion in a medium with complex geometry. However, no general theory is available for computing the tortuosity of a given porous medium based on path lengths for diffusion (Nicholson and Sykova 1998). Studies in several different regions of the brain have led to tortuosity estimates close to 1.6 (Gardner-Medwin 1980; Lehenekuhler et al. 1993; Nicholson 1992a,b, 1995; Nicholson and Phillips 1981), and this value will be assumed here. The diffusivity $D_0$ of $K^+$ in water at 25°C (a moderate environmental temperature) is $1.96 \times 10^{-5}$ cm$^2$/s (Hille 1984). Substitution of Eq. 2 into Eq. 1 gives

$$\frac{\partial C}{\partial t} = \frac{D_0}{\phi} \nabla^2 C + \frac{q}{\phi}$$

(3)

**Estimation of $K^+$ release**

The resting value of $\left[ K^+ \right]$ within the extracellular space of a glomerulus from *Manduca* is approximately 3 mM (Pichon et al. 1972). The release of $K^+$ into the extracellular space was assumed to result from a train of action potentials in the receptor axons filling the
hemisphere of a glomerulus opposite to the mouth opening (Fig. 1). Each action potential was assumed to release approximately \( A = 4 \times 10^{-12} \) moles of \( K^+ \) per \( cm^2 \) of membrane into the extracellular environment, based on data from squid axons (Adelman et al. 1973); variations in the amount of potassium released per action potential were not explored but would lead only to a proportional increase or decrease in the net accumulation of extracellular \( K^+ \). If each receptor axon in a glomerulus is electrically active over its entire surface area, the resulting rise in \( K^+ \) concentration in the extracellular space is \( A/(w/2) \), where \( w \) is the width of the extracellular spaces, since the membranes bounding both sides of the space release \( K^+ \).

The width of the extracellular spaces was estimated using the relationship \( \phi = (\pi/2)Nw \), where \( N \) is the mean number of extracellular spaces intersected per unit length of a randomly placed line in a cross-section of the structure. The factor \( (\pi/2) \) is required according to the Buffon Principle (Weibel 1979), because extracellular spaces are intersected at random angles, whereas the width \( w \) is measured perpendicular to the direction of the space, as observed in the cross-section. A line with scaled length \( 2 \mu m \) was placed at 90 randomly chosen locations across 9 electron micrographs of intraglomerular neuropil in thin sections of 3 Epon-Araldite embedded brains from pharate adult moths (magnifications of \( \times36,450 \) and \( \times45,900 \), from the study of Oland and Tolbert 1987) (Fig. 2), and \( N \) was found to be 2.5 per \( \mu m \), implying that \( w = 0.05 \mu m \) if \( \phi = 0.20 \). Therefore the estimated amount of \( K^+ \) released into the extracellular space per action potential is 1.6 mM.

Average firing rates of 10–20 action potentials per second have been recorded in olfactory receptor neurons in Manduca in response to puffs of odor (Marion-Poll and Tobin 1992). Instantaneous rates, however, can be much higher: odorant dose-response relationships show that the peak impulse frequency of moth receptor neurons ranges between 10 and 300 action potentials per second (Kaisissling 1996). Here, we assume a representative volley of 20 action potentials over a 500-ms period. Receptor neuron responses vary with concentration of the odor (Harrison and Scott 1986; Kaissling 1996), but the number of receptor neurons recruited to respond to an odorous stimulus is not known. For simplicity, we assume that all the axons are activated during a volley, giving an increase of \([K^+]_{out}\) of 1.6 mM per action potential. Because the governing equations are linear, activation of some but not all of the axons would simply lead to a proportional reduction in the increase of \([K^+]_{out}\) above its baseline level.
**Porosity and effective diffusivity of the glial envelope**

The porosity of the glial layer was assumed to be equal to that of the glomerulus, i.e., \( \phi_g = 0.20 \). The glial envelope is a highly anisotropic, layered structure (Oland et al. 1999; Tolbert and Hildebrand 1981). We based our estimates of the effective diffusivity of this layer on electron micrographs of the cross-sections of glial borders obtained from brains from two pharate adult animals, prepared specifically for this study using the fixation procedure outlined under **Morphological measurements**. Four montages were made from multiple micrographs taken at \( \times6,000 \) along four interglomerular borders from one section. In each montage, the extracellular pathways surrounding the glial processes in the glial layer were traced (Fig. 3). The glial border between adjacent glomeruli varied between 2 and 16 µm in thickness, with approximately 5 elongated layers of extracellular space per micrometer of thickness of the border at its thinnest parts. Measurements from a thin region were used, with a thickness \( h = 3 \) µm, to obtain an upper bound on the diffusion through the glial layer.

The electron micrographs show that the glial processes form sheets aligned with the boundary of the glomerulus, and that pathways for solute diffusing through the glial layer are typically much longer than the thickness of the layer, because a solute molecule must travel some distance along the gap between two sheets before reaching the edge of a sheet. To obtain an order-of-magnitude estimate of the effective diffusivity \( D_g^\text{eff} \), perpendicular to the glial border, including this effect, a two-dimensional analysis was used. In three dimensions, additional diffusional pathways would be available, but they would not be significantly shorter on average, because the layer is formed of nonaligned sheets as described above.

In the present analysis, the effective diffusivity was estimated by relating the observed structure to one that was geometrically deformed or “stretched” until it appeared isotropic (Fig. 4). The criterion for isotropy was that the distribution of the extracellular pathway lengths was uniform with respect to orientation angle in the plane. If a given difference in concentration is imposed across the original and the stretched structures, then

\[
\frac{\partial C}{\partial t} = \frac{h}{h_s} \left( \frac{\partial C}{\partial y} \right),
\]

where \( h \) and \( h_s \) represent the widths of the original and stretched structures. The path widths are assumed to be the same in both structures. However, path lengths are lower in the original structure by a factor of \( \cos \theta \), where \( \theta \) is the angle between the segment and the \( x \)-axis in the stretched structure, assuming that \( h_s \gg h \). Since the distribution of segment angles is assumed to be uniform in the stretched structure, the average ratio of path lengths in the original and stretched structures is \( 2/\pi \) (the average of \( \cos \theta \) on the interval 0 to \( \pi/2 \)). Therefore

\[
J = \frac{\pi}{2} J_s \quad \text{and} \quad \phi_g = \frac{2}{\pi} \phi_s
\]

where \( J \) and \( J_s \) are the fluxes under these conditions in the two structures and \( \phi_g \) and \( \phi_s \) are the glial layer porosities. For the isotropic stretched structure, the effective diffusivity is \( D_g^\text{eff} = D_g / \lambda^2 \) as in Eq. 2. Since in general \( J = -D \frac{\partial C}{\partial y} \), the effective diffusivity through the glial layer can be estimated using Eqs. 5 and 6 as

\[
D_g^\text{eff} = \frac{h}{h_s} \frac{\pi \phi_s}{2 \phi_g} D_g^s = \frac{\pi^2}{4} \left( \frac{h}{h_s} \right)^2 D_g^s
\]

Thus \( D_g^\text{eff} \) is proportional to \( (h/h_s)^2 \), indicating that a layered structure can provide a much larger barrier to diffusion than an isotropic one, for given values of \( \phi_g \) and \( D_g \). The numerical coefficient depends on the assumptions of the analysis. Because this is a two-dimensional...
analysis, the tortuosity $\lambda$ cannot be assumed to equal that observed experimentally in three-dimensional structures (i.e., 1.6). For the numerical results, a conservative estimate for the effect of tortuosity was used by setting $\lambda^2 = 2$. Higher tortuosity would further limit diffusion through the glial layer.

In the portion of the glial envelope that was examined, 98 extracellular path segments were identified, meeting at 73 nodal points (Fig. 4A). These diffusional pathways were represented by line segments, yielding the arrangement shown in Fig. 4B. This structure was geometrically stretched by factors $(h/h_s)^{-1}$ ranging from 1 to 40. The variance of the distribution density of segment angle, which measures the deviation from a uniform distribution with angle, is shown in Fig. 5 as a function of $(h/h_s)^{-1}$. The variance was minimal when $(h/h_s)^{-1} = 14$, and the resulting structure is shown in Fig. 4C. From Eq. 7, an estimate of the effective diffusivity of $K^+$ ions perpendicular to the glial layer is $D_{glia}^0 = 1.2 \times 10^{-7} \text{cm}^2/\text{s}$.

**Boundary conditions at the glial envelope**

The concentration was assumed to vary continuously at the boundary between the glial layer and the glomerulus. The boundary condition for the outer edge of the glial layer was assumed to have a form similar to Eq. 4

$$\left. \frac{\partial C}{\partial r} \right|_{r=R+h} = k_s (C - C_0)$$

(8)

The constant $k_s$ was estimated assuming a spherically symmetric decay of concentration, approaching the baseline value at infinity, resulting in $k_s = 1/(R + h)$. The results are not very sensitive to this assumption. The alternative assumption that $[K^+]_1$ reaches its baseline value at twice the radius of the glial border ($r = 2R + 2h$) was found to result in $<5\%$ decrease in the average concentration within the glomerulus at 3 s.

For comparison, three other hypothetical boundary conditions were also considered. The “no-glia boundary” represents the case when no glial layer is present. In this case, the glomerulus was assumed to be embedded in a larger sphere, with twice the diameter of the glomerulus, of material of the same porosity as the glomerular neuropil. The “impermeable boundary” represents the case in which the glial envelope is impermeable to $K^+$ ions, and so $\partial C/\partial r = 0$ there. The “buffer” represents the case in which the glial cells instantaneously regulate $[K^+]_{1\text{out}}$ to a constant level $C_p$, so that $C = C_0$ there at all times. These boundaries conditions are not considered to be realistic, but illustrate how the presence of the actual partially permeable layer affects the spread of $K^+$.

**Numerical methods**

The diffusion equation in spherical coordinates $(r, \theta, \phi)$ with axial symmetry takes the following nondimensionalized form, where $r$ is non-

$$\frac{\partial C}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 D \frac{\partial C}{\partial r} \right) + q'$$

(9)

where $q' = q/\phi$. Due to the symmetry of the problem, $\partial C/\partial \theta = 0$ when $\theta = 0$ and $\theta = \pi$. The alternating direction implicit method (Strikwerda 1989) was chosen for its low error and unconditional stability. If

$$\frac{\partial C}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 D \frac{\partial C}{\partial r} \right)$$

then Eq. 9 becomes

$$\frac{\partial C}{\partial t} = \frac{1}{r} \frac{\partial}{\partial r} \left( r D \frac{\partial C}{\partial r} \right) + q'$$

(10)

Equation 10 was expanded in Taylor series by using a Crank-Nicholson approximation, and the resulting equation was solved numerically using two alternating steps, one implicit in $r$ and the other implicit in $\theta$. Time steps of 0.001 s and a $20 \times 20$ grid in $(r, \theta)$ were used. Numerical simulations continued until the solution approached a
steady state. Because the diffusion equation is singular at the origin, a different treatment was required there. Following Smith (1985), the right-hand side of the time-dependent diffusion equation at the origin was approximated by

$$\nabla^2 C = \frac{6(C_M - C)}{\Delta r^2}$$

where $\Delta r$ is the increment in $r$, $C_M$ represents the mean concentration value at a distance $\Delta r$ from the origin, and $C_0$ is the concentration at the origin. The accuracy of the numerical scheme was tested by comparison with exact solutions in special cases, and the resulting maximal numerical errors were on the order of $10^{-4}$. In the general case, the governing equations could also be solved in terms of infinite series (Crank 1975). However, the discontinuous boundary conditions at the edge of the mouth would lead to poor convergence of such series solutions.

R E S U L T S

In simulated odor activation, all axons innervating a hemisphere of the glomerulus were assumed to produce 20 action potentials in a 500-ms period, each action potential causing $[K^+]_{out}$ to increase by 1.6 mM in the extracellular space. Predictions of $C_{avg}$, the spatial average of $[K^+]_{out}$ over the interior of the glomerulus, are shown in Fig. 6A as a function of time. The sawtooth fluctuations reflect the periodic release of $K^+$. Highest concentration levels are predicted when the glial envelope is impermeable to $K^+$, with average concentration above 10 mM after 10 s. If the glial envelope is permeable, the rise in concentration is slightly reduced, but still above 9 mM at 10 s. Much lower levels of $K^+$ are predicted if the glial envelope is absent or if it instantaneously buffers the $K^+$ concentration to its resting value.

Figure 6B shows the net amount of extracellular $K^+$ that escapes from the glial layer as a function of time. The spatial distribution of the resulting extracellular $K^+$ in the region outside the glomerulus depends on the arrangement of the neighboring glomeruli and cannot be predicted with the present model. The top curve indicates the total amount of $K^+$ released. With no glial layer, $K^+$ can spread to neighboring glomeruli with very little time delay. A partially permeable glial layer not only hinders the increase above baseline of extracellular $K^+$ values outside the glomerulus, but also delays this build up. The “impermeable” and “buffer” cases were excluded from this figure since in these two models, $K^+$ could spread only to neighboring glomeruli through the mouth region.

To investigate how the size of the mouth influences the results, the effect of increasing the mouth opening angle from $\pi/4$ to $\pi/2$ was considered (Fig. 7). For the larger mouth opening, the flux coefficient $k_m$ was found to be 0.02 $\mu m^2/s$. The diffusive flux of $K^+$ through the mouth is then increased and the decay of concentrations within the glomerulus is more rapid.

Predicted distributions of $[K^+]_{out}$ within the glomerulus are shown in Fig. 8. When the glial envelope is permeable as estimated above (Fig. 8A), $[K^+]_{out}$ is significantly elevated for several seconds following $K^+$ release. In the hypothetical absence of a glial envelope, $[K^+]_{out}$ would be continuous with neighboring glomeruli, and Fig. 8B implies that $[K^+]_{out}$ would increase significantly in neighboring glomeruli during the first second after the start of $K^+$ release. The decline of $[K^+]_{out}$ within a glomerulus would be much more rapid than it is in the presence of a glial layer, and $[K^+]_{out}$ would return to near baseline levels within 3 s. If the glial envelope is modeled as an impermeable barrier to $K^+$ (Fig. 8C), $[K^+]_{out}$ remains significantly elevated for more than 5 s following release, and decays relatively slowly as a result of diffusion through the mouth. Finally, if the glial cells instantaneously buffer $[K^+]_{out}$ holding it fixed at 3 mM (Fig. 8D), the glial cells would quickly remove the released $K^+$, and $[K^+]_{out}$ would return to near baseline within 1 s.

FIG. 6. A: variation with time of the average concentration ($C_{avg}$) over the glomerulus in mM. B: the increase of extracellular $K^+$ above its baseline value outside the glomerular/glial structure in units of femtomoles for the “no glia” and “partially permeable” cases.

FIG. 7. Variation with time of the average concentration ($C_{avg}$) over the glomerulus. Curves are labeled according to the mouth size for the partially permeable glial boundary.
2) An approximate analysis of diffusion through such a layer shows that the effective diffusivity for ions diffusing across the layer is much less than the diffusivity within the glomerulus itself.

3) Numerical simulation of the diffusion of K\(^+\) released by odor-stimulated receptor axons terminating in a glomerulus shows that the glial envelope can profoundly reduce the spread of K\(^+\) from the glomerulus in which it is released.

4) In the simulation most closely reflecting the biological situation (Fig. 8A), [K\(^+\)]\(_{\text{out}}\) in an activated glomerulus is predicted to be maintained, for seconds, at levels high enough to affect neural signaling in the glomerulus.

Our findings are likely to apply, at least qualitatively, to other species as well as Manduca, given the striking similarities in cellular organization of glomeruli across species. As reviewed by Boeckh et al. (1990) and Hildebrand and Shepherd (1997), glomeruli in a wide variety of species, both vertebrate and invertebrate, are between 20 and 200 \(\mu\)m diam and are surrounded by glial processes. Each olfactory receptor axon terminates in a single glomerulus; in the species in which olfactory receptor genes have been identified, the receptor neurons innervating a particular glomerulus express the same particular olfactory receptor genes (above reviews, and Gao et al. 2000; Vosshall et al. 2000). Measures of activation have revealed that individual glomeruli or small sets of glomeruli are activated by stimulation with particular odors. The glomeruli in Manduca fall in the middle of the size range for glomeruli, and their glial envelope is no thicker, but may be more complete (continuous) than those in mammals, where the cell bodies of small neurons are interspersed among the layers of glial processes (Pinching and Powell 1971; Willey 1973). In mammals, unlike Manduca, a small number of glial cell bodies reside in the glomeruli (Pinching and Powell 1971), but even here, the glial investment of neural processes is less than in other neuropil areas, and receptor axons branching within glomeruli are devoid of any glial coverings (Valverde and Lopez-Mascaraque 1991).

Assumptions

As in any theoretical model, our predictions depend on the underlying assumptions and bases for estimation of values for biological parameters. We chose values based on experimental data, where they could be taken directly from histological, electron microscopical, or electrophysiological observations in Manduca, or from published results in the other systems most closely related to Manduca. The assumptions for the simulations we present are that 1) the glomerulus is a sphere containing a homogeneous, isotropic system of extracellular pathways; 2) the glial envelope surrounding each glomerulus is a thin, highly anisotropic, multilayered structure; 3) the mouth of the glomerulus is a particular size and offers a path for diffusion that has the same porosity as that of glomerular neuropil; 4) receptor axons terminate in one-half of the glomerulus; 5) all receptor axons providing input to a particular glomerulus are activated by a physiologically relevant level of odor concentration; and 6) all release a particular amount of K\(^+\) per action potential. Also, we argue that 7) a glial boundary that presents primarily a physical barrier for K\(^+\) diffusion, with no significant cellular uptake of K\(^+\) on the time scale of fractions of...
seconds to seconds, provides the most biologically relevant simulation.

In general, as outlined below, our assumptions and estimations of values for parameters were designed where possible to err on the side of minimizing the possible effects of the glial border on $K^+$ movement between glomeruli. Thus any predicted effect would be a conservative estimate.

1) Glomeruli in many species are roughly spherical, and in Manduca they are especially rounded. In the central part of the glomerulus, no systematic variation in the appearance of the cross-sections of neural processes has been seen (Tolbert 1988; Tolbert and Hildebrand 1981). Therefore isotropy of the extracellular pathways was assumed for simplicity. In fact, the fine branches of olfactory receptor axons and of the dendrites of antennal-lobe neurons (Sun et al. 1993, 1997) are preferentially oriented near the poles of the glomerulus where axons and dendrites enter and exit. Such an orientation will tend to bias flow of $K^+$ toward the mouth of the glomerulus, enhancing the inhibition of spread laterally to adjacent glomeruli. However, there is evidence for only short, incomplete tight junctions between glial processes (Oland et al. 1990; Tolbert and Hildebrand 1981), suggesting that a tight barrier is not able barrier would greatly reduce the spread of ions (Fig. 8D). A number of experiments in other species have revealed that potassium buffering by glial cells is a much slower process. In the optic nerve of the mudpuppy, the internal glial $[K^+]$ reached a plateau value a full 30 s after the $[K^+]$ of the bathing solution was increased abruptly, and returned to its resting level only after an additional 2 min (Kuffler et al. 1966). Studies in other species have also indicated that the uptake of $K^+$ by glial cells has too slow a time course to affect neuronal excitability significantly after a single 500-ms barrage of action potentials (Ballanyi et al. 1987; Gardner-Medwin et al. 1979; Walz and Hinks 1985; Wuttke 1990). Therefore $K^+$ uptake by glial cells was neglected in the "partially permeable" model. Neuronal $K^+$ uptake within the glomerulus was similarly neglected, because the primary $K^+$ uptake mechanism in neurons, the $Na^+/K^+$ pump, begins to absorb released $K^+$ ions after several seconds and produces a maximum effect only after tens of seconds (Amédée et al. 1997; Gardner-Medwin et al. 1986; Newman 1995). Future use of the model to study $K^+$ accumulation in situations mimicking more prolonged odor stimulation should incorporate both neuronal and glial $K^+$ uptake.

Implications for information processing in olfactory glomeruli

Fluctuations in $[K^+]_{out}$ have been postulated by many investigators to mediate ephaptic communication between neurons, in which currents originating in one cell directly alter the membrane potential in nearby cells (Grundfest 1959; Jefferys 1995). For example, such $K^+$-mediated ephaptic communication between vestibular hair cells and their ciliary nerve endings is thought to affect transmission from the hair cell to the calyx (Goldberg 1996), and between neurons in the mammalian hippocampus and areas of cerebral cortex is thought to underlie epileptic seizures (Demir et al. 1998; McCormick and Contreras 2001). Elevations of $[K^+]_{out}$ may increase excitability by depolarizing neurons, thereby bringing them closer to threshold, or may decrease neuronal excitability, via changes in threshold that may follow an effect on sodium channel inactivation (see Demir et al. 1998; Khayari et al. 1988b; McCormick and Contreras 2001).

Glial cells in many parts of the brains in many species form loose sheaths around synapses or synaptic complexes (Peters et al. 1991) that may influence synaptic function (Bacci et al. 1999). An exaggerated example of this type of sheath occurs at the "axon cap" of the Mauthner neuron in the goldfish, where glial processes encapsulate the axon hillock. Activation of fine axons that spiral around the Mauthner axon beneath the cap leads to a focal hyperpolarization of the Mauthner axon membrane and a rise in its threshold for excitation (Furukawa and Furshpan 1963), apparently due to trapping of extracellular $K^+$ by the glial capsule (Korn et al. 1978).

How large a change in $[K^+]_{out}$ is needed to affect neuronal excitability? In the giant axon of the cockroach, which has a resting $[K^+]_{out}$ of approximately 5 mM, a blockage of neuronal excitability occurred when $[K^+]_{out}$ reached 14 mM (Grossman and Gutnick 1981; Hendy and Djamgoz 1987). Reduction in the efficacy of synaptic transmission was shown to occur in rat hippocampal slices at 5 mM (where the resting value of $[K^+]_{out}$ was 3 mM) (Newman 1995).

If the same percentage increase in $[K^+]_{out}$ were to decrease
the excitability of neuronal processes in *Manduca* olfactory glomeruli, a reduction of the excitability of processes in the glomeruli would be seen when \([K^+]_{\text{out}}\) reached 5 mM, and a conduction blockage would be seen when \([K^+]_{\text{out}}\) reached 9 mM. Figure 8 shows that the predicted levels of \([K^+]_{\text{out}}\) in the glomerulus probably far exceed 9 mM in response to an input volley of 20 action potentials in 500 ms. Therefore a blockage in the conduction of action potentials by olfactory receptor axons innervating glomeruli would be expected as early as 500 ms after the initial input, and lasting for several seconds. The finding of Wachowiak and Cohen (1999) that action potentials failed to propagate into olfactory receptor axon terminals in the turtle and lobster when paired pulses were given at 300- to 400-ms interstimulus intervals may be explained by this type of phenomenon. In moths, however, there is experimental evidence that receptor axons are able to fire at high frequency for longer than 10 s during high levels of olfactory stimulation (Kaisling 1996; Kaisling et al. 1989; Marion-Poll and Tobin 1992). It seems more likely therefore that high \([K^+]_{\text{out}}\) in *Manduca* serves to increase excitability in these axons. If so, the effect of glial envelopes on the accumulation of \(K^+\) in glomeruli may be to enhance the firing of axons in the stimulated glomerulus and therefore to enhance sensitivity to the stimulating odorant.

Effects on excitability could be mediated via an influence of high levels of \([K^+]_{\text{out}}\) on \(E_K\). We can assume that in axons, \([K^+]_{\text{in}}\) does not change significantly with a volley of action potentials because the volume of the axons is large compared with that of the extracellular space. Taking \([K^+]_{\text{in}}\) to be 150 mM in *Manduca* (Hayashi and Levine 1992), \(E_K\) changes by a factor of approximately 0.5 when \([K^+]_{\text{out}}\) is increased from 3 to 8 mM. Thus at rest, \(E_K = -98.5\) mV and at peak \(E_{Kc} = -53\) mV.

Output from the glomeruli is carried to higher brain centers by projection neurons. Individual projection neurons in *Manduca* have different maximum following frequencies and therefore convey different aspects of the timing of odorant stimulation. Whereas some projection neurons continue to fire intermittently in response to pulsed odorant stimuli delivered at 10/s, others lose their intermittency of firing at much lower pulse rates (Christensen and Hildebrand 1989; Christensen et al. 1996). Perhaps different subtypes of projection neurons have different sensitivities to accumulation of extracellular \(K^+\) ions. In addition, perhaps some projection neurons extend processes deeper into the portion of the glomerulus that comprises mainly receptor axons, and therefore are exposed to the highest values of \([K^+]_{\text{out}}\).

Whether elevated \([K^+]_{\text{out}}\) raises or lowers neuronal excitability, a general role for localized elevations of \([K^+]_{\text{out}}\) may be to increase synchronization of neuronal firing in response to stimulation. Using multunit recording methods in the antennal lobe of *Manduca*, Christensen and Hildebrand (1989) found that correlation between action potentials in different antennal-lobe interneurons is high early in the response to a pulse of odor, when olfactory receptor axons fire at the highest rates, and that pulsatile stimuli evoke greater degrees of coactivity in ensembles of neurons than do continuous stimuli. This synchrony may enhance the representation of the eliciting stimulus over representations of other stimuli, assisting in discrimination of stimuli (Buracas and Albright 1999). Synchronous firing of neurons also undoubtedly underlies the approximately 50-Hz oscillatory activity ubiquitous in both vertebrate and invertebrate olfactory centers (Wehr and Laurent 1996) following odor stimulation; the synchronization appears to be important for olfactory sensitivity (Stopfer et al. 1997), but mechanisms for the synchronization are still poorly understood.

In summary, we have shown that the presence of a specialized envelope of glial processes around olfactory glomeruli might have significant effects on the levels of extracellular \(K^+\) occurring in the glomerulus and in the surrounding tissue in response to activation of olfactory receptor neurons by a specific odor. These effects include an increase in the duration of elevated \([K^+]_{\text{out}}\) within the glomerulus following release, and a decrease in the amount reaching other glomeruli. Since extracellular \(K^+\) influences neuronal function, such effects may contribute to enhancement of sensitivity and discrimination in the olfactory system. The results suggest a need for further experimental work to measure levels of extracellular \(K^+\) in glomeruli, and to determine the effects of elevated \(K^+\) levels on the excitability of olfactory receptor axons and their target neurons in the glomeruli.

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REFERENCES


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